Iraqi Journal of Market Research and Consumer Protection
IJMRCP

SCIENTIFIC REFERRED JOURNAL (Semi-annual)
Publishes subjects related to the market research and consumer protection

Iraqi Journal of Market Research and Consumer Protection
Published by
Market Research and Consumer Protection Center
University of Baghdad – Iraq

Vol. (16) No. (2) Year (2024)

ISSN: 2071-3894
E-ISSN: 2523-6180
DOI prefix: 10.28936
Baghdad, 1373/2010

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وزارة التعليم العالي والبحث العلمي
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IRAQI JOURNAL OF MARKET RESEARCH AND CONSUMER PROTECTION

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Information of...
Iraqi Journal of Market Research and Consumer Protection

About:

Scientific refereed journal, accredited for the purposes of scientific promotions, published by market research and consumer protection center, university of Baghdad, republic of Iraq by two numbers per year.

Specialty:

The journal publishes scientific researches in the field of social sciences that are directly related to the market and consumer in various fields including public health, marketing, economics, environment, agriculture, pure science and media.

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The journal was established on 26/8/2008 as one of the scientific refereed journals of the university of Baghdad, and since that date the journal has taken important steps through continuous development until it took its form and its current organizational structure as a specialized journal that publishes applied researches and studies in the field of social sciences that are directly and indirectly related to the market and consumer.

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To enhance the level of scientific research in the field of market research and consumer protection and to achieve a distinguished international position through publish of scientific research in this field that aimed at to serving and developing the society.

Message:

To provide the appropriate journal and scientific references for researchers which working in the field of market research and consumer protection through the publication of scientific research in this field in English languages with emphasis on strict adherence to the standards used in scientific publishing.

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(ISSN: 2071-3894):
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(E-ISSN: 2523-6180):
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Iraqi Journal of
Market Research and Consumer Protection
IJMRCP
Vol. 16 No. 2 Year 2024

Issue contents.

Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 1-10
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(1)
Saleh & Al Bahrani

المجلة العراقية لبحوث السوق
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RESPONSE OF CABBAGE PLANTS TO APPLICATION OF NATURAL ZEOLITE, ORGANIC AND MINERAL FERTILIZERS

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Received 26/ 4/ 2023, Accepted 14/ 5/ 2023, Published 31/ 12/ 2024

ABSTRACT

This experiment was conducted in one of fields belong to College of Agricultural Engineering Sciences/ University of Baghdad –Station Research (F) in Jadriya. to find out effect of natural zeolite, organic fertilizer (poultry manure) and mineral fertilizers and their interactions, "Dounia" cabbage growth and yield, a field experiment was carried out in fall season 2021and the number of plants in the experimental unit was 20 plants. Treatments were replicated three times at factorial design in a RCBD. Factors of study experiment included addition of three levels of natural zeolite (without addition (Z0), 5 Mg.ha^{-1} (Z5), and 10 Mg.ha^{-1} (Z10)), while poultry manure was added with three levels; without addition (O_0) , 5 Mg.ha^{-1} (O5), and 10 Mg.ha^{-1} (O10), As for mineral fertilizer treatments, they were added in three levels (50%, 75%, and 100% of complete fertilizer recommendation), which were 100 kg ha^{-1} N, 150 kg ha^{-1} P, and 150 kg ha^{-1} K, which are denoted by symbols M50, M75, and M100, respectively. The experimental results showed that natural zeolite at 10 Mg. ha^{-1} (Z10) significantly increased in shoot dry weight of 113.74 gm plant^{-1}, root dry weight of 37.07 gm plant^{-1} and total yield of 27.66 Mg. ha^{-1}. Results also shows that poultry manure at 10 Mg. ha^{-1} (O10) gave highest shoot dry weight of 105.33 g. plant^{-1}, highest root dry weight of 31.38 g. plant^{-1}, highest leaf chlorophyll content of 174.37 mg 100 g plant^{-1} and highest total yield of 27.98 Mg ha^{-1}. As for addition of mineral fertilizers, data cleared that, addition at 100% of complete fertilizer recommendation (M_{100}) gave highest shoot dry weight of 109.24 g. plant^{-1}, highest root dry weight of 33.02 g. plant^{-1} , highest leaf chlorophyll content of 172.07 mg 100 g plant^{-1} and highest total yield of 27.94 Mg ha^{-1}.

Keywords: Cabbage Plants, Natural Zeolite, Mineral Fertilizers.

استجابة محصول اللهانة لأضافة الزيولايت الطبيعي والأسمدة العضوية والمعدنية

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الخلاصة

أجريت هذه التجربة في أحد الحقول التابعة لكلية علوم الهندسة الزراعية، جامعة بغداد- محطة البحوث (F) في الجادرية. لمعرفة تأثير الزيولايت الطبيعي والسماد العضوي (مخلفات الدواجن) والأسمدة المعدنية وتداخلاتها في بعض خصائص التربة ونمو وحاصل اللهانة صنف "دنيا" أجريت تجربة حقلية في فصل الخريف 2021. صممت التجربة تحت تصميم القطاعات الكاملة المعشاة وبتصميم التجربة العاملية RCBD. وبثلاث مكررات اذ تضمنت عوامل تجربة

الدراسة إضافة ثلاثة مستويات من الزيولايت الطبيعي (بدون إضافة (Z_0) ، 5 ميكاغرام هـ$^{-1}$ (Z_5) ، 10ميكاغرام هـ $^{-1}$ (Z_{10})، بينما أضيفت مخلفات الدواجن بثلاثة مستويات. بدون إضافة (O_0) و 5 ميكاغرام هـ $^{-1}$ (O_5) و 10 ميكاغرام هـ$^{-1}$ (O_{10}) أما معاملات الأسمدة المعدنية فقد تمت إضافتها على ثلاثة مستويات (50% ، 75% ، 100%) من التوصية السمادية الكاملة، والتي كانت 100 كغم N هـ $^{-1}$و 150 كغم هـ $^{-1}$و 150 كغم Kهـ$^{-1}$، والتي يشار إليها بالرموز M50 وM75 و M100 بالتتابع. أظهرت نتائج التجربة أن الزيولايت الطبيعي عند 10 ميكاغرام هكتار (Z_{10}) زاد معنوياً في الوزن الجاف للنبات عند 113.74 غرام نبات $^{-1}$ والوزن الجاف للجذور 37.07 غرام نبات $^{-1}$ والحاصل الكلي 27.66 ميكا غرام هكتار$^{-1}$. أظهرت النتائج أيضًا أن مخلفات الدواجن عند 10 ميكاغرام هكتار (O_{10}) أعطى أعلى وزن جاف للمجموع الخضري بمقدار 105.33 غم نبات $^{-1}$، وأعلى وزن جاف للجذور بلغ 31.38 غم نبات $^{-1}$، وأعلى محتوى من الكلوروفيل للأوراق بلغ 174.37 ملغم 100 غم. نبات $^{-1}$ وأعلى حاصل بلغ 27.98 ميكاغرام هـ$^{-1}$. أما بالنسبة لإضافة الأسمدة المعدنية ، فقد بينت النتائج أن الإضافة بنسبة 100٪ من التوصية الكاملة للأسمدة (M100) أعطت أعلى وزن جاف للنبات 109.24 غم نبات $^{-1}$، وأعلى وزن جاف للجذور 33.02 غم نبات$^{-1}$، وأعلى محنوى من الكلوروفيل 172.07 ملغم 100 غم نبات $^{-1}$ وأعلى محصول إجمالي 27.94 ميكاغرام هكتار $^{-1}$

الكلمات المفتاحية: نبات اللهانة، الزيولايت الطبيعي، الاسمدة المعدنية.

INTRODUCTION

The Cabbage (*Brassica oleracea var. capitata* L.) is one of the main winter vegetable crops in Iraq. It is grown in most of its regions and belongs to the cruciferous family, as the part that is eaten from it is the wrapped leaves, which are fresh and may be used in making pickles and salad. Zeolite is a natural material that was discovered in 1756, it is one of sedimentary minerals, and it consists of aluminosilicates that are distinguished by their crystalline structure in form of three dimensions. Natural zeolite used as a natural fertilizer in traditional and protected agriculture, it improves soil properties and provides a good environment for plant growth and increased productivity, it has an importance in slow liberation for nutrients adsorbed and thus ensures continued supply of nutrients to plant during its growth stages, it also preserves soil moisture and increases microorganisms activity and decomposition of organic matter added to soil, and addition of zeolite helps plant to get rid of free radicals that form as a result of plant's exposure to stress (**Karami *et al.*, 2020**) .natural zeolite affect the growth and yield of plants, **Hasin *et al.*, (2021**) found a significant increase in yield of tomato (*lycopersicon esculentum* Mill), jasmine cultivar, when adding three levels of zeolite (0, 5, and 10 ton ha^{-1}), especially when adding at level 10 ton ha^{-1}. **Nooprom *et al.*, (2022**) conducted a study on broccoli plants (*Brassica oleracea* var. italica), Top Green variety grown in field, by treating plants with five levels of zeolite (0, 1,56, 3,12, 4,68, and 6,24 ton ha^{-1}) in which it was concluded that addition of levels 4.68 and 6.24 ton ha^{-1} led to an increase in growth and yield and a significant increase in stem diameter and a 50% reduction in flowering time, head diameter and total yield, compared to control treatment. Organic farming is one of most important modern practices in plant production, since organic fertilization is one of important ways to supply plants with needs of nutrients without any negative impact on environment and increase from it does not lead to plant damage that occurs when fertilizing with mineral fertilizers in large quantities. It also results from organic matter disintegration (Humic acid), which is important in plant nutrition by increasing soil mineral availability, and developing root system, which encourages its absorption and increases enzymatic activity and cell division. The organic matter also has a role in plant growth and yield, whether it is added to soil or foliar spray (**Muslat & Musleh, 2015; Nardi *et al.*, 2016**). Poultry manures are used widely and in large quantities compared to other animal wastes, because they are high in

nutrients, especially nitrogenous, compared to other animal wastes, and they are a source of macro and micronutrients, also, these remains do not contain slow-dissolving substances such as lignin and therefore, it facilitates the transformation of nitrogen into a ready-to-absorb form, which makes it a source that continues to provide the plant with this element throughout the growing season. Poultry manure content of high organic material and therefore will work when added to an increase of soil ability to retain water and nutrients and reduce their losses by washing (**Ali, 2012; Al-Hadethi, 2019**). Poultry manures affect the growth and yield of plants, Asomah et al (2021) found in an experiment that included adding poultry manure alone at a rate of 40-ton ha^{-1} or in combination with chemical fertilizers N, P, K to soil in cabbage growth, a significant superiority was obtained in vegetative plant growth characteristics and the studied yield characteristics. **Yeasmin *et al.*, (2021)** found in an experiment to find out effect of adding different organic fertilizers (poultry and cow waste and mustard husk residue and triple interaction between them) as well as control treatment on growth and yield of cauliflower plant, level of adding poultry waste was 15 ton ha^{-1}, The results showed that addition of poultry manure, either alone or by triple interaction, caused a significant superiority in all vegetative growth characteristics, and yield compared to control treatment.

The readiness of nutrients required for plants in sufficient quantities ensures a high amount of yield per unit area, as addition of chemical fertilizers may cause an increase of 50% or more in yield, provided that other growth factors are fully available. Because of this main effect of chemical fertilization, use of these fertilizers has increased, especially in irrigated agriculture, without thinking of any other considerations. This unscientific use of chemical fertilizers will be uneconomical and negatively affect plants. Due to side effects that chemical fertilizers may cause, they may be negative for humans, environment and living organisms. On which they live, alternative materials have been resorted to from natural compounds that can perform an effect similar to that of mineral fertilizers. The aim here is to reduce their use by relying on various mechanisms and strategies, including the addition of organic fertilizers (**Ali *et al.*, 2014**). Several studies have been conducted to determine role of chemical fertilizers in growth and yield of plants, **Ali &Al-Bahrani (2022**) mentioned that 100% of the fertilizer recommendation caused a significant effect in total yield of Cauliflower, Megha cultivar. **Al-Temimi & Al-Hilfy (2022)** studied the effect of five fertilizer treatments including 100 % and 50 % of the fertilizer recommendation on three varieties of maize (bohooth 5018, Baghdad 3 and Sumer) and found the treatment of 100% mineral fertilizer was superior in weight of 500 grains. The research aims to determine best level of zeolite for its significant effect on some soil properties (as a natural improver) and its reflection on growth and yield. And effectiveness of zeolite and organic fertilizers in reducing mineral fertilizers added to the soil.

MATERIALS AND METHODS

This experiment was conducted in one of fields belong to College of Agricultural Engineering Sciences / University of Baghdad –Station Research (F) in Jadriya. To find out effect of natural zeolite, organic fertilizer (poultry manure) and mineral fertilizers and their interactions on some soil characteristics, "Dounia" cabbage growth and yield, a field experiment was carried out in mixed clay soil classified at level of Typic Torrifluvent for fall season 2021. Treatments were replicated three times at factorial design in a RCBD. Factors of study experiment included addition of three levels of natural zeolite (without addition (Z0), 5

Mg.ha^{-1} (Z5), and 10 Mg.ha^{-1} (Z10)), while poultry manure was added with three levels; without addition (O_0) , 5 Mg.ha^{-1} (O5), and 10 Mg.ha^{-1} (O10), and added organic fertilizer and natural zeolite before planting, mixed with the soil, As for mineral fertilizer treatments, they were added in three levels (50%, 75%, and 100% of complete fertilizer recommendation), which were 100 kg ha^{-1} N, 150 kg ha^{-1} P, and 150 kg ha^{-1} K, which are denoted by symbols M50, M75, and M100, respectively. Nitrogen was added in urea form (46% N) in two batches, Ten days after planting, and second batch 20 days after first batch. Phosphate fertilizer was added in triple superphosphate (20% p) in one batch when planting and by feeding method. Potassium fertilizer was added in form of potassium sulfate (42% K) and two batches 10 days after planting and second batch 20 days after first batch. Drip system was used to irrigate the field. The following parameters were determined in experimental season:

1. Shoot and root dry weight (g): 5 plants were taken from each experimental unit and shoot was separated from root, after which shoot and root were washed well, then samples were cut and placed in paper bags to be dried in electric oven at 65 ° C until the weight was stable, then dry weight was calculated (**Al Sahaf, 1989**).
2. Leaf chlorophyll contents (mg.100g-1 fresh weight): content of fresh leaves of chlorophyll a and b was calculated, and then total chlorophyll in shoots before maturity was calculated. A weight of 5 gm was taken from the fresh leaves (fourth and fifth), fully ripe and free from any physiological injury, and cut into small pieces and mashed in a clean ceramic mortar by adding acetone at a concentration of 85% in the range of 2-10 ml, then the volume was added to 50 ml. filtrate was separated using a centrifuge for 10 minutes, process was repeated, extract was collected in tubes covered with opaque paper to block light from chlorophyll to prevent photo-oxidation of the dye. Then optical density of the filter was measured using a spectrophotometer at two wavelengths of 663 and 645 nm according to mackinny (1941).
3. Yield (µg ha^{-1}) Total yield of one experimental unit was calculated by calculating harvest yield cumulatively for each experimental unit and then attributed to hectares by following formula: Product of experimental unit (µg) x 10000 m^2 / experimental unit area (m^2).

The obtained results were subjected to analysis of variance according to (**Elsahookie & Wuhaib, 1990**) using L.S.D 0.05 for comparing differences between various treatment means.

RESULTS AND DISCUSSIONS

Effects of Natural Zeolite, Organic and Mineral Fertilizers and their interaction on shoot and root dry weight:

Data concerning effect of treatments on shoot and root dry weight are listed in (Tables 1 and 2). The data cleared that, natural zeolite at 10 µg. ha^{-1} (Z10) significantly increased in shoot dry weight of 113.74 gm plant^{-1} and root dry weight of 37.07 gm plant^{-1}, while lower values of these traits was in Z_0 treatment. Tables (1 and 2) also shows that poultry manure at 10 µg. ha^{-1} (O10) superiority of control treatment and gave highest shoot dry weight of 105.33 g. plant^{-1} and highest root dry weight of 31.38 g. plant^{-1}. Also, lower values of these traits were in control treatment (O_0). As for addition of mineral fertilizers, data cleared that, addition at 100%

of complete fertilizer recommendation (M_{100}) superiority of M_{50} and gave highest shoot dry weight of 109.24 g. plant^{-1}and highest root dry weight of 33.02 g. plant^{-1}. The interactions between poultry manure and natural zeolite significantly affected in shoot and root dry weight especially interaction treatment ($Z_{10}M_{10}$). The interactions between natural zeolite and mineral fertilizers significantly affected especially when interaction treatment ($Z_{10}M_{100}$) and gave 122.95 g. plant^{-1} as shoot dry weight and 42.62 g. plant^{-1} as root dry weight. As for adding poultry manure and their interaction with mineral fertilizers, data in tables (1 and 2) cleared that ($O_{10}M_{100}$) treatment gave highest shoot dry weight of 114.45 g. plant^{-1} and root dry weight of 35.81 g.plant^{-1}, while lower values of these traits was in O_0M_{50} treatment. Triple interactions between study factors had a significant effect in shoot and root dry weight specially $Z_{10}O_{10}M_{100}$ treatment.

Table (1): effect of natural zeolite, organic and mineral fertilizers on shoot dry weight (g. plant^{-1}) of cabbage plants.

Zeolite	Poultry manure (O)	Mineral fertilizer (M)			Z × O
		M_{50}	M_{75}	M_{100}	
Z_0	O_0	85.22	86.50	90.31	87.34
	O_5	85.72	90.80	91.70	89.40
	O_{10}	86.11	89.15	95.00	90.08
Z_5	O_0	86.58	88.90	105.02	93.50
	O_5	92.71	100.61	112.62	101.98
	O_{10}	94.43	105.63	119.70	106.58
Z_{10}	O_0	98.54	112.83	117.12	109.49
	O_5	95.33	118.81	123.07	112.40
	O_{10}	109.05	120.33	128.67	119.35
L.S.D 0.05		12.06			6.96
Z × M					Z
Z_0		85.68	88.81	92.33	88.94
Z_5		91.24	98.38	112.44	100.68
Z_{10}		100.97	117.32	122.95	113.74
L.S.D 0.05		6.96			4.02
O × M					O
O_0		90.11	96.07	104.15	96.77
O_5		91.25	103.40	109.13	101.26
O_{10}		96.53	105.03	114.45	105.33
L.S.D 0.05		6.96			4.02
M		92.63	101.50	109.24	
L.S.D 0.05		4.02			

This increase in dry weight may be due to added nitrogen content of soil as a result of zeolite retaining nitrogen and preventing its loss from soil, and then this led to an increase in its uptake by plant, which led to an increase in growth rate (**Sarkar & Naidu, 2015**). As nitrogen has a fundamental importance in building plant protein, thus increasing dry weight of vegetative and root system. The reason is also attributed to fact that enhancing nutrition with mineral fertilizers leads to an increase in root surface area, and then an increase in nutrients and water absorption, and this leads to an increase in total root dry mass. These results are consistent with the findings of **Al-Mharib *et al.*, (2020)** on kohlrabi and with **Al-Ubaidy *et al.*, (2019**) on red cabbage.

Table (2): effect of natural zeolite, organic and mineral fertilizers on root dry weight (g. plant^{-1}) of cabbage plants.

Zeolite	Poultry manure (O)	Mineral fertilizer (M)			Z × O
		M_{50}	M_{75}	M_{100}	
Z_0	O_0	18.48	19.96	22.81	20.41
	O_5	18.93	21.73	25.31	21.99
	O_{10}	20.52	24.18	27.19	24.14
Z_5	O_0	19.42	22.84	27.72	23.23
	O_5	23.67	24.52	31.39	26.52
	O_{10}	26.32	29.21	34.94	30.15
Z_{10}	O_0	25.34	36.12	38.76	33.43
	O_5	32.44	37.06	43.79	37.67
	O_{10}	35.35	39.43	45.32	40.03
L.S.D 0.05		4.11			2.37
Z × M					Z
Z_0		19.31	21.95	25.10	22.12
Z_5		23.13	25.52	31.35	26.66
Z_{10}		31.07	37.53	42.62	37.07
L.S.D 0.05		2.37			1.37
O × M					O
O_0		21.11	26.30	29.67	25.72
O_5		25.01	27.77	33.49	28.75
O_{10}		27.39	30.94	35.81	31.38
L.S.D 0.05		2.37			1.37
M		24.50	28.33	33.02	
L.S.D 0.05		1.37			

Effects of Natural Zeolite, Organic and Mineral Fertilizers and their interaction on leaf chlorophyll content and total yield: The data in Tables (3 and 4) cleared that, addition of zeolite did not significantly affect leaf chlorophyll content, while natural zeolite at 10 µg. ha^{-1} (Z10) significantly increased in total yield of 27.66 µg. ha^{-1}, while lower values of total yield was in Z_0 treatment. Tables (3 and 4) also shows that poultry manure at 10 µg. ha^{-1} (O10) superiority of control treatment and gave highest leaf chlorophyll content of 174.37 mg 100 g $plant^{-1}$ and highest total yield of 27.98 µg ha^{-1}. Also, lower values of these traits were in control treatment (O_0). As for addition of mineral fertilizers, data cleared that, addition at 100% of complete fertilizer recommendation (M_{100}) superiority of M_{50} and gave highest leaf chlorophyll content of 172.07 mg 100 g $plant^{-1}$ and highest total yield of 27.94 µg ha^{-1}. The interactions between poultry manure and natural zeolite significantly affected in leaf chlorophyll content and total yield especially interaction treatment ($Z_{10}M_{10}$). The interactions between natural zeolite and mineral fertilizers significantly affected especially when interaction treatment ($Z_{10}M_{100}$) and gave 176.39 mg.100 g. $plant^{-1}$ as leaf chlorophyll content and 32.50 µg ha^{-1} as total yield. As for adding poultry manure and their interaction with mineral fertilizers, data in tables (3 and 4) cleared that ($O_{10}M_{100}$) treatment gave highest leaf chlorophyll content of 178.25 mg 100 g $plant^{-1}$ (Abdel Rahim and Mohammed, 2020) and highest total yield of 31.01 µg ha^{-1}, while lower values of these traits were in O_0M_{50} treatment. Triple interactions between study factors had a significant effect in leaf chlorophyll content and total yield specially $Z_{10}O_{10}M_{100}$ treatment, and it's gave 186.89 mg 100 g $plant^{-1}$ and 34.90 µg ha^{-1}, respectively.

Table (3): effect of natural zeolite, organic and mineral fertilizers on Leaf chlorophyll contents (mg.$100g^{-1}$ fresh weight) of cabbage plants.

Zeolite	Poultry manure (O)	Mineral fertilizer (M)			Z × O
		M_{50}	M_{75}	M_{100}	
Z_0	O_0	152.29	161.89	163.05	159.07
	O_5	164.71	167.81	171.89	168.14
	O_{10}	163.41	170.64	175.21	169.75
Z_5	O_0	154.81	159.11	166.54	160.15
	O_5	165.11	162.49	170.10	165.90
	O_{10}	169.71	174.50	172.66	172.29
Z_{10}	O_0	154.65	156.68	170.96	160.76
	O_5	153.49	159.32	171.31	161.37
	O_{10}	176.32	180.01	186.89	181.07
L.S.D 0.05		9.54			5.51
Z × M					Z
Z_0		160.14	166.78	170.05	165.66
Z_5		163.21	165.37	169.77	166.12
Z_{10}		161.49	165.34	176.39	167.74
L.S.D 0.05		5.51			N.S
O × M					O
O_0		153.92	159.23	166.85	160.00
O_5		161.10	163.21	171.10	165.14
O_{10}		169.81	175.05	178.25	174.37
L.S.D 0.05		5.51			3.18

M	**161.61**	**165.83**	**172.07**	
L.S.D 0.05	**3.18**			

The increase in yield is due to zeolite role as an improver of some fertile and physical soil characteristics, and then an increase in yield, thanks to its unique properties, the ion exchange properties of zeolite can be used in agriculture due to its large porosity and high ability to exchange cations (**Kavvadias *et al.*, 2018**). This superiority is also due to organic fertilizers role in increasing readiness of nitrogen and phosphorus in soil and preserving them from fixation processes due to secretion of some enzymes and organic acids through activity of microorganisms, which was reflected in leaves chlorophyll content and thus increased the total yield (**Shilan & Hama, 2022**). This result is consistent with what was found by **Al-Amery *et al.*, (2020**) on potato plants. The reason may be explained by effect of mineral fertilizer on nutrients availability needed by plant and supplied directly from soil, as well as effect of zeolite on improving some of soil physical, chemical and fertility properties, which in turn leads to an increase in spread of root system and an increase in water and nutrients absorption. Organic matter has a role in improving nutrients availability for plant, The interaction between organic and mineral fertilizers lead to increase the plant productivity through increasing water holding capacity (**Al-Halfi & Al-Azzawi, 2022**), and these factors help in the formation of a good root system that reflects positively on absorption of nutrients and on processes that take place inside plant, and then plant total yield (**Sarkar & Naidu, 2015**).

Table (4): effect of natural zeolite, organic and mineral fertilizers on total yield (µg ha $^{-1}$) of cabbage plants.

Zeolite	Poultry manure (O)	Mineral fertilizer (M)			Z × O
		M_{50}	M_{75}	M_{100}	
Z_0	O_0	17.97	20.60	20.20	19.59
	O_5	20.13	19.53	23.70	21.12
	O_{10}	23.30	24.53	26.43	24.75
Z_5	O_0	19.67	22.30	22.87	21.61
	O_5	20.43	24.63	29.13	24.90
	O_{10}	20.77	30.40	31.70	27.62
Z_{10}	O_0	20.17	24.83	30.97	25.32
	O_5	21.17	25.40	31.63	26.06
	O_{10}	28.50	31.33	34.90	31.58
L.S.D 0.05		3.42			1.97
Z × M					Z
Z_0		20.47	21.55	23.44	21.82
Z_5		20.29	25.78	27.90	24.66
Z_{10}		23.28	27.19	32.50	27.66
L.S.D 0.05		1.97			1.14
O × M					O
O_0		19.27	22.58	24.68	22.17
O_5		20.58	23.19	28.15	23.97
O_{10}		24.19	28.75	31.01	27.98
L.S.D 0.05		1.97			1.14
M		21.35	24.84	27.94	
L.S.D 0.05		1.14			

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 11-25
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(2)
Mohamed & Ahmed

المجلة العراقية لبحوث السوق
وحماية المستهلك

EXTRACTION AND PARTIAL PURIFICATION OF ACTINIDIN FROM KIWI FRUITS AND ITS EFFECT ON AGED CHICKEN MEAT

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Received 9/ 4/ 2023, Accepted 8/ 6/ 2023, Published 31/ 12/ 2024

ABSTRACT

The aim of this study was to extract the enzyme actinidin from kiwi fruits and purify it partially and determine the pH and the optimum temperature for the effectiveness and stability of the enzyme. Three concentrations of the purified enzyme were used for treatment of aged poultry meat for a period of 2, 4 and 6 h for the purpose of softening it. Some variables used as indicators of oxidation were monitored. The results showed that the crude enzyme had activity of 23 units/ml and specific activity of 19.65 units/mg, and the purification steps resulted in obtaining the enzyme with specific activity of 52.80. The results indicated that the optimal pH for enzyme activity and stability was 7, and the optimum temperature for enzyme activity was 40°C, and the enzyme retained its activity in the range of 20-60 °C. Treating aged chicken breast meat with enzyme led to a significant decrease in peroxide value with increasing enzyme concentration, and it increased with incubation period. The value of total volatile nitrogen was at the lowest value in the control treatment (5.44%) and increased with increasing enzyme concentration and incubation period. The values of Thio barbituric acid showed a significant increase in the control treatment and reached its highest value in the longest incubation period, and actinidin enzyme decreased the values of Thio barbituric acid at all concentrations used. The values of free fatty acids decreased in the enzyme-containing treatments, compared to the control, which gave the highest value (0.68 for an 8-h incubation period).

Key words: kiwi fruit, protease, enzyme extraction, ammonium sulfate, meat quality.

الاستخلاص والتنقية الجزئية للأكتينيدين من فاكهة الكيوي وتأثيرها على لحوم الدجاج المسنة

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الخلاصة

هدفت هذه الدراسة إلى استخلاص إنزيم الأكتينيدين من ثمار الكيوي وتنقيته جزئياً وتحديد الأس الهيدروجيني ودرجة الحرارة المثلى لفعالية وثبات الإنزيم. استخدمت ثلاث تراكيز من الانزيم المنقى لمعالجة لحوم الدواجن المسنة لمدة 2 و 4 و 6 ساعات لغرض تليينها. تم رصد بعض المتغيرات المستخدمة كمؤشرات للأكسدة. أظهرت النتائج أن الإنزيم الخام له نشاط 23 وحدة / مل ونشاط نوعي 19.65 وحدة / ملجم ، وخطوات التنقية أدت إلى الحصول على الإنزيم بنشاط نوعي 52.80. أشارت النتائج إلى أن درجة الحموضة المثلى لنشاط الإنزيم وثباته كانت 7 ، ودرجة الحرارة المثلى لنشاط

الإنزيم كانت 40 درجة مئوية، واحتفظ الإنزيم بنشاطه في نطاق 20-60 درجة مئوية. انخفاض معنوي في قيمة البيروكسيد مع زيادة تركيز الإنزيم وزيادة مع فترة الحضانة. كانت قيمة النيتروجين الكلي المتطاير أقل قيمة في المعاملة الضابطة (5.44٪) وتزداد مع زيادة تركيز الإنزيم وفترة الحضانة. أظهرت قيم حامض الثيوباربيتوريك زيادة معنوية في المعاملة الضابطة ، ووصلت إلى أعلى قيمته في أطول فترة حضانة ، وخفض إنزيم الأكتينيدين قيم حمض الثيوباربيتوريك بكافة التركيزات المستخدمة. انخفضت قيم الأحماض الدهنية الحرة في المعاملات المحتوية على الإنزيم مقارنة بالمجموعة الضابطة التي أعطت أعلى قيمة (0.68 لمدة حضانة لمدة 8 ساعات).

الكلمات المفتاحية: فاكهة الكيوي ، البروتييز ، استخلاص الإنزيم ، كبريتات الأمونيوم ، جودة اللحوم.

INTRODUCTION

Kiwi is the most popular crop of the genus Actinidia which have fruits with unique flavor, high nutritional value, and beneficial health effects **(Wu *et al.*, 2019)**. China is the richest natural resource in production of kiwi fruits, accounting for nearly 50% of the total world production **(Madhusankha & Thilakarathna, 2021)**. The fruits of the Actendines produce fifteen types, but the commercially available and edible kiwis are three types, which are the green kiwi A. deliciosa, the yellow kiwi A. chinensis, and the hard kiwi A. arguta **(Kaur& Boland, 2013)**. Actinidin enzyme is the dominant enzyme and is abundantly present in kiwi species, with a ratio of (10.7-1.6 mg / 100 gm of fruit juice) **(Gong *et al.*, 2020)**. Previous studies have shown through *in vivo* experiments that this enzyme improves the digestion of protein in the stomach and small intestine. Kiwi contains a simple but important protein that consists of both soluble and insoluble forms **(Montoya *et al.*, 2014; Bagheri *et al.*, 2019)**.

Actinidin is a protein extracted from edible kiwi fruit and Chinese gooseberry **(Dhiman *et al.*, 2021).** The enzyme is also obtained commercially from a variety of organisms **(Chao, 2016)**. It is part of the family of sulfhydryl proteases and its main use is as a meat tenderizer. It used than the rest proteases in meat tenderization. Actinidin forms up to 30-60% of the soluble protein in kiwi fruit, and the immunological detection results showed that kiwi fruit contains 3800 times more actinidin than leaves **(Peyrot des & Breslin, 2016)**. Actinidin has a high potential for commercial use in meat tenderization and milk curdling, and is included in health-promoting diet supplements (**Bagheri *et al.*, 2019)**, and this enzyme is used to help the digestive system in digesting protein food in particular. It is also involved in the production of compounds that help in the process of digesting food, especially in humans with digestive problems such as enteritis **(Peyrot des & Breslin, 2016).**

Considering that actinidin protease is capable of digesting many enzymes, including alpha-amylase, which is an essential enzyme found in saliva and pancreatic juice, responsible for the decomposition of large, insoluble starch into simple parts of sugar, and this affects the rate of carbohydrate digestion and absorption **(Martin *et al.*, 2017)**, and treatment Type 2 diabetes which involves inhibition of the alpha-amylase enzyme by the enzyme actinidin **(*Mahdian et al.*, 2022; Bagarinao, 2020)**. It is a globular protein that accounts for 50-40% of soluble proteins **(Aworh & Nakai, 1986).**

The aim of the recent study was to extract the enzyme actinidin from kiwi fruit, partially purify it, determine the optimum pH and temperature for the activity.

MATERIALS AND METHODS

Kiwi fruits used in the experiment.

Fresh kiwi fruit was purchased from the local markets in the capital, Baghdad, and was used in the experiment after peeling, washing with distilled water, drying it with blotting paper, and then cutting it into small pieces, about 2 cm in size.

Enzyme extraction

In the extraction process, the method described befor was adopted. One hundred g of kiwi fruit pulp was taken and cut into small pieces of 2 cm in size. The pieces were soaked with the extraction solution previously prepared, consisting of (6% sodium chloride + 2% boric acid) in a ratio 4:1 (v/v). After 24 h at room temperature (20°C), the soaked pieces were macerated in electrical mixer for 5 minutes and the mixture was filtered through cotton. The pulp was re-extracted and the whole filtrate was centrifuged at 3000 × g for 20 min. Supernatant was used raw extract.

Enzyme activity and protein concentration

In a test tube 0.95 mL of previously prepared cysteine solution and 0.1 mL of enzyme extract was mixed, left at 35 °C for 3 min, 0.95 mL of previously prepared casein solution was added, and the mixture was kept at a temperature 35°C for 10 min. The reaction was stopped by adding 3 ml of TCA solution at a concentration of 5% at room temperature for 60 min. Centrifugation at 3000 × g for 20 min was done, the liquid was separated quietly, and the absorbance was measured at a wavelength of 280 nm. Blank solution was prepared by following the aforementioned steps, except that the TCA solution was added to the substrate solution before adding the enzyme **(Chao, 2016)**. The enzyme activity was measured according to the equation below:

Enzyme activity (unit / mole) = Absorbance at 280nm / (0.1×10 × 0.01)

The unit of enzyme activity was defined as the amount of enzyme in milliliters that gives an increase of 0.01 in the absorbance at a wavelength of 280 nm per minute under the experimental conditions.

For Estimation of protein concentration, the method mentioned priviously **(Segel, 1976)**.

Enzyme purification

The enzyme was concentrated by the gradual addition of ammonium sulfate to the crude enzyme extract with continuous stirring on the magnetic stirrer in an ice bath until reaching 20% saturation. Centrifugation was carried out at 11000 × g for 20 min. The lytic activity of the enzyme in the filtrate and the sediment was estimated. Then the filtrate was taken, and a new weight of ammonium sulfate was added to the filtrate in order to reach a saturation percentage at which the enzyme activity in the filtrate decreases **(Horwitz, 1975)**. A saturation percentage was used to concentrate the actinin enzyme from (20%, 40%, 60% and 80%) respectively.

The precipitate was dissolved in a small amount of previously prepared sodium phosphate buffer solution. The precipitate representing the crude enzyme extract was collected and the dialysis process was performed using sodium phosphate solution at 4°C for 24 h with the replacement of the solution (phosphate buffer) every 4 hours after which the methylated

extract was collected and the final volume and analytical activity of the enzyme were measured. The enzymatic activity of the remaining enzyme was measured using of method of **Horwitz, (1975)**.

Factors affecting enzyme production.

For determination of the optimum pH for the activity of the purified enzyme, several tubes of buffer solutions with different pH numbers ranging from (4-9) were prepared previously by adding 3 ml of all solutions in different tubes. 0.1 ml of purified enzyme solution was added to tubes containing 0.3 ml of buffer solutions with different pH numbers. The tubes were incubated for 20 min at a temperature of 37°C, then 0.95 ml of casein solution prepared at a concentration of 1% and a pH of 7 was added. Then the reaction was stopped, the enzyme activity was estimated, and the relationship between the pH and the remaining activity of the enzyme was drawn to determine the optimal pH for enzyme activity. The tubes were incubated for another 20 min at a temperature of 37 °C for determination of the optimum pH for the stability of the purified enzyme **(Horwitz, 1975)**. The same procedures with certain modifications were adopted for determination of optimum temperatures for enzyme activity and stability.

Treatment of chicken breast meat with enzyme solution

After lyophilization, the partially purified enzyme was dissolved and diluted with an amount of deionized water for the purpose of immersing the chicken breast pieces, and the dilution ratio was (0.25,0.75 and 1) %, where 200 g of chicken breast was immersed for every 100 ml of diluted enzyme The samples were incubated on the optimum temperature for enzyme action for different periods of time (0, 2, 4, 6) h. After the incubation period, these samples were placed directly in an electric oven for 20 minutes to inactivate the enzyme at 120 °C, cooled and kept in the refrigerator for the purpose of conducting tests.

Qualitative tests

Peroxide value (PV)

The peroxide value was estimated according to the A.O.A.C method **(Witte *et al.*, 1970)** using the following equation:

Peroxide number (meq) = S×N×1000 / g

Where S = ml of $Na_2S_2O_3$ sodium thiosulfate, N = sodium thiosulfate molar and g = number of grams of oil.

Thio barbituric acid (TBA)

Thio barbituric acid was estimated for the purpose of measuring lipid oxidation for each sample separately and it was accomplished according to (**Bekhit *et al.*, 2021)** using the equation:

TBA Value (mg MDA/kg) = A530 × 5.2

Where: A530 is the absorbance of reaction mixture at 530 nm.

Total Volatile Nitrogen (T.V.N)

The method adopted by (**Ahamed & AL-Abadi, 2015)** was used for estimation of the percentage of total volatile nitrogen in chicken breast meat samples treated with the enzyme in addition to the control sample. 100 g was taken from each sample, homogenized with 300 ml of 5% trichloroacetic acid (TCA) solution, filtered to obtain a clear extract and transferred to 5

mL of a 2 M sodium hydroxide solution. The mixture was heated until distillation into a receiving flask containing 4% boric acid. Few drops of methyl red dye indicator and bromocresol green was added. Then titration was performed for the mixture using hydrochloric acid with a concentration of 0.01 M. The amount of volatile nitrogen was calculated according to the following equation.

Amount of volatile nitrogen (mg nitrogen / 100g) = 500 / v (300 + mo) × 14

Free Fatty Acid (FFA)

Free fatty acids were estimated according to (**Cunniff, 1995)**. the method **(Pizarro *et al.*, 2013)**. The method is summarized by extracting the fat by the cold method, taking 10 g of fat and adding 25 ml of ethanol at a concentration of 95% neutral and one ml of phenolphthalein reagent and treating it with 0.1 M sodium hydroxide until the solution becomes pink. The percentage of free fatty acids was calculated as oleic acid:

Acid value = NaOH (ml) × 5.61/ Sample weight (g)

% FFA = Acid value/2

Non-protein Nitrogen (NPN)

Non-protein nitrogen was estimated in chicken breast samples according to (**SAS, 2018**). The method mentioned in 1995. Five g of each sample was weighed and placed in a 100 ml glass container, 5g of pure sand was added to it, the sample was mixed well using a glass rod and 25 ml of pure sand was gradually added to it. Distilled water is warm while continuing to mix, then 25 ml TCA 24% was added, and the solution was left for 10 min. The solution was filtrated through Whatman No.1 filter paper. One ml was transferred to the digestion vial of the microkeldahl device, and 10 ml of concentrated H_2SO_4 was added to it. The digestion process was completed using the Buchi 430 apparatus and distillation using the Buchi 320 apparatus.

Statistical analysis

The statistical program System Statistical Analysis (**SAS- 2018)** was used to analyze the data to study the effect of different factors (concentration and time) on the studied traits according to a complete random design (CRD). Significant differences between means were compared with a less significant test (Least significant difference –LSD).

RESULTS AND DISCUSSION

Enzyme extraction

The aforementioned extraction solution was used to extract the actinidin enzyme, and this solution was composed of sodium chloride and boric acid, as it gave an enzyme activity of 23 units/ml and a specific activity of 19.6 units/mg protein, and the protein concentration was 1.17 mg/ml as shown in (Table ,1). This result was similar to the results obtained by **(Chao, 2016)**, when the enzyme actinidine was extracted using a phosphate buffer with a pH7, as the enzymatic activity reached 59.67 units / ml and the specific activity was 56.82 units/ mg. Sharma and Vaidya **(Zhu *et al.*, 2018)** used a phosphate buffer to extract the enzyme actinidin, as the enzymatic activity was 0.22 units/ml, the specific activity was 0.55 units / mg protein, and the protein concentration was 0.4 mg / mol. If a phosphate buffer with a pH8 was used, the

enzymatic activity was 220 unit / ml and the specific activity was 0.52 mg: protein and the protein concentration was 0.42 mg / ml.

The difference in the specific activity values from one method to another may be due to the difference in the solubility of the proteins present in the fruit or due to the difference in the extraction solutions that were reflected in the protein concentration, which affected the specific activity of the enzyme under study. All enzymes are extracted and purified at 4°C, although the temperature reduces the solubility of proteins, but in return it reduces the loss of enzymatic activity to the maximum extent, and then preserves the enzyme with the highest activity **(Dhaher *et al.*, 2022)**.

Shows Table,1 the step of gradual saline precipitation of the crude extract using gradual saturation rates of ammonium sulfate that ranged from 20% to 80%, as the enzymatic activity reached the highest value (52.4 units / ml) at a saturation rate of 60%. The sediment resulting from centrifugation was collected, and membrane aspiration was carried out in against phosphate buffer every 6 hours for 24 h and at 4 °C. After that, the enzymatic activity was estimated to be 84 units/ml. The specific activity was 52.8 units/mg, the protein concentration was 1.6 mg/ml, purification folds 2.69, and the enzyme yield was 73.04%, as shown in Table1.

The results differed according to the different sources when using ammonium sulfate for the purpose of enzyme concentration and sedimentation. The results were similar with Sharma and Vaidya. **(Zhu *et al.*, 2018)**, when using ammonium sulfate with a saturation of 60% to precipitate and purify the enzyme actinidin from kiwi fruit, then membrane permeability, as it obtained a specific activity of 0.86. mg/protein, enzymatic activity of 190 units/ml, enzyme yield of 86%, and purification folds of 1.65. It also agreed with (**Chao, 2016)**, when using ammonium sulfate with a saturation of 60% to precipitate and purify the actinidin enzyme, and then membrane permeability, as it obtained a specific efficiency of 88.54 units / mg, enzymatic activity 244 units / ml, enzymatic yield 23% and purification folds 1.55. Ammonium sulfate is used in most studies with different saturation rates because of its positive advantages, including its high solubility, cheap price, and lack of toxicity **(Seifter & Englard, 1990**). and that the reason for the difference in the results in different sources is due to the use of different concentrations of ammonium sulfate used to modify the charges on the surface of the enzyme and to cause disruption of the water layer surrounding the protein, and hence reduce the solubility of the protein and its precipitation **(Zou *et al.*, 2018; Nidhal, 2013)**.

Table (1): Steps of purification of actinidin enzyme from kiwi fruit.

Purification step	volume (ml)	activity units/ml	protein mg/ml	Specific activity Units/mg	Total activity units	Purification folds	Yield %
Crude extract	1000	23	1.170	19.65	23000	1	100
Precipitation with Ammonium sulfate (20–80%) and Dialysis	200	84	1.592	52.8	16800	2.693	73.04

Optimum pH for the activity of the partially purified enzyme

The optimal pH for the activity of the purified actinidin enzyme from kiwi fruit was set with a range of pH numbers ranging from 4-9. It was shown from the results shown in Figure1

that the optimum pH of the purified actinidin from kiwi fruit was 7, as it was found that the highest activity of the enzyme 30 units / ml, and it is noted that the activity decreases at pH numbers 8-9. The reason of the decrease in the pH in the acidic and basic ranges is due to the effect of the groups present in the active site of the enzyme or the substrate or both, and the reason for the change in the ionic state of these groups and its reflection on the ability of the enzyme to bind to the subject matter **(Al-Zubaidy, 2017)**. This result was consistent with what was found by **(Al-Mahdawi, 2022)**, as the optimum pH of the purified actinidin enzyme from kiwi fruit is 7, and identical to **(Chao, 2016)**, where the optimum activity of the actinidin enzyme was at a pH7.

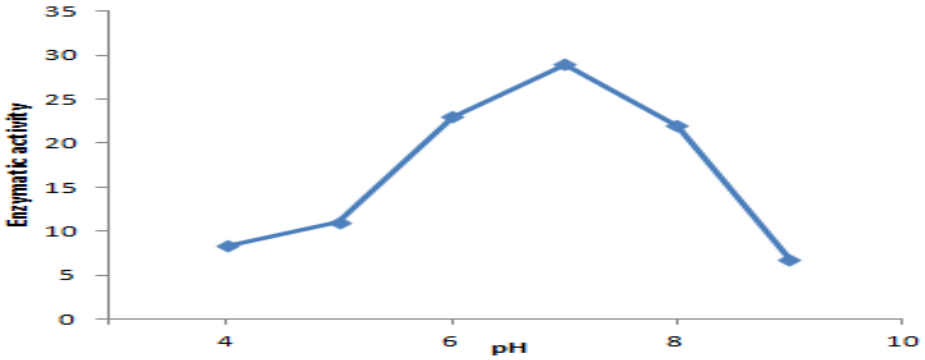

Figure (1): Optimum pH curve for activity of purified actinin enzyme from kiwi fruit

Optimum pH for the stability of the purified enzyme

The results shown in Figure 2 showed that the optimal pH for enzyme stability is between 6 and 8, as it was observed that the enzyme retained its effectiveness at pH 6 by 95%, while at pH 8 it was 91.1%, and the highest stability of the enzyme was at pH 7, while the enzyme activity was low in the extreme values of acidic and basic pH. The reason for the decrease in the enzymatic activity at the acidic and basic pH values is due to the change in the secondary and tertiary structure of enzyme that leads to the push amino acids in the active site away from each other **(Zhang *et al.*, 2017)**. These results were also close to the results of **(Kazeem *et al.*, 2013)** as they indicated that the optimal pH for the stability of the enzyme extracted from kiwi fruit is between 3 and 7. Also, the results of **(Chao, 2016)** showed that the purified actinidin enzyme showed high activity within a pH range between 6 and8 for sodium phosphate buffer solution.

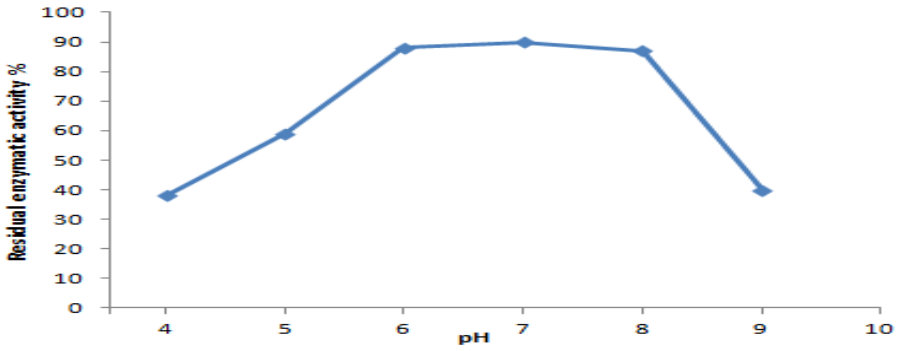

Figure (2): Optimum pH curve for the stability of purified actinidin enzyme from kiwi fruit

Optimum temperature for enzyme activity

The reaction of purified actinidin enzyme from kiwi fruit was done at different temperatures to determine the optimum temperature for the enzyme activity, as these temperatures ranged between 20 and 60 for 10 minutes. Figure 3 shows that at a temperature of 40, the enzymatic activity reached 27 units/ml, then the enzymatic activity decreased to 25.5 units/ml at a temperature of 50, and reached the lowest level of activity at 60, reaching 4.6 units/ml. The increase in temperature increases the speed of enzymatic reactions, and the reason is due to the increase in the thermal energy of the molecules, and then the increase in the collisions between the enzyme molecules and the molecules of the substrate, as it reaches the degree at which the reaction speed reaches its maximum value. After that the enzymatic activity decreases due to the denaturation of protein at high temperatures which changes the secondary, tritiary and tetratiary structures of protein **(Horwitz, 1975)**. These results agreed with **Kazeem, *et al.*, (2013)** who found that the optimum temperature for the action of the purified enzyme extracted from kiwi fruit was 40 degrees Celsius, after which the activity lost a large percentage at 60 degrees Celsius**(Lee *et al.*, 2010)** stated that the optimum temperature for the activity of the actinidin enzyme extract is 65 °C, and this contradicts what was confirmed by **Zhang *et al.*, (2017)** who stated that the optimum temperature for the actinidin enzyme is 40°C.

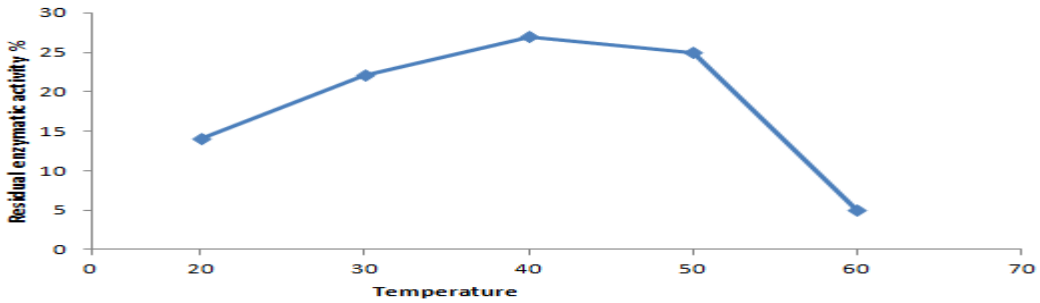

Figure (3): Optimum temperature curve for activity of purified actinin enzyme from kiwi fruit.

Optimum temperature for enzyme stability

The results in Figure 4 showed that the temperatures of enzyme stability ranged between (20-40) for a period of 20 minutes, where the enzyme retained its maximum activity, which ranged from (96.3-93.0)%, after which the enzyme activity decreased with the increase in temperature to reach 51.6% at a temperature 50 ºC, and the effectiveness continued to decrease with the increase in temperatures, to drop to 23% at 60 ºC. These results agreed with what was indicated by other authors **(Kazeem, *et al.*, 2013; Lee *et al.*, 2010**), as the enzyme retained its full activity at a temperature of 20-40 °C, then lost it by 50% at 50°C. The activity continued to decrease with the increase in temperature, dropping to only 10 % at 60°C.

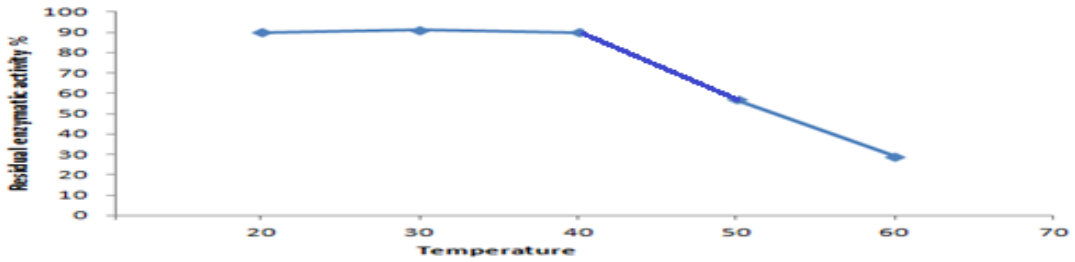

Figure (4): Optimum temperature curve for the stability of purified actinin enzyme from kiwi fruit

Qualitative tests

Peroxide value (PV)

Table 2 shows the effect of partially purified actinidin enzyme on peroxide values of aged chicken breast fortified with three concentrations (0.25, 0.75 and 1.00) % during incubation periods (0, 2, 4 and 6) h at 40 °C. Significant decreases ($p \leq 0.05$) were noted in peroxide values for the three treatments compared to the control. The higher the concentration, the lower the value of peroxide in the treatment samples during the same incubation period. The average of PV values for the control was 3.65, then it decreased after an incubation period of 2 hours to become (2.85, 3.05, 3.22), for concentrations (0.25, 0.75,1.00) %, respectively, but when comparing the control with one concentration during different incubation periods (0, 2, 4, 6) hours, higher PV values were recorded for the enzyme-treated samples compared to the control. It reached the highest value at a concentration of 0.25% and during the longest incubation period of 6 h, reaching 4.98. Through these results, it was found that the PV value is affected by two important factors, namely the incubation period and concentration. The higher the enzyme concentration, the lower the PV value in chicken breast samples treated with the purified enzyme. At the same time PV increases with increase the incubation period, as the average value of peroxide for the treatment at a concentration of 0.25% and for an incubation period of 6 h was 4.98, which is the highest value compared to other treatments except control. These results agreed with what was stated by Poona **(Abdelrahman *et al.*, 2023)** which applied kiwi juice applied to the chicken breast meat and observed significant differences in the peroxide values between the treatments compared to the control.

Table (2): Effect of actinidin enzyme on peroxide value in chicken breast samples at incubation periods (0, 2, 4, 6) hours with three concentrations (0.25, 0.75 and 1.00%).

Time	Control	0.25%	0.75%	1%
2	3.65	3.22	3.05	2.85
4	4.54	4.11	3.40	3.19
6	5.66	4.98	4.25	4.02
LSDvalue	**0.994***			
***(p≤0.05)**				

Total volatile nitrogen (TVN)

Table 3 shows the effect of three concentrations (25, 0.75, 1.00) % for different storage periods (0, 2, 4, 6) h at 40°C on TVN values for chicken breast samples treated with partially purified actinidin enzyme. Significant increases (p≤0.05) in the values of TVN for the samples treated with the enzyme, where the value of TVN for the control sample was 5.44, while it was in the three treatments at concentrations (0.25, 0.75, 1.00) % (5.87, 6.00, 6.25) during the same period 2 h, respectively. It was also observed a rise in the values of TVN with increasing concentration and with the increase of the incubation period, where the highest value of TVN was 8.09 at the incubation period of 6 hours at a concentration of 1%. These results agreed with **(Abd Elrahman *et al.*, 2023)** who found an increase in TVN values of chicken breast meat treated with kiwi juice at a concentration of 5-7 + pineapple juice 5-7) % and (kiwi juice 5% + pineapple juice 5%) during a storage period of 3 months.

Table (3): Effect of actinidin enzyme on the value of total volatile nitrogen in chicken breast.

Time	Control	0.25%	0.75%	1%
2	5.44	5.87	6.00	6.25
4	5.80	6.08	6.33	7.00
6	6.05	6.44	6.74	8.09
LSDvalue	2.061*			
***(p≤0.05)**				

Thio barbituric acid (TBA)

Table 4 shows the effect of three concentrations of the enzyme (0.25, 0.75 and 1.00)% on TBA value of chicken breast samples treated with partially purified actinidin enzyme for different storage periods (0, 2, 4, 6) h at 40 °C. Significant decreases (p≤0.05) in the TBA values of the enzyme-treated samples were observed, where the TBA value for the control sample was 0.036, while it was (0.030, 0.026, 0.021) for the treatments at concentrations (0.25, 0.75, 1.00)% during the same incubation period 2h, respectively It was also observed a rise in the TBA values with a decrease in concentration with the incubation period, as the highest value in the treatment reached 0.050 during the longest incubation period of 6 hours at the lowest concentration 0.25%. Through these results, it was found that the value of TBA was affected by two important factors, namely the incubation period and concentration. The lower enzyme concentration the lower TBA value in chicken breast samples treated with purified enzyme.

As shown by **(Abdel-Naeem & Mohamed, 2016)**, there was a significant decrease in the TBA values when treating camel meat with ginger extract and papain compared to the

control sample, and there was no significant difference between the same treatments after soaking the meat with ginger extract at a concentration of 7% and papain at a concentration of 0.01% and a mixture of ginger extract and papain at a concentration of 0.01%. The decrease in the value of TBA is attributed to the activity of ginger extract in inhibiting the action of the peroxidase enzyme, which can reduce the release of unsaturated fatty acids and the oxidation of unsaturated fatty acids.

These results were confirmed by **(Naveena *et al.*, 2001)** when using ginger extract on smoked chicken meat. From these results it can be conclude that proteolytic enzymes possess antioxidant activities **(Richardson *et al.*, 2018).**

Table (4): The effect of actinin enzyme on the value of Thio barbituric acid (TBA) in chicken.

Time	Control	0.25%	0.75%	1%
2	0.036	0.030	0.026	0.021
4	0.052	0.035	0.030	0.027
6	0.069	0.050	0.042	0.035
LSDvalue	**0.022***			
***(p≤0.05)**				

Free fatty acids (FFA)

(Table, 5) shows the effect of partially purified actinidin enzyme on the values of free fatty acids in chicken breast during the incubation period of (0, 2, 4, 6) h at 40 °C for three concentrations (0.25, 0.75 and 1.00) %. Significant decreases ($p \leq 0.05$) were observed in the value of free fatty acids (FFA) for meat samples at three concentrations and at the same incubation period compared to the control sample, where its value was 0.35, while the averages of the treatments were (0.31, 0.26, 0.22) for the three concentrations (0.25, 0.75, 1.00) %, respectively. However, when comparing the control sample to a single concentration during different incubation periods, an increase in the FFA value of the enzyme-treated samples can be seen compared to the control sample. These data showed that the highest average of FFA was when the concentration treatment was 0.25% and for an incubation period of 6 h.

These results agreed with **(Pooona & Singh 2020)**, when soaking chicken breast pieces in kiwi juice and during different refrigerated storage periods, as the value of free fatty acids decreased during the storage period.

Free fatty acids are among the main evidence for judging the quality of fat in meat and its various products. It is an indicator of spoilage, especially the volatile ones. There may be a decomposition of glycerides by the action of the lipase enzyme and the production of fatty acids. This decomposition is rapid in the presence of light and air and is accompanied by rancidity **(Auda& Khalifa, 2019; Hassan, *et al.*, 2022; Ibrahim, 2022; Fradi, 2022).**

Table (5): The effect of actinidin enzyme on the value of free fatty acids (FFA) in chicken.

Time	Control	0.25%	0.75%	1%
2	0.35	0.31	0.26	0.22
4	0.47	0.37	0.31	0.27
6	0.68	0.48	0.40	0.35
LSDvalue	0.219*			
***(p≤0.05)**				

CONCLUSION

The enzyme actinidin can be extracted and partially purified from kiwifruit with good activity. This enzyme acts as an antioxidant beside its basic function which is tenderizing agent. These results suggest the potential use of this enzyme in tenderization of aged meat. Further studies may be required to improve extraction and purification steps and to apply the enzyme in other processing treatments which utilize enzymes.

CONCLUSIONS

1. Actinidine enzyme is one of the most important enzymes present in kiwi fruit. It constitutes 60% of the protein. It can be extracted, precipitated, and purified with ammonium sulfite salt at a temperature of 4 °C.
2. The results of the qualitative tests confirmed that the enzyme actinidine is a natural antioxidant that can provide protection for the treated mites according to several factors, the most important of which are concentration, incubation period and temperature.
3. It was found that the optimum pH for enzyme activity was 7 and the stability ranged between 6-8

RECOMMENDATIONS

1. A study of the effect of actinidine as an anti-cancer and anti-inflammatory
2. Study of the effect of partially purified actinidine as an antimicrobial agent for food contaminants
3. The use of the enzyme in the manufacture of easy-to-digest protein foods for sensitive groups

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 26-34
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(3)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

ESTIMATING RENAULT TRACTOR COSTS USING DIFFERENT DEPTHS AND AGRICULTURAL MACHINES

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Received 26/ 4/ 2023, Accepted 2/ 5/ 2023, Published 31/ 12/ 2024

ABSTRACT

The experiment was carried out in one of the fields of the Agricultural Research Department affiliated to the Ministry of Agriculture in Abu Ghraib in the year 2022 for the purpose of calculating the total costs of the Renault type tractor and studying the effect of three different equipment represented by the disc softener, masher and seeder and three levels of depths of 5 cm, 10 and 15 cm on the performance of the type tractor (Renault has been studied) overall economic costs, The Randomized Completely Block Design (RCD) system was used, and the results were analyzed using the Least Significant Difference (LSD) method at the level of 0.05 and compared with the averages of the coefficients. The results showed the following:

1- Changing the type of machine from disc harrows to packers and then to seeders led to a significant decrease in the total costs from 24119 to 24055 and then to 19793 (D.ha -1).

2- Increasing the depth from 5 to 10 and then to 15 led to a significant increase in the total cost of the mechanical unit from 22521 to 22678 and then to 22768 (D. hectare -1).

3- The overlap between the disc harrows and the depth of 15 cm led to recording the highest value in the total costs, which was 24245 (D. Ha-1).

As for the overlap between the seed and the depth of 5 cm, it led to recording the lowest value in the total costs, and it was 19639 (D. ha- 1).

Keywords: Costs, Disc Harrows, Packers, Seeder.

تقدير تكاليف الجرار نوع Renault باستخدام أعماق والالات زراعية مختلفة

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الخلاصة

نفذت التجربة في أحد حقول دائرة البحوث الزراعية التابعة الى وزارة الزراعة في ابي غريب في شهر العاشر لسنة 2022 لغرض حساب التكاليف الجرار الكلية نوع Renault ودراسة تأثير ثلاث معدات مختلفة متمثلة بالمنعمة القرصية والهارسة والباذرة وثلاثة مستويات من الأعماق 5سم و10 و15 سم في أداء جرار نوع (Renault) وتم دراسة التكاليف الاقتصادية الكلية، استعمل نظام تصميم القطاعات العشوائية الكاملة (Randomized Completely

[*] The research is taken from a master's thesis by the first researcher.

Block Design (RCBD، وتم تحليل النتائج باستعمال طريقة اقل فرق معنوي (LSD) عند مستوى 0.05 ومقارنتها مع متوسطات المعاملات، وأظهرت النتائج ما يلي:

1- إن تغيير نوع الآلة من الامشاط القرصية الى الهارسة ومن ثم الى الباذرة ادى الى انخفاض معنوي في التكاليف الكلية من 24119 الى 24055 من ثم الى 19793 (دينار. هكتار -1).

2- أدت زيادة العمق من 5 الى 10 ومن ثم الى 15 الى زيادة معنوية في التكاليف الكلية للوحدة الميكنية من 22521 الى 22678 ثم الى22768 (دينار. هكتار -1).

3- أدى التداخل الحاصل بين الامشاط القرصية والعمق 15سم الى تسجيل أعلى قيمة في التكاليف الكلية كانت 24245 (دينار. هكتار-1).

أما التداخل بين الباذرة والعمق 5 سم فقد أدى الى تسجيل اقل قيمة في التكاليف الكلية وكانت 19639(دينار. هكتار -1)

الكلمات المفتاحية: التكاليف، الباذرة، الكلفة الكلية.

INTRODUCTION

Agricultural mechanization is the main pillar of agriculture in our modern area, and it plays a fundamental and effective role in increasing agricultural production and providing food for the world's population, which amounted to 7.4 billion, according to the United Nations estimate in 2015 **(Alain Parant, 2016).** With the increase in population numbers and urban progress, the need to provide food increased, which It led to an increase in cultivated areas and an increase in the attempt to raise productivity per unit area in order to provide the largest possible amount of food and effort. **(Laibi, 2022)** Despite this, the quantities produced remained insufficient to meet the needs of the increasing numbers of the population for two main reasons, the first of which is the insufficiency of cultivated land to meet the need, and the second is the low production per unit area.

The speed of conducting the plowing process is of great importance in increasing productivity, but increasing the speed above a certain limit led to increased stress on the puller and the machine, as well as the lack of control over the depth of plowing leads to poor plowing conditions and depths. It results in low productivity (uneconomical) and reduces the efficiency and depth of the tillage process as well. The forward speed is an important and direct factor in improving plant productivity in quantity and quality.

MATERIALS AND METHODS:

1. Experimental Procedure

The experiment was carried out in one of the fields of the Agricultural Research Center in Abu Ghraib in the tenth month of the year 2022 for the aim of Estimating Renault tractor costs using different depths and agricultural machines.

2. Executing the experiment:

The experiment was carried out using Statistical Analysis System (SAS, 2012) in data analysis to study the effect of different factors on the studied traits according to an experiment (3 * 3), (totally 27 treatment) applied with a complete randomized design (RCBD Randomized Completely Block Design).

the significant differences between the averages were compared with the least significant difference test (LSD) 0.5%.

The research relied on a study of two factors, namely:

The first factor: the type of implements with three levels: disc harrows by (I1) as shown in Figure No. (1), the packers by (I2) and as shown in Figure No. (2), and the seeders by (I3) as shown in Figure No. (3).

The second factor: the depth in three levels: 5 cm (D1), 10 cm (D2), 15 cm (D3).

Image (1): Disc Plow.

Image (2): Packers.

Image (3): Seeder**.**

1. Total costs (D. ha^{-1}):
The total costs were calculated according to the method adopted from (**Al-Tahan *et al.*, 1991),** Al-Ani (2020) and **(SAHAY, 2010).**

2. Depreciation or consumption:
Depreciation or depreciation can be calculated from the following equation:
Amortization or depreciation costs = tractor purchase price - selling price / (operating life in hours)
D = P-S/

3. Interest
It is calculated from the following equation.
Int= (P+s / 2) / L *0.1

4. Taxes, insurance and shelter:
Its value was calculated from the following equation:
Ist = P/L * 2%

5. Variable costs:
Variable costs are defined as the costs that change relatively with the amount of work produced on the machine, or in other words, they are the costs as a result of operating the machine, so they increase with increasing operation and decrease as the operation of the machine decreases **(Al-Tahan 1991).**

6. Fuel costs:
It is calculated using the following equation **(Al-Khafaji 2001).**
Fu.c=BHP * 60% * 25% * FP

7.Oil cost:
It is calculated using the following equation **(Al-Khafaji 2001).**
OC=BHP * 60% * 25% * 0.03 * Fo

8. Maintenance and repair costs:
It is calculated using the following equation **(Al Tahan 1991):**
M.R.C=P/h*Ppr *(M.R.) Rate

9. Labor cost:
It is calculated using the following equation: **(Al-Khafaji 2001)**
L.c=Dl/d*Pr
The variable costs of the tractor are calculated according to the following equation:
V.c = Fu.c + O.c + M.R.c + Lc

10. Management costs:
Management costs are calculated as a percentage (10%) of the total fixed and variable costs of the tractor, using the following equation:
Ma.c=(F.c V.c)*0.10

11. Total costs of the tractor:
It is the sum of the fixed, variable and administrative costs of the tractor and is calculated using the following equation:
T.t.c = F.c + V.c + Ma.c

12. The total costs of the equipment used in the experiment:
The fixed, management and total costs of the equipment were calculated using the same equations that were used in calculating the economic costs of the tractor.
With the exception of the variable costs of the equipment, which were calculated as a percentage of (80%) of the fixed costs for each equipment, using the following equation **(Al-Tahan, 1991)**
P.V.c=P.F.c*0.08

13. Total unit costs:
It is the sum of the total costs of the tractor and the total costs of each equipment (plow + packers+ seeder) and is calculated using the following equation
T.c1=T.Tc+P.Tc1
T.c2 = T.t.c + P.T.c2

RESULT AND DISCUSSION:

Total costs (D. ha- 1)

Table (1) shows the effect of each of the type of machine and the depth on the characteristic of the total costs, as it is clear from Table (1) that changing the type of machine had a significant effect on the characteristic of the total costs, as it was found that the total costs of the disc harrows were 24119 D. ha-1 and for the masher were 24055 D. ha-1 and when using the seeder , the total costs decreased to 19793 D. ha-1 The reason is attributed to the increase in practical speed due to the decrease in weight, which leads to an increase in productivity as a result of it, and this leads to a decrease in costs. This is consistent with **(Jassim and Al-Hashemi, 2015)** and **(2003 Mosad and Foudy)**.

Table (1): Represents the effect of each of the machine type and depth on the total costs (D. ha-1).

Implement	Depth			Mean Implement
	D1 (5سم)	D2	D3	
I1(المنعمة القرصية)	23957	24156	24245	24119
I2 (الهارسة)	23966	24152	24046	24055
I3(الباذرة)	19639	19726	20013	19793
LSD I*D	300.21 غ.م			156.77
Mean depth	22521	22678	22768	
LSD D	199.26			

From the same Table No. (1) that increasing the working depth from 5 to 10 and then to 15 cm for the seeder led to an increase in total costs from 22521 to 22678 and then to 22768 D. ha-1, the reason for the increase is due to the increase in the percentage of slippage, and this leads to a decrease in the practical speed, which is one of the components of productivity, and these results are consistent with **(Mankhi 2012; Jassim & Al Hashemi 2015).**

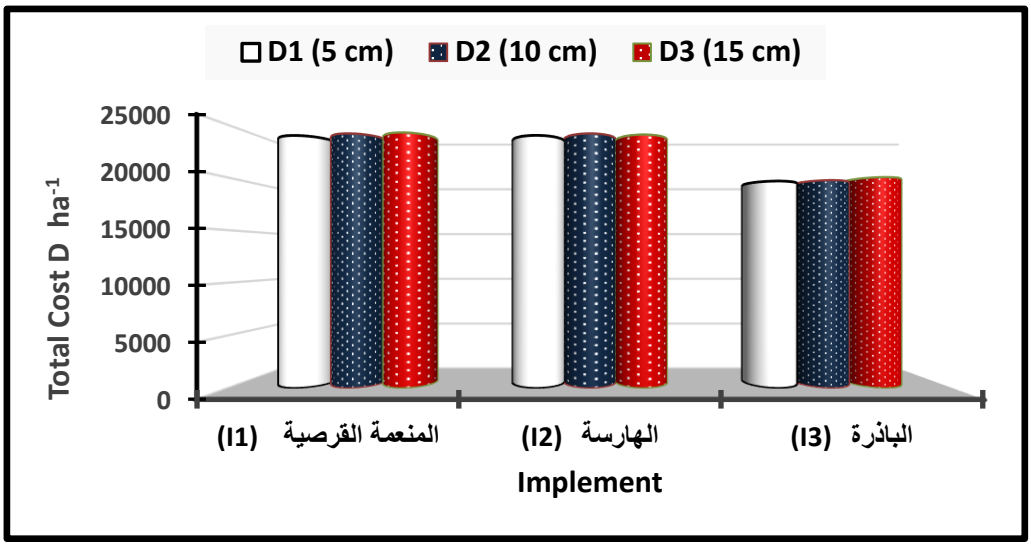

Figure (4): The effect of each type of machine and depth on the total costs.

As for the bilateral overlap between the plowing depth and the machine, it was also clear from the same table above that the highest total costs were using disc harrows with a depth of 15 cm, which was 24245 D. ha-1, while the lowest total costs were recorded when working at a depth of 5 cm using the seeder 19639 D. ha-1.

CONCLUSIONS:

The results showed that the overlap between the lowest rate of the total costs for the used puller, and the highest rate of field productivity and field efficiency.

RECOMMENDED:

We recommend using different depths and measuring the studied characteristics in the research.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 35-49
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(4)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

LEVEL OF SPREAD OF PALM TRUNK INJECTION TECHNOLOGY FOR FERTILIZATION AND CONTROL PURPOSES IN ALTARMIYA DISTRICT

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Received 27/ 4/ 2023, Accepted 31/ 5/ 2023, Published 31/ 12/ 2024

ABSTRACT

The research aimed to identify some of the personal characteristics of the respondents and their agricultural systems (educational level, age, experience in palm cultivation, tenure, the importance of palm in income) and determine the level of spread of palm trunk injection technology for fertilization and control in the Tarmiya district, the research community included palm farmers, who numbered 1708 and distributed as follows (1000 Tarmiya, 495 viewing, 213 Al-Abaiji) A proportional random sample was taken by 7% and by 120 respondents and distributors on (15,35,70) researcher, and to achieve the objectives of the research prepared a questionnaire consisting of two parts, the first part included some of the personal characteristics of the respondents, the second part included (11) paragraph and distributed on 4 areas and 8 axes, was measured according to a bilateral scale (yes, no), was given the following weights (0.1) respectively, used many statistical means of frequencies and arithmetic mean, percentages, and the equation of alpha Cronbach, weighted mean, percentage weight, The results of the research showed that most of the respondents are from the age group of 46-58 years and holders of an intermediate certificate, and have experience of 22-38 years, and own small areas distributed between (3-35) dunums, plant palm trees with small areas distributed between (3-35) acres, and the number of palms is estimated between 20-280 palm trees, and the respondents rely on palm cultivation as the main source of their income, which is 72.5%, and the results also showed that farmers apply technology to all the area of the orchard, which is 95.8%, and that The number of years of application ranges between 4-6 years and by 47.5%, and that the respondents continue to apply the technology by 95.8%, and the results showed that the respondents want to continue to apply the technology by 95.8%, and that the technology of injection of the palm trunk is compatible with the experiences of palm growers and appropriate with their farm systems and by 96.6%, and compatible with the values and customs prevailing in the region by 92.5%, as well as that the respondents have sufficient experience and skills to apply the technology and by 95.8%, and the researcher recommended to Continuing to spread this injection technology because of its role in preserving humans, animals, the environment and beneficial insects such as bees in all governorates of the country, and Increasing interest in technology and increasing government support for farmers by providing this technology and training farmers on how to use it.

Keywords: Technology, Injection Mechanism, Level of Spread.

***The research is extracted from a master's thesis for the first researcher**

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(2024) 16(2): 35-49

مستوى انتشار تقانة الحقن بجذع النخلة لأغراض التسميد والمكافحة في قضاء الطارمية

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الخلاصة

هدف البحث الى التعرف على بعض الخصائص الشخصية للمبحوثين ونظمهم الزراعية والمتمثلة (المستوى التعليمي، العمر، الخبرة في زراعة النخيل ، الحيازة، أهمية النخيل في الدخل) وتحديد مستوى أنتشار تقانة الحقن بجذع النخلة لأغراض التسميد و المكافحة في قضاء الطارمية ، شمل مجتمع البحث مزارعي النخيل و البالغ عددهم 1708 والموزعين على كما يلي (1000 الطارمية، 495المشاهدة، 213العبايجي) تم أخذ عينة عشوائية تناسبية بنسبة 7% وبواقع 120 من المبحوثين و الموزعين على (70،35،15) مبحوث، ولتحقيق أهداف البحث أعدت أستبانه متكونة من جزئين، الجزء الاول تضمن بعض الخصائص الشخصية للمبحوثين، أما الجزء الثاني تضمن (11) فقرة و الموزعين على 4 مجالات و 8 محاور، تم قياسها وفق مقياس ثنائي(نعم ،لا)، أعطيت له الاوزان الاتية (0.1) وعلى التوالي، استخدمت العديد من الوسائل الاحصائية المتمثلة بالتكرارات والمتوسط الحسابي، النسب المئوية، ومعادلة الفا كرونباخ، الوسط المرجح، الوزن المئوي. أظهرت نتائج البحث بأن أغلب المبحوثين هم من فئة العمر 46-58سنة وحاملين شهادة المتوسطة، ويمتلكون خبرة من 22-38 سنة، ويمتلكون مساحات صغيرة موزعة بين 3-35دونم، يزرعون أشجار النخيل بمساحات صغيرة موزعة بين بين (3-35) دونم، وتقدر أعداد النخيل بين 20-280نخلة، والمبحوثين يعتمدون على زراعة النخيل كمصدر رئيسي لدخلهم و البالغ نسبتهم 72.5%، كما أظهرت النتائج بأن المزارعون يطبقون التقانة على كل مساحة البستان و البالغ نسبتهم 95.8%، وأن عدد سنوات التطبيق تتراوح بين 4-6 سنوات و بنسبة 47.5%، وان المبحوثين مستمرين بتطبيق التقانة وبنسبة 95.8% ،كما أظهرت النتائج بأن المبحوثين يرغبون بالاستمرار في تطبيق التقانة وبنسبة 95.8%، و ان تقانة الحقن بجذع النخلة متوافقة مع خبرات زراع النخيل وملائمة مع نظمهم المزرعية وبنسبة 6. 96%، ومتوافقة مع القيم والعادات السائدة في المنطقة وبنسبة 92.5%،فضلاً عن ان المبحوثين يمتلكون خبرات ومهارات كافية لتطبيق التقانة وبنسبة 95.8% ، وقد اوصى الباحث بالاستمرار في نشر تقانة الحقن لما لها من دور في الحفاظ على الانسان والحيوان والبيئة والحشرات النافعة كالنحل في كل محافظات البلد، وزيادة الاهتمام بالتقانة وزيادة الدعم الحكومي للمزارعين من خلال توفير هذه التقانة وتدريب المزارعين بكيفية استخدامها.

الكلمات المفتاحية: التقانة، الية الحقن، مستوى انتشار.

INTRODUCTION

The agricultural sector is one of the most important sectors in the developing world and in Iraq as a **whole (Al-Shibawi, Al-Wasiti, 2022).** The development of the agricultural sector and the development of production methods are not only sought by developing countries but have become an urgent need for the overall success of agricultural development. **(Salhi, 2016) (Nema, 2019)** Agriculture is the main financier of national production in most developing countries and an area of employment and has an impact on the population and their lives. **(Al Bayati *et al.*, 2016)** Food security is one of the number one major challenge facing global agriculture **(FAO, 2015),** and to achieve this, widespread strategic objectives require increases in plant and animal agricultural production, which must be achieved at this stage and in the future to meet the widespread and increasing demand for food and agricultural products resulting from continued intensive growth. for the population, which is estimated at between 50.5 million and 2025. **(Al-Hakim, 2013)**. The development of agricultural production and productivity by achieving self-sufficiency in the country and agricultural production is a primary goal sought by all countries, including Iraq, and has contributed significantly to achieving sustainable food security and the national economy, because it is considered an important source of the national economy in many countries of

the world, by exporting large quantities to the countries of the world and providing hard currency, in addition to contributing to the development of GNP along with contributing to the development of the national economy while developing the contributing agricultural sector as a percentage of GDP **(Fatlawi, 2018).** Date palm cultivation needs a lot of preventive cultivation activities to protect it from pests and diseases in order to increase date palm production and achieve this development **(Al-Shuwaili, 2022)**. Agricultural technology and modern technologies are only a means and not an end themselves, and therefore achieving the desired goals requires a large-scale launch of this program. E techniques in the field of farmers and their application according to scientific advice, as well as the so-called green revolution that the world is witnessing, especially in Asia, America and Europe, the development of agricultural techniques characterized by high productivity and as a result of their widespread spread in the fields of millions of farmers **(FAO, 2011) (Shaaban, 2016**). The need for modern technologies has increased a lot in our time **(Al-Khafaji, Al-Badri, 2016)** and scientific and technological progress and the creation of modern technologies are among the main reasons for the occurrence of economic and social development **(Abdul Razzaq and Salman, 2018)** Modern technology has become a standard that determines the progress of nations and countries, but more than that, it has become seen as a source of wealth and an important factor for Promote social and economic development.(**Abdul wahab,b,2012)**. The dissemination of agricultural technologies is an activity and a basic service of increasing interest and impact in the agricultural sector in most countries, including Iraq, because it is related to meeting the technical needs of farmers, addressing the problems of their agricultural activities, increasing productivity and agricultural production, improving its quality, increasing its economic returns and meeting the needs of society from food security. **(Al-Taie, 2009)**. As the dissemination of technologies is an organized process consisting of three main activities (research, extension, processing) and includes a series of events, namely the selection of the appropriate technology for the targeted agricultural systems, introducing farmers to the technology and its advantages, convincing them to adopt it, providing them with the knowledge and skills necessary for its application, equipping them with it and the requirements for its application, and following up the application to achieve its spread in the targeted agricultural systems **(Al-Tai, 2013).** Therefore, achieving the effective spread of these technologies requires the existence of plans and strategies as well as planned programs involving the relevant authorities for research, processing, agricultural and extension services, and some institutions can be governmental and private in many developing countries or can be the private sector or agricultural companies and have a role in this process, especially through the supply of technologies or the provision of extension activities, and this is a marginal trend. There is an increasing increase in the field of agricultural development work in order to ensure the achievement of the desired goals **(Al-Hilfi, 2014)**. The level of spread achieved in any agricultural development, including the technology of palm injection in farmers' orchards - and the speed of the technology's spread, sustainability and productivity (the area on which it is deployed, the number of adopters, the time it takes) **(Rogeres, 2003)**. It is the result of the interaction of interrelated factors, including the characteristics of technology, farmers, the publishing plan, their farm systems, the organization of the publishing process, the level of effectiveness of providing technology, guidance, equipping farmers with it, services, follow-up, evaluation, and the characteristics of government support and lending **(Al-Tai, 2006)**. Modern agricultural technologies aim to change the style and practices of

farms from traditional practices and provide them with advanced agricultural knowledge and skills and positive trends, and all these factors contribute to increasing the rate of adoption and application of these technologies. **(Hatem, 2016)** Based on the importance and advantages of this technology (palm trunk injection technology) and the country's need to develop palm tree service operations and increase its productivity and sustainability of dates as one of the basic components in achieving sustainable food security for members of society, the Ministry of Agriculture, through a number of formations, has deployed this technology in 2014 in many governorates where palm cultivation is widespread, which represents an activity for youFarmers and their families and a main source of income **(Public Authority for Prevention 2016)**. Agricultural extension is also a system that ensures that farmers and their organizations have access to sources of information, knowledge and technologies and facilitate their communication and interaction with the relevant authorities**. (Al-Hafiz, Al- Tai, 2022)** Due to the importance of this technology, this research came to determine the spread of this technology, the number of farmers adopting this technology, and the agricultural areas in the research area.

Therefore, this research came to answer the following questions, including:

What are the characteristics of the respondents and the nature of their agricultural systems?

- What is the level of spread of palm stump injection technology for fertilization and control purposes in Tarmiya district / Baghdad governorate?

Research aims:

1. Identifying some of their agricultural characteristics and systems (educational level, age, agricultural experience in palm cultivation, tenure, and the importance of palm trees in income).
2. Determining the level of spread of the injection technology of the palm trunk for the purposes of fertilization and control of palm pests in the district of Al-Tarmiya.

Operational definitions:

Technology: An agricultural innovation designed to facilitate the implementation of date palm control and fertilization in the Tarmiya district in a faster and accurate manner.

Injection technology: A machine used by palm farmers in the Tarmiya district to control and fertilize palm trees.

Injection mechanism: A mechanism used to control diseases that affect palm trees in the district of Tarmiyah and to add nutrients.

Dissemination of injection technology: The process of facilitating the access of technology to the target audience in order to adopt and apply it on a large scale.

First - the research method

I used the field survey method to achieve the objectives of the current research because it is appropriate in obtaining data related to this field **(Saleh, 2004)**, and it branches out from the descriptive approach in social research **(Melhem, 2000).**

Secondly, the research community

Al-Tarmiyah District was chosen from Baghdad province as an area to conduct the research for two reasons:

1- The presence of a large number of palm groves in this district.

2- The technique of injection with the trunk of the palm tree was applied in this area.
The research included all date palm farmers in the province of Baghdad (Al-Tarmiyah district), who numbered (1708) farmers, distributed among three divisions within the Directorate of Agriculture of the province (Al-Abayji, Al-Mashada, Al-Tarmiyah).

Third - the research sample

Proportional random sample of 7% was selected from the total number of 1708 date palm farmers in Baghdad / Al-Tarmiya district, distributed among three agricultural divisions within the district (Al-Tarmiya Center *, Al-Abaiji *, Al-Mashahid *) so that the sample size was 120 respondents, as shown in the table number. (1).

The sample size would be 120 respondents, as shown in the following table.

Table (1): Distribution of the respondents among the agricultural divisions of Al-Tarmiya district.

district	Agriculture Division	Farmer numbers	percentage (7%	Final numbers after rounding	%
Altarmiya	Al-Taramia Division	1000	70	70	58.4
	Al-Abayji division	495	34.6	35	30
	Almushahada division	213	14.9	15	11.6
	Average	1708	120	120	100

Fourth - Data Collection Tool:

The questionnaire was adopted as a means of obtaining information from the respondents in order to suit it with the nature of the research and the data required to obtain it, as the questionnaire is considered an appropriate tool for collecting data because it is more objective than other means of collecting data to achieve the objectives of the research.

1. Scientific literature related to the dissemination of agricultural technologies.
2. The annual reports of the palm tree injection campaign issued by the Ministry of Agriculture
3. Meetings with the workers in the campaign (administration and researchers) affiliated with the General Authority for Plant Protection
4. Opinions of a group of field specialists in plant and soil protection, horticulture and agricultural extension, as experts are considered one of the main sources in building the scale.
5. View the studies in agricultural extension and research in the protection of crops and horticulture

In the light of the above, the researcher prepared a preliminary questionnaire that includes two parts, the first part includes a description of the respondents and their agricultural

systems, which includes (8) paragraphs, and the second part includes (11) paragraphs to determine the level of spread of the injection technology of the palm trunk in Al-Tarmiya district h, in the light of the meaning of the spread of agricultural technologies. And the prevalence indicators mentioned in some studies, including (Al-Taie 2013 study), four indicators of prevalence were identified, which are:

1. Number of implementers of agricultural technology (%).
2. The area where the technology is applied (%).
3. Sustainability of technology application (years of technology application).
4. The future direction of technology sustainability.

Fifth - the scale validity

The apparent validity of the scale was examined by presenting it after the completion of the measurement tool to a group of experts in agricultural extension to measure the apparent validity and experts and specialists in horticulture and prevention to measure the validity of the content. A preliminary test (pre-test) was conducted in (11) months. /2022) on a sample consisting of (15) respondents taken from Al-Tarmiya district- Baghdad provainc. The stability of the questionnaire was measured using Cronbach's alpha equation, and the stability coefficient was (0.90), where all the statements were characterized by high stability. Data were collected during a period from (12/2022 to 1-2023) using the personal interview method. The following statistical tools were used (frequencies, percentages, arithmetic mean, Cronbach's alpha equation, weighted average, weight percentage).

Sixth: quantifying the scale

The researcher used a binary scale (yes, no), and the following weights (0, 1) were given, respectively, for all (11) paragraphs to determine the level of spread of the palm stem injection technology in Al-Tarmiyah district.

Results and discussion

The first aim: - to identify the social and economic characteristics of the respondents: -

1_ The educational level: The results of the research showed that the majority of the respondents are middle school graduates with a rate of 30%, then college graduates with a rate of 27.4 %.

Then middle school graduates with a rate of 22.5 %, then reading and writing with a rate of 8.5 %, then the honest respondents had a percentage of 6.6%, while the respondents had high school diplomas with 5%, and this percentage is very low, as in Table No. (2)

Table (2): Distribution of respondents according to educational level

Educational level	Number of farmers	percentage
Illiterate	8	6.6
Reads and writes	10	8.5
Medium	36	30
Prep	27	22.5
College	33	27.4
Master's Degree	6	5
Average	120	100

We conclude from the above table that the majority of the respondents are educated and are good at reading and writing, and this is a positive factor that helps the farmer to read the instructions for using pesticides and fertilizers and the quantities specified by the producing companies and how to use the injection technology correctly on palm trees and to learn about the advantages and benefits of technology in the fields of control and fertilization as well On the environmental effects resulting from the use of technology.

2- Age: The results showed that the category of respondents whose ages ranged from (33-45) years came in the first place with a rate of (33.5 %), then the category of respondents (20-32) years came in the second place with a rate of (32.7 %), then the category The respondents (46-58) years came in third place with a rate of (27.6 %), then the category of respondents (59-71) came in the last place with a rate of (6.2%), as in Table. (3).

Table (3): Distribution of respondents according to age groups.

No.	age (year)	Number of farmers	percentage	Arithmetic average	standard deviation
1	20-32/year	39	32.7	22	6 .2
2	33-45/year	40	33.5		
3	46-58/year	33	27.6		
4	59-71/yea	8	6.2		
average		120	100		

We conclude from the above table that most of the respondents are young and middle-aged, and these groups are characterized by their acceptance of new ideas and developments, and they accept everything new in their field of work and application in order to develop their agricultural production.

3- Experience in palm cultivation and orchard management: The results showed that the category of respondents with experience ranging between (22-37) years came in the first place with a rate of (60%), then the category of respondents (38-53) years ranked second with an average of (27.5%) Then the category of respondents (5-21) years, with a rate of (12.5%), as shown in Table (4).

Table (4): Distribution of respondents according to categories, years of experience in palm cultivation and orchard management.

Years of experience in palm cultivation and orchard management	Number of farmers	percentage	Arithmetic average	standard deviation
5-21/year	15	12.5	32	2
22-37/year	72	60		
38 - 53 / year	33	27.5		
Average	120	100		

Years of experience in palm cultivation and orchard management, We conclude from this that the majority of the respondents have sufficient experience to know the diseases that affect date palms, methods of controlling them, types of fertilizers, various methods of control, fertilization, and palm service, in addition to sufficient information on how to use injection technology in the control and fertilization processes because of their role in preserving human and animal health in particular. environment in general.

4- A- Possession: The results showed that the category of respondents who have possession ranging between (3-35) dunums came in the first place with a rate of (61%), then the category of respondents (36-68) dunums ranked second with a rate of (33%), then the category of respondents (69-101) dunums ranked last, at a rate of (6%), as shown in Table (5).

Table (5): Distribution of respondents according to categories of possession.

categories	The total area of the orchard/dunum	Number of farmers	percentage	Arithmetic average	standard deviation
possession	(3-35)	73	61	43	5.7
little area	(36-68)	40	33		
medium area	(69-101)	7	6		
	average	120	100		

It appears in the above table that most of the respondents are holders of small- and medium-sized holdings.

4-b- The area planted with palm trees: The results showed that the category of respondents who have an area planted with palm trees ranging between (3-35) dunums ranked first, with a percentage of (45.8 %), then the category of respondents (36-68) dunums came in the second rank, with a percentage of (37.6 %) Then the category of respondents (69-101) dunums came last with a rate of (16.6 %) as in Table (6).

Table (6): Distribution of the respondents according to the categories of the area planted with palm trees.

tenure categories	Area cultivated with palm trees / dunum	Number of farmers	percentage	Arithmetic average	standard deviation
little area	(3-35)	55	45.8	41	10
medium area	(36-68)	45	37.6		
A large area	(69-101)	20	16.6		
	average	120	100		

We conclude that the majority of the respondents are the owners of small and medium holdings, and that a large percentage of the respondents own orchards of medium size and somewhat large, and this indicates that most palm farmers need modern technologies to facilitate the process of palm service due to the role of these technologies in reducing effort and time in addition to costs.

4- C- The number of palm trees planted: the results showed that the category of respondents who have palm trees ranged between (20-280) palm trees came first with a percentage of (58.4%), then the category of respondents (281-540) palm trees came second with a percentage of (25.8 %) Then the category of respondents (542-802) palm trees with a rate of (15.8 %) ranked last as in Table (7).

Table (7): Distribution of respondents according to the number of palm trees planted in their orchards.

Number of palm trees / dunum	Number of farmers	percentage	Arithmetic average	standard deviation
20-280 palm trees	70	58 .4	35 .2	31
281-540 palm trees	31	25.8		
542-802 palm trees	19	15.8		
average	120	100		

We conclude from the above table that two-thirds of the respondents own palm trees in their orchards in large numbers, and the greater the number of palm trees in the orchard, the more the farmer is encouraged to use all modern methods that facilitate the palm service process.

5- The importance of date palms in agricultural income: The results showed that palm cultivation is a major source for (87) respondents with a rate of (72.5%) and is a secondary source for (27) respondents with a rate of (22.5%) and is considered a source of little importance for (6) Respondents with a rate of (5%) as shown in the table. (8)

Table (8): Distribution of respondents according to the importance of palm cultivation in agricultural income.

Importance	Number of farmers	percentage
main source	**87**	**72.5**
secondary source	**27**	**22 .5**
An insignificant source	**6**	**5**
Average	**120**	**100**

We conclude that palm cultivation is a basic and important source of agricultural income on which the vast majority of the respondents depend, which calls for intensifying agricultural extension efforts towards it and intensifying awareness of the injection method of fertilization and control in order to increase the number of palm trees and increase their productivity.

The second aim:

The researcher determined the level of spread of the injection technology of the palm trunk for the purposes of fertilization and control, according to the indicators of the spread of modern technologies, as follows:

First: The number of technology applicants

The results showed that the majority of the (120) respondents apply the technology of injecting the palm trunk, and this indicates the widespread spread of this technology in the district, and this indicates that the technology meets the needs of farmers and contributes to achieving a continuous increase in agricultural productivity and production and improving the quality of dates, in addition to improving farm income and its role in preserving natural and environmental resources.

Second: The area in which the technology is applied

The results showed that the majority of the (115) respondents applied the injection technique to the palm trunk on the whole orchard at a rate of 95.8%. As for the respondents who applied to a part of the orchard, their number was (5) at a rate of 4.2%, as shown in Table (9):-

Table (9): Distribution of the respondents who applied the technology to the entire area of the orchard and to part of the area of the orchard.

Technology application	Farmers numbers	percentage
All	115	95.8
Part	5	4.2
Average	120	100

We conclude from this that the majority of the respondents apply the technology to all orchards because of its availability at reasonable prices and contribute to reducing time and effort as well as being safer than the traditional methods used in fertilization and control.

Third: the sustainability of the application

1- Number of years of application:

The results showed that the category of respondents who had a number of years of technology application ranging between (4-6) years ranked first with a percentage of (47.5), then the category of respondents (7-9) years ranked second with a percentage of (28.3), then the category of respondents (1- 3) A year in the last rank, with a percentage of (24.2), as shown in Table (10).

Table (10): Distribution of respondents according to the number of years of applying the injection technique of the palm trunk.

Number of years of application	Farmers numbers	percentage
1-3 / year	29	24.2
4-6/year	57	47.5
7-9/year	34	28.3
Average	120	100

We conclude that the number of years the respondents applied the injection technology to the trunk of the palm tree was a good period for adopting this technology. of wastage and can be applied in various conditions such as wind and rain.

2- Continuity in the application of technology

The results showed that the majority of the (115) respondents continued to apply the palm stem injection technology with a rate of (95.8%), while the respondents who did not continue to apply the palm trunk injection technology were (5) with a rate of (4.2%) as in Table (11).

Table (11): shows the distribution of the respondents according to the continuous application of the injection technique of the palm trunk.

Continuity of application	Farmers numbers	percentage
continuous	115	95.8
not continuous	5	4.2
Average	120	100

We conclude that the majority of the respondents continue to apply the injection technology because of the positive results achieved in fertilization and control of the contribution of this technology in reducing the rate of environmental pollution because the pesticide is injected in a closed system and the efficiency of the chemicals used for this method increases and its effect is significant against many types of insects that infect palm trees.

Fourth: the future direction of sustainability and application

A- Desire to continue applying injection technology

The results showed that the majority of the respondents (115) wished to continue applying the injection technology in the trunk of the palm tree at a rate of (95.8%), while the respondents who did not continue to apply the palm trunk injection technology were (5) with a rate of (4.2%) as in Table (12).

Table (12): Distribution of respondents according to their desire to continue applying injection technology in Al-Tarmiya district.

Desire for continuity	Farmers numbers	percentage
I want to continue	115	95.8
I do not want	5	4.2
Average	120	100

We conclude the desire of the respondents to have a desire to continue applying the injection technology with the trunk of the palm, because of the efficiency of the injection technology with the trunk of the palm and achieving the desired goals of adopting it, which is a more economical and less polluting method for the environment, and achieving a high rate of killing insects for palm trees treated by the injection method, more than 85% of palm trees treatment by other methods

b- The technology is compatible with the experiences of palm farmers.

The results showed that the majority of the respondents, amounting to (116), that the technology is compatible with their experience, with a rate of (96.6%), while the respondents

whose technology is not appropriate for their experience was (4), with a rate of (3.4%), as shown in Table (13).

Table (13): Distribution of respondents according to the compatibility of technology with their experiences in the district of Al-Tarmiya.

The compatibility of technology with the experiences of farmers	Farmers numbers	Percentage
compatible	116	96.6
non compatible	4	3.4
average	120	100

We conclude that the technology is compatible with the experiences of the respondents because of its ease and can be used in the most difficult conditions and does not cause damage to palm trees.

Adaptation of technology to agricultural systems

The results showed that the majority of the respondents, who numbered (116), were suitable for their farming system, with a percentage of (96.6%), while the respondents whose technology is not appropriate for their experience was (4), with a rate of (3.4%), as shown in Table (14).

Table (14): Distribution of the respondents according to the suitability of the injection technique of the palm trunk with the agricultural system in the Al-Tarmiya district.

suitable Technique	Farmers numbers	percentage
suitability	116	96.6
Non suitable	4	3.4
Average	120	100

We conclude that the technology is suitable for the agricultural systems of the respondents because of its ease and efficiency of application and the possibility of achieving the highest productivity in quantity and quality, and it is used in dense orchards that are densely populated with imaginary trees.

D- View the results of the application

The results showed that the majority of the respondents, whose number is (115), can see the results of the technology at a rate of (95.8%). As for the respondents who do not see the results of the technology, their number was (5) at a rate of (4.2%), as shown in Table (15).

Table (15): shows the distribution of the respondents in observing the results of the application of the injection technology with the palm trunk in the Al-Tarmiya district.

View the results of applying the technology	Farmers numbers	percentage
	115	95 .8
I watch	5	4 .2
I do not watch	120	100

We conclude that the majority of the respondents can see the results of injection technology in fertilization, control and application time are less compared to other methods, as well as other advantages.

E- Compatible with the prevailing values and customs in the region

The results showed that the majority of the respondents, who numbered (111), that technology is compatible with the prevailing values and customs in the region, with a percentage of (92.5%), while the respondents were not compatible with the prevailing values and customs in the region, so their number was (9), with a rate of (7.5%), as in Table (16)

Table (16): Distribution of respondents according to the compatibility of the injection technique with the trunk of the palm tree with the prevailing values and customs in the Al-Tarmiya district.

Compatible with prevailing values and customs	Farmers numbers	percentage
Compatible	111	92 .5
not compatible	9	7.5
Average	120	100

We conclude from this that the majority of the respondents are compatible with technology with the prevailing values and customs in the region because it contributes to preserving productive and fruitful agricultural crops and trees as well as its contribution to reducing production costs, effort and time, protecting the environment and reaching the highest productivity in quantity and quality.

F- Possess sufficient expertise and skills to apply the technology.

The results showed that the majority of the (115) respondents had experience and skills to apply technology at a rate of (95.8%), while the respondents who did not have experience and skills to apply technology were (5) at a rate of (4.2%) as in the following table (16).

Table (16): Distribution of the respondents according to their experience and skills to apply the injection technique with the trunk of the palm tree in Al-Tarmiya district.

Possession of the respondents experiences and skills	Farmers numbers	Percentage
I have	115	95.8
I dont have	5	4.2
Average	120	100

We conclude from this that the majority of the respondents have sufficient skills and experience to apply the injection technique of the palm trunk for ease of application in the two processes used in fertilization and control.

CONCLUSIONS

1- The injection technology of the palm trunk has achieved a high level of spread in Al-Tarmiya district in injecting pesticides and fertilizers into the trunks of palm trees, as it is one of the very effective and economical methods when compared to other methods, as well as reducing environmental pollution and ensuring consumer protection.
2- The extension activities provided in the field of date palm service are rather few and the focus is on the control aspect at the expense of the rest of the aspects.

RECOMMENDATIONS

1- Continuing to spread this injection technology because of its role in preserving humans, animals, the environment, and beneficial insects such as bees in all governorates of the country.

2- Increasing extension activities in the field of fertilization and intensifying efforts in the field of control

3- Increasing interest in technology and increasing government support for farmers by providing this technology and training farmers on how to use it.

4- Educating farmers about the importance of this technology in preserving the environment from pollution

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 50-64
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emad & Al-Bayati

المجلة العراقية لبحوث السوق
وحماية المستهلك

PREPARATION AND ANALYSIS OF MOLECULARLY IMPRINTED SOLID-PHASE EXTRACTION FOR DIAZEPAM AND ITS PHARMACEUTICAL APPLICATIONS BASED ON ACID MONOMERS USING A BULK POLYMERIZATION METHOD

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Received 8/ 5/ 2023, Accepted 5/ 7/ 2023, Published 31/ 12/ 2024

ABSTRACT

A molecularly imprinted polymer was used to make two electrodes (MIP). MIP was manufactured using Diazepam (DZP) as the template, 2-acrylamido-2-methyl-1-propan sulfanic acid as the monomer, and N.N-methylene bis acrylamide as the cross-linker. Non-imprinting polymers (NIP) were created using the identical composition, minus the template (Diazepam). Tritolyl phosphate (TTP) and dibutyl sebacate (DBS) are examples of plasticisers utilised in the PVC matrix to produce films. DZP-MIP electrode slope, detection limit, durability, and linearity range are evaluated. The outcomes of the selectivity measurements on the interfering cations (Al^{+3}, Ca^{+2}, and K^{+1}) indicate that they do not inhibit Diazepam. The produced electrode exhibited favorable response, including to conduct research on pharmaceuticals.

Keywords: Molecularly imprinted electrode, diazepam, potential metering, 2-acrylamido-2-methyl-1-propan sulfanic acid monomer, and different plasticizers (DBS) (TPH).

تحضير وتحليل الطور الصلب المستخلص المطبوع جزيئيا للديازيبام وتطبيقاته الصيدلانية المعتمدةعلى المونمرات الحامضية باستخدام طريقة البلمرة الثقيلة

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الخلاصة

تم استخدام بوليمر مطبوع جزيئيًا لصنع قطبين (MIP). تم تصنيع MIP باستخدام Diazepam (DZP) كقالب، 2-acrylamido-2-methyl-1-propan sulfanic acid as monomer، و N.N-methylene bis acrylamide باعتباره الرابط المتقاطع. تم إنشاء البوليمرات غير المطبوعة (NIP) باستخدام تركيبة متطابقة، باستثناء القالب (Diazepam). يعتبر تريتوليل فوسفات (TTP) وثنائي بيوتيل سيباكات (DBS) أمثلة على الملدنات المستخدمة في مصفوفة PVC لإنتاج الأغشية. يتم تقييم منحدر القطب Dia-MIP، وحد الكشف، والمتانة، والمدى الخطي. تشير نتائج قياسات الانتقائية على الكاتيونات المتداخلة (Al + 3 و Ca + 2 و K +1) إلى أنها لا تثبط الديازيبام. أظهر القطب الناتج استجابة إيجابية، بما في ذلك إجراء البحوث على المستحضرات الصيدلانية. على المستحضرات الصيدلانية.

الكلمات المفتاحية: قطب كهربائي مطبوع جزيئيًا ، ديازيبام ، قياس الجهد ، مونومر 2-acrylamido-2-methyl-1-propan sulfanic acid، والملدنات المختلفة (DBS) (TTP).

INTRODUCTION

Diazepam, 7chloro1, 3dihydro1methyl5phenyl2H1, 4benzodiazepin2one, is the most often prescribed benzodiazepine hypnotic, tranquilliser, anticonvulsant, and muscle relaxant (**Hosseini &Motaharian, 2015**). Rapid and dependable screening procedures for drugs and poisons in highly intricate biological specimens (urine, serum)for use in forensic and clinical toxicology (Liu *et al*., 2013).Diazepam is one of the most often prescribed 1,4-benzodiazepines and is commonly marketed under Valium (**Honeychurch *et al*., 2013**).

The effects of sleeping aids. DIA is a benzodiazepine that provides a positive allosteric regulation receptor for gamma-aminobutyric acid, increasing receptor-binding GABA molecules. This modification will induce repolarisation of GABA receptors (channel ligand) and have a calming effect(**Omar & Eesa, 2017**) . The receptors are located in the central nervous system and the mechanism explanation by which DZP induces drowsiness and reduces stress . In Indonesia, DZP is considered a substance class of psychoactive medications, and its prescription is subject to government regulation. To obtain DZP, one must visit a physician and get a prescription (**Hasanah *et al*., 2021**).

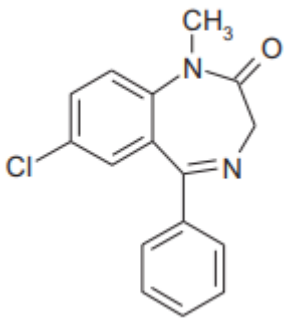


Figure (1): Chemical structure of Diazepam (**Casarrubea *et al*., 2012**).

Experimental

Preparation of MIP and NIP

- To prepare Diazepam molecularly imprinted polymer (DZP-MIP1), (0.2847g) of Diazepam was combined with 1 ml of 2-acrylamido-2-methyl-1-propan sulfanic acid as the monomer. Next, 3.08 g of N.N-methylene bis acrylamide was included in the mix as the cross-linker, and (0.3g) of benzoyl peroxide was added as the initiator. The amalgamation was agitated for 5 minutes to achieve a homogenous solution, and then oxygen was removed from the mixture by passing N_2 over it for 20 minutes. The resulting tube was inserted. a container filled with water at 65°C. The molecularly imprinted polymer resulted as soon as the procedure ended completely solidified, and following the polymerisation process, it disintegrated into a tiny polymer particle. Sonication was used on this material. in a 9:1 mixture of CH_3OH and CH_3COOH, eliminating the MIP sample document. How big are DZP-MIP particles (75-125 m) non-molecularly imprinted polymers can be made in the same way components and under the same circumstances as molecularly imprinted polymers. DZP-MIP, but without the Diazepam (template).

Instruments

This study utilised a WTW model ion analyser, a WTW model pH 720 pH metre, and a calomel electrode that has reached saturation (Gallenkamp, USA). The BHH-MIP was an electrode fabricated in a test tube, and every potentiometric experiment was performed at room temperature. In conjunction with the Ag-AgCl and the reference electrode, the diazepam-MIP electrode was a 0.1 M diazepam dissolved in the internal fluid. Placing the PVC tube (1-4 cm in length) in a clear dish and soaking it in THF caused it to become flattened and polished. A membrane was trimmed to match the outer diameter of the PVC tubing and adhered to the precise result. The opposite orientation of the PVC and, finally, the tubing was fastened towards the electrode device. The electrodes were enhanced by good soaking them in 0.1 M diazepam solution for a minimum of three hours prior to use.

Materials and chemicals

Daizepam was acquired to do with the State Corporation for Pills Industry and Healthcare Equipment (IRAQ- SID- Samara). Commercial diazepam pills bought from nearby pharmacies include VALIAPAM 10 tablets 500 mg from (SDI-lraq), Valium 10 tablets 5 mg from (Australian), and. Plasticizers, Tritoly phosphate 90% was utilised as received and dibutyl sebacate (DBS) (purity of 97.0%), were acquired from Sigma-Aldrich. As a monomer, allyl chloride was used; ethylene glycol di methacrylate (EGDMA) and benzoyl peroxide (BPO)(78%) were acquired from Sigma-Aldrich. The chemicals utilised were of the purest concentration of reagents and were used without extra cleansing.

The creation of standard solutions

Producing a common fluid of 0.01 M Diazepam by breaking down 0.1423 g of legal Diazepam in methanol and then filling a 50 mL volumetric flask with the resulting solution. Using the same method, the additional solutions were produced in 50 mL at concentrations ranging from ($5x10^{-5}$ to 10^{-2}) M. All competing cations (Al^{+3}, Ca^{+2}, and K^{+1}) were made as a 0.01 M stock solution at concentrations ranging from ($5x10^{-5}$ to 10^{-2}) M and then reduction of to 100 ml.

Synthesis of Membrane Molecularly Imprinted Polymers Electrode

According to Thomas and Moody (Moody & Thomas, 1988) , the Diazepam membrane was immobilised within the PVC tube (17) DZP-MIP (0.036g) was combined with other plasticisers (0.45g) employed in this study, including TTP(electrode M1), DBS, and others (electrode M2). Then, 0.2g of It was PVC powder. Added 7 mL of tetra hydro furan and mixed until a thick, viscous liquid was obtained. The fluids were then stirred until the combination was complete and homogenous. The whole combination was poured into a glass ring (30-35 mm in diameter) and set on a flat glass with a superimposed ribbon filter. At ambient temperature, the solvent was permitted to vaporise for at least (24-48) hours. The obtained membrane had a varied thickness than other membranes, ranging between (0.4) and (0.7) mm. This membrane size was appropriate for preparing electrodes.

Construction of Ion-Selective Electrodes

Electrode body structure and immobilisation were accomplished as Mahajan *et al* **(Mahajan & Sood, 2007**). described. The glass tube had been filled. With 0.1 M diazepam fluid as an internal fluid. Membrane electrode requirements include soaking the membrane in a standard (0.1) M diazepam solution for at the very least two to three hours prior actual metrics **(Aljabari & Al-Bayati, 2023**).

Pharmaceutical Sample Preparation

Extract the active ingredients from pharmaceutical samples by grinding the crushing tablets using a mortar and pestle. Then, take an acceptable quantity required in advance for use in 50 mL of solutions. Utilised a correct methanol concentration (CH_3OH) for dissolving pharmaceutical samples and filled the volumetric flask to 50 mL with methanol while stirring for more than 30 minutes. After filtering the fluid via 0.07m cellulose filter paper, amounts or proportions of $5x10^{-3}$ M and $5\ x10^{-4}$ M diazepam were achieved.

3. Results and Discussion

The polymer molecular imprint of Diazepam was identified after it was determined using UV. Via the drug's wavelength. As demonstrated in the figures, a preliminary diagnosis was made to confirm the presence of the drug in this imprint**.**

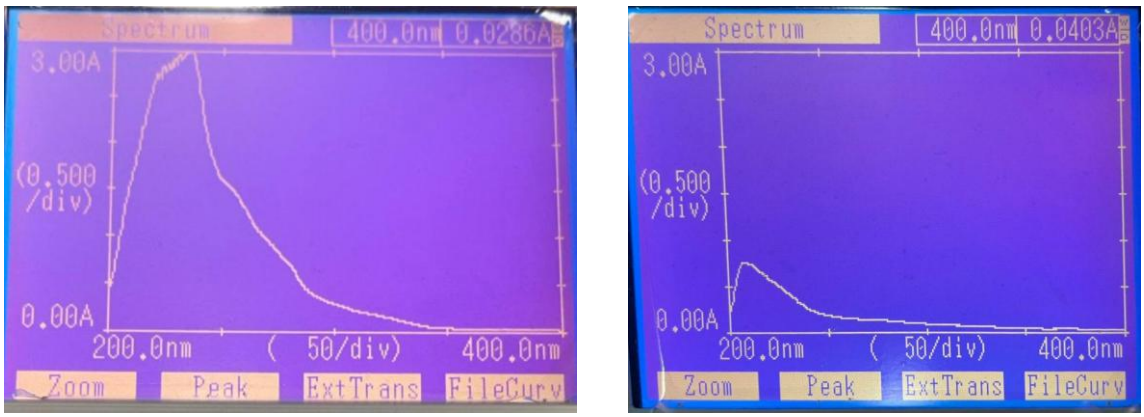


Figure (2): A and B the absorption of diazepam drug at 235.5 nm before and after extraction . MIP of Ate was synthesised using bulk polymerisation (non-covalent). A functional monomer played a crucial role in researching interactions with the template. The MIP and NIP were made using allyl chloride as the monomer. FTIR analysis FTIR is an essential chemical characterisation technique for detecting functional groups in a molecule.

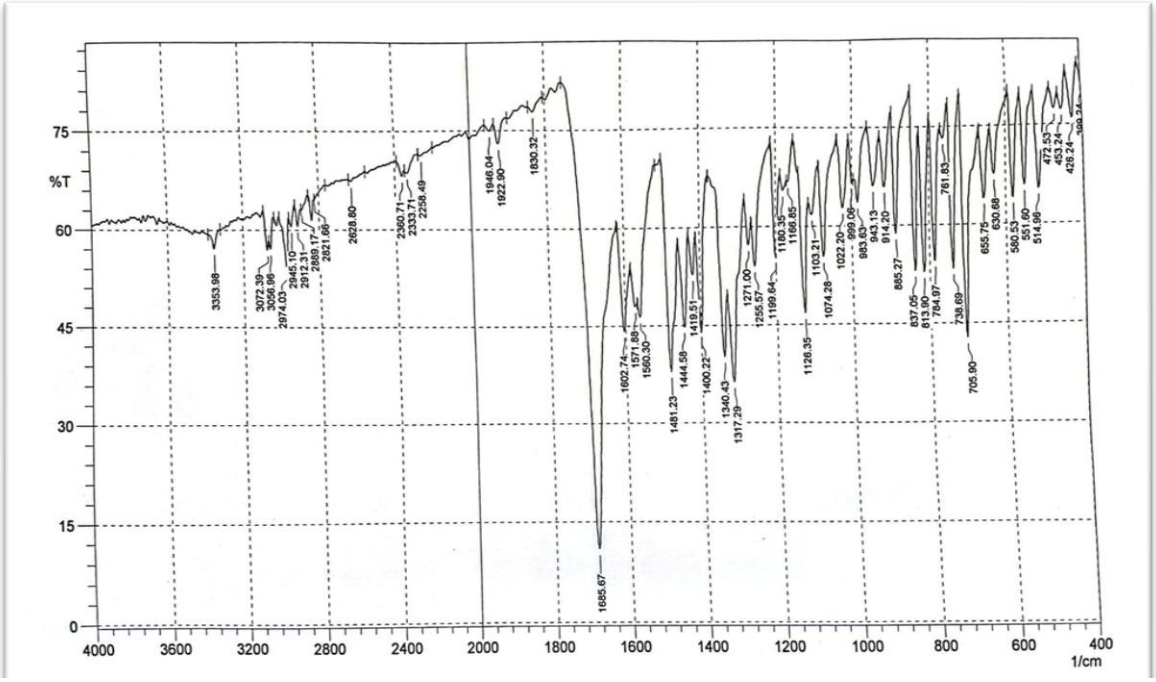


Figure (3): FTIR spectrum of diazepam standard.

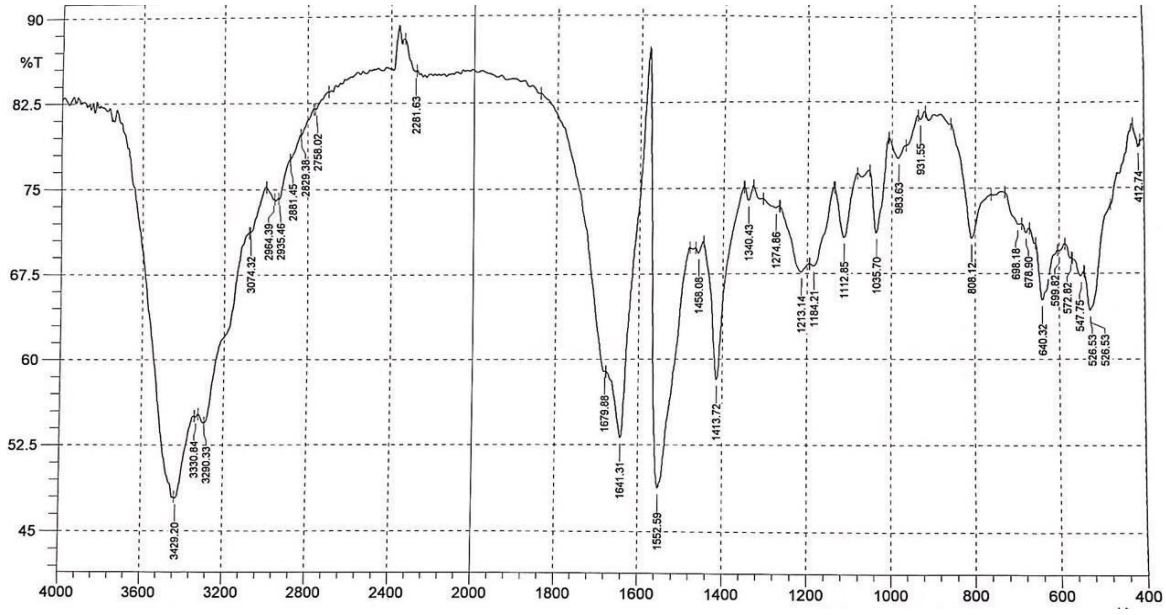


Figure (4): FTIR spectrum of DZP-MIP prior to and following elimination(before removal of the template Diazepam).

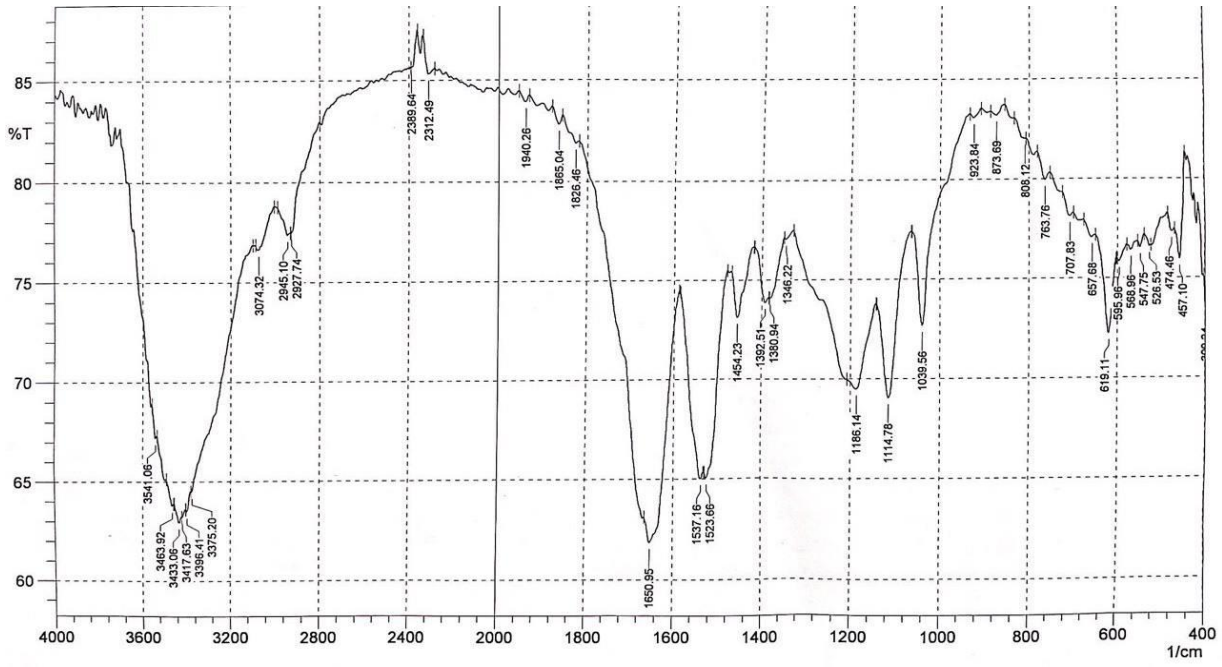


Figure (5): FTIR spectrum of DZP-MIP prior to and following elimination (after removal of the template Diazepam).

Table (1): The structures of the main three compositions of Dia-MIP and the bands indicate MIP before &after the removal template.

Template (Diaspam)	**Monomer (Allyl chloride)**	**Cross linker (Ethylene glycol di amethacrylat)**	
Band	**Drug(Template)**	**MIP before extraction**	**MIP after extraction**
N C-H aliph. str.	2945,2912cm^{-1}	2964,2935 cm^{-1}	-
N C-H aromatic str.	3056 cm^{-1}	3074 cm^{-1}	2945,2927 cm^{-1}
N O=C-N str. amid	1685 cm^{-1}	1641 cm^{-1}	-
N C=C aromatic. str.	1602 cm^{-1}	1552 cm^{-1}	-
N C-Cl str.	813 cm^{-1}	808 cm^{-1}	-
NN-H str.	-	3330 cm^{-1}	3396 cm^{-1}
N-S=O str.	-	1340 cm^{-1}	1380 cm^{-1}
O=S-OH str.	-	3424,3074 cm^{-1}	3417,3433 cm^{-1}
N-C=O carbonyl.	-	1679 cm^{-1}	1650 cm^{-1}

The FTIR spectra of Ate displayed the following bands: (2945, 2912, 3056, 1685, 1602, 813) cm-1 for NC-H aliph. str, NC-H aromatic str.,N O=C-N str. amid, NC=C.aromatic. Str. N-C-CL str. N N-C=O carbonyl. Before template removal, the FTIR spectra of the Diazepam MIP(DZP) displayed the following bands: 2964, 2935, and 1641 cm-1 for N C-H alph str., NO=C-N str. amid, and N C=C aromatic str. The absence of N C-H alph str, NO=C-N amid str, and N C=C aromatic str in the FTIR spectrum of the MIP(DZP), following template removal indicates that the drug has been extracted from the template. Using allyl chloride as a monomer for synthesising other MIPs for Diazepam (DZP), the FTIR spectra of the MIPs before and

after removing the template and NIP are shown in Table. The values of the band **(Abbas *et al.*, 2020)** Ion-selective electrodes (ISE) are among the most widely used types of band electrodes. commonplace sensors based on voltage analysis. Utilised in laboratory experiments, industrial applications, process control, physiological assessments, and ecological monitoring. Membranes of electrodes that respond to Reaction analysis of concentration that generate ions that may be detected using an ion-selective electrode (**Aljabari & Al-Bayati, 2021)**. Electrodes with a membrane are divided into two primary categories: ion-selective, which are sensitive to ions, and molecular-selective, which is sensitive to molecules which are utilised for measuring molecular analytes (**Al-Nisani *et al.*, 2021, Ameen *et al.*, 2015**). Electronic current travels along electrons in metals but along ions in liquids. Ion-selective electrodes operate based on these two distinct forms of electrical conductivity **(Ismaeel & Al-Bayati, 2021**). It is possible to do electrical analysis using one of these galvanic cells, electrolysis, and used to assess the conductivity of each electrochemical reaction **(Muhammad, 2013**).

These cells must be in touch, having fluid across the membrane and inside the cell. There are further ISE configurations where wires are attached to the membrane on only one side. Conventional cell composition consists of:

Outer ref. Test solution | membrane | internal ref. |

Or Outer ref. Test solution |ion-selective electrode |

a device's internal electric current An electrolytic cell is required equal zero. Based on this need, the cell is created based on the terms of the underlying principle behind the construction of electrolytic cells.

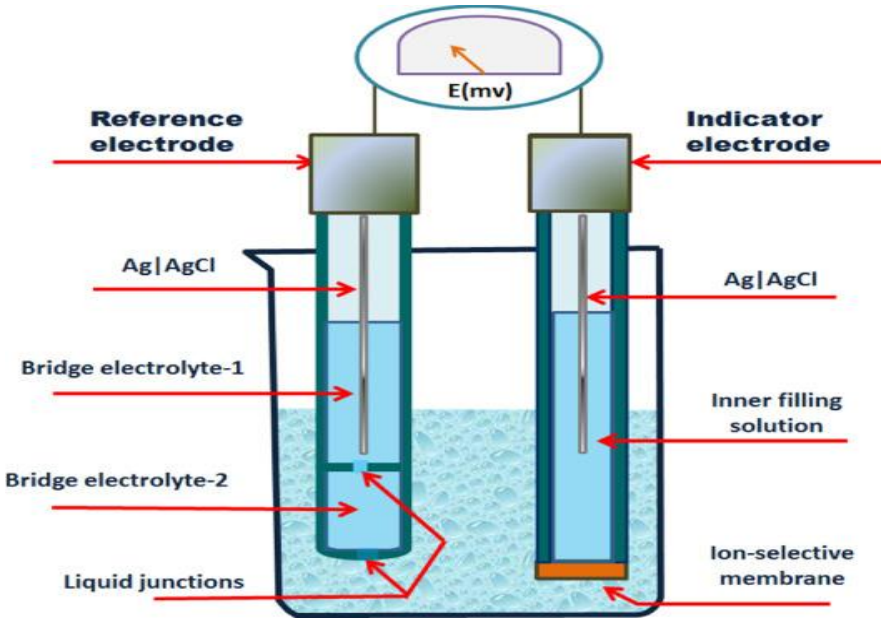


Figure (6): Diagram in schematic form. The typical potentiometric cell is depicted here using an ion-selective electrode in the accompanying(**Abd El-Rahman & Salem, 2015**).

Using Diazepam as a template, 2-acrylamido-2-methyl-1-propan sulfanic acid and N.N-methylene bis acrylamide as monomers and cross-linkers, respectively, as well as the benzoyl peroxide as an initiator, two electrodes were produced. Plasticisers are indispensable to ISE membranes. Membranes, including polymer and other materials compatibility ingredients, offer a membrane-homogeneous environment when plasticisers are utilised as membrane solvents. However, leaching of the plasticiser during The ISE membrane should see some practical use. be prevented because it would harm the electrode's performance over time. Because of a PVC matrix, four electrodes have been manufactured. The plasticisers Tritolyl phosphate (TTP) and dibutyl sebacate(DBS) are examples. The linearity range, correlation coefficients, detection limit (M), and life duration of the DZP-MIP1 (M1, M2) membrane-based electrodes were evaluated (day). The gathered information is presented in table 1 and figure 5.

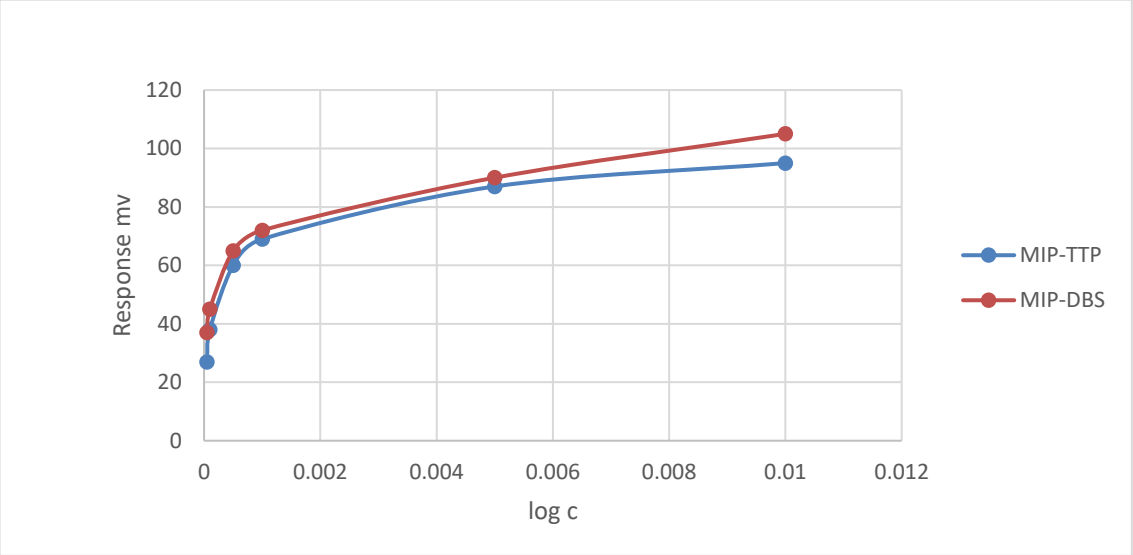


Figure (7): For DZP-MIP membrane electrodes, calibration curve**.**

Table (2): Characteristics of the diazepam-MIP electrode built with several functional plasticizers and monomers.

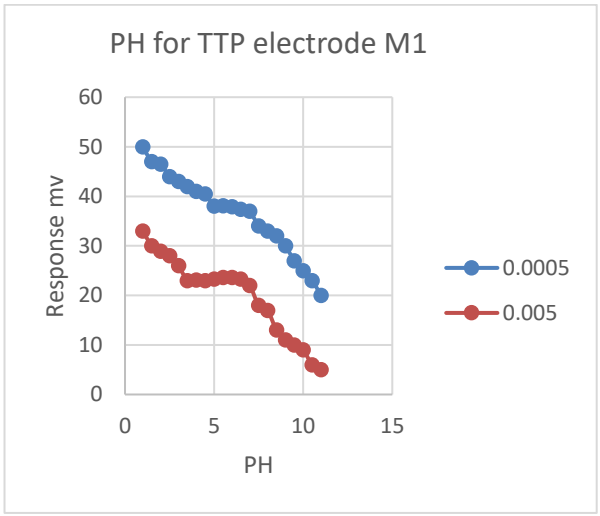
Membrane composition	DZP-MIP1+ TTP (M1)	DZP-MIP1+DBS (M2)
Slop (mV/decade)	27.113219	28.41902
Linearity range (M)	5×10^{-5}-1×10^{-2}	5×10^{-5}-1×10^{-2}
Correlation coefficient	0.9663	0.9932
The detection limit (M)	5×10^{-6}	5.5×10^{-6}
Lifetime (day)	19	16

Effect of pH on electrodes response:

Two electrodes were fabricated using Diazepam as a template and allyl chloride as a precursor. A pH investigation was conducted on DZP membrane electrodes with varying concentrations of Dia (5×10^{-3} and 5×10^{-4}). In pH investigations, pH testing (1-11) uses HCLacid(0.1M,1M) or NH4OH (0.1M,1M). As indicated in Table (3) and Figure 8, the end result achieved by including the proper amount of HCl/NH4OH the composition of electrodes is responsible for the difference in pH-value-related potential (**Mahdi & Al-Bayati, 2020**).

Table (3): Working pH range for Selective diazepam electrode.

Number and design of MIPs	Membranes	Membrane design	pH range	
			5×10^{-3}	5×10^{-4}
MIP DZP+ AMPS +N,N-MDAA	M1	DZP-MIP+TTP	3.5-7	5-7
	M2	DZP-MIP+DBS	3-4.5	2-5.5



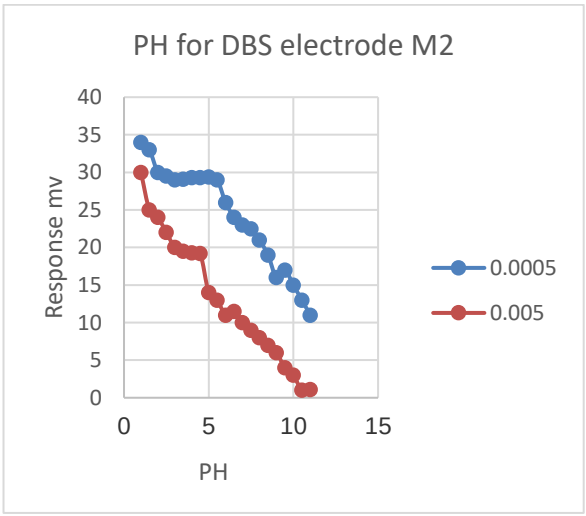


Figure (8): Effect of pH on the Diazepam [DZP-MIP+ TTP(M1) and DZP-MIP +DBS (M2)] electrodes at concentrations 5×10^{-3} and 5×10^{-4}.

Interference studies

Two electrodes were fabricated using Diazepam as a template and 2-acrylamido-2-methyl-1-sulfanic acid as a precursor. The pH For measuring the selectivity coefficient, a different solution approach was used. Utilise the unique equation required for these parameters, as shown in the following equation.

Log K pot= [(EB − EA)/(2.303RT/z F)] + (1 –zA/zB) log aA (**Al-Safi & Al-Bayati, 2018**)
EA, EB, zA, zB, and aA are, in order as well as the charges, potentials, and actions of the primary A ions and the interfering B ions when aA = aB.

The findings for principal ion selectivity coefficients and interference from other ions, such as (K^{+1}, $Ca^{+2}$and Al $^{+3}$), have been obtained in this investigation. Primary ion charge and secondary ion interference charge, as well as the concentration and composition of the electrodes, all have a role in determining the selectivity coefficients. All selectivity coefficient values were presented in Figures 9,10. Using several electrodes of the DZP membrane Tables 4,5 and

Table (4): Selectivity coefficients for (DZP-MIP+TTP) electrode at different concentrations of Diazepam.

Con.	**Concentrations of Diazepam (M): concentrations of interference ions (M)**					
	Interfering ions					
	K^{+1}		Ca^{+2}		Al^{+3}	
	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$
10^{-2}	90	8.5169×10^{-2}	61.5	2.3934×10^{-3}	65.2	2.2325×10^{-3}
5×10^{-3}	88	1.0989×10^{-1}	60	1.0189×10^{-3}	63	6.1006×10^{-4}
1×10^{-3}	82	2.3601×10^{-1}	63	1.4862×10^{-3}	63	4.6987×10^{-4}
5×10^{-4}	75	2.7970×10^{-1}	65	1.1963×10^{-3}	51.6	8.2556×10^{-5}
1×10^{-4}	69	7.1196×10^{-1}	66	1.745×10^{-3}	52	7.7961×10^{-5}
5×10^{-5}	66	3.0167×10^{-1}	76	7.0533×10^{-3}	45.5	5.2863×10^{-5}

Table (5): Selectivity coefficients for (DZP-MIP+DBS) electrode at different concentrations of Diazepam.

Con.	**Concentrations of Diazepam (M): concentrations of interference ions (M)**					
	Interfering ions					
	K^{+1}		Ca^{+2}		Al^{+3}	
	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$
10^{-2}	39	4.7600×10^{-3}	43	2.0814×10^{-3}	61	6.0959×10^{-3}
5×10^{-3}	33	9.8696×10^{-3}	39	1.6048×10^{-3}	51	1.9691×10^{-3}
1×10^{-3}	30	3.3275×10^{-2}	31	1.1410×10^{-3}	44	1.0343×10^{-3}
5×10^{-4}	27	4.6012×10^{-2}	24	3.6083×10^{-4}	32	1.4860×10^{-4}
1×10^{-4}	25	1.9781×10^{-1}	20	4.1716×10^{-4}	27	1.079×10^{-4}
5×10^{-5}	23	3.2164×10^{-1}	18	2.1450×10^{-4}	20	2.5212×10^{-5}

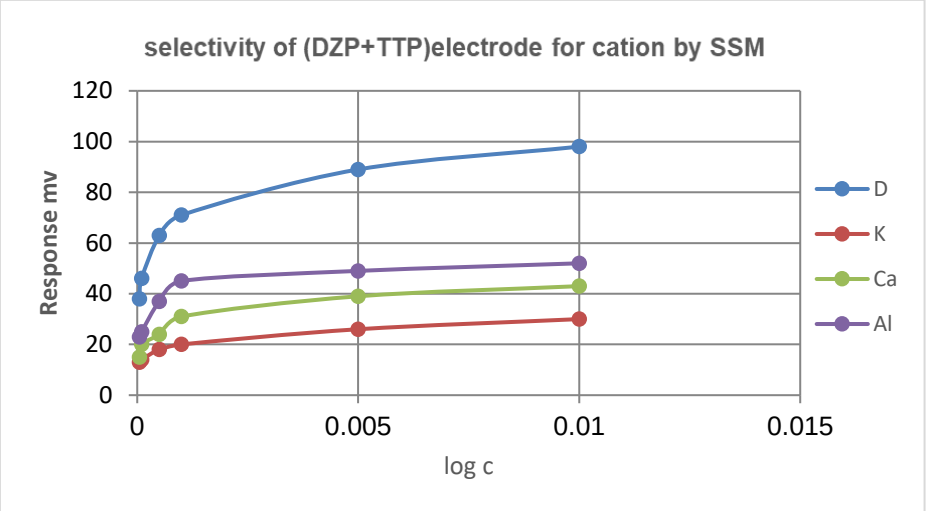


Figure (9): Selectivity of (DZP+TTP) ions at the electrodes using the Separation Solution Method.

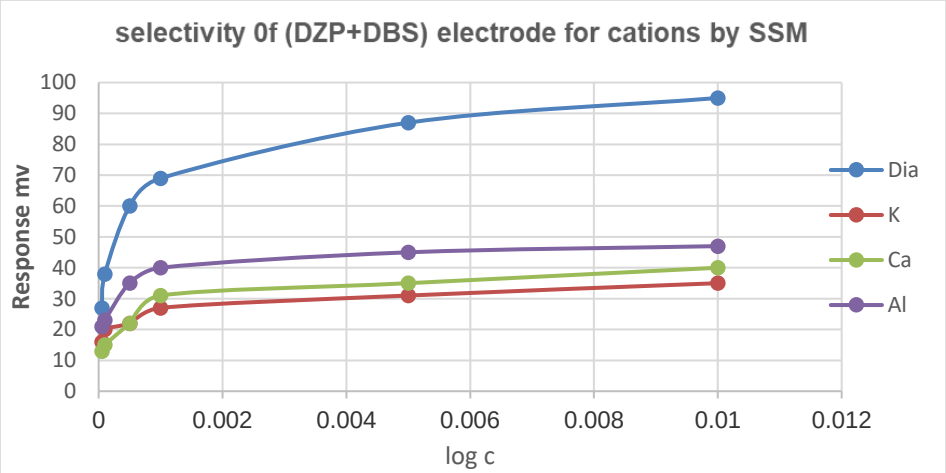


Figure (10): Selectivity of (DZP-DBS) ions at the electrodes using the Separation Solution Method.

Calculation by Multiple Standard Addition Method (MSA)

The concentrations employed in this method ($5x10^{-3}$ and $5x10^{-4}$) for graphing the antilog E/S (Y-axis) versus the normal level diazepam were investigated (X-axis). Figs. (9,10) depict what we found of diazepam ratios computed based on electrodes on DZP-MIP+ TTP, DZP-MIP+DBS.s performed on electrodes of DZP membrane utilising various concentrations of DZP.

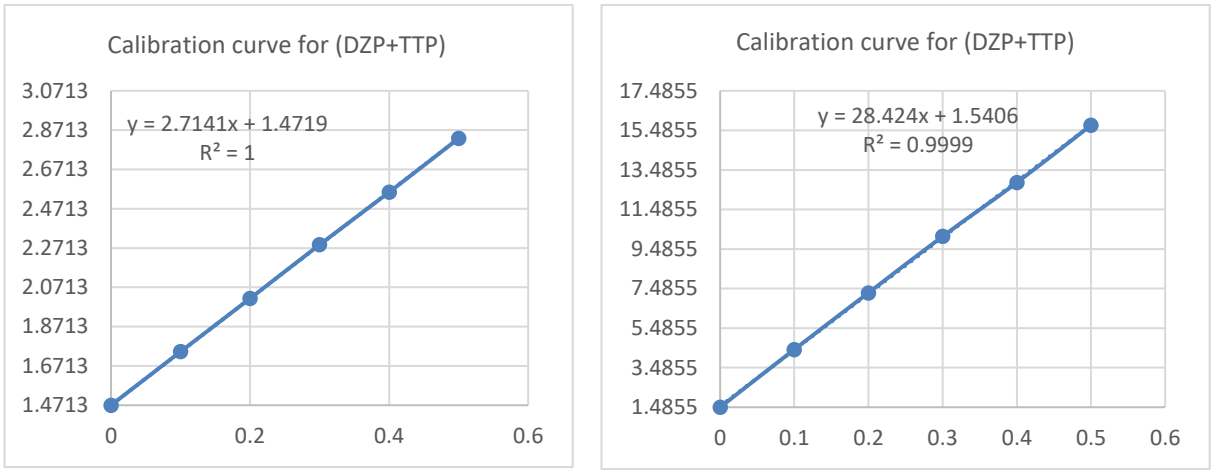


Figure (11): Antilog (E / S) in comparison to the total volume of the increased standard for the purpose of determining diazepam solution (5×10^{-3} and 5×10^{-4}) by MSA using (DZP–MIP + TTP) electrode**.**

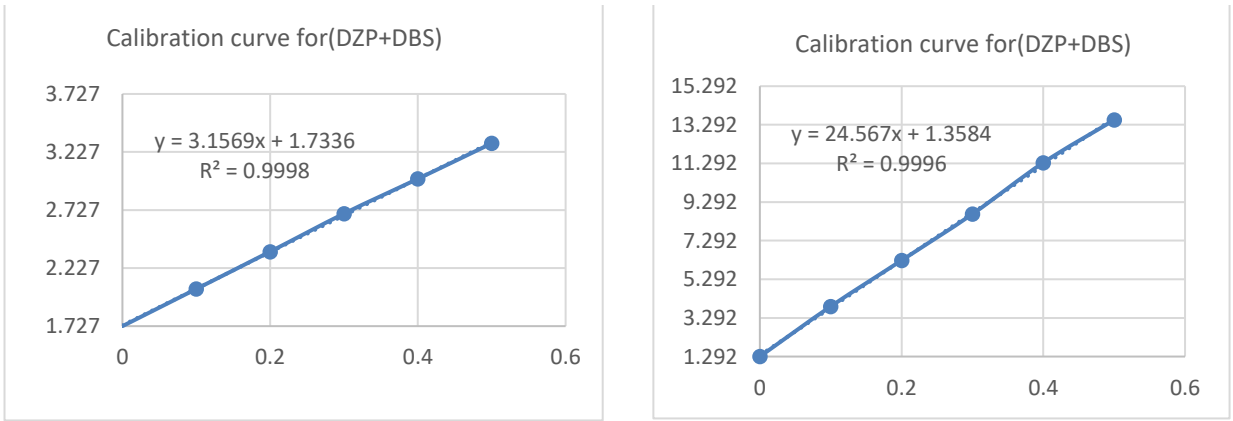


Figure (12): Antilog (E / S) in comparison to the total volume of the increased standard for the purpose of determining Diazepam solution (5×10^{-3} and 5×10^{-4}) by MSA using (DZP–MIP+ DBS) electrode.

Applications of pharmaceuticals.

Molecularly imprinted polymer-based ion selective electrodes were utilised to determine Diazepam in medicines. This ISE includes standard addition, direct, Gran plot, and multiple standard addition measurements. They were preparing solutions of Diazepam at $5x10^{-3}$and $5x10^{-4}$ M concentrations. The RE%, RC%, and RSD% of Diazepam in medicinal use were calculated. The outcomes achieved are shown in Table (7).

Table (6): Determination of Diazepam Samples by Ion Selective electrodes (ISEs) techniques based on PVC membranes.

Electrode No.	Concentration (M)			
	Sample	Measurement using potentiometric methods		
		Direct	SAM	MSA
DZP-MIP+TTP	5×10^{-3}	5.0159×10^{-3}	4.9933×10^{-3}	4.9943×10^{-3}
	RSD%	0.85	2.89	…….
	RC%	100.32	99.866	99.89
	RE%	0.32	-0.134	-0.11
	5×10^{-4}	5.0052×10^{-4}	4.9911×10^{-4}	4.9891×10^{-4}
	RSD%	0.4845	0.37	…….
	RC%	100.40	99.822	99.78
	RE%	0.10	-0.178	-0.22

Electrode No.	Concentration (M)			
	Sample	Measurement using potentiometric methods		
		Direct	SAM	MSA
DZP-MIP+DBS	5×10^{-3}	5.0250×10^{-3}	4.9881×10^{-3}	4.9893×10^{-3}
	RSD%	0.82	2.76	…….
	RC%	100.50	99.762	99.79
	RE%	0.50	-2.38	-0.21
	5×10^{-4}	4.9871×10^{-4}	4.9856×10^{-4}	5.0092×10^{-4}
	RSD%	0.911	0.52	…….
	RC%	97.94	99.712	100.18
	RE%	-2.06	-0.288	0.18

Table 7: Sample analyses of pharmaceutical Diazepam using DZP-MIP+TPH electrode.

pharmaceutical	(Iraq)		
Concentration prepared	Direct	SAM	MSA
	5×10^{-3}	5×10^{-3}	5×10^{-3}
found	4.9176×10^{-3}	4.9933×10^{-3}	4.9649×10^{-3}
RC%	98.35	99.867	99.30
RSD%	1.2674	2.89	……..
RE%	-1.65	-0.134	-0.70
pharmaceutical	Direct method	SAM	MAS
Concentration prepared	5×10^{-4}	5×10^{-4}	5×10^{-4}
Found	5.0198×10^{-4}	5.0341×10^{-4}	4.9624×10^{-4}
RSD%	1.6953	1.47	……..
RC%	100.40	100.682	-0.75
RE%	0.40	0.628	99.25

pharmaceutical	**(Iraq)**		
Concentration prepared	Direct	SAM	MSA
	5×10^{-3}	5×10^{-3}	5×10^{-3}
found	4.9577×10^{-3}	4.9881×10^{-3}	4.9855×10^{-3}
RC%	99.15	99.762	99.71
RSD%	1.1893	2.76	……..
RE%	-0.85	-0.238	-0.29
pharmaceutical	Direct method	SAM	MAS
Concentration prepared	5×10^{-4}	5×10^{-4}	5×10^{-4}
Found	4.818×10^{-4}	4.9838×10^{-4}	5.0473×10^{-4}
RSD%	2.249	3.6907	……..
RC%	96.36	99.676	100.95
RE%	-3.64	-0.324	0.95

***each measurement was carried out three times.**

CONCLUSION

By combining different plasticisers with Diazepam membranes, selective electrodes can be created. TTP and DBS plasticisers were employed to manufacture PVC-based diazepam membrane electrodes. The results obtained for all electrodes applied to standard and medicinal solutions were excellent. Developing electrodes for use in pharmaceutical analytical determination is intended to serve this purpose.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 65-76
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(6)
Nayyef& et al.,

المجلة العراقية لبحوث السوق
وحماية المستهلك

THE EFFECT OF GOVERNMENTAL EXPENDITURE IN THE AGRICULTURAL DOMESTIC PRODUCTION IN IRAQ 1990-2020/ECONOMIC ANALYSIS

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Received 4/ 5/ 2023, Accepted 15/ 6/ 2023, Published 31/ 6/ 2024

ABSTRACT

the aim of the study is determining the effect of agricultural investment and operation expenditure in agricultural domestic product in Iraq for period(1990-2020) . The public expenditure is include of investment and operation expenditure .the investment expenditure increase production capacities it's so effect in agricultural growth ,but it's unsteady because economic and security state of the Country on other side reduction in public and private agricultural investment but the operation expenditure features with increase it's size because expansion the employment in public sector. It was obtained on time series data of variables in model after test stability of the variables in the first different.We used Autoregressive Distributed Lag (ARDL) to analysis the data to measurement the effect of operation and investment expenditure at agricultural domestic product ,the results explained when operation expenditure increase by% 0.01 due to reduce in agricultural product by%0.10 it's refer to un employment in ministry of agriculture and their institutions , The higher operational expenditure compared to investment expenditure, the last have significant on Agricultural product, when it's increase% 0.01 cause increase% 0.06 in agricultural product, we recommend, restructured public sector to reduce operation expenditure and raise investment, efficiency workers in agricultural public sector .

Key words: Investment Expenditure, Operation Expenditure, Autoregressive Distributed Lag.

تحليل اقتصادي لأثر الانفاق الحكومي في الناتج المحلي الزراعي في العراق للمدة 1990-2020

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الخلاصة

استهدف البحث تحديد أثر الانفاق الزراعي العام في الناتج المحلي الزراعي بالعراق للمدة 1990- 2020 يتضمن الانفاق الزراعي العام كل من الانفاق الاستثماري والانفاق التشغيلي. يعمل الانفاق الاستثماري على توسيع الطاقات الانتاجية وزيادة رؤوس الاموال الثابتة وبالتالي التاثير في النمو الزراعي، الا ان هذا الانفاق يتسم بالتقلب والتذبذب نتيجة الاوضاع الاقتصادية والامنية للبلد هذا من جانب ومن جانب اخر انخفاض حجم الاستثمار الزراعي العام والخاص . اما الانفاق التشغيلي فانه يتسم بزيادة حجمه نتيجة توسيع الاستخدام في القطاع العام, تم الاعتماد على بيانات سلسلة زمنية للمتغيرات التي تضمنها الانموذج وبعد فحص استقرارية المتغيرات التي استقرت بعد اخذ الفرق الاول لها , تم استخدم انموذج الانحدار الذاتي للأبطاءات الموزعة (ARDL) لتحليل البيانات لقياس تأثير كل من الانفاق التشغيلي والاستثماري في الناتج المحلي الزراعي, وقد بينت النتائج أن الانفاق التشغيلي كان له تأثير سالباً في الناتج إذ أشار الى

وجود بطالة مقنعة في الجهاز الاداري لوزارة الزراعة والدوائر التابعة لها وان ارتفاع حجم الانفاق التشغيلي يكون على حساب الانفاق الاستثماري الذي كان تأثيره إيجابياً ومعنوياً حيث ان زيادة الانفاق الاستثماري بنسبة 0.01% يؤدي الى زيادة الناتج المحلي الزراعي بنسبة 0.06% لذا ويوصي البحث بزيادة حجم الانفاق الاستثماري العام بما يتلاءم وأهميته الاقتصادية والاجتماعية و ينبغي اعادة هيكلة القطاع العام لتقليص الانفاق التشغيلي ورفع كفاءة العاملين في القطاع الزراعي العام.

الكلمات المفتاحية: الانفاق الاستثماري، الانفاق التشغيلي، الانحدار الذاتي للابطاءات الموزعة.

INTRODUCTION

The public expenditure is defined sum of money the state employ it for fulfill social benefit **(Al-far, 2008)**. as well It defined as sum of money that give off from state treasury by different types institutions ,ministries for satisfy social benefit (**Abu Ahmed 2002;Al-Ugaili&Salih 2017;Barbaz *et al.*, 2020)**, The activates of this sector have been related to the provision of basic human needs and the achievement of food security through adoption by the state of appropriate policies, development of agricultural production in general and the development of human food in particular is a major concern for agricultural economic policy planners, especially in developing countries so the investor aims is obtain the highest return and the lowest degree of risk **(Nagham & Aysar, 2020**), so must be the real challenge for any organization as the ability to employ modern technologies in any field of its activities to achieve the excellence (**Al-Heali,2022**), in our study there is the operation &investment agricultural expenditure of money policies that the state use it to obtain development in agricultural sector , the investment expenditure for agricultural sector have evident effect not just at agriculture domestic product but at general domestic product, the investment expenditure is sum of money get it from financial budget of agriculture ministry for investment it is in economic development project for the country as infrastructure project ,dams erection ,split rivers and else stimulate the economic for growth (**Ali &Ali, 2020**), Development prepare the needs of the present people without harming the needs of future people. Agricultural activity is one of the main basic of economic developmentand Agricultural development have an important status because of its important role in economic and social life so Achieving Agricultural development make food security**. (Al-Affoun2017; Al-Janabi &Ahmed2017; Hamad & Muhammad2011; Hussein 2017).** aim of the study is effect both of the operation &investment agricultural expenditure at agriculture domestic product, problem of the study is run down in agriculture domestic product connect with study hypothesis that find overflowing in agricultural operation expenditure, we obtain at data of the government institutions by questionnaire form, utilization human, natural and financial capabilities is the optimum solution of most economists to crossing economic problems. The Investment find production ,self-sufficiency, employment and improve the level of living ,political constancy, The higher the growth rates of domestic production and Limitedness of the market is one of the main impediments to development, and it has become clear that the growth and increase of external demand for export products leads to the stimulation and channeling of investment in them to introduce better methods in the production and marketing of their products (**Khadrawi 2013; Al-Jubory & Al-Badri 2017**) **,** as well investment increase the productivity of the producer, so developing productive sectors, as new employment, renew buildings and agricultural facilities, reclaiming agricultural areas, using technical methods of agricultural mechanization, and developing productive capacities, if it was plant or animal (**Ahmed &Hamza, 2017**), and that the agricultural sector is one of the leading sectors in Economic growth (**Al-Sudani &Al-Hayali, 2021**), so agricultural investment projects have an important

function in obtain agricultural and economic growth, investment projects are of great importance by which countries turn from stagnation to growth (**Mahmoud *et al.*, 2019**). The agricultural sector has a linear effect on agricultural growth and investment (**Nuri & Al-Hayali 2019**). investment is a positive economic function with it increase in fixed and working capital, especially investment in infrastructure such as agricultural areas reclamation (**Shukr &Hussein, 2013**).

MATERIALS AND METHODS

We are obtain at data of the variables in economic function as investment and operation expenditure , agriculture domestic product from its secondary sources, the model was estimated by ARDL method, as this model takes a sufficient number of lag periods to obtain the best set of data by the general framework model so find best results for the parameters at long run, make diagnostic tests (Enders 1995;Al-Hani&Ahmed 2016) objective of the unit root test is to measure the stability of time series , and diagnostic tests were conducted for standard problems and a period was chosen Optimal lag period of the model and the program Automatically choose the best lag period that the best statistical and econometric model, (Kahli , 2017). we used ARDL model after accentual that the variables are not integrated of the second order, using unit root tests. And the bounds test was carried out to see if there was evidence of a long-run relationship between the variables, and stability was made for the variables included in the economic model, as the variables were dynamically stable in both tests (Phillips-perron and Dickey Fuller).

Table (1): data of Agricultural domestic product, agricultural operation and investment expenditure (thousand Iraqi dinar) 1990-2020.

AGP	Y1	OP	YEAR	AGP	Y1	OP	YEAR
288030	6195900	57220000	2006	254000	3447800	101000	1990
14320	4479900	73805000	2007	51000	2877200	102000	1991
20020	3889000	84823000	2008	125000	3531903	179000	1992
46020	4020700	487896000	2009	137000	3492402	417000	1993
1918000	4036700	557379000	2010	96730	3741001	550000	1994
1770016	4739700	812091000	2011	52080	4188200	1940000	1995
15116000	4941400	794432000	2012	19100	4498300	1284000	1996
39307000	6123800	837847000	2013	108070	4133800	2039000	1997
12964000	6000600	3248000000	2014	92070	4475100	2583000	1998
9470000	3787400	808788000	2015	103060	5188300	4018000	1999
164000	3775700	801650000	2016	139060	4589000	5043000	2000
10670000	3171700	609498000	2017	287058	4644000	3680000	2001
12033000	3811900	619066000	2018	228050	5432600	5469000	2002
13858000	3217800	631659000	2019	119045	3850300	33419000	2003
14366000	3513800	1424000	2020	10040	4521800	182042000	2004
				70032	5939600	65178000	2005

Source: Ministry of agriculture, planning and follow-up data, Statistics Department, Baghdad, Iraq,2020.

*OP: refer to agricultural operation expenditure.
*Y1: refer to agriculture domestic product.
*AGP: refer to agriculture investment expenditure.
We are formulated economic model for explain the economic relationship as follows:
$LnY_1=b_0+B_1lnOP+B_2LNAGP$
LNY_1: The natural logarithm of agricultural domestic product in Iraq for the period(1990-2020).
LNOP: The natural logarithm of agricultural operation expenditure in Iraq for the period (1990-2020).
LNAGP: The natural logarithm of agricultural investment expenditure in Iraq for the period (1990-2020).

RESULTS AND DISCUSSION

By using the ARDL model, the results of the quantitative analysis of the effect the sum of variables on the agricultural domestic product through the duration 1990-2020 before find the effect of independent variables as agricultural operation expenditure and agricultural investment expenditure on agricultural domestic product we make tests Phillips-perron and Dickey Fuller for model variables that were Static at the first difference, where the economic variables were not constant at the level, but they static at the first difference, where the calculated value of T was less than the critical or tabular T value, where the economic tests assume that the variables were not constant at the level, but become constant at the first difference, as in the table (2).

Table (2): results of unit root of test (PP).

UNIT ROOT TEST TABLE (PP)

		At Level		
		LNOP	LNY1	LNAGP
With Con...	t-Statistic	-1.6960	-2.5453	-1.5524
	Prob.	*0.4230*	*0.1154*	*0.4939*
		n0	n0	n0
With Con...	t-Statistic	3.9997	-2.2928	-3.0418
	Prob.	*1.0000*	*0.4248*	*0.1382*
		n0	n0	n0
Without C...	t-Statistic	0.0438	0.0183	0.7142
	Prob.	*0.6890*	*0.6808*	*0.8641*
		n0	n0	n0
		At First Difference		
		d(LNOP)	d(LNY1)	d(LNAGP)
With Con...	t-Statistic	-2.6695	-6.6550	-8.4425
	Prob.	*0.0915*	*0.0000*	*0.0000*
		*	***	***
With Con...	t-Statistic	-3.0534	-15.1156	-10.8250
	Prob.	*0.1359*	*0.0000*	*0.0000*
		n0	***	***
Without C...	t-Statistic	-2.8137	-6.7994	-7.6172
	Prob.	*0.0066*	*0.0000*	*0.0000*
		***	***	***

Source: Calculated based on output of program Eviews10.

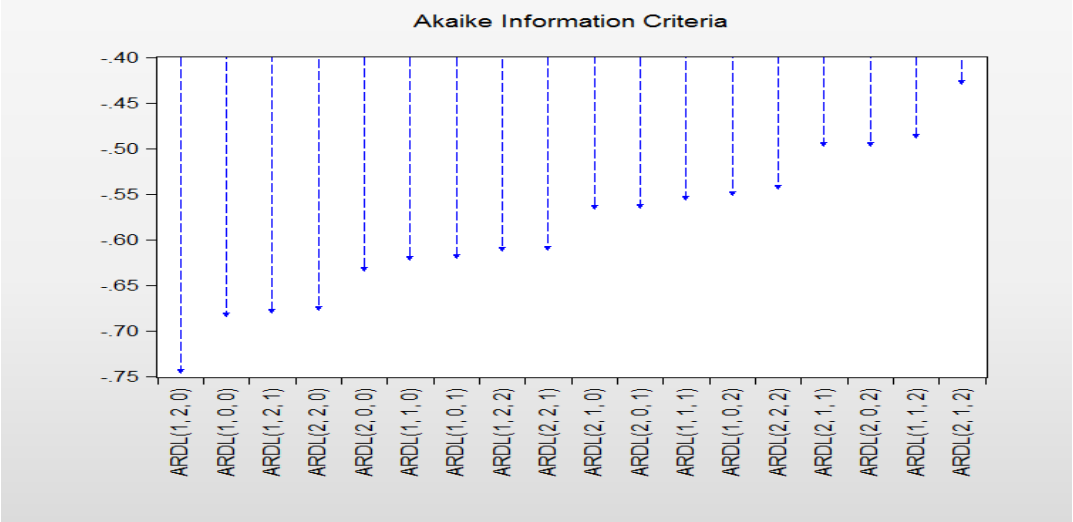

Figure (1): possible models according akaike criteria
Source: Calculated based on output of program Eviews 10.

From Fig.1. explain The Akaike criteria is a tool utilized to determine the optimal lag period and estimate the rank of a model. The Akaike criteria is used to select the model (212) with the best fit among a set of (20) models.

To determined a relationship between the dependent variable and the independent variables, the cointegration test and the BOUND Test were adopted for the model through (table, 3):

Table (3): results cointegration test for the model:

F-Bounds Test		Null Hypothesis: No levels relationship		
Test Statistic	Value	Signif.	I(0)	I(1)
F-statistic	3.895626	10%	2.63	3.35
k	2	5%	3.1	3.87
		2.5%	3.55	4.38
		1%	4.13	5

Source: Calculated based on output of program Eviews10

Table (3) explain the calculated value of F amounted to 3.89, which is higher than the tabular value at a significant level of 5%, rejecting the null hypothesis, there is no cointegration, and confirming and sure of cointegration. After confirming a model is free of econometric variables affecting the agricultural domestic product problems was estimated the short-run function using the ARDL method and the agricultural operational expenditure parameter explain a negative effect at a significant level of 1%. When operating expenditure increased by 1%, the agricultural domestic product decreased by % 0.10. but the agricultural investment expenditure parameter, it had a positive and significant effect at a significant level of 1%,which indicates an increase in agricultural investment expenditure by 1% due to an increase in agricultural produce by %0.06, which is consistent with economic logic, as an increase in capital invested in implementing strategic agricultural projects, it was animal or plant have a function in raise production capacities and thus increasing the agricultural domestic product. The value of R^2 was 0.66, indicating that 66% of the changes in the dependent variable cause of the independent variables included in the model, and that 34% of the variables were not included in the model, the effect of which was absorbed by the random variable, explain in the results of the short-term as shown in table (4).

Table (4): short run function.

ARDL Error Correction Regression
Dependent Variable: D(LNY1)
Selected Model: ARDL(2, 1, 2)
Case 2: Restricted Constant and No Trend
Date: 01/31/23 Time: 19:01
Sample: 1 31
Included observations: 29

ECM Regression
Case 2: Restricted Constant and No Trend

Variable	Coefficient	Std. Error	t-Statistic	Prob.
D(LNY1(-1))	0.361479	0.200404	1.803748	0.0914
D(LNOP)	-0.102379	0.030923	-3.310801	0.0048
D(LNAGP(-1))	0.063385	0.020373	3.111236	0.0072
CointEq(-1)*	-0.358069	0.105593	-3.391019	0.0040

R-squared	0.660333	Mean dependent var	0.000226
Adjusted R-squared	0.509369	S.D. dependent var	0.178765
S.E. of regression	0.125216	Akaike info criterion	-1.056351
Sum squared resid	0.282223	Schwarz criterion	-0.624405
Log likelihood	23.26074	Hannan-Quinn criter.	-0.927911
Durbin-Watson stat	2.114972		

* p-value incompatible with t-Bounds distribution.

Source: Calculated based on output of program Eviews10.

But long run function was explain that agricultural operational expenditure have a negative effect on the level of 1%, and its value was 0.07, but its value is less than its value in the short term, so cause the policies followed in decreasing disguised unemployment in the Ministry of Agriculture and its institutions but the value of agricultural investment expenditure, it amounted to 0.32, which had a positive and
Significant effect At the level of 1% as shown in Table (5).

Table (5): long run function.

Levels Equation
Case 2: Restricted Constant and No Trend

Variable	Coefficient	Std. Error	t-Statistic	Prob.
LNOP	-0.077650	0.024872	-3.121981	0.0056
LNAGP	0.320267	0.115506	2.772730	0.0074
C	15.66960	0.870634	17.99793	0.0000

EC = LNY1 - (-0.0776*LNOP + 0.3202*LNAGP + 15.6696)

Source: Calculated based on output of program Eviews10

Econometric tests of the ARDL model

It is sum of criteria and tests used in econometrics for evaluate the efficiency of the model. These tests as the Lagrangian factorial test for the serial correlation of the residuals and the homogeneity of variance test for Breusch Codfrey, Harvey and Arch the model was free of econometric problems Table (6) indicates that the model has passed standard tests, such as the absence of Autocorrelation using the LM test with a probability value of (0.2346). Therefore, we can accept the Null hypothesis of no autocorrelation problem in the model. Table (7) The test showed no issue of heteroscedasticity using a probability value of (0.1374) and the tests of Harvey and ARCH showing no problem in heteroscedasticity with a probability values (0.3224), (0.9892) In sequence,as in the following tables(6) and (7).

Table (6): Lagrangian factorial test for the serial correlation between the residuals LM)

Breusch-Godfrey Serial Correlation LM Test:

F-statistic	0.782051	Prob. F(2,13)	0.4778
Obs*R-squared	2.899646	Prob. Chi-Square(2)	0.2346

Source: Calculated based on output of program Eviews10.

Table (7): Heterogeneity tests.

Heteroskedasticity Test: Breusch-Pagan-Godfrey

F-statistic	2.014776	Prob. F(11,15)	0.1034
Obs*R-squared	16.10193	Prob. Chi-Square(11)	0.1374
Scaled explained SS	3.464893	Prob. Chi-Square(11)	0.9830

Heteroskedasticity Test: Harvey

F-statistic	1.187774	Prob. F(11,15)	0.3704
Obs*R-squared	12.56948	Prob. Chi-Square(11)	0.3224
Scaled explained SS	6.208973	Prob. Chi-Square(11)	0.8591

Heteroskedasticity Test: ARCH

F-statistic	0.000169	Prob. F(1,24)	0.9897
Obs*R-squared	0.000183	Prob. Chi-Square(1)	0.9892

Source: Calculated based on output of program Eviews10

The normal distribution of residuals in Fig. (2) achieved by using the Jarque-Bera(JB) test with a probability value (0.770) which is greater than 0.05 and we accept the Null hypothesis that the residuals have normal distribution.

To ensure that the data used in this study is free from any structural changes, it is necessary to employ appropriate tests such as Cumulative Sum of Recursive Residuals (CUSUM) and Cumulative Sum of Squares of Recursive Residuals (CUSUM of Squares). These tests are considered important in this field detecting the presence of any structural changes in the data and assessing the long-term stability and coherence of parameters with short-term parameters the graphical representation of both CUSUM and CUSUM of Squares tests falls within the critical boundaries at a significance level of 5%, it indicates structural stability. Based on the findings of most of these studies, we conducted the tests CUSUM in Figure (3). CUSUM of squares in Fig. (4) There is harmony and stability in both the short-term and long-term models.

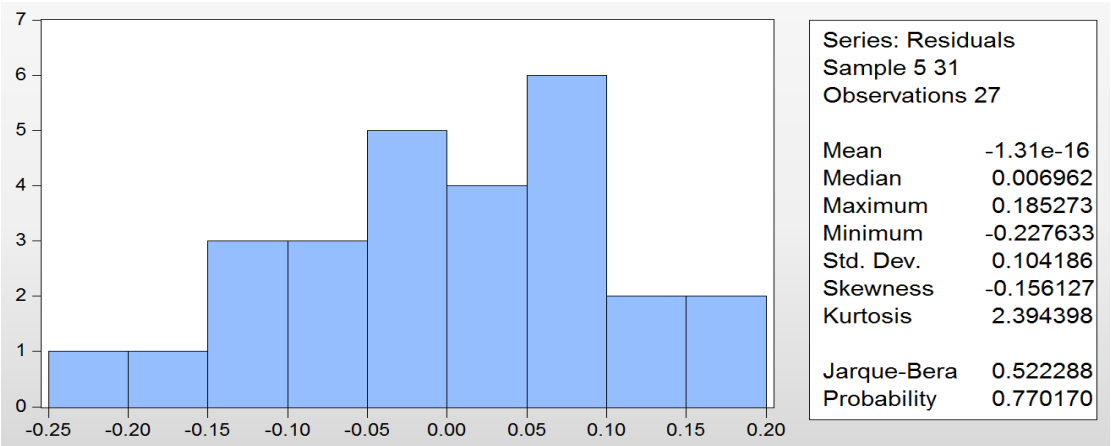

Figure (2): test of random error distribution
Source: Calculated based on output of program Eviews10

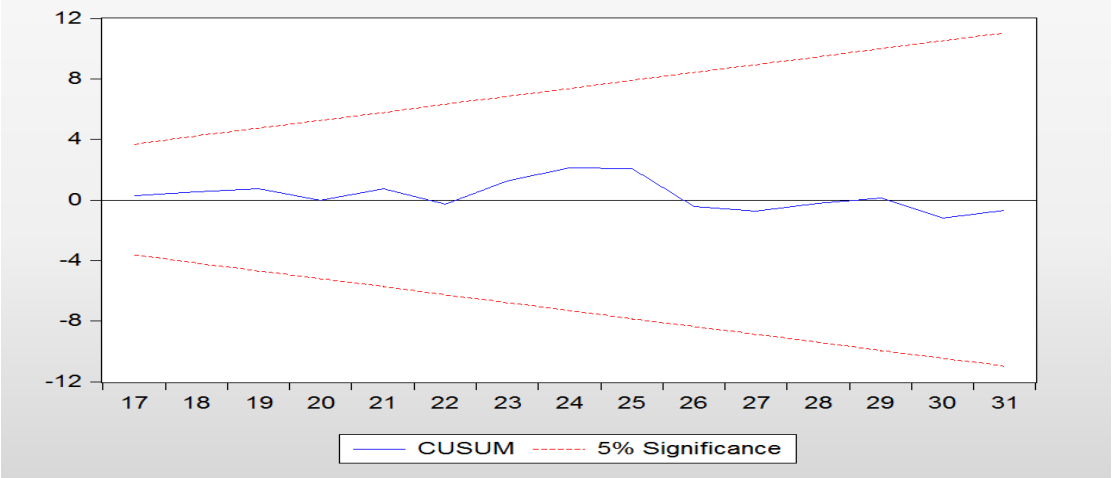

Figure (3): structural stability CUSUM

Source: Calculated based on output of program Eviews10.

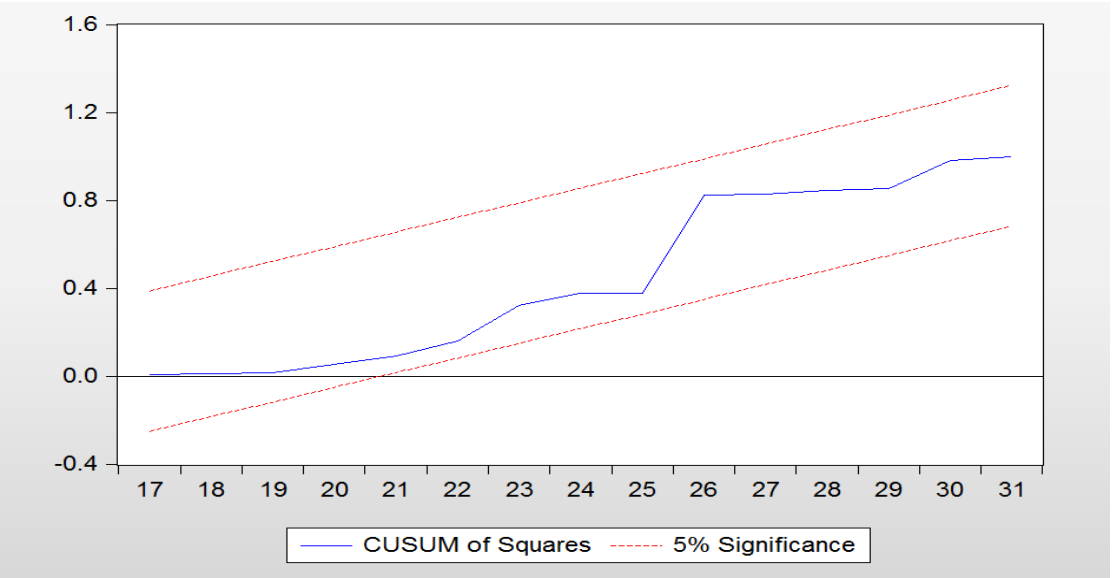

Figure (4): structural stability CUSUM of squares.
Source: Calculated based on output of program Eviews10

CONCLUSIONS AND RECOMMENDATIONS

The study is concluded the disguised unemployment in the Ministry of Agriculture and its institutions, so increase of operational expenditure and prefer on investment expenditure, which is negatively reflected in the agricultural domestic product and make the Ministry to finance agricultural investments in the budget by lending, also payment of interest, which increases the state of disability in finance investment expenditure, so the study recommend restructure the ministry of agriculture and their institutions ,employment certificates and technicians and review the employment policy and the distribution of jobs for find the benefit to farmers and the agricultural sector . motivation it is role in private sector in agricultural product investment agricultural ,so must make an expenditure policy in relation to operational and agricultural investment expenditure to get financial and monetary stability to reach the levels of development and economic and agricultural growth also put importance to unemployment because it is one of the serious economic problems that threaten the economic situation, its stability and development, and the provision of job opportunities by development projects related to the agricultural sector, the employment, and the absorption and curing disguised unemployment.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 77-85
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(7)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

PERFORMANCE OF THE SAKALAK SK-FM SEED DRILL IN SILTY CLAY LOAM SOILS

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Received 23/ 5/ 2023, Accepted 25/ 6/ 2023, Published 31/ 12/ 2024

ABSTRACT

The experiment was conducted in agricultural fields of the Agricultural Research Department in Abu-Ghraib region during November 2022 to study three levels of depths: 2, 4, and 6 cm, and three speed 5, 6.5, and 7.5 km h^{-1} on the performance of the fertilized seed drill, SAKALAK SK-FM, with the Renault tractor as a mechanical unit in the silty clay loam soil. The following characteristics were studied: the percentage of slippage, actual field productivity, fuel consumption, and number of plants per square meter. The experiment was conducted using a randomized complete block design, with split plot arrangement with three replications. The depth allocated to the main plots, and the forward speed allocated to the sub-plots. The results indicated that highest slip ratio7.06% and pulling force 12 KN at the interaction between depth 6 cm and forward speed 7.5 km/h , the lowest slip ratio 3.55 and pulling force 8 KN at the interaction between depth 2 cm and forward speed 5 km/h. The lowest fuel consumption 6.39 and the number of plants per square meter 4.33 at the interaction between depth 2 cm and forward speed 7.5 km/h, The highest fuel consumption 10 and the number of plants per square meter 5.67at the interaction between depth 2 cm and forward speed 5 km/h.

Keywords: Actual Field productivity, fuel consumption, Seed drill, Slippage, Pulling force.

اداء الباذرة SAKALAK SK-FM في الترب المزيجة الطينية الغرينية

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الخلاصة

نفذت التجربة في أحد الحقول الزراعية التابعة لدائرة البحوث الزراعية في منطقة ابي غريب في شهر الحادي العاشر سنة 2022 لغرض دراسة ثلاث مستويات من الأعماق 2 و4 و6 سم وثلاث مستويات من السرع المتمثلة بالقيم 5 و6.5 و7.5 كم.ساعة$^{-1}$ في أداء الباذرة المسمدة الزارعة SAKALAK SK-FM مع الجرار رينلت كوحدة ميكنية في الترب المزيجة الطينية الغرينية وتم دراسة الصفات التالية: النسبة المئويه للانزلاق، الإنتاجية الحقلية الفعلية، الوقود المستهلك وعدد النباتات في المتر المربع نفذت التجربة وفق تصميم القطاعات العشوائية الكاملة وبترتيب الالواح المنشقة وبثلاث مكررات. اشارت النتائج الى أعلى نسبة انزلاق 7.06% وقوة سحب 12 كيلو نيوتن سجلت عند التفاعل بين العمق 6 سم والسرعة الأمامية 7.5 كم/ساعة، وأدنى نسبة انزلاق 3.55%وقوة سحب 8 كيلو نيوتن سجلت عند تداخل بين العمق 2 سم والسرعة الأمامية 5 كم/ساعة . أقل استهلاك للوقود 6.39 وعدد النباتات لكل متر مربع 4.33 سجلت عند تداخل بين العمق 2 سم والسرعة الأمامية 7.5 كم/ساعة، وأعلى استهلاك للوقود 10 وعدد النباتات لكل متر مربع 5.67 سجلت عند التفاعل بين العمق 2 سم وسرعة أمامية 5 كم / ساعة.

الكلمات المفتاحية: الإنتاجية الحقلية استهلاك الوقود، عدد النباتات، الانزلاق، قوة السحب.

INTRODUCTION

Humans began using modern agricultural machinery and their accessories in order to keep up with technological advancements in various fields. When designing agricultural machinery, consideration is given to minimizing energy consumption while maximizing productivity, in order to provide the highest possible fuel efficiency. Even slight differences in fuel consumption should be taken into account, especially when working on large areas. **(Frank, *et al*., 2012).** Seed drill is specialized agricultural equipment used for seed planting. The process of sowing grains is an important agricultural operation that follows soil preparation processes, including plowing, harrowing, and seedbed preparation. Seeds can be sown manually, which is one of the oldest methods of sowing and is still widespread in many regions of Iraq, especially in small fields owned by farmers. However, this method is not efficient in evenly distributing seeds and requires significant time and labor, resulting in additional costs in crop production and significant loss in yield due to delayed planting. The second method used in sowing is the use of mechanical seed drill, which has become widely popular in recent years, particularly for large areas. These mechanical seeders have shown positive results in terms of speed and minimizing seed wastage, making them indispensable in large-scale agricultural production operations. Similarly, the fertilization process has shown high economic success when using fertilization equipment to increase agricultural productivity. Fertilization equipment often has a similar design to seed drill, and combined seed drill and fertilizers have been used in one vehicle **(Al-Tahan & Al-Na'mah, 2000).**

Corn is considered one of the most important crops worldwide due to its multiple uses, allowing it to thrive in a wide range of agricultural environments **(Al-Aridhee & Mahdi, 2022).** Corn is a strategic crop that plays a significant role in ensuring food security in Iraq and contributes to the development of agro-industries. It also plays a crucial role in animal production by providing green and concentrated fodder **(Al-Janabi, *et al*., 2023**), especially with the increasing population. The projected population increase, currently at 7.2 billion, is expected to reach approximately 9.2 billion by 2050 **(Tawfiq, 2019).**

Study Objective

To examine the performance of the SAKALAK SK-FM seed drill and find the optimal combination of depth and speed with the lowest overall costs, as well as to provide recommendations through the result obtained to achieve the best performance.

MATERIALS AND METHODS

Experiment Field Implementation

The experiment was conducted at the Abu Ghraib Research Station, Agricultural Research Department, Ministry of Agriculture in Baghdad during the 2022/2023 growing season. Corn variety (Al-Maha) was planted at the experimental site due to the availability of suitable water and soil for cultivation. The soil texture at the site was a silty clay loam, and the location was characterized by a moderate climate according to the Agricultural Meteorological Center, Ministry of Agriculture, Iraq (2022). The purpose was to study three levels of depths and three levels of speeds and evaluate the performance of the Spanish-made SAKALAK SK-FM seed drill.

Table (1): Fertilized seed specifications.

machine type	Seed Drill
the weight	1020 kg
Tank number	4
Tank capacity	25 kg
Fertilization tank number	2
Composting tank capacity	200kg
Number of bottles for sowing and fertilizing	4
The distance between the vials	75cm
Seed drop mechanism	4 perforated discs that differ from one crop to another The number of holes on the disc is 20
origin	Spanish
manufacturing year	2019

Characteristics Studied in the Experiment and Calculation Methods:
Slippage Percentage:

It is calculated using the following equation: **(Laibi & Al- Ani, 2022).**

$$SP = \frac{VT-VP}{VT} X\ 100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where:
SP = Slippage percentage (%)
VT = Theoretical speed without load (km/h)
VP = Operational speed with load (km/h)
The theoretical speed (km/h) is calculated by dividing the distance traveled by the theoretical

time, using the following equation:

$$VT = \frac{D}{Tt} X\ 3.6 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where:
D = Length of the plowed line (m)
Tt = Theoretical time without load (seconds)

The operational speed (km/h) is calculated using the following equation:

$$VP = \frac{D}{TP} X\ 3.6 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where:
D = Distance of the plowed line (m)
Tp = Operational time with load (seconds)

Fuel Consumption Rate:
The fuel consumption rate is calculated using the following equation**: (Al-Jarrah, 1998).**

$$QF = \frac{Qd \times 10000}{D \times WP \times 1000} \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where:
QF = Fuel consumption per hectare (liters/hectare)
Qd = Fuel consumption during treatment (milliliters)
D = Length of the treatment (meters)
WP = Actual plow width (meters).

Pulling Force:
It is measured using the following equation: **(Laibi, 2022)**
FT = Fpu - FRM..... ……. (**5**)
Where:
FT = Pulling force (kN)
Fpu = Pulling force where the plow almost touches the ground
FRM = Rolling resistance where the plow almost touches the ground

Number of Plants per Square Meter:
The number of plants per square meter was calculated after germination, randomly within each treatment, and the average was taken.

RESULTS AND DISCUSSION

Slippage Percentage (%)

Table (2) shows the effect of both each of the depth and forward speed of the tractor on the slippage percentage. It is evident that when increasing the working depth from 2 to 4 and then to 6 cm resulted in an increase in the slippage percentage from 4.10 to 4.75 and then to 6.10%. The reason for this increase is attributed to the fact that increasing the plowing depth increases the load on the plow, leading to an increase in the pulling force, which in turn increases the slippage percentage. These results are consistent with (**Menkhi, 2012; Al-Shujairy, 2008).**

The increase in speed from 5 to 6.5 and then to 7.5 km/h led to an increase in the slippage percentage from 4.12 to 4.88 and then to 5.95%. However, all slippage percentages at all speeds were within the permissible limits for tracked tractors, which is 15%. This is consistent with **(Al-Ani, 2012)**, and the reason for the increase in the slippage percentage with increasing operational speed is that at lower speeds, there is greater adhesion between the contact area of the tractor tires and the soil surface. The adhesion decreases as the operational speed of the mechanical unit increases, due to the soil being well smoothed before the seeding process, resulting in the soil being finely fragmented, making it difficult for the tractor tires to maintain cohesion with it. This is in line with (**Hachim & Jebur, 2022; Al-Suhaibani & Ghaly, 2010; Al-Azzawi & Zeinaldeen, 2023; Jassim,2019).**

The result indicate non-significant interaction between plowing depth and forward speed, it is also evident from Table 3 that the highest slippage rate was recorded at the third depth (6 cm) with the third speed (7.5 km/h), with a value of 7.06%. The lowest slippage rate was recorded when working at the first depth (2 cm) with the first speed (5 km/h), with a value of 3.55%

Table (2): Effect of depth and forward speed on slippage percentage.

Depth cm	Forward speed km h^{-1}			Mean depth
	(5)V1	(6.5)V2	(7.5)V3	
2 cm(D1)	3.55	3.83	4.92	4.10
4 cm(D2)	4.15	4.23	5.88	4.75
6 cm (D3)	4.57	6.67	7.06	6.10
LSD	N.S			0.32
Mean speed	4.12	4.88	5.95	
LSD	0.82			

Fuel consumption (liter ha^{-1})

Table (3) shows the effect of both each of the depth and forward speed of the tractor on the fuel consumption. When increasing the working depth from 2 to 4 and then to 6 cm led to an increase in fuel consumption from 7.04 to 7.69 and then to 7.96 L ha^{-1}. respectively The reason for this increase is attributed to the increased disturbed soil volume and the need for greater energy to perform the work. These results are consistent with (**Alwash et al., 2022; Abtan, 2000).**

It is evident from the results that increasing the speed from 5 to 6.5 and then to 7.5 km h^{-1} resulted in a decrease in fuel consumption from 8.89 to 6.85 and then to 6.94 L ha^{-1} Respectively. The decrease in fuel consumption is due to the fact that increasing speed leads to a decrease in energy consumed and the time required completing the work. This is in line with (**Hamzah *et al.*, 2021; Alsharifi & Ameen, 2018; Mankhi & Jebur, 2022).**

The highest fuel consumption rate was recorded at the interaction of a depth of 6 cm and the first forward speed of 5 km h^{-1}, with a value of 10.00 L ha^{-1}. The lowest fuel consumption rate was recorded when working at a depth of 2 cm and the third forward speed of 7.5 km/h, with a value of 6.39 L ha^{-1}.

Table (3): Effect of depth and forward speed on fuel consumption (liter ha^{-1}).

Depth cm	Forward speed km h^{-1}			Mean depth
	(5)V1	(6.5)V2	(7.5)V3	
2 cm(D1)	7.78	6.94	6.39	7.04
4 cm(D2)	8.89	6.95	7.22	7.69
6 cm (D3)	10.00	6.67	7.22	7.96
LSD	0.57			0.54
Mean speed	8.89	6.85	6.94	
LSD	0.27			

Pulling Force (KN)

Table (4) shows the effect of both each of the depth and forward speed of the tractor on the pulling force. It is evident from that when increasing the working depth from 2 to 4 and then to 6 cm resulted in an increase in the pulling force from 9.11 to 10.22 and then to 11.33. The reason for this increase is attributed to the fact that increasing the plowing depth increases

the load on the plow, leading to an increase in the pulling force. These results are consistent with (**Taha &Taha, 2019; Naderloo *et al*., 2009).**

Furthermore, it is evident that increasing the speed from 5 to 6.5 and then to 7.5 km/h led to an increase in the pulling force from 9.78 to 10.00 and then to 10.89 kN. The reason for this increase is that higher speed leads to increased acceleration force of soil particles, increased vertical force on the plow shares, increased frictional resistance, and increased kinetic energy of the soil, resulting in an increase in the pulling force. This is supported by (**Alsharifi *et al.*, 2019; Jebur & AL-Halfi 2022).**

The highest pulling force rate was recorded at the interaction of a depth of 6 cm and the third forward speed of 7.5 km h^{-1}, with a value of 12.00 kN. The lowest pulling force rate was recorded when working at a depth of 2 cm and the first forward speed of 5 km h^{-1}, with a value of 8.67 KN.

Table (4): Effect of depth and forward speed on pulling force (KN).

Depth cm	Forward speed km h^{-1}			Mean depth
	(5)V1	(6.5)V2	(7.5)V3	
2 cm(D1)	8.67	9.00	9.67	9.11
4 cm(D2)	9.67	10.00	11.00	10.22
6 cm (D3)	11.00	11.00	12.00	11.33
LSD	N.S			0.54
Mean speed	9.78	10.00	10.89	
LSD	0.28			

The number of seeds per square meter:

Table (5) shows the effect of both each of the depth and forward speed of the tractor on the number of seeds. It is evident from that when increasing the working depth from 2 to 4 and then to 6 cm resulted in a statistically non-significant increase in the number of seeds from 4.78 to 5.11 and then to 5.22 plants m^{-2}. The reason for this increase is attributed to the increased depth occupied by the roots, allowing for a larger water distribution area and increased water and nutrient absorption. This provides more favorable conditions for the plants and enhances the efficiency of the photosynthetic process, resulting in higher growth and yield. This is in line with (**Jassim *et al*., 2018**).

Furthermore, it is evident from Table (4) that increasing the speed from 5 to 6.5 and then to 7.5 km h^{-1} led to a non-significant decrease in the number of seeds from 5.56 to 4.89 and then to 4.67 plants m^{-2}. The increase in speed disrupts the proper seed placement, causing the grains to fall at different depths, some of which may be too deep for surface emergence, making them susceptible to bird, rodent, and ant predation, or they may be buried too far from the surface, making it difficult for them to germinate. This is consistent with (**Al-Rajabu, 2002; Al-Khafaji & Al-Sabbagh, 2010; Rajabu & Qadu, 2018).**

Table (5): Effect of depth and forward speed on the number of seeds per square meter.

Depth cm	Forward speed km h^{-1}			Mean depth
	(5)V1	(6.5)V2	(7.5)V3	
2 cm(D1)	5.33	4.67	4.33	4.78
4 cm(D2)	5.67	5.00	4.67	5.11
6 cm (D3)	5.67	5.00	5.00	5.22
LSD	N.S			N.S
Mean speed	9.78	10.00	10.89	
LSD	N.S			

CONCLUSIONS

- The results showed that increasing the working depth from 2 to 4 and then to 6 cm led to an increase in slippage percentage, fuel consumption, and it also led to a significant decrease in both field productivity and field efficiency.
- The results also showed that an increase in practical speed led to an increase in both the slip percentage and field productivity, and it also led to a significant decrease in both field efficiency and fuel consumption.
- We recommend using the first depth of 2 cm and the third speed of 7.5 km * h^{-1} to record the best technical results for the mechanical unit, as it recorded the lowest fuel consumption rate and the overall best vegetative characteristics of the germination rate.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 86-100
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(8)
Khalaf & Al-Mashhadani

المجلة العراقية لبحوث السوق
وحماية المستهلك

PROBLEMS OF USING DIGITAL AGRICULTURAL EXTENSION IN PROVIDING AGRICULTURAL EXTENSION SERVICES FROM THE VIEWPOINT OF AGRICULTURAL EXTENSION AGENTS

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Received 28/ 5/ 2023, Accepted 13/ 7/ 2023, Published 31/ 12/ 2024

ABSTRACT

The research has aimed to determine the problems of using digital agricultural extension in providing agricultural extension services from the viewpoint of agricultural extension agents. The research population has included all agricultural extension agents who work in the Agricultural Extension and Training Department, the Training and Rehabilitation Center, the Agricultural Extension Center for Training, Al-Tarmiya Farm, as well as Al-Rashidiya Farm in Baghdad Governorate. The population consist of (100) agricultural extension agents, thus, a random sample has been taken from the research population at a rate of (83%). Accordingly, the proposed sample size has included (83) agricultural extension agents (from male and female). To achieve the goal of the research, a questionnaire has prepared that included two axes: the axis of administrative and financial problems, and the axis of technical and human problems, and the approval of the agricultural extension agents (male and female) has measured by asking them about the extent of the problem or non-existence of the problem, using the phrases (yes, no) and assigning degrees (0,1), and then identifying the extent of the impact of these problems (if they exist) using the phrases (low, medium, big) and grades were assigned to them (1,2,3), respectively. The results of the research have shown that most of the agricultural extension agents have indicated the existence of these problems as well as their direct impact on the use of digital agricultural extension in providing agricultural extension services to target audience, as the general impact rate of administrative and financial problems was (2.41) degree, and the rate of technical and human problems was (2.32) degree, so, these results may reflect the realism and objectivity of the problems addressed in the research, as well as their direct impact on the use of digital agricultural extension. Accordingly, the failure to confront and solve these problems will turn into a threat that prevent or fails the entire process, and in return, seeking to solve these problems and overcome them by the decision-making authorities in the Iraqi Ministry of Agriculture and the Agricultural Extension and Training Department in particular, will provide an appropriate environment and achieves a satisfactory level of efficiency and effectiveness in the process of using digital agricultural extension in providing agricultural extension services to the target audience.

Keywords: Administrative and financial problems, Technical and human problems, Digital agricultural extension, Agricultural extension service.

[*]**The research is taken from a master's thesis by the first researcher.**

المجلة العراقية لبحوث السوق وحماية المستهلك

Iraqi Journal of Market Research and Consumer Protection

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(2024) 16(2): 86-100

مشكلات استخدام الإرشاد الزراعي الرقمي في تقديم الخدمة الإرشادية الزراعية من وجهة نظر المُرشدين الزراعيين

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الخلاصة

يستهدف البحث تحديد مشكلات استخدام الإرشاد الزراعي الرقمي في تقديم الخدمة الإرشادية الزراعية من وجهة نظر المُرشدين الزراعيين، وقد شمل مجتمع البحث جميع المُرشدين الزراعيين العاملين في دائرة الإرشاد والتدريب الزراعي، ومركز التدريب والتأهيل، والمركز الإرشادي التدريبي الزراعي، ومزرعة الطارمية النموذجية، وكذلك مزرعة الراشدية النموذجية في محافظة بغداد، وقد ضم مجتمع البحث (100) مرشد زراعي، وأُخِذت عينة عشوائية بسيطة من مجتمع البحث وبنسبة (83%)؛ وعليه بلغ حجم العينة المقترح (83) مُرشداً زراعياً (من الذكور والإناث)، ولتحقيق هدف البحث أُعدت استبانة ضمت محورين، وهي: محور المشكلات الإدارية والمالية (7) فقرات، ومحور المشكلات التقنية والبشرية (11) فقرة، في عملية استخدام الإرشاد الزراعي الرقمي؛ وتم قياس موافقة المُرشدين الزراعيين بسؤال المُرشد الزراعي (من الذكور والإناث) عن مدى تواجد المشكلة من عدمها بأستخدام عبارات (نعم ، كلا) وحددت لها الدرجات (0,1)، ومن ثم التعرف على مدى تأثير تلك المشكلات (في حال تواجدها) باستخدام العبارات (منخفض، متوسط، كبير) وحددت لها الدرجات(1,2,3) على الترتيب. وقد أظهرت نتائج البحث أن أغلب المرشدين الزراعيين قد أشاروا إلى تواجد هذه المشكلات فضلاً عن تأثيرها المباشر على استخدام الارشاد الزراعي الرقمي في تقديم الخدمة الارشادية الزراعية لجمهور المسترشدين، أذ بلغ معدل التأثير العام للمشكلات الادارية والمالية (2.41) درجة، ومعدل المشكلات التقنية والبشرية (2.32) درجة، وقد يعكس ذلك واقعية وموضوعية المشكلات التي تم تناولها في البحث فضلاً عن تأثيرها المباشر على استخدام الارشاد الزراعي الرقمي؛ وعليه فأن عدم مواجهة وحل هذه المشكلات من شأنه أن يتحول إلى تهديد يعيق أو يفشل العملية بأكملها، وفي المقابل فأن السعي نحو حل هذه المشكلات وتذليلها من قبل الجهات المتخذة القرار في وزارة الزراعة العراقية ودائرة الإرشاد والتدريب الزراعي على وجهة الخصوص، من شأنه أن يوفر أرضية ملائمة ويحقق مستوى مُرضي من الكفاءة والفاعلية في عملية استخدام الإرشاد الزراعي الرقمي في تقديم الخدمة الإرشادية الزراعية إلى جمهور المسترشدين.

الكلمات المفتاحية: المشكلات الإدارية والمالية، المشكلات التقنية والبشرية، الارشاد الزراعي الرقمي، الخدمة الارشادية الزراعية.

INTRODUCTION

Today, the world countries are facing great challenges in the field of agricultural work and production (including Iraq), and at the forefront of these challenges is the issue of achieving sustainable food security, which in turn requires good management of the country's natural resources; to achieve increases in the national (local) agricultural production and to meet the needs of the growing population for both plant and animal agricultural products **(Mhaibes & Al-Taiy, 2019); (Mohammed, 2016)**.

So, in order to achieve this increase in plant and animal production, it is necessary to develop strategic plans that aim to achieve behavioral changes in the target audience, as well as adopting the use of modern agricultural technologies in various fields of agricultural production, which in turn, can be achieved by investing in agricultural extension efforts. Accordingly, using the efforts of the Agricultural Extension Agency, as one of the development agencies in the agricultural sector, will achieve the desired increase in agricultural production, and that will be done through the agricultural extension services which provide the target audience, including: transferring modern agricultural technology, agricultural training, developing skills, correcting knowledge, and improving attitudes **(Najris & Arsan, 2023); (Ajayi & Fapojuwo, 2014); (Kaegi, 2015)**.

Despite the importance of the Agricultural Extension Service role in agricultural work, the reality indicates that agricultural extension services in general, and the central administration for Agricultural Extension in Iraq in particular, are facing many criticisms at the present time, because of the poor effectiveness and efficiency in transferring and disseminating agricultural information, technical recommendations and agricultural innovations, as well as the poor sustainability of the solutions which provided by the Agricultural Extension Authority **(Al-Ajili & Novan,2019)**.

In fact, these criticisms are considering as a result of the many problems that the agricultural extension organization suffers from, including: limited training for agricultural extension agents in general, poor communication between them and the target audience, decrease in the number of agricultural extension agents compared to the numbers of the target audience, and lack of funding, which has led to a gradual reduction in public agricultural extension services over the past decades (**Bhattacharjee & Raj, 2016**)**;** (**Al-Hafidh & Al-Taiy, 2022)**. As well as the difficulty of dealing with the target audience who disparity in terms of culture, experience, awareness, etc., which has made agricultural extension agents use various methods and means when providing agricultural extension services to confront this problem (**Al-Mashhadani, 2013**). On the other hand, it is noted that the agricultural extension departments suffer from a lack of good qualifications for agricultural extension agents that enable them to keep abreast of developments in the use of modern digital information and communication technology (**Jassim & Jumaili, 2021**).

Accordingly, all of the above represents great pressure on agricultural extension organization and its workers, which can be overcome by following right and successful steps in developing agricultural extension work, and at forefront of these steps is work to support traditional agricultural extension methods and means, by providing agricultural extension services using information and communications technology or what is known as digital agricultural extension (**Bhattacharjee & Raj, 2016**)**;** (**Al-Hafidh & Al-Taiy, 2022)**. As the traditional methods alone are no longer feasible or appropriate in providing, analyzing and transferring data and information, whether to the target audience or to decision makers alike, so it is necessary to introduce modern technology in dealing with data and information, and the gradual transformation into digital organizations instead of routine work **(Al, Awadi,2019)**.

The world today is witnessing a great revolution in communication and information systems as a result of technological developments in various fields, which can be used by agricultural extension agents in providing agricultural extension services (**Al-shamry, 2021**). Since digital applications have become modern methods of communication, which can be employed and used in the activities of various organizations (including agricultural extension organizations) and service their audiences from inside or outside the organization **(Shubar & Zidane, 2021**)**; (Al-Heali, 2022).** Thus, digital applications these days are able to arouse or urge interest and obtain feedback on the information, technologies and expertise which provided by the organization, and obtain the behavioral changes that organizations aspire to achieve in general **(Ismael, 2021).** Therefore, modern digital applications can contribute to supporting the desired agricultural development in various agricultural organizations, including the Agricultural Extension Organization **(Najris & Arsan, 2023).**

The agricultural extension organization, in turn, depends on many factors to achieve effectively its goals, the most important of which is the performance of the agricultural

extension agents who are working as a link or bridge between the extension organization and the target audience, and at the present time they have the task of using modern digital extension methods and means (as well as traditional extension methods and means) to deal with the huge and diverse amount of renewable and constantly evolving agricultural information and technologies in the field of the agricultural sector, as well as to benefit from them in providing extension services to the target audience **(Al-Lazzawy & Al-mashadany, 2017); (Najris & Arsan, 2023);** (**Al-Salihi, 2016**).

Despite the importance of digital agricultural extension in improving the effectiveness and efficiency of the level of agricultural extension services, but unfortunately, it is not without problems that may hinder the continuity of its work or prevent its full use (**Suleiman, Siddiq, & Mohsen, 2018**). Therefore, not knowing, analyzing, diagnosing, and confronting these problems that may face agricultural extension agents when using digital agricultural extension, may lead to their transformation into a threat that hinders or fails the entire process (**Ahmadpour & Mirdamadi, 2010); (Dharmawan *et al.*, 2021**).

Based on the foregoing, and as a result of the lack of Iraqi studies that show the extent of the problems as well as the diagnosis of their impact, which may hinder or prevent the use of digital agricultural extension in providing agricultural extension services, this current research came to answer the following research question:

_ What are the problems of using digital agricultural extension in providing agricultural extension services from the viewpoint of agricultural extension agents?

Research Aims

The research has aimed to determine the problems in the use of digital agricultural extension, as well as indicating the extent of their impact from the viewpoint of agricultural extension agents, as follows:

1. The administrative and financial problems and its impact.
2. The technical and human problems and its impact.

Research Hypothesis

The process of identifying the problems facing the use of digital agricultural extension in the process of providing the agricultural extension service will reveal in the existence of many problems with big impact, which must be resolved and overcome in a manner that ensures the achievement of the highest effectiveness and efficiency when using digital agricultural extension in the present and future.

Research Importance

The importance of the research is related to the necessary need to provide officials (or decision makers) in the Iraqi Ministry of Agriculture and the Agricultural Extension and Training Department with potential administrative, financial, human and technical problems, as well as an indication of their impact; which may hinder the process of using digital agricultural extension in providing the agricultural extension service to the target audience or threaten the success of the entire process, so, identifying these problems and indicating the extent of their impact will help officials to work on their part to solve these problems and overcome them in a way that guarantees the investment of efforts, times, funds and the available resources in an optimal manner, as well as guarantees the continuous using of digital agricultural extension in providing the agricultural extension service to the target audience with the highest possible effectiveness and efficiency.

MATERIALS AND METHODS

Research Methodology

The research mainly aimed at identifying the problems of using digital agricultural extension in providing the agricultural extension service and indicating the extent of its impact from the viewpoint of agricultural extension agents; accordingly, the appropriate research approach is the descriptive approach, and the most appropriate research method of this approach is the analytical diagnostic method, which was adopted in the research to achieve accurate diagnosis and objective identification of the problems and their impact in using of digital agricultural extension, and then interpret results in light of possible reasons. This type of research has proven its effectiveness in many areas, including political, economic, social and others, by using data collection methods and interpreting information through questionnaires and personal interviews **(Kandilji,2008).**

Research Population and Sample:

The research population included all agricultural extension agents (agricultural extension agents service providers) who were existed in the Agricultural Extension and Training Department, the Extension Training and Rehabilitation Center, and the Agricultural Extension Center for Training, in addition to Agricultural Extension Farms in Baghdad governorate, thus, research population included (100) agricultural extension agents (from male and female), as shown in Table (1).

Table (1): distribution of agricultural extension agents (of both sexes) according to their Places.

Research Population	Research Area	Number
Agricultural Extension Agents	Agricultural Extension and Training Department	80
	Training and Rehabilitation Center	4
	Agricultural Extension Center for Training	6
	Agricultural Extension Farm in Al-Tarmiya	5
	Agricultural Extension Farm in Al-Rashidiya	5
Total		**100**

Then, a random sample took from research population which consist of (83%) from the five agricultural extension places, so, the random sample has included (83) agricultural extension agents (from male and female) which used to carry out research and achieve its aims.

Research Tool

In order to build a measure to determine the problems of use digital agricultural extension from the viewpoint of agricultural extension agents, dependence has been made on a group of scientific sources specialized in agricultural extension, as well as scientific sources related to computers and information technology, in addition to the opinions of a group of experts specialized in agricultural extension in the College of Agricultural Engineering Sciences \ University of Baghdad, as well as experts specialized in computer and information technology who work in the Iraqi Ministry of Agriculture. Hence, two axes were identified for the research in order to identify the problems of using digital agricultural extension from the viewpoint of the agricultural extension agents, which were measured by asking them (from male and female) about the extent of his/her agreement or disagreement with the paragraphs

constituting the two axes, which namely: administrative and financial problems (7) paragraphs and technical and human problems (11) paragraphs; and this was done by identifying the existence or not existence of the problem by using the phrases (yes, no) and assigning degrees (0,1), respectively, and then identifying the extent of the problem impact (if it exists) using the phrases (low, medium, big) and assigning them with degrees (3, 2, 1), respectively.

Questionnaire Validity and Reliability:

The method of content validity and face validity **(Bhattacherjee, 2012)** was used to measure the validity of the questionnaire by presenting the questionnaire (in its initial form) to (10) arbitrators (judges) from faculty members at the University of Baghdad / College of Agricultural Engineering Sciences / Department of Agricultural Extension and Technology Transfer, as well as arbitrators in the field of computer and digital applications, then the necessary modifications were made to the questionnaire, and after completing the questionnaire in its final form, a Pre-Test was conducted in October of the year (2022) using the final questionnaire on a random sample consisting of (12) agricultural extension agents from the Agricultural Extension and Training Department, the Training and Rehabilitation Center, and the Agricultural Extension Center for Training[1]. The purpose of the Pre-Test was to verify the consistency (reliability) and stability of the results, as well as to reveal the validity of the content and the extent of clarity and distinction of the paragraphs in the questionnaire, then Cronbach's Alpha (α) equation was used to examine the reliability of the results because it is suitable for obtaining a stability coefficient from the data set for a graduated scale of expressions[2] and this method is characterized by being short in effort and avoiding the effect of transferring the effect of training or avoiding an effect of remembering and trying to avoid some of the previous answers, all of which are disabled features in the Retest method for example **(Sami,1999)**. Thus, the results showed that the coefficient of reliability was high for the two axes of problems of use digital agricultural extension, which ranged between (0.791) as a minimum and between (0.888) as a maximum, so, this indicates that the questionnaire has a high degree of reliability, and it can be relied upon in field application to achieve the objectives of the research. This is according to the Nunnally & Bernstein scale, which adopted (0.7) as a minimum for reliability **(Nunnally & Bernstein, 1994)**. Then, Pearson Linear Correlation Coefficient was used to examine the validity of the internal consistency between each paragraph of the axis and the total score of the same axis. The results showed that all Pearson's correlation coefficients between the axis' paragraphs and the total score of the axis were statistically significant at a significant level (0.01) and (0.05), where the minimum for the correlation coefficients was (0.582), while the upper limit was (0.886), accordingly, all the paragraphs of each axis were internally consistent with the axis to which they belong, which proves the validity of the internal consistency of the axes and paragraphs of each axis.

Data Collection:

After completing all stages of the process of preparing and developing the questionnaire, the researcher proceeded with the process of collecting data, and the data was

[1] Pretest participants were excluded from the final data collection process.
[2] The same applies to the split-half method or the Guttman method, which gives almost the same results.

collected in the questionnaire through a personal interview with the agricultural extension agents (from male and female), as well as communication and follow-up with them using social media to answer questions about the nature and objectives of the research or questionnaire, so, the data collection process took about two months, which extended from 1/11/2022 to 29/12/2022, noting that all the questionnaires were obtained from the agricultural extension agents (the research sample).

Statistical Processors:

Appropriate statistical methods were used to achieve the aims of the research, which are weighted average, numbers and percentages, simple linear correlation law (Person), Cronbach's alpha coefficient, and (Steven K. Thompson) equation to determine the required sample size from the target population.

RESULTS AND DISCUSSION

This part deals with a presentation of the determining of the problems existence as well as an indication of the extent of their impact on the use of digital agricultural extension in providing the agricultural extension service, as follows:

1. Administrative and Financial Problems:

The research results have shown that the answers of the agricultural extension agents have been somewhat similar in determining the existence of administrative and financial problems that related to the use of digital agricultural extension, so, the problem of (difficulty of following up the target audience and correcting their levels (including the process of measurement and evaluation) during and after doing digital educational activities, due to the small number of agricultural extension agents compared to the huge number of target audience) has ranked first in terms of the existence, and the problem of (weak encouragement and interest by the central administration about the use of digital agricultural extension in providing extension services) has ranked in the last place of existence, also the results have shown that the overall average effect of this axis has reached (2.41) degrees, which has described as a big (or high) effect on the process of using digital agricultural extension, as shown in table (2).

Table (2): Distribution of agricultural extension agents according to their answers about administrative and financial problems.

NO.	Administrative and Financial Problems	Freq. & Perc.	Problem Existence		Freq. & Perc.	Problem Effect			Weighted Average	Rank
			No	Yes		Low	Medium	High		
1.	Lack of symposiums, lectures and training courses for agricultural extension agents in the use of digital agricultural extension.	Freq.	11	72	83	8	21	43	2.49	2
		%	13.3	86.7	100	9.6	25.3	51.8		
2.	Poor coordination between research centers and agricultural extension departments on the one hand, and between relevant organizations such as colleges of agriculture, agricultural associations, and civil society organizations to provide the available digital agricultural extension websites with priority agricultural information that needed by the target audience.	Freq.	12	71	83	12	21	38	2.37	5
		%	14.5	85.5	100	14.5	25.3	45.8		
3.	Weak and insufficient funding to train agricultural extension agents to use digital extension in providing extension services.	Freq.	6	77	83	14	22	41	2.35	6
		%	7.2	92.8	100	16.9	26.5	49.4		
4.	Difficulty of following up the target audience and correcting their levels (including the process of measurement and evaluation) during and after doing digital educational activities, due to the small number of agricultural extension agents compared to the huge number of target audience	Freq.	4	79	83	8	32	39	2.39	4
		%	4.8	95.2	100	9.6	38.6	47.0		
5.	The weakness of the integrated infrastructure of the agricultural extension departments, including halls, equipment, electronic platforms, digital applications, and high-quality Internet services.	Freq.	7	76	83	7	25	44	2.49	2
		%	8.4	91.6	100	8.4	30.1	53.0		
6.	Absence of funding allocated for the purchase of digital devices and applications, and the establishment and design of extension platforms or websites to provide digital extension service.	Freq.	7	76	83	6	27	43	2.49	2
		%	8.4	91.6	100	7.2	32.5	51.8		
7.	Weak encouragement and interest by the central administration about the use of digital agricultural extension in providing extension services.	Freq.	14	69	83	12	25	32	2.29	7
		%	16.9	83.1	100	14.5	30.1	38.6		
Overall Average Effect = 2.41 **Low Effect (1 – 1.66), Medium Effect (1.67 – 2.33), High Effect (2.34 - 3)**										

Also, it can be concluded from the above table that the most administrative and financial problems affecting the use of digital agricultural extension in providing agricultural extension services from the viewpoint of agricultural extension agents were the problem of (Lack of symposiums, lectures and training courses for agricultural extension agents in the use of digital agricultural extension), and the problem (The weakness of the integrated

infrastructure of the agricultural extension departments, including halls, equipment, electronic platforms, digital applications, and high-quality Internet services), as well as the problem (Absence of funding allocated for the purchase of digital devices and applications, and the establishment and design of extension platforms or websites to provide digital extension service), with a weighted average of (2.49) degrees, so, this may be attributed to the presence of great awareness among agricultural extension agents about the seriousness of these problems and their direct impact on the success or failure of using digital agricultural extension in providing extension service.

In addition, the problem (Weak encouragement and interest by the central administration about the use of digital agricultural extension in providing extension services), came in the last place in terms of impact, with a weighted average of (2.29) degrees, and this may lead to the reluctance (unwillingness) of agricultural extension agents to use digital agricultural extension, or it may cause a total or partial suspension of digital agricultural extension activities when applied in the future, especially if the matter is stemming (originated in) from the conviction and certainty of central administration officials (from the beginning) of the futility or uselessness of digital agricultural extension in providing the agricultural extension service.

Thus, based on the above, the matter requires serious action and hard work to overcome all the administrative and financial problems that the research results have shown their existence and direct impact; this is to ensure the highest effectiveness and efficiency in the use of digital agricultural extension in the providing the agricultural extension services.

2. Technical and Human Problems:

The research results have shown that the answers of the agricultural extension agents have been somewhat similar in determining the existence of technical and human problems that related to the use of digital agricultural extension, so, the problem of (The spread of illiteracy in its general sense (the inability to read and write) among the target audience, as well as electronic illiteracy (the inability to use and deal with electronic devices)) has ranked first in terms of the existence, and the problem of (The difficulty of providing and achieving security and digital data protection for participants in digital agricultural extension) has ranked in the last place of existence, also the results have shown that the overall average effect of this axis has reached (2.32) degrees, which has almost described as a big (or high) effect on the process of using digital agricultural extension, as shown in table (3).

Table (3): Distribution of agricultural extension agents according to their answers about technical and human problems.

NO.	Technical and Human Problems	Freq. & Perc.	Problem Existence		Freq. & Perc.	Problem Effect			Weighted Average	Rank
			No	Yes		Low	Medium	High		
1.	The difficulty of providing and achieving security and digital data protection for participants in digital agricultural extension	Freq.	16	67	83	15	25	27	2.18	10
		%	19.3	80.7	100	18.1	30.1	32.5		
2.	Some agricultural extension agents are not convinced of the using the digital applications for fear of reducing their role in the field extension process, and transfer or decline of their role in designing and following up electronic extension programs.	Freq.	12	71	83	16	28	27	2.15	11
		%	14.5	85.5	100	19.3	33.7	32.5		
3.	Weakness in the direct interaction between the agricultural extension agents and the target audience during the implementation of some counseling activities.	Freq.	7	76	83	14	27	35	2.28	7
		%	8.4	91.6	100	16.9	32.5	42.2		
4.	Limited availability of a culture of participation, discussion and constructive criticism among the users of digital agricultural extension, which must be far from attacking or offending others.	Freq.	8	75	83	12	31	32	2.27	8
		%	9.6	90.4	100	14.5	37.3	38.6		
5.	Some members of the rural community consider digital agricultural extension as less efficient than traditional agricultural extension.	Freq.	7	76	83	12	23	41	2.38	3.5
		%	8.4	91.6	100	14.5	27.7	49.4		
6.	The widening gap between the digital knowledge and skill required to use digital agricultural extension, and between the traditional knowledge and skill that most agricultural extension agents have it.	Freq.	9	74	83	8	33	33	2.34	5
		%	10.8	89.2	100	9.6	39.8	39.8		
7.	Weakness or limited possibility of covering all plant and animal topics at the beginning of using digital extension, especially those that need application and performance evaluation.	Freq.	9	74	83	11	33	30	2.26	9
		%	10.8	89.2	100	13.3	39.8	36.1		
8.	Lack of information that explaining the mechanisms of operation or use the digital agricultural extension applications by the target audience.	Freq.	9	74	83	8	30	36	2.38	3.5
		%	10.8	89.2	100	9.6	36.1	43.4		

NO.	Technical and Human Problems	Freq. & Perc.	Problem Existence		Freq. & Perc.	Problem Effect			Weighted Average	Rank
			No	Yes		Low	Medium	High		
9.	Speed and magnitude of change in information and communication technology, and the difficulty of keeping pace (pro-cyclical) with it according to the available financial, material and human resources.	Freq.	9	74	83	7	27	40	2.45	2
		%	10.8	89.2	100	8.4	32.5	48.2		
10.	Some agricultural extension agents fear accountability (questioning) when errors or malfunctions occur in the devices used in digital agricultural extension.	Freq.	10	73	83	8	33	32	2.33	6
		%	12.0	88.0	100	9.6	39.8	38.6		
11.	The spread of illiteracy in its general sense (the inability to read and write) among the target audience, as well as electronic illiteracy (the inability to use and deal with electronic devices)	Freq.	2	81	83	7	28	46	2.48	1
		%	2.4	97.6	100	8.4	33.7	55.4		
Overall Average Effect = 2.32 Low Effect (1 – 1.66), Medium Effect (1.67 – 2.33), High Effect (2.34 - 3)										

Following the above table, it can be concluded that the most technical and human problems affecting the use of digital agricultural extension in providing agricultural extension services from the viewpoint of agricultural extension agents was the problem of (The spread of illiteracy in its general sense (the inability to read and write) among the target audience, as well as electronic illiteracy (the inability to use and deal with electronic devices)), with a weighted average of (2.48) degrees, so, this may be due to the presence of great awareness among agricultural extension agents of the great impact of this problem, which may threaten and hinder the use of digital agricultural extension. If some of the target audiences are unable to deal with electronic platforms or applications, this will undoubtedly threaten the spread of the use of digital agricultural extension, and will negatively affect its level of success.

In addition, the problem (Some agricultural extension agents are not convinced of the using the digital applications for fear of reducing their role in the field extension process, and transfer or decline of their role in designing and following up electronic extension programs), came in the last place in terms of impact, with a weighted average of (2.15) degrees, and this may show the need to educate some agricultural extension agents that the use of digital agricultural extension is a complementary and supportive means to other agricultural extension methods and means, and it's not a substitute for it.

Hence, based on the foregoing, the matter requires a serious action and hard work to overcome all the technical and human problems that the research results have shown their existence and direct impact; this is to ensure the continuity and sustainability of the use of digital agricultural extension in the providing the extension services, and to achieve the feasibility and desired goals behind its use.

CONCLUSIONS

Based on the research results that have been reached, the following are concluded:

1. The research results have shown that the answers of the agricultural extension agents were somewhat similar in determining the existence of administrative and financial problems related to the use of digital agricultural extension, which are described as having a significant impact on the process of using digital agricultural extension in the provision of agricultural extension service.
2. The research results have shown that the highest administrative and financial problems affecting the use of digital agricultural extension were the problem of (Lack of symposiums, lectures and training courses for agricultural extension agents in the use of digital agricultural extension), and the problem (The weakness of the integrated infrastructure of the agricultural extension departments, including halls, equipment, electronic platforms, digital applications, and high-quality Internet services), as well as the problem (Absence of funding allocated for the purchase of digital devices and applications, and the establishment and design of extension platforms or websites to provide digital extension service).
3. The research results have shown that the answers of the agricultural extension agents were somewhat similar in determining the existence of technical and human problems related to the use of digital agricultural extension, which are described as having a significant impact on the process of using digital agricultural extension in the provision of agricultural extension service.
4. The research results have shown that the highest technical and human problems affecting the use of digital agricultural extension was (The spread of illiteracy in its general sense (the inability to read and write) among the target audience, as well as electronic illiteracy (the inability to use and deal with electronic devices)).

RECOMMENDATIONS

1. The need for the Ministry of Agriculture to provide the necessary financial allocations for the implementation of symposium, lectures and training courses for agricultural extension agents in the field of using digital agricultural extension, which is also necessary to provide hardware devices, digital software applications and other infrastructure necessary to provide digital agricultural extension service, and enables the Agricultural Extension and Training Department and its affiliated departments to perform their work with the highest possible quality, in addition to solving problems that they may face in the future.
2. The Agricultural Extension and Training Department must provide a group of agricultural extension agents who are able to implement digital educational activities; and this is done either by hiring people with experience and skill in the field of information and communication technology in addition to experience in the field of agricultural extension, or by implementing training courses for agricultural extension agents in agricultural departments (especially young ones) on the use of digital applications in providing agricultural extension services to the target audience.
3. The Agricultural Extension and Training Department must carry out educational campaigns targeting mainly rural youth (male and female) to encourage and urge them to use digital applications facilely and easily, and to benefit from the agricultural extension

services provided by the Agricultural Extension Departments, thus, these young people, in turn, encourage, edification, and educate their families and neighbors, as well as the rest of their community, to use and benefit from digital applications.

4. The Ministry of Agriculture must (as a first step) create a digital application that includes agricultural information, agricultural experiences, as well as the possibility of obtaining electronic agricultural consultations by the target audience, then assess the benefit achieved and the problems that may appear and find appropriate solutions to them, and after that (and as a second step) starting to create an integrated electronic platform that provides all agricultural extension services to the target audience.
5. The Ministry of Agriculture should work on solve all other problems which have indicated by the results of the research in some detail in terms of existence or impact; This is by solving it directly through the departments of the Ministry of Agriculture or in coordination and cooperation with other relevant ministries, to guarantees achieving the highest efficiency and effectiveness in the process of using digital agricultural extension in providing agricultural extension service to the target audience.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 101-113
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(9)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

THE ROLE OF FOLIAR APPLICATION AND LIGHT INTENSITY ON THE FRESH AND DRY LEAVS YIELD OF PEPPERMINT

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Received 29/ 5/ 2023, Accepted 30/ 10/ 2023, Published 31/ 12/ 2024

ABSTRACT

A field experiment was conducted in a research station affiliated to the College of Agricultural Engineering Sciences - University of Baghdad, from March to Juley 2021. The study's objective was to investigate the effect of foliar application of the urea, nano-amino acids, and yeast extract, under two different lighting intensities on the growth and yield of leaves. The experiment included two factors: the environment type, involving an open field (100% lighting) and 50% shadow (50% lighting). The second factor included foliar spray of the yeast extract 5 and 7.5 g L^{-1}, 1000 and 2000 mg L^{-1} (as urea), and nano-amino acids 1 and 1.5 ml L^{-1} with half the amount of nitrogen and the entire recommended amount of N, P, K and treatment (full amount of N,P,K fertilizers). The experiment was conducted using the Randomized Complete Block Design (RCBD) according to the Nested system with three replicates. the results observed that cultivation under shade achieved a significant increase in plant height, number of branches, leaf area, and total chlorophyll (83.6 cm, 10.63 branch $plant^{-1}$ and 15.45 dm^2, 1.05 mg g^{-1} respectively), while the result was reversed in The fresh and dry yield of the leaves reached the highest at 100% lighting (1902.6 and 877.6 kg ha^{-1}, respectively),the concentration of N,P,K leaves. Results showed that all foliar spray treatments increased significantly compared to the control. The highest significant increase was from the two spray treatments, urea of 2000mgN L^{-1} and nano-amino acids of 1.5ml L^{-1} at the shadow plants for the traits, the plant height, number of branches, leaf area, and total chlorophyll content, while in the open field environment, recorded the highest fresh and dry yield of leaves(2007.8, and 995.2; 1955.1 and 957.7 kg ha^{-1} for the two treatments, respectively).

Keywords: peppermint, Foliar spray, Fresh leaves, Dry leaves, lighting intensity.

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(2024) 16(2): 101-113

دور الرش الورقي وشدة الإضاءة في النعناع الفلفلي وحاصل الأوراق الطري والجاف

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الخلاصة

أجريت تجربة حقلية على نبات النعناع الفلفلي في أحدى المحطات البحثية لكلية علوم الهندسة الزراعية- جامعة بغداد – للمدة من آذار- تموز 2021 بهدف دراسة دور الرش الورقي لليوريا والأحماض الأمينية النانوية ومستخلص الخميرة تحت شدتي إضاءة مختلفة في النمو وحاصل الأوراق. تتكون التجربة من عاملين، الأول يمثل نوع البيئة: الحقل المفتوح (100% إضاءة) ، (50% ظل 50%إضاءة) وتضمن العامل الثاني الرش الورقي مع إضافة الموصى به من التسميد الأرضي من الفسفور والبوتاسيوم ونصف الكمية من النتروجين مقارنة مع معاملة القياس اما معاملات الرش الاخرى فهي رش مستخلص الخميرة 5 و 7.5غم لتر$^{-1}$ ، نتروجين 1000و 2000 ملغم N لتر$^{-1}$ (بهيأة يوريا)، أحماض امينية نانوية 1 و 1.5 مل لتر$^{-1}$. نفذت التجربة باستعمال تصميم القطاعات العشوائية الكاملة(RCBD) وفق نظام Nested بثلاثة مكررات. اظهرت النتائج أن الزراعة تحت الظل (50%إضاءة) حققت زيادة معنوية في ارتفاع النبات وعدد الأفرع والمساحة الورقية والكلوروفيل الكلي (83.6 سم و10.63 فرع نبات $^{-1}$و15.45 دسم^{2} ، 1.05 ملغم غم$^{-1}$ بالتتابع) بينما ظهر العكس في الحاصل الطري والجاف للأوراق وبلغ أعلاه عند 100% اضاءة (1902.6 ، 877.6 كغم هـ$^{-1}$بالتتابع) وكذلك تراكيز مغذيات (N,P,K). لوحظ زيادة معنوية في معاملات الرش الورقي مقارنة بمعاملة القياس عند معاملتي الرش باليوريا(2000 ملغم N لتر$^{-1}$) والأحماض الامينية النانوية(1.5 مل لتر$^{-1}$) في نباتات الظل لارتفاع النبات وعدد الأفرع والمساحة الورقية والكلوروفيل الكلي ، وفي بيئة الحقل المفتوح لحاصل الأوراق الطري والجاف(2007.8، 995.2 و 1955.1، 957.7 كغم هـ$^{-1}$ للمعاملتين بالتتابع).

الكلمات المفتاحية: النعناع الفلفي,الرش الورقي، الوزن الطري للاوراق، الوزن الجاف للاوراق، شدة الاضاءة.

INTRODUCTION

Medicinal plants are the primer source and basis for treating various diseases, directly as herbs or indirectly, through their involvement in manufacturing medicines used in traditional pharmacies or as a guide for constructing chemical compounds similar to that found in plants in terms of physiological and pharmacological effects. Among the medicinal plants that are characterized by many therapeutic and industrial benefits is peppermint (*Mentha piperita* L.), which belongs to the family, *Labiatae*. It is a perennial evergreen herbaceous plant, and the nature of its growth is creeper or erect. The Mediterranean basin is the original home of the plant, especially the southern part of the European continent and the northern part of the continent of African continent. The essential part of the plant is leaves, the main source of aromatic oil, which is medically industrially necessary, as the plant participates in medicines for colds and coughs. The aromatic oil is useful in expelling gases because it is rich in a menthol compound and industry, it is used in the preparation of perfumes, soaps, and some food products, such as sweets, to give them taste and flavor **(Halabo *et al.*,1995 ; Franz *et al* 2007)**.Because of the oil content of other compounds such as limonene, cineole, and others, it is added to medicines that cause diarrhea to prevent colic, and one of the most prominent

industries that the plant is famous for is kinds of toothpaste for its anti-inflammatory effect on throat and pharynx infections**(Heikal, & Omar, 2012).**

Because of the importance of medicinal plants, it became necessary to develop the plant performance and its production under different light intensity conditions for its relation to the process of photosynthesis. Determining the optimal light intensity level is necessary because each plant grows ideally in light limits that differ from others. A high level of light intensity leads to a decrease in net photosynthesis, so shading is used **(Berenschot & Quecini, 2014)**. On the other hand, exposing plants to the low intensity of lighting leads to a decrease in the efficiency of photosynthesis and metabolism **(Wang *et elle*, 2013)**. Based on the aforementioned, several studies were conducted on this. At growing mint (*Mentha spicata* L.) under shade and sun, the plants grown under shade showed significant superiority in the percentage of chlorophyll a and b, total chlorophyll and carotenoids (0.89, 0.40, and 1.29 mg g^{-1}, respectively) compared to the plants grown in the sun, amounted to 0.80, 0.31, and 0.25 mg g^{-1}, respectively **(Patra *et al*., 2003). Ade-Ademilua *et al*. (2013)** noticed in their experiment when growing African basil (*Ocimum gratissimum* L.) under full sunlight and shade (44.2-26.7% of sunlight), that plants grown under shade and sun did not show significant differences in the plant height or leaf area, while the plants grown under the sun were significantly superior in increasing the fresh and dry weight of the plant, reaching 37 and 10.4 g $plant^{-1}$ respectively, compared to the plants grown under shade (24 and 4.5 g $plant^{-1}$, respectively).

Chemical fertilization is one of the necessary treatments for increasing leafy crops production, but the increased use of chemical fertilizers has become a source of environmental pollution, especially when they are overused, in particular, the leafy crops fertilized with nitrogen, so it is necessary to ration the quantities with preserving the yield in quantity and quality, for achieving this aim, several studies were conducted using nutrients and stimulants as a foliar spray in limited quantities leading to encouraging results. **Mahmoodabad *et al*. (2014)** observed in their experiment on mint (*Mentha spicata* L) through foliar spraying with different concentrations of urea (1, 2, and 3%) that the 3% concentration had a significant superiority in the highest value of stem diameter (3.65 mm) compared to the control (2.7 mm), while the 2% concentration recorded a significant increase in the traits of leaf area and fresh plant weight (88.60 cm^2 and 942 $g.m^{-2}$ respectively) compared to the control plants (77.3 cm^2 and 570 g. m^{-2} respectively).

The results obtained by **Al-Amrani *et al*., (2018)** confirmed the existence of significant differences between the peppermint plants (*Mentha piperita* L) treated with urea (1000 and 2000 mg L^{-1}) and the plants fertilized with whole soil fertilization (N, P, K), the plants treated with spraying 2000 $mg.L^{-1}$ nitrogen plus whole soil P, K, and ¼ N were significantly superior in the plant height and number of branches per plant (46.50cm and 20.86 $branches.plant^{-1}$, respectively) compared with the control (44.39 cm and 20.39 $branch.plant^{-1}$ respectively), while the treatment of spraying the concentration 1000 mg L^{-1} nitrogen plus soil P,k recorded

the highest average in leaf area, and dry weight (15.19 dm^2 and 15.2 gm $plant^{-1}$, respectively) compared to the control treatment (10.03 dm^2 and 12.95 g $plant^{-1}$, respectively).

Matter & ElSayed (2015), in their experiment on caraway plants (*Carum carvi*L.) sprayed with dry yeast extract (2 and 4 g L^{-1}), observed the plants with a concentration of 4 g L^{-1} were significantly superior in the plant height, number of branches, fresh and dry weight compared to control treatment. Hamed (2018) showed, in his experiment on mint (*Mentha spicata*L.Siwa), when spraying the plants with dry yeast extract (*Saccharomyces cerevisiae*) at different concentrations (0, 2, 4, and 6 g L^{-1}) that the plants sprayed with the highest concentration (6 gm L^{-1}) were significantly superior in the fresh and dry weight of plants (491.08, 390.77, 231.13 and 189.47 gm $Plant^{-1}$) and (127.60, 109.13, 71.05 and 56.80 gm $Plant^{-1}$) respectively for the first and second seasons of four cuts compared to the control treatment. (84.45, 103.33, 202.06, and 317.46 gm $Plant^{-1}$) and (25.04, 32.10, 56.08, and 82.04 gm $Plant^{-1}$), respectively. **Ostadi *et al.* (2020)** found a response of the mint plant (*Mentha piperita* L.) to spraying with nano-amino acids. they obtained a significant superiority in the plant height and number of leaves and branches (46.4 cm, 588 leaves $plant^{-1}$ and 19.3 branches $plant^{-1}$, respectively) compared to control plants. (40.3 cm, 440 leaves $plant^{-1}$, and 16 branches $plant^{-1}$, respectively). When spraying the Vinca plant (*Catharanthu srosus*) with two concentrations of amino acids (1 and 1.5 g L^{-1}), **Fetouh *et al.* (2016)** found that the concentrations 1.5 g L^{-1} recorded the highest average of the plant height and fresh and dry weight respectively compared to the control.

To increase the qualitative and quantitative yield of the peppermint plant with reducing the use of chemical fertilizers added to the soil, the study aimed to:

1- Find out the optimum light intensity for the growth of peppermint under the conditions of the central region of the country.

2- Increasing the quantitative and qualitative production through foliar spraying of urea, yeast and nano-amino acids in limited quantities with reducing soil nitrogen fertilizer by half to reduce environmental pollution and economic costs.

MATERIALS AND METHODS

The study was conducted at an agricultural station in College of Agricultural Engineering Sciences - University of Baghdad, during the period, from March to July 2021,In which environmental conditions were recorded (Table 1).The cuttings(semi-soft) were prepared prior to planting within plots, after successful growth of cuttings selected according to the homogeneity in length and root size, they were planted in the corridor (1 m width) with a distance of 40 cm between plants getting to 8 plants per the experimental unit . The field soil was analyzed to determine the chemical and physical characteristics (Table 2). Available nitrogen in the soil was determind to be subtracted from the fertilizer recommendation(243 kg N, 121 kg P, 60 kg K per hectare) according to the recommendation mentioned by Abu Zeid

(1992), Having the nitrogen amount modified, the whole amount of the adjusted nitrogen with phosphorus and potassium was added to the plants of the control treatments, and half of the amount of nitrogen (after modification) with the whole amount of phosphorus and potassium for foliar spray treatments.The experiment was factorial of two factors (14 treatments) conducted based on the Randomized Complete Block Design (RCBD) according to the Nested system with three replicates. The first factor (symbolized by E) was planting in two environments of lighting intensity: E1, planting in an open field (100% lighting), and E2 planting under shade (50% lighting); the second factor was the foliar spray of nitrogen (as urea), 1000 (U1) mg L^{-1} and 2000 (U2) mg L^{-1}, yeast extract 5 (Y1) g $L^{-1}$and 7.5 (Y2) g L^{-1}, nano-amino acids 1 (Am1) ml L^{-1} and 1.5 (Am2) ml L^{-1}, with adding the recommended amount of soil fertilization of whole of P and K and half amount of nitrogen compared to the control (full soil fertilization of NPK). The plants were sprayed accompanied with a Surfactants agent in the early morning (4 times) during the vegetative growth period (10 days between one spray and another), reaching the harvest (beginning of flowering). Soil nitrogen fertilizer was added to the soil in two batches, the first with P and K after planting the seedlings and the second 21 days after the first one.

Bread yeast was prepared by dissolving 5 and 7.5 g in a liter of distilled water at 32°C (for the two concentrations of the experiment) with 1g of sucrose to increase the yeast activity , then kept in an incubator at 25 °C for two hours before spraying. Urea solution was prepared by dissolving 2.14 g of urea in 1 L of water to prepare 1000 mg N L^{-1} and the double quantity of urea (4.34 g) in 1 L of water to prepare 2000 mg N L^{-1}. Nano-amino acids (produced by the Commercial Agricultural Sciences Company Izmir - Turkey) were prepared by adding 1 ml to 1 liter of water to prepare the first concentration and adding 1.5 ml to 1 liter of water to prepare the second concentration.

The following parameters were measured: plant height (cm), number of branches (branch plant $^{-1}$), leaf area (dm^2. plants^{-1}), total chlorophyll (mg .g^{-1} fresh weight), N, P, K concentration in leaves, Fresh and dry leaf yield (kg ha^{-1}).

Table (1). Environmental conditions of the experiment field

Sun					Shade			
Month	Mean of 10 days	Maximum temperature °C	Minimum temperature °C	Solar illumination hours QF	Month	Mean of 10 days	Maximum temperature °C	Minimum temperature °C
March	1-10	24.9	9.24	8	March	1-10	23.1	8.21
	11-20	25.13	12.42			11-20	24.7	10.20
	21-31	26.01	12.61			21-31	24.1	10.31
April	1-10	30.6	14.01	9	April	1-10	29.6	13.11
	11-20	31.78	14.97			11-20	29.63	13.43
	21-30	38.76	21.22			21-30	37.38	20.10
May	1-10	38.29	21.24	11.3	May	1-10	36.14	20.12
	11-20	42.01	22.9			11-20	39.55	20.45
	21-31	41.72	23.06			21-31	39.37	22.01
June	1-10	42.15	22.9	12.1	June	1-10	41.7	21.3
	11-20	40.6	24.65			11-20	38.3	22.33
	21-30	46.51	25.12			21-30	44.26	24.01

Table (2): Chemical and physical properties of field soil

Measure Characteristics	Value	Measruing unit
Electrical Conductivity (EC)	2.23	ds m^{-1}
pH1:1	7.20	----------
Available nitrogen	40	mg kg^{-1}
Available phosphorous	20.7	
Available Potassium	73	
Organic matter O.M.	3.67	gm kg^{-1}
Mineral carbonate $CaCO_3$	79.5	g Kg^{-1}
Dissolved Calcium Ca^{-2}	12.8	meq L^{-1}
Dissolved MagnesiumMg^{+2}	6.7	
Dissolved SodiumNa^{+}	3.75	
Bicarbonate	1.45	
Dissolvedchlorine	16.02	
dissolved potassiumK	0.87	
Sand	740.8	Soil g kg^{-1}
Clay	135.0	
Silt	124.2	
Texture	sandy loam	----------

RESULTS AND DISCUSSION

Results in Table 3 refer to the prominent significant role of the study factors in the, plant height, number of branches, and leaf area. The plants grown under 50% lighting (E2) recorded the highest values of plant height, number of branches and leaf area (83.67 cm, 10.63 branches. $plant^{-1}$, and 15.45 dm^2, respectively) compared to the open field E1 (50.10 cm, 9.10 branches.$plant^{-1}$, and 10.63 dm^2, respectively).

As for the foliar spray treatments, there were significant differences among them. The highest values of the traits above were from the two treatments, urea (U2) and nano-amino acids Am2 (76.08 and 72.81 cm; 10.48 and 10.22 branches.$plant^{-1}$; 13.63 and 13.42 dm^2, respectively) compared to the lowest values of the plant height and number of branches was recorded at the control (58.77 cm and 9.22 branches.$plant^{-1}$, respectively) and the leaf area recorded by control treatmentsand the yeast (Y1) (12.6 and 12.58 dm^2, respectively).

The significant differences among the treatments of lighting intensity and foliar spray led to a significant effect of the interaction on the studied traits. The highest values of the plant height, number of branches and leaf area were recorded from the interaction of each of urea (U2) and nano-amino acids (Am2) with the shadow E2 (94.65 and 89.58 cm; 11.6 and 10.91 branches. $plant^{-1}$; 16.22 and 15.96 dm^2, respectively) compared to the lowest values obtained from the two of control and yeast (Y2).

Table (3): Effect of the environment type and spraying with yeast extract, urea and nano-amino acids on the plant height, number of branches and leaf area of *Mentha piperita* L.

Trait	Plant height (cm)			Number of branches (branches. plant^{-1})			Leaf area (dm^2)		
Foliar spray	Environment type		Mean	Environment type		Mean	Environment type		Mean
	E1	E2		E1	E2		E1	E2	
Control	45.07	72.48	77.58	8.23	21.10	9.22	10.66	14.66	12.66
Y1	45.22	75.33	28.60	8.63	10.35	9.49	10.19	14.98	12.58
Y2	55.61	84.42	70.02	9.16	10.64	9.90	10.58	15.40	12.99
U1	45.80	86.90	66.35	9.35	10.78	10.06	10.72	15.78	13.25
U2	57.59	94.56	76.08	9.89	11.06	10.48	10.05	16.22	13.63
Am1	45.41	82.39	63.90	8.90	10.49	9.69	10.32	15.14	12.73
Am2	56.03	89.58	72.81	9.53	10.91	10.22	10.88	15.96	13.42
L.S.D	5.96			0.04			0.26		
Mean	50.10	83.67		9.10	10.63		10.63	15.45	
L.S. D	5.14		3.35	0.02		0.07	0.15		0.18

Among the traits studied in our research correlated to the foliar spray treatments was the variance in nutrient elements (N, P and K) percentage. Results in Table 4 show that nutrient elements percentage were affected by the lighting intensity as the plants grown under the sun (open field E1) recorded the highest percentage of them, reaching 1.73, 0.38, and 2.69%, respectively, compared to the shadow plants (1.47, 0.30 and 2.49 %, respectively).

Concerning the foliar spray treatments, the highest of N, P, K was recorded at the treatment of spraying urea U2 (1.71, 0.45 and 2.72%, respectively) followed by the nano-amino acid (Am2) (1.67, 0.42 and 2.69%, respectively).

Regarding the interaction treatments, the plants grown in the open field (E1) treated with urea (U2) recorded the highest percentage of the elements (1.84, 0.49 and 2.83 %, respectively) followed by the plants treated with the nano-amino acids (Am2) at the same environment (1.80, 0.45, and 2.78% respectively) while, the lowest percentages of them were recorded in the control plants grown in the shadow environment E2 (1.34, 0.19 and 2.35 %, respectively)

Table (4). Effect of the environment type and spraying with yeast extract, urea, and nano-amino acids on the percentage of nitrogen, phosphorus, and potassium in the leaves of *Mentha piperita* L.

Trait	Nitrogen (%)			Phosphorus (%)			Potassium (%)		
Foliar spray	Environment type		Mean	Environment type		Mean	Environment type		Mean
	E1	E2		E1	E2		E1	E2	
Control	1.62	1.34	1.48	0.26	0.19	0.23	2.55	2.35	2.45
Y1	1.66	1.39	1.53	0.31	0.22	0.27	2.59	2.38	2.49
Y2	1.73	1.47	1.60	0.38	0.31	0.35	2.68	2.59	2.59
U1	1.76	1.51	1.63	0.42	0.34	0.38	2.72	2.63	2.63
U2	1.84	1.58	1.71	0.49	0.41	0.45	2.83	2.74	2.74
Am1	1.70	1.42	1.56	0.35	0.26	0.31	2.64	2.53	2.53
Am2	1.80	1.54	1.67	0.45	0.38	0.42	2.78	2.69	2.69
L.S.D	0.015			N.S			0.014		
Mean	1.73	1.47		0.38	0.30		2.69	2.49	
L.S.D	0.012		0.009	0.009		0.009	0.011		0.008

It is clear from the results in Table 5 that the plants grown under 50% shade (E2) had the highest content of total chlorophyll (1.05 mg. g^{-1}) compared to the plants grown in the open field (E1) recording 0.68 mg. g^{-1} .On the contrary, in the yield of fresh and dry leaves, the open field plants(E1) recorded a significant superiority reached 1902.6 and 877.6 kg ha^{-1} compared to the plants of shade 50% (E2) that recorded 1650.9 and 639.3 kg ha^{-1}.

Regarding the foliar spray treatments, there were obvious significant differences. Urea spray treatment (U2) recorded the highest total chlorophyll content, fresh and dry yield of leaves (1.11 g.mg^{-1}, 1879.3 kg.ha^{-1} and 857.0 kg.ha^{-1} respectively) followed by the spraying nano-amino acids Am2 (1.03mg.g^{-1}, 1809.7kg.ha^{-1}, and 820.1kg.ha^{-1} respectively).

Results of the same table refer to the superiority of the foliar spray treatments at various environmental conditions (lighting intensity). The plants in 50% shadow (E2) recorded the highest chlorophyll content at the two foliar spray treatments, spraying urea (U2) and nano-amino acids Am2 (1.31 and 1.22 g.mg^{-1} respectively). The highest values of the fresh and dry yield of leaves were recorded at the interaction of the plants grown in the open field (E1) with urea spraying U2 (2007.8 and 995.2 kg.ha^{-1} respectively) and with Am2 (1955.1 and 957.7

kg.ha^{-1} respectively), while the lowest values of the fresh and dry leaf weight were at the control in the shadow environment E2 (1559.5 and 522.0 kg ha^{-1} respectively) .

Table (5): Effect of the environment type and spraying with yeast extract, urea, and nano-amino acids on the total chlorophyll content the fresh and dry weight of leaves of *Menthapiperita* L.

Trait	**Total chlorophyll**			**fresh Leaf weight (kg.ha^{-1})**			**dry Leaf weight (kg.ha^{-1})**		
Foliar spray	**Environment type**		**Mean**	**Environment type**		**Mean**	**Environment type**		**Mean**
	E1	E2		E1	E2		E1	E2	
Control	0.41	0.77	0.59	1814.2	1559.5	1686.9	749.3	522.0	635.7
Y1	0.54	0.85	0.69	1835.0	1621.8	1728.4	811.2	602.1	706.7
Y2	0.69	1.05	0.87	1921.2	1636.8	1779.0	910.6	632.3	771.5
U1	0.76	1.14	0.95	1886.5	1674.4	1780.5	863.3	662.2	762.8
U2	0.91	1.31	1.11	2007.8	1750.8	1879.3	995.2	718.7	857.0
Am1	0.61	0.98	0.79	1898.4	1648.7	1773.5	856.0	655.0	755.5
Am2	0.85	1.22	1.03	1955.1	1664.2	1809.7	957.7	682.5	820.1
L.S.D	0.019			47.2			32.1		
Mean	0.68	1.05		1902.6	1650.9		877.6	639.3	
L.S.D	0.007		0.014	36.9		29.2	18.5		22.4

From the aforementioned, we notice that plants in the shade (50%) were superior in plant height, which occurred due to the phytochrome, which participates in the photosynthesis process, as a form of phytochrome red(pr) at a light and when there is a lack of light or shading, it turns into phytochrome over red cytochrome(pfr) affecting the growth of plants so that their stems elongate and reach the more light to perform the rest plant functions in food synthesis **(Taiz & Zeiger, 2010)** accompanied by an increase in the number of branches and the leaf area affected by shadow may be due to the increase in the leaf cell size, which in its turn affects through increasing the leaf tissue area to expand the area leaves intercepting the light to increase photosynthesis and also to compensate for the deficiency in the photosynthesis products**(Deraman *et al*, 2019)** .. Also, the increase in the chlorophyll content in the plants grown in shadow is due to the increase in substances that are directed to synthesize chlorophyll in a low-light environment to use the available light to the maximum extent, so that the plants' absorption for light is improved by increasing the pigment density per leaf area unit as

physiological responses at the level of leaves and chloroplasts **(Wittmann *et al*, 2001).** The situation was reverse in the fresh and dry yield of the leaves, as it increased in the open field plants (100%), which may be due to the higher net metabolism due to the greater light abundance and the appropriate environmental conditions to increase the fresh and dry yield (Table 1).

From the previous results, we notice that all foliar spray treatments were superior in the studied traits tables (3,4,5), except for the leaf area that decreased in the yeast treatment (Y1). The increase in these traits at treated with urea (especially U2) may be attributed to the role of nitrogen in structure chlorophyll using four N atoms with an Mg atom, leading to an increase in chlorophyll content (chlorophyll association with photosynthesis) and reflecting on the traits of vegetative growth as well as the N participating in structure cell membranes and vitamins such as vitamin B, which contributes to increasing the leaf area and the number of lateral branches **(Ghareeb, 2019)**. Among the physiological effects of nitrogen which involvement in Synthesis nucleic acids RNA and DNA, organic compounds such as amino acids, stimulation of enzymatic systems, as well as cell division and elongation**(Al-Amrani *et al*., 2018; Burhan & AL-Hassan 2019)**,which are essential for the development of vegetative traits, including leaf areas associated with photosynthesis, especially at the increase of chlorophyll content, leading to increasing the plant height and number of branches. The N importance comes from its involvement in synthesizing growth hormones, auxins, and cytokinin's, as well as proteins, as they are essential in regulating tissue building and the development of vegetative growth through cell division and elongation which increases growth **(Al-Khafaji, 2014)** represented by plant height, number of branches, leaf area, and fresh and dry weight of the plant. Spraying urea also has a role in increasing the nutrient elements by providing the leaves with nitrogen directly through leaves because urea is characterized by its small molecule size, high solubility, non-polarity, and high nitrogen content **(Taleb &Al-Amiry, 2017**), or indirectly by increasing the vegetative growth resulting in increasing the need for absorbing more amounts of nutrient elements to build the plant tissues and increasing the percentage of N, P, and K .

In amino acid treatments, the increase in growth traits may be due to their role in protein and enzymes synthesis **(Faraj & Shaker, 2011)**.and preserving metabolism products from degradation, Glycine (an amino acid), for instance, inhibits photorespiration in C3 plants (including mint), asparagine and glutamine are essential in linking the two metabolic cycles in plants (carbon and nitrogen), they affect both sugars and proteins **(Taiz & Zeiger, 2010).**

Amino acids also improve the absorption of plant nutrients, stimulate plant growth, regulate biological processes, as well as enhance enzymatic activity, and increase metabolism **(Talaat *et al*. 2014, Al-asadi & Al-jebory, 2020)**, which reflects on increasing the content of elements (N, P, K) and vegetative growth traits, especially the fresh and dry yield of leaves, as well as the indirect effect of participating amino acids in providing nitrogen and constructing

proteins necessary for the construction of plant hormones and their role in plant growth and development **(Mohamed *et al*., 2020).**

Regarding the effect of yeast, especially the second concentration, Y2 (7.5 gm L^{-1}), the reason behind the increase in the study traits may be attributed to the yeast content of hormones, in particular cytokines, vitamins, enzymes and amino acids, as well as the content of minerals (P, K, Mg, Ca, and Fe) and their role with other compounds in synthesizing proteins and in cell division and elongation and chlorophyll formation **(Shehata *et al*., 2012; Bevilaqua *et al* 2008; Abdul-Qader & Rabie,2019)**, which leads to increasing growth and developing the vegetative traits and plant yield of leaves. Because of the yeast content of gibberellin, which, along with cytokinins, plays a prominent role in cell division and elongation, resulting in an increase in plant height; moreover, the yeast contains the enzyme Trehalose-6-phosphate, which manufactures Trehalose, which is necessary for plant growth **(Marzauk *et al* 2014; Ateah *et al*., 2010)**.Concerning the increase in the accumulation of elements affected by yeast, it may be due to the elements that the yeast supplies in addition to growth hormones, which work to attract nutrients and increase photosynthesis, which reflects in improving the growth characteristics **(Al-Badri, 2019).**

CONCLUSION

Determining the lighting intensity is necessary for obtaining the best plant production, especially leafy crops, as it is closely related to the photosynthesis process, furthermore foliar spraying, according to our study, can compensate for soil fertilization by 50% with an encouraging increase in yield, as well as reducing environmental pollution and economic costs.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 114-123
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(10)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

SOIL ORGANIC CARBON AND AGGREGATE STABILITY AS AFFECTED BY SOIL MANAGEMENT PRACTICES

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Received 4/ 6/ 2023, Accepted 2/ 8/ 2023, Published 31/ 12/ 2024

ABSTRACT

A field experiment was in research station Aljadriya at the College of Agricultural Engineering Sciences - University of Baghdad– Iraq (33° 16′ 02″ N. 44° 22′ 33″ E) during spring season - 2022. The purpose of the investigation was to evaluate the effect of Tillage, Crop Rotation and Crop Residues Management Practices on soil organic carbon (SOC) and active soil carbon (ASC), Aggregate stability and saturated hydraulic conductivity. The experiment was laid out on Randomized Complete Block Design (RCBD), with Split –Split Plot Arrangement. Factors were: Crop Residues (0% Residues (0%R) and 100% Residues (100%R)), Tillage (Minimum Tillage (MT) and Conventional Tillage (CT)) and Crop Rotation (Clover – Maize and Clover – Mung Bean) with four replicates. Results of the trial indicated that the best results were with the treatment (100% R + MT + Crop Rotation Clover – Mung Bean) giving (12.78 g SOC Kg^{-1} Soil , 178.92 mg AC Kg^{-1} Soil, 44.72% Aggregate stability, and 2.79 cm h^{-1} for Saturated hydraulic conductivity) compared with results of the Treatment (0% R+ CT + Crop Rotation Clover - Maize) giving (8.08 g SOC Kg^{-1} Soil, 88.88 mg AC Kg^{-1} Soil, Aggregate stability 39.05%, Saturated hydraulic conductivity 2.32 cm h^{-1}). So, it can be concluded that adopting good management practices can improve many soil properties like Organic Carbon and Soil aggregate Stability leading to healthy and productive soil.

Keywords: Soil health indicators, Minimum Tillage, Conventional Tillage, Saturated hydraulic conductivity , SOM.

كاربون التربة العضوي وثباتية التجمعات وتأثرهما بعمليات إدارة التربة

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الخلاصة

نُفذت تجربة حقلية في المحطة البحثية التابعة لكلية علوم الهندسة الزراعية– جامعة بغداد في الجادرية، بغداد– العراق (33° 16′ 02″ N . 44° 22′ 33″ E) خلال الموسم الربيعي 2022 لتقييم تأثير الحراثة والتعاقب المحصولي وبقايا المحصول في كاربون التربة العضوي والإيصالية المائية المشبعة للتربة. نفذت التجربة بتصميم القطاعات الكاملة المعشاة وبترتيب الألواح المنشقة– المنشقة. تضمنت التجربة ثلاثة عوامل هي: بقايا المحصول السابق (0% بقايا و 100% بقايا) والحراثة (حراثة دنيا وحراثة تقليدية) اما العامل الثالث فهو التعاقب المحصولي (برسيم– ذرة صفراء) و (برسيم– ماش) بأربعة مكررات لتقييم تأثير هذه العوامل في كاربون التربة العضوي (الكاربون العضوي والكاربون النشط (الفعال) وبعض الصفات الفيزيائية (ثباتية تجمعات التربة والإيصالية المائية المشبعة). كانت افضل النتائج (12.78 غم كاربون عضوي

[*] **The research is extracted from the doctoral thesis of the first researcher.**

كغم$^{-1}$ تربة و 178.92 ملغم كاربون نشط (فعال) كغم$^{-1}$ تربة) و ثباتية تجمعات التربة 44.72% وإيصالية مائية مشبعة 2.79 سم ساعة$^{-1}$) للمعاملة (100% بقايا المحصول+ حراثة دنيا+ تعاقب محصولي برسيم– ماش) بالقياس الى معاملة (0% بقايا+ حراثة تقليدية+ تعاقب محصولي برسيم– ذرة صفراء) التي حققت (8.08 غم كاربون عضوي كغم$^{-1}$ تربة و 88.88 ملغم كاربون نشط (فعال) كغم$^{-1}$ تربة) و ثباتية تجمعات التربة 39.05% وإيصالية مائية مشبعة 2.32 سم ساعة$^{-1}$). لذا يمكن الاستنتاج ان تبني ممارسات إدارية جيدة يمكن ان تحسِّن، من كاربون التربة العضوي وثباتية تجمعات التربة مما تؤدي الى تكوين تربة صحية ومنتجة.

الكلمات المفتاحية: دلائل صحة التربة، الحراثة الدنيا، الحراثة التقليدية، الإيصالية المائية المشبعة، مادة التربة العضوية.

INTRODUCTION

Soil Organic Carbon (SOC) can be considered one of the most soil criteria that affect other soil properties, crop production, soil health, and the environment. Soil organic carbon is the part that soil Microorganisms depend it in their growth. At the same time, Soil microorganisms can play an important role in organic carbon formation, preservation, and loss **(Tao *et al.*, 2023)**. Soil Organic carbon is very important in determining soil fertility, increasing biodiversity and productivity, and soil health. A lot of SOM can be lost due to soil and environmental effects, especially in arid and semi-arid regions. Soil organic carbon can play a very important role in carbon dioxide (CO_2) sequestration and decreasing Global warming **(Magdoff & Es, 2021; Ali *et al.*, 2022; Al-Halfi & Al-Azzawi, 2022a,b)** So soil health institute considered soil organic carbon as the main indicator for soil health **(Soil Health Institute, 2023)**. Identifying agricultural management practices that minimize loss or even enhance SOC stores, is very important for sustaining soils and food production and security systems, and improving the environment by minimizing global warming **(Amelung *et al.*, 2020)**. Soil aggregates, and their stability (i.e. soil structure) is other important criteria for good and healthy soil. Soil with good soil structure can hold more water, good aeration, drain extra water, and have good crop production **(Ali, 2015; Magdoff & Es, 2021; Ali, *et al.*, 2022; Masood & Ali, 2022; Jassim & Hamied, 2022).**

Integrating good soil's physical, chemical, and biological properties can improve soil health and productivity **(Van Eerd *et al.*, 2021)**. Several studies reported greater formation SOC under minimum or no tillage compared to conventional tillage **(Magdoff & Es, 2021; Nyambo *et al.*, 2021; Ali *et al.*, 2022).** Many studies indicated that good Soil Management Practices such as using crop rotation, minimum tillage, and crop residues improved physical, chemical, biological, and fertility of soil and enhanced soil health and quality, **(Nath *et al.* 2021; Riahinia & Emami, 2021; Saurabh *et al*, 2021; Al-Halfi, & Alazzawi, 2022a,b; Mohammed & Hasan, 2022a,b; Masood & Ali, 2023; Morrisville, 2023; Jasim & Hamid, 2023)**, besides, enriching the soil, they enhance life on our planet **(Morrisville, 2023)**. Zero tillage was better than minimum and conventional tillage in maize grain yield and other yield components, **(Al-Rubaie & Al-Ubaidi, 2018)**. **Nafawaah, & Mageed, (2019)** mentioned that different harrowing systems affected soil organic matter decomposition as well as some soil and plant properties. **Othman *et al.*, (2020)** indicated that conservation agriculture in the presence of crop rotation. Using the optimum crop combination (crop rotation) together with conservation agriculture can maximize the profits and the agricultural income of farms **(Alnassr, 2019)**.

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Research Station of the College of Agricultural Engineering Sciences - University of Baghdad in Aljadriya, Baghdad – Iraq (33° 16′ 02″ N. 44° 22′ 33″ E) during two seasons Fall and Spring of 2021-2022. Trial were conducted in a randomized complete block design with four replicates, to investigate the effect of Tillage, Crop Residues, and crop rotation Management Practices on organic carbon and aggregate stability. In the 1st experiment, two factors were used: the first was the residues of the previous crop (Alfalfa) (*Medicago sativa* L.), 100% residues (100% R) and 0% residues (0%R), the 2nd factor was tillage (minimum tillage MT and conventional tillage CT) in split-plot arrangement planted with Clover (*Trifolium repens* L.). The 2nd experiment followed the 1st one in the spring season using Tillage, Crop Residues, and crop rotation Clover - Maize (*Zea mays* L.) and Clover - Mung bean (*Vigna radiate* L.) on Maize and Mung bean Productivities. Both crops were sawn at the same plots of the previous Clover crop, in a split – split plot arrangement. Soil samples were collected before and after each trial to estimate some chemical, physical and fertility properties of soil. At the end, of every experiment samples of soils were collected for measuring SOC, SAC, Aggregate stability, and Soil hydraulic conductivity, The estimation results for the studied field soil pre-planting are shown in table (1).

Table (1): Chemical, Physical, Biological and Fertility properties of the Soil before planting*

Characteristics		Value	Unit
Potential Hydrogen (pH) (1:1)		8.25	-
Electrical Conductivity (EC) (1:1)		1.85	dS m^{-1}
Available Nitrogen		28.00	mg kg^{-1} Soil
Available Phosphorus		13.25	
Available Potassium		174.01	
Available Iron		5.65	
Available Zinc		3.75	
Carbonate minerals		345.0	g kg^{-1}Soil
Soil Organic matter		16.13	
Soil Organic Carbon		9.35	
Active Carbon		128.44	mg kg^{-1}Soil
Cations	Ca^{2+}	8.95	m mol L^{-1}
	Mg^{2+}	4.55	
	K^{1+}	2.35	
	Na^{1+}	3.47	
Anions	SO_4^{2-}	5.1	
	Cl^{1-}	21.5	
	HCO_3^{1-}	2.95	
	CO_3^{2-}	Nill	
CEC		19.45	C mol_+ kg^{-1} soil
Soil Aggregate Stability		26.45	%
Saturated Hydraulic Conductivity		1.96	cm h^{-1}
SPD		g kg^{-1}	Soil texture Class
Sand		353.0	Silty Loam
Silt		519.0	

Clay	128.0	
water content		
at 33 kPa	23.4	%
at 1500 kPa	12.0	
Available Water	11.4	
Biological Properties		
Total Bacteria Count	$4.5 * 10^9$	CFU g^{-1} dry Soil
Total Fungi Count	$3 * 10^3$	
Alkaline Phosphatase Enzyme Activity	108.49	Microgram p-nitro phenol g^{-1}dry soil h^{-1}
*Measurements done according to method mentioned (Black *et al.*, 1965 ; Aoda & Mahdi , 2017; Salim & Ali, 2017)		

It should be noted that Although there are two experiments but the 2nd experiment represented the collective effect of both trials, so the results of the 2nd trial will be presented and discussed in this paper.

RESULTS AND DISSCUSION

1- SOIL ORGANIC CARBON (SOC) (SOC g kg^{-1} Soil)

Table 2 showed the effect of crop residues, tillage and crop rotation on soil organic carbon, results emphasized the best value of SOC was 12.78 g SOC kg^{-1} soil for treatment (100% R + MT + Crop Rotation Clover – Mung bean) with increasing of 58.17% compared with 8.08 g SOC kg^{-1} soil for treatment (0% R + CT + Crop Rotation Clover – Maize). Results indicated the best values were with 100% R, minimum tillage and crop rotation clover – mung bean individually and double interaction with (100% R + MT) , (100% R + crop rotation clover – mung bean) and (MT + crop rotation clover – mung bean),with significant differences .

Table (2): Effect of crop residues, two tillage systems and crop rotation on Soil Organic Carbon (SOC g kg^{-1} Soil).

Crop Residues	**Tillage System**	**Crop Rotation**		**Crop Residues and Tillage**
		Clover - Maize	**Clover – Mung bean**	
0% Residues	Conventional T.	8.08	9.20	8.64
	Minimum T.	9.29	10.43	9.86
100% Residues	Conventional T.	10.95	11.50	11.22
	Minimum T.	11.74	12.78	12.26
LSD 0.05		0.382		0.395
Crop Residues		Crop Rotation		Residues Mean
		Clover - Maize	Clover – Mung bean	
0% Residues		8.68	9.81	9.25
100% Residues		11.34	12.14	11.74
LSD 0.05		0.397		0.414
Tillage Systems		Clover - Maize	Clover – Mung bean	Tillage Systems Mean
Conventional T.		9.52	10.35	9.93
Minimum T.		10.51	11.60	11.06
LSD 0.05		0.122		0.103
Crop Rotation Mean		10.01	10.98	
LSD 0.05		0.084		

2- ACTIVE SOIL CARBON (ASC) (ASC mg kg^{-1} Soil)

Table 3 showed the significant effect of using crop residues, tillage and crop rotation in ASC. The best value of ASC was 178.92 mg ASC kg^{-1} Soil with triple interaction treatment (100% R + MT + Crop Rotation Clover – Mung bean) with increment of 101.31% compared with 88.88 mg ASC kg^{-1} Soil for treatment (0% R + CT + Crop rotation Clover – Maize). The double interactions and the individual treatment all were significant.

Table (3): Effect of crop residues, two tillage systems and crop rotation on Active Soil Carbon (ASC mg kg^{-1} Soil).

Crop Residues	**Tillage System**	**Crop Rotation**		**Crop Residues and Tillage**
		Clover - Maize	**Clover – Mung bean**	
0% Residues	Conventional T.	88.88	105.80	97.34
	Minimum T.	111.45	130.32	120.88
100% Residues	Conventional T.	145.09	155.19	150.14
	Minimum T.	161.39	178.92	170.16
LSD 0.05		4.926		5.080
Crop Residues		Crop Rotation		Residues Mean
		Clover - Maize	Clover – Mung bean	
0% Residues		100.17	118.06	109.11
100% Residues		153.24	167.06	160.15
LSD 0.05		5.120		5.344
Tillage Systems		Clover - Maize	Clover – Mung bean	Tillage Systems Mean
Conventional T.		116.99	130.50	123.74
Minimum T.		136.42	154.62	145.52
LSD 0.05		1.621		1.398
Crop Rotation Mean		126.70	142.56	
LSD 0.05		1.085		

3- SOIL AGGREGATE STABILITY (%)

Table (4): Effect of crop residues, two tillage systems and crop rotation on Soil Aggregate Stability (%).

Crop Residues	**Tillage System**	**Crop Rotation**		**Crop Residues and Tillage**
		Clover - Maize	**Clover – Mung bean**	
0% Residues	Conventional T.	39.05	39.82	39.43
	Minimum T.	40.41	40.62	40.52
100% Residues	Conventional T.	42.08	42.54	42.31
	Minimum T.	43.81	44.72	44.26
LSD 0.05		0.974		0.916
Crop Residues		Crop Rotation		Residues Mean
		Clover - Maize	Clover – Mung bean	
0% Residues		39.73	40.22	39.97
100% Residues		42.95	43.63	43.29
LSD 0.05		0.909		0.985
Tillage Systems		Clover - Maize	Clover – Mung bean	Tillage Systems Mean
Conventional T.		40.56	41.18	40.87
Minimum T.		42.11	42.67	42.39
LSD 0.05		0.540		0.442
Crop Rotation Mean		41.34	41.93	
LSD 0.05		0.391		

Table 4 showed the significant effect of using crop residues, tillage systems and crop rotation as a soil management practices in soil aggregate stability. The best value was 44.72% for treatment (100% R + MT + Crop Rotation Clover – Mung bean) with increment of 14.52% compared with 39.05% for treatment (0% R + CT + Crop rotation Clover – Maize). The double and individual interactions treatments all were significant.

4- SOIL SATURATED HYDRAULIC CONDUCTIVITY (cm h^{-1})

Table 5 showed the significant effect of using crop residues, tillage systems and crop rotation as a soil management practices in soil saturated hydraulic conductivity. The best value was 2.79 (cm h^{-1}) for triple interaction treatment (100% R + MT + Crop Rotation Clover – Mung bean) with increasing of 20.26% comparing with 2.32 (cm h^{-1}) for treatment (0% R + CT + Crop rotation Clover – Maize). The double and individual interactions treatments all were significant.

Table (5): Effect of crop residues, two systems tillage and crop rotation on Soil Saturated Hydraulic Conductivity (cm h^{-1}).

Crop Residues	Tillage System	Crop Rotation		Crop Residues and Tillage
		Clover - Maize	Clover – Mung bean	
0% Residues	Conventional T.	2.32	2.37	2.34
	Minimum T.	2.34	2.38	2.36
100% Residues	Conventional T.	2.51	2.67	2.59
	Minimum T.	2.72	2.79	2.75
LSD 0.05		0.098		0.076
Crop Residues		Crop Rotation		Residues Mean
		Clover - Maize	Clover – Mung bean	
0% Residues		2.33	2.37	2.35
100% Residues		2.61	2.73	2.67
LSD 0.05		0.076		0.076
Tillage Systems		Clover - Maize	Clover – Mung bean	Tillage Systems Mean
Conventional T.		2.41	2.52	2.47
Minimum T.		2.53	2.58	2.56
LSD 0.05		0.068		0.054
Crop Rotation Mean		2.47	2.55	
LSD 0.05		0.051		

Tables (2, 3, 4, 5) showed the Effect of Tillage, Crop Rotation and Crop Residues Management Practices on some soil properties as an indicator of Soil Health and Quality. These tables presented the differences in Organic Carbon Sources and Some Physical Properties as affected by such treatments. The increments in soil carbon, soil aggregate stability, soil hydraulic conductivity were very clear as well **(Abbasi *et al*., 2009; Alam *et al*., 2014; Ali & Albayati, 2018)**. These results reflect the role of residues of the last crop (clover) in increasing soil organic carbon parameters (Table 2). Soil organic carbon can be considered one of the best indicators for soil health **(Magdoff, & Es 2021; Morrisville, 2023; Higashi *et al.., 2014*)** due to the carbon role in biodiversity especially soil microorganisms. These Microorganisms have a very important role in nutrient cycling **(Ali, *et al*., 2022)** Besides, soil

organic matter has a very important role in nutrient availability. Crop residues at the same time enhance physical soil properties like soil aggregate stability (table 4) and saturated hydraulic conductivity (Table 5) through the activity of bacteria which secrete or provide soil polysaccharides that keep particles of soil together and that lead to creating new soil aggregates and keep another aggregate and resistance of soil degradation, so that leads to enhance physical soil properties like aggregate stability and saturated hydraulic conductivity and soil structure. Fungi have a very important function represented by gathering particles of soil by hyphae so it helps to form new aggregates of soil and increasing of aggregates stability.

Minimum tillage leads to the same result due to reducing oxidation of organic matter and conserves organic carbon from being lost especially in an arid-semi arid climate of Iraq. At the same time, minimum tillage can reduce broken colonies of bacteria and fungi and keep them healthy .

Crop rotation especially one containing legumes in sequences is very important in providing nutrients, especially nitrogen **(Magdoff & Es, 2021**). The results of this experiment confirmed the results of **Jeghata & Muhawish (2021**) and the results of **Mohammed & Hasan (2022 a, b**), and results of **Jasim & Hamid (2023)**; **Masood & Ali (2023**).

It can be concluded that adopting best management practices can improve soil properties and soil health. Healthy soil will produce better yield with good quality as have been seen from the results of **(Alhalfi & Alazzawi, 2022a; Al-Furaiji & Ali, 2023; ITPS, 2015; Mooleki *et al*., 2016; Sommer *et al*., 2014)**.

CONCLUSION

Using integrated management practices: crop rotation, residues of previous crop and minimum tillage can have a very clear impact on Soil Organic Carbon and Soil Aggregate Stability and can lead to soil health, so using such practices can be recommend.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 124-135
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(11)
Ahmed & *Hasan*

المجلة العراقية لبحوث السوق
وحماية المستهلك

IMPACT OF AIR POLLUTION ON SERUM AND SALIVA NITRIC OXIDE, PEROXY NITRITE AND ISCHEMIA MODIFIED ALBUMIN IN THE WORKERS EXPOSED TO HEAVY FUEL OIL COMBUSTION'S FUMES

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Received 5/ 6/ 2023, Accepted 13/ 8/ 2023, Published 31/ 12/ 2024

ABSTRACT

The object of this study was to measure the effect of air pollution on the health of the workers exposed to the heavy fuel oil (HFO) combustion fumes. This was achieved by testing the alteration in the levels of saliva and serum nitric oxide (NO), peroxynitrite ($ONOO^-$) and ischemia modified albumin (IMA). The participants enrolled in this study were male workers (N= 59) at heavy fuel oil combustion unit in Dora station of electricity in Iraq. Healthy individuals with matched age and body mass index (N=53) were also enrolled in the study to be used as a control group. The results detected a significant increase in the level of serum nitric oxide (NO) and peroxy nitrite ($ONOO^-$) ($p<0.005$), meanwhile a non- significant alteration of these levels was found in the saliva ($p>0.005$) of the workers group when compared with that of control group. The measured parameters also included measurement of serum and salivary level of ischemic modified albumin (IMA), which showed a highly significant increases ($p<0.001$) in both types of the tested fluids. The influence of different factors such as the age of the workers, mode of working, smoking habit and using the safety equipment on the levels of the measured biochemical parameters were also checked in this study.

Keywords: Nitric Oxide, Peroxynitrite, Ischemia-modified albumin, Air pollution, Heavy fuel oil combustion.

تأثير تلوث الهواء على اوكسيد النتروجين والبروكسي نتريت والالبومين المعدل بنقص التروية في مصل الدم ولعاب العاملين المعرضين لأبخرة احتراق زيت الوقود الثقيل

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الخلاصة

كان الهدف من هذه الدراسة قياس تأثير تلوث الهواء على صحة العاملين المعرضين للبخرة المنبعثة من احتراق زيت الوقود الثقيل. تم ذلك عن طريق فحص التغيرات في مستويات كل من اوكسيد النتروجين والبروكسي نتريت والالبومين المعدل بنقص التروية في مصل الدم و لعاب المشاركين في الدراسة من الذكور العاملين (N= 59) في وحدة احتراق زيت الوقود الثقيل في محطة الدورة الكهربائية. تم تضمين الأفراد الأصحاء (N=53) المتطابقين في العمر ومؤشر كتلة الجسم مع العاملين في الدراسة كمجموعة ضابطة. كشفت النتائج عن زيادة معنوية (P>0.005) في مستوى(NO) و $ONOO^-$) في مصل الدم واللعاب. تم ايضا ضمن القياسات التي اجريت قياس مستوى الالبومين المعدل وكشفت النتائج عن وجود زيادة معنوية عالية (P>0.001) في مصل ولعاب العاملين مقارنة مع المجموعة

الضابطة. كذألك تم في هذه الدراسة ايضا فحص تأثير العوامل المختلفة مثل عمر العاملين وطريقة العمل وعادة التدخين واستخدام معدات السلامة على المعايير المقاسة.

الكلمات المفتاحية: أكسيد النيتريك، البيروكسي نتريت، الألبومين المعدل بنقص التروية، تلوث الهواء.

INTRODUCTION

Based on the most recent data from the Environmental Statistics Report, air pollution is viewed as a fundamental environmental health challenge across the world **(Planning, 2020).** Airborne particulates constitute a heterogeneous complex mixture that differ according to their source that varied with time, and the condition of the atmosphere **(Brook *et al.*, 2010)**. A number of factors affect the toxicity of particles, including their size, shape, structure, surface reactivity, solubility and ‘‘leachable’’ components **(Elder *et al.*, 2010)**. The most common used method for characterizing the particulate matter (PM) is dependent on their size. Particulate matter <10 µ*M* (PM_{10}), particulate matter <2.5 µ*M* ($PM_{2.5}$), and those of <0.1 µ*M* ($PM_{0.1,}$ which also called ultrafine particles, UFPs) are the most important sizes-fractions. In Iraq the annual mean concentration of $PM_{2.5}$ is 62 g/m^3, which exceeds the World Health Organization (WHO) Air Quality Guidelines for health–harmful pollution levels of 10 g/m^3. In 2016, about 4.2 million premature deaths worldwide was reported to be resulted from ambient air pollution in both rural areas and cities; this mortality has been reported to be attributed to $PM_{2.5}$ exposure **(WHO Europe, 2016)**. According to the WHO Global Health Estimates database.

Heavy Fuel oil is the oil that make up all the distillation residue, including those obtained by blending of fuel oils. Its kinematic viscosity, at 80 ℃ is above 10 CSt, with a flash point always above 50 ℃ and a density > 0.90 kilogram/liter **(Levy *et al.*, 1971)**. Upon burning of HFO at power plants, it creates emissions of carbon dioxide (CO_2), nitrogen oxides (NOx), sulfur dioxide (SO_2), particulate matter (PM), mercury (Hg), and other pollutants. NO_X and SO_2 emissions involve in the formation of fine PM and ground level ozone. These pollutants emit when the combustion is incomplete. The term complete combustion is applied if all carbon in the fuel burns to carbon dioxide, all hydrogen burns to water and all sulfur (if any) burns to sulfur dioxide. Conversely, combustion is incomplete if the products of combustion contain components such as H, CO, C& OH, or any unburned fuel and the main reason for such incomplete combustion is the presence of insufficient oxygen **(Cato *et al.*, 1997).** Exposure to air pollution were found by many researchers such as (**Cohen *et al*., 2017**) to have adverse health effects and air pollution has been documented to be linked to many types of the illnesses, including cardiovascular disease, respiratory disorders, impaired lung function, renal disease, preterm birth, dementia, autism, male infertility and overall mortality. **(Schraufnagel *et al.*, 2019: Hassani *et al*., 2016).**

Nitric oxide (NO) is an important molecule, in mammals it involves in many physiological and pathological processes. It can be protective, or hazardous for tissues or organs in where it exists (**Zetterquist *et al.*, 1999)**. In human body, it may be produced from both dietary substances and metabolic pathways, followed by its transport via the blood to the salivary glands **(HFO, 2016)**. Furthermore, its harmful effect in the human body is due to its production of peroxynitrite (the ugly free radical) upon its reaction with superoxide **(Zelnickova *et al.*, 2008).** The generation of peroxynitrite, in a moderate flux, over long periods of time lead to substantial oxidation and potential destruction of the different host

cellular constituents, that results in dysfunction of critical cellular processes, cell signaling pathways' disruption, and critical cellular processes' dysfunction, then, through both apoptosis and necrosis, induction of cell death **(Radi *et al.*, 2013)**. Several studies dealing with different types of air pollution measured the levels of blood (NO and $ONOO^-$) in their studied participants (**Sandrah *et al.*, 2015).**

Ischemia-modified albumin (IMA) is a protein formed due to damage of blood albumin final amino acid terminal as a result of presence of free radicals, which damage albumin and reduces its capacity to bind to heavy transition metals (nickel, cobalt) IMA forms about 1%–2% of total serum albumin levels **(Kaefer *et al.*, 2010).** Though IMA is an ischemia-sensitive marker, recent studies have shown that it increases in situations such as late stage renal failure and obesity and diseases such as diabetes and chronic liver disease, hypercholesterolemia, cancer and multiple myeloma **(Ellidag *et al.*, 2013).**

Generally, nowadays, saliva has been reported to be suitable for diagnosis of many local and systemic diseases. It generating considerable interest in term of its economic, easy noninvasive sampling method that needs no experience, or special equipments to be collected compared with that required for the blood collection **(Pink *et al.*, 2009)**. It is worth to mention, no literatures have been found that dealing with the study the variation of the present study measured biochemical parameters in saliva.

In a previous study carried out in our laboratory, oxidative stress was reported to be present among the workers at the HFO combustion unit **(Ahmed & Hasan, 2022)**. The main objective of the current study was to check the impact of the fumes emitted from HFO combustion, at molecular level, on the health of the workers in the electricity Dora station/ Baghdad/ Iraq , by measuring the variations in the level of serum and saliva of some related oxidative stress parameters including the good free radical: nitric oxide (NO), the ugly free radical: peroxy nitrite $(ONOO)^-$ and ischemia modified albumin (IMA), of the workers at the combustion unit of heavy fuel oil in this station.

PARTICIPANTS AND METHODS

Study participants, Ethical approval, **Exclusion criteria**, **Serum and saliva sampling*:***
All details concentering the participants of the present work were the same as mentioned in our previous publication (**Ahmed & Hasan, 2023**).

Participants and methods

Study participants:

This study participants were males (N= 59) who are working at the HFO combustion unit in the electricity Dora station/ Baghdad/ Iraq ,as well as age, gender and body mass index (BMI) matched apparently control healthy individuals (N=53). Biological samples were collected during the period of December 2021 to February 2202.

Ethical approval

The ethics Committee of the College of Science/ University of Baghdad had approved this study protocol.

Exclusion criteria

All workers and healthy individuals who were alcohol drinker, and those presented acute or other chronic diseases such as high pressure, diabetic, cardiac disease, cancer or any immune dysfunction were excluded from the study.

Serum and saliva sampling: -

The initial number of the workers from whom the saliva and serum specimens were collected was 83. Twenty-four of them were excluded. Therefore, the final participants of the present study were 59. In order to perform the required measurements of this study, a volume of 5ml of venous blood was collected from each healthy individual and worker and left for ten-twenty minute at the room temperature, then was centrifuged at 2000xg for 10 minutes, and any hemolyzed sample was discarded. The obtained sera were transferred immediately to test tubes and frozen at – 20 °C for subsequent analysis. Meantime the workers and healthy individuals were asked to rinse their mouths with saline before a volume of about 2 ml_of unstimulated whole saliva was collected. The collection time was always between 8.0-10.0 a.m. and the collection period was approximately twenty minutes. The collected saliva was centrifuged at 2000xg for 10 minutes, this was done within one hour after collection to eliminate debris and cellular matter. The resulting supernatant was stored at – 20 °C until used for different parameters assays.

Measurement of the **concentration of nitric oxide**

Method (**Jose *et al.*, 1998**) was used to measure the nitric oxide concentration. This method is based on that cadmium reduces nitrate to nitrite and then the produced nitrite level was determined using Griess reaction. The azo dye formation was detected via its absorbance at the wavelength =540 nm. In order to construct the standard curve, the following different concentrations (0, 50, 100, 150, 200, and 250μM) of sodium nitrite (NaNO2) were prepared from the stock $NaNO_2$ solution and were used instead of the tested samples. The nitric oxide concentration in the serum and saliva samples was calculated using the equation derived from this constructed standard curve.

Measurement of the concentration of peroxynitrite

The [Peroxynitrite] was determined by using to **(Vanuffelen *et al.*, 1998)** procedure. In which the nitration of phenol that is mediated by peroxynitrite results in the formation of nitrophenol, which its absorbance (A) can be measured at λ=412 nm.

Calculation

Peroxynitrite concentration (mM) = $\frac{\textbf{A test – A blank}}{\boldsymbol{Molar\ extinction\ coefficient(\varepsilon)}} \times \mathbf{10^3}$

Where:

A $_{\textbf{test}}$ was referred to the absorbance of the test, **A** $_{\textbf{blank}}$ was referred to the absorbance of blank solution

ε was referred to the nitrophenol molar extinction coefficient which is equal to 4400 M^{-1} cm^{1}**.**

Measurement of the concentration of ischemia modified albumin

Ishemic modified albumin [IMA] was measured using an indirect measurement method (Gurumurthy et al 2014). By using this method, the unbound cobalt to albumin is measured**.**

RESULTS AND DISUSSION

The results of this work was expressed in the form of mean value (M) ± the standard deviation (S.D.). The data were compared using SPSS version 26 (Independent samples T-Test), where the difference ($P < 0.001$) was considered as highly significant, ($P < 0.05$) significant and ($P > 0.05$) as a nonsignificant.

The general detailed characteristics of the used participants in the current study were illustrated in Figure (1)

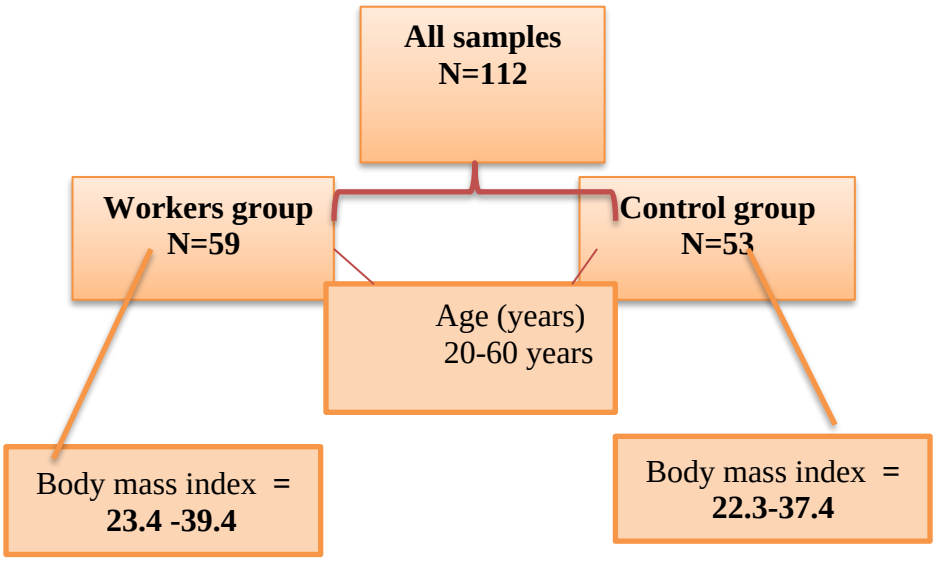


Figure (1): The participant characteristics of the present study.

The above Figure showed that the number of the workers occupationally exposed to the fumes of the fuel's combustion that were enrolled in the present study was 59 and the number of the control group was 53. All of them were males with age ranged between 20-60 years. Their weights and heights were measured, and their body mass index (BMI) was calculated. The workers' group were further divided into sub-groups, according to their ages, the period of the service at the HFO combustion unit and if they were smokers or using safety protection equipment's.

The level of nitric oxide (NO) and peroxy nitrite ($ONOO^-$) were measured in serum and saliva of the exposed workers to the fumes emitted from HFO combustion and control groups and the results were presented in Figure (3).

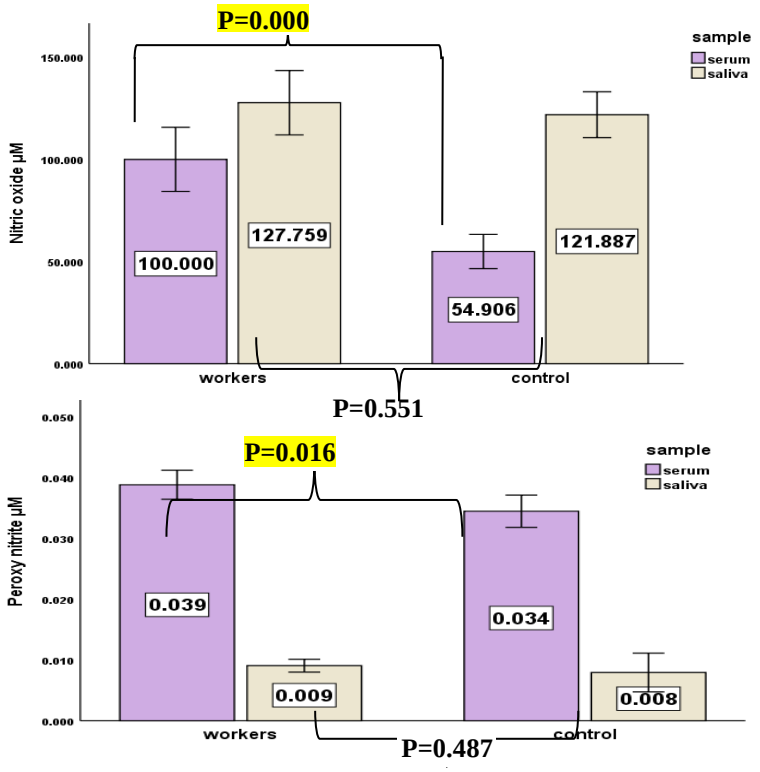


Figure (2): Comparison between levels of nitric oxide, peroxy nitrite in serum and saliva of both the workers and control groups.

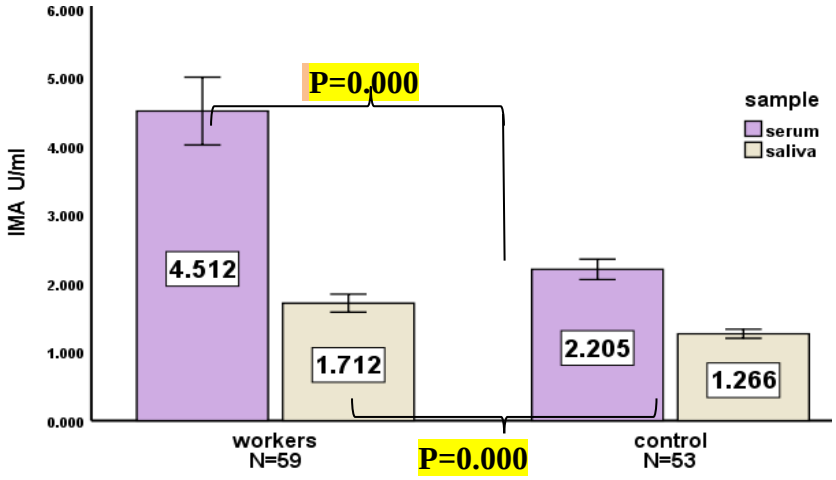
It is clear from the Figure (2) that a highly significant increase in serum level of nitric oxide and peroxynitrite ($p<0.005$) was detected in serum of the workers when compared with that of the control, while a non-significant increase in these levels ($p>0.05$) was measured in the saliva samples of the workers in comparison with that of the control group.

Nitric oxide (NO) is an intercellular messenger, with a short life, that was shown to play important roles in a wide range of biological functions such as many related processes to pathophysiology of pulmonary and cardiovascular systems such as inflammation; host defense, neurotransmission and immune function, (**Bogdan, 2001).** In the lung, neutrophils, macrophages, endothelial cells, epithelial cells, and non-adrenergic noncholinergic neurons are capable of nitric oxide production **(Barnes &Liew, 1995)**. Various isoforms of nitric oxide synthase (NOS) control the endogenous production of this molecule. These isoforms broadly are classified as constitutive and inducible NOS (iNOS). A hypoxia is reported to be a synergistic inducer of iNOS expression that favors the reaction between NO and oxygen, resulting in nitrosative modifications. Therefore, the measured elevation in [nitric oxide] in the workers group (Figure 2) may be a result of cytokines release by inflammatory mediators due to presence of pollution, or may be due to the presence of hypoxia, a condition that was detected to exist in the enrolled workers of the current study (the result not shown), such condition was reported to result in over expression of iNOS synthesis **(Holgate, 2012).** In the current study a significant increase in serum with a non-significant alteration in saliva, was measured in the [peroxynitrite] of the workers compared to the control group (Figure 2) **(Pryor**

& Squadrito, 1995). reported that peroxy nitrite produce upon the reaction of superoxide and nitric oxide, each can modulate the effects of the other and the ratio of their concentrations, is important in systems in which both of them are produced. Superoxide dismutase (SOD) is the usual scavenger of superoxide. This enzyme is a metalo proteins presents in all aerobic metabolizing cells. The reaction of nitric oxide with superoxide anion competes the reaction of scavenging of this ion by SOD, thus the presence of SOD in various compartments of human body enables it to get rid of superoxide radicals immediately as soon as it forms, this protects all the different biomolecules in the cells from its oxidative damage **(Hasan & Rashid, 2004)**. Many studies observed a decrease in this enzyme activity upon exposure to air pollution such as the study by (**Hasan *et al.*, 2001)** who measured a decline in SOD activity and thus suggested that the depression in SOD activity may result in cellular injury by superoxide radical.

This study included the measurement of Ischemia modified albumin (IMA) level. IMA level was reported, would be influenced by the albumin concentration in the used samples (Lee *et al*, 2007), therefore recently another term: (IMA index) albumin-adjusted ischemia-modified albumin index has been introduced as a new marker that compensates the effect of serum albumin and reported to be more accurate and sensitive marker than the conventional IMA value, therefore IMA index was calculated according to the following formula:

Ischemia Index IMA= IMA χ [induvial.[alb.]/median [alb.], furthermore the ratio IMA/Albumin was calculated in the saliva and serum samples of the exposed workers to the fumes emitted from HFO combustion as well as of the control group and the results were presented in Figure (3).



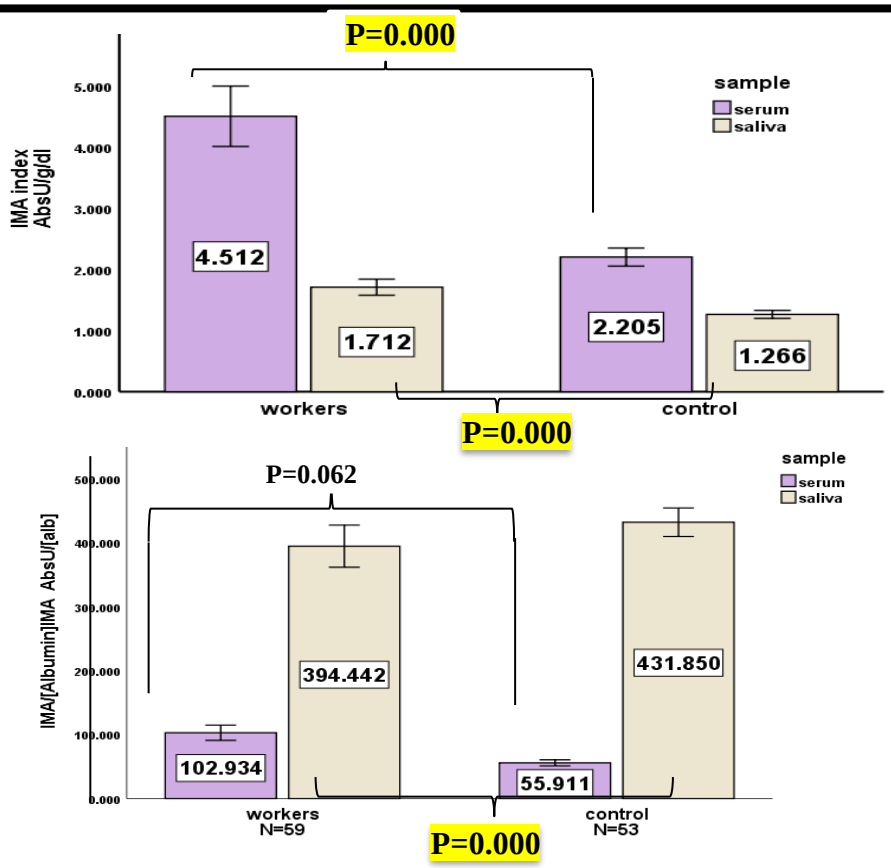


Figure (3): Comparison between levels of Ischemia modified albumin, Ischemia modified albumin index and ratio (IMA/Albumin) in serum and saliva of both the workers and control groups.

It is clear that a high significant increase ($p<0.001$) in IMA, IMA index and the ratio (IMA/Albumin) IMAR was detected in the saliva and serum of the workers group compared with the control group. IMA is a modified form of serum albumin in which its nitrogen-terminal amino acids are modified and become unable to bind to transition metals. IMA is produced as a result of the presence of free radicals where the presence of oxidative stress and acidosis during ischemia are the major determinants of its formation **(Sahin *et al.*, 2018).** The measured increase in [IMA] in the workers group thought to be either due to an increase in its production, or a decrease in its clearance (**Gafsou *et al.*, 2010**). Under normal conditions, the [IMA] was reported to correlate negatively with the [albumin]. The presence of inflammation, hypoxia, the severity and duration of OS with its related oxidative damage, in addition to tissue hypo- perfusion and the accompanying disorders affect the serum level of IMA, all of these has been reported by Sahin *et al* to be involved in the different types of mechanisms that cause an increased [IMA] in serum (**Sahin *et al.*, 2018)**. In a previous study carried on in our laboratory, [albumin] was found to elevate significantly in the same workers group (**Ahmed & Hasan 2023**). This result with the current ones lead us to the suggest that the high OS status and the

hypoxia measured in the workers group are among the causes of the increased [IMA] and its related parameters in the worker included in the present study.

Most individuals who are exposed to harsh working environments are unaware of the impact of long exposure to such environment on their health, therefor in order to study the possible influence of the different time of exposure to pollution on the level nitric oxide and peroxynitrite, The variations in these parameters were checked in saliva and serum of those workers who were working in an alternative mode (seven hours/ day for two successive days, followed by one day off) and those who worked for seven hours/ day for five days weekly. The results were presented in Table: 1. When the influence of the different factors (age, period of working, mode of working, smoking and using safety equipment) were checked on the above observed variations on serum and saliva levels of NO, $ONOO^-$, IMA and its related parameters. The results showed that the only factor that was found to affect these variations was the mode of work (as it was clear from Table 1).These results showed presence of a significant elevation ($p<0.005$) in the level of both (NO) and ($ONOO^-$) in serum of those workers who worked in an alternative mode than those who worked for 7 hours/day for successive 5 days. But such influence was not present, in saliva samples where a non-significant difference was observed between both subgroups. It is worth to mention, that all the above-mentioned studied factors showed no influence on the above variations (the results were not shown).

Table (1): The effect of the working mode on serum and saliva nitric oxide and peroxy nitrite of the workers.

parameters	Mean±SD			
	Serum		Saliva	
	Alternative mode of work (N=24)	Working 7 hours/day for successive 5 days followed by 2 days off. (N=35)	Alternative mode of work (N=24)	Working 7 hours/day for 5 successive days followed by 2 days off. (N=30)
Nitric oxide µM	80.238±31.681	109.428±68.048	130.714±77.302	123.142±48.447
	0.034		0.653	
Peroxy nitrite µM	0.042±0.008	0.036±0.009	0.009±0.004	0.008±0.003
	0.017		0.239	

The non-significant variations that observed in the levels of nitric oxide and peroxy nitrite with the observed alteration in [IMA] may be explained as follows: even though, in the body the saliva is considered as first defense line against active oxygen species (**Zukowski *et al.*, 2018**), it seems that the alteration in saliva nitric oxide and peroxynitrite are independent of that of the circulation system, and a different nature of disturbances occur in saliva than that of serum during the exposure to the air pollution.

Inhalation generally was reported to be the main route of exposure to the harmful contaminates present in pollutant air. Previously many studies reported an association between air pollution and chronic and acute respiratory & systematic inflammatory responses **(Odewabi *et al.*, 2012)**. How could the measured changes in the present study parameters contribute to

the adverse respiratory outcomes associated with HFO fume emissions in the workers group? This may be explained as follows: Lung epithelial dysfunction is considered central to development of many respiratory diseases such as asthma; with insults such as air pollutants serving not simply as triggers for disease exacerbation but also as playing critical roles in the origin and progression of airway and lung pathology **(Fitzpatrick *et al.*, 2012)**. A growing body of literature further implicates impaired antioxidant defenses and disturbances in oxidation/ reduction (redox) balance as risk factors for development of this type of diseases Accumulating *in vitro* and *in vivo* experimental studies have shown that PM exposure is associated with increased lung oxidant burden related to increased ROS such as $O_2^{\bullet-}$ **(Sugimoto *et al.*, 2005).** Other studies of Diesel exhaust particles (DEP):-exposed rodents revealed concomitant increases in NO and ONOO- in BAL fluid cells **(Zhao *et al.*, 2006).**

CONCLUSIONS

The presence of oxidative stress index, which was previously measured in these workers, and the presence of a high concentration of albumin, resulting in an elevation of ischemia modified albumin (IMA) in all its forms, as well as increase in peroxy nitrite($ONOO^-$) as a result of the increase in nitric oxide(NO) and the decrease in superoxide dismutase (SOD) activity, which were recorded in several studies in people exposed to air pollution, this confirm the presence of OSI that was reported to be statistically significant in the working individuals, while it was not significant in their saliva except for IMA and related parameter.

Further studies which included larger samples size are required to further confirm the present study obtained results, especially which obtained with the saliva parameters.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 136-148
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(12)
Ali & et al.,

المجلة العراقية لبحوث السوق
وحماية المستهلك

DETERMINATION OF OPTIMUM CONDITIONS FOR BIOREMEDIATION OF IMIDACLOPRID BY *RHIZOBIUM PUSENSE* STRAIN IHB 1(OP218458.1) AND *PSYCHROBACTER CELER* STRAIN IHB2(OP672320.1)

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Received 5/ 6/ 2023, Accepted 2/ 8/ 2023, Published 31/ 12/ 2024

ABSTRACT

Imidacloprid (1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine), is a recent systemic and contact insecticide with high activity against a wide range of pests. Continuous dispersion of this pesticide in the environment and its stability in soil results in environmental pollution which demands remediation. Used in this research *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2(OP672320.1). which were previously isolated from botanical fields soil of greenhouses which has been using imidacloprid pesticides for many years to determine optimum condition and degradation ability for imidacloprid by tested in minimal salt medium (MSM) for a duration of 21 days. The temperature, pH number, and concentration of the pesticide were determined for the growth of bacteria. The best growth of *Psychrobacter celer* strain IHB2(OP672320.1) was at 28°C, pH 6, and pesticide concentration 50ppm, while Rhizobium pusense strain IHB1 (OP218458.1) had the best growth at 24°C, pH 7, and pesticide concentration 75ppm.Levels of imidacloprid in MSM medium were analyzed by high-performance liquid chromatography (HPLC). *Rhizobium pusense* strain IHB 1 (OP218458.1) was able to degrade 50.2% and *Psychrobacter celer* strain IHB2(OP672320.1) was able to degrade 59.01% of the initial amount of imidacloprid at the concentration of 25 mg /L in MSM media. All bacteria introduced in this study were among the first reports of imidacloprid degrading isolates in MSM-limited media from greenhouse soil. Therefore, the results of this study demonstrate the effectiveness of using soil bacteria for microbial degradation of imidacloprid. These findings suggest that these strains may be promising candidates for bioremediation of imidacloprid-contaminated soils.

Keywords: MSM, *Psychrobacter*, *Rhizobium*, HPLC.

[*]**The article is taken from the doctoral thesis of the first researcher.**

المجلة العراقية لبحوث السوق وحماية المستهلك

Iraqi Journal of Market Research and Consumer Protection

Ali & et al.,

(2024) 16(2): 136-148

تحديد الظروف المثلى للمعالجة الحيوية للإيميداكلوبريد بواسطة *سلالة* *Rhizobium pusense* IHB 1 (OP218458.1) وسلالة *Psychrobacter celer* IHB2(OP672320.1)

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الخلاصة

يعرف Imidacloprid (1-[(6-chloro-3-pyridinyl) methyl] -N-nitro-2- imidazolidinimine بانه مبيد حشري جهازي ويعمل بالملامسة أيضا وذو فعالية عالية ضد مجموعة واسعة من الآفات. ويؤدي التشتت المستمر لهذا المبيد في البيئة واستقراره في التربة إلى تلوث بيئي يتطلب معالجة. استخدمت في هذا البحث بكتيريا *Psychrobacter celer* strain IHB2 (OP672320.1) و *Rhizobium pusense* strain IHB 1 (OP218458.1) التي تم عزلها سابقًا من ترب البيوت البلاستيكية، والتي تستخدم مبيدات الآفات الاميداكلوبريد لسنوات عديدة وذلك لتحديد الظروف المثلى لها وقدرتها على تحليل الاميداكلوبريد عن طريق اختبارها في الوسط الملحي (MSM)لمدة 21 يومًا. جرى تحديد كل من درجة الحرارة والرقم الهيدروجيني وتركيز المبيد في نمو البكتريا. كان أفضل نمو لبكتريا *Psychrobacter celer* strain IHB2 (OP672320.1) في درجة 28 مئوية و 6pH وتركيز المبيد 50ppm، اما بكتريا *Rhizobium pusense* strain IHB 1 (OP218458.1) فكان أفضل نمو في درجة 24مئوية و 7pH وتركيز المبيد 75.ppm تم تحليل مستويات إيميداكلوبريد في الوسط الملحي MSM بواسطة تحليل الكروماتوجرافي السائل عالي الأداء (HPLC)، واستطاعت سلالة *Rhizobium pusense* IHB 1 (OP218458.1) أن تحلل بنسبة 50.2% وسلالة *Psychrobacter celer* IHB2 (OP672320.1) أن تحلل بنسبة 59.01٪ من الكمية الأولية من إيميداكلوبريد بتركيز 25 ملغم/ لتر في الوسط الملحي MSM. كانت أنواع البكتريا التي تم إدخالها في هذه الدراسة من بين التقارير الأولى للعزلات المحطمة للإيميداكلوبريد في الوسط الملحي MSM المعزولة من ترب البيوت البلاستيكية، لذلك أظهرت نتائج هذه الدراسة فاعلية استخدام بكتيريا التربة في التحلل الحيوي لمادة الاميداكلوبريد. تشير هذه النتائج إلى أن هذه السلالات قد تكون مرشحة واعدة للمعالجة الحيوية للتربة الملوثة بالاميداكلوبريد.

الكلمات المفتاحية: وسط ملحي MSM، *Psychrobacter*، *Rhizobium* ، كروماتوجرافي سائل عالي الأداء.

INTRODUCTION

Pesticides are a significant environmental risk where three million metric tons of pesticides are used globally each year because of their enduring effects on the environment. The organic decomposition of many of these pesticides in the soil is incomplete and enter the food chain through bioaccumulation and biomagnification, having an effect on both the target and non-target organisms, including people. Since farmers who spray pesticides in greenhouse fields are also affected by them, pesticide residues are viewed as a significant risk factor in society **(Awad *et al.*, 2018; Othman & Kakey, 2018)**. One million of the three million individuals who are fatally poisoned by pesticides each year do not even know it, according to the World Health Organization (WHO) **(Miccoli *et al.*, 2016)**. Neonicotinoid insecticides are widely used because insect pests are a widespread issue. accounting neonicotinoid around 25% of the global pesticide market **(Zhang *et al.*, 2019)**. These compounds are becoming more and more well-liked as an alternative to pyrethroid and organophosphate due to their unique mechanism of action **(Abdel-Ghany *et al.*, 2016)**. More and more academics have been focusing on the effects on organisms, the environment and activities using these substances through Studies on aquatic creatures **(Chen *et al.*, 2019)**, birds **(Humann-Guilleminot *et al.*, 2019)**, bees **(Zhu *et al.*, 2019; Strobl *et al.*, 2021)** and mammals (including humans) have shown that exposure to neonicotinoid pesticides may cause acute or

chronic toxicity **(Ali *et al*., 2018; Saqer *et al*., 2018).** As of late. The residual duration after pollution has been removed is an important indication. The amount of time that neonicotinoids stay in the soil varies greatly, from a few hundred to several thousand days **(Schaafsma *et al*., 2016)**. Imidacloprid, the first half-life of one neonicotinoid insecticide is 28–1250 days, while the half-life of clothianidin is 148–6931 days. There hasn't been much investigation into the precise reasons behind the significant inter-annual variation in neonicotinoid residual durations in the environment. The most popular insecticide is imidacloprid, also known as (1-[(6-chloro-3-pyridinyl) methyl] The first neonicotinoid pesticide on the market was N-nitro-2-imidazolidinimine. It is used in more than 100 countries and more than 140 different types of crops **(Jeschke & Nauen, 2018)**. More than two-thirds of the world's imidacloprid is produced in China, with 12,000 tons produced in 2012. 2019 China exported 31,595 tons of imidacloprid-related goods in 2017 **(Chen *et al*., 2019).** IMI has been used heavily and widely, but this has led to several problems, including environmental contamination, insect resistance, and the extinction of natural adversaries **(Liu *et al*., 2013)**. Because of the increased attention being paid to environmental issues, IMI's microbial degradation has been studied to eliminate IMI residues in ecosystems. It was discovered that IMI can remain in the soil for up to 156 days before it starts to lose its effectiveness **(Jeschke & Nauen, 2018)**. The most common method for converting synthetic chemicals into inorganic compounds is biodegradation, and both biotic and abiotic components of soil, such as elements, sunlight, and microbes, aid in this process. Soil microorganisms have been shown to degrade Heavy Metals and imidacloprid in several studies **(Al-Soufi *et al*., 2015; Garg *et al*.,2021)**, also found the ability of pseudomonas sp. To biodegradation of imidacloprid, hydrocarbons and Phenol in soil **(Hatit *et al*., 2013; Nafal & Abdulhay, 2020; Kridi *et al*., 2021; Dhari & Hetite, 2019)** and bioremediation of imidacloprid using Azospirillium biofertilizer and Rhizobium biofertilizer **(Kulkarni *et al*., 2022)**. This research aimed to ability of *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2(OP672320.1) the best degrading of imidacloprid in communities' soil samples for greenhouses and identify the best.

MATERIAL AND METHODS

Chemicals and organisms

By the National Center for Pesticide Control in Baghdad provided imidacloprid (purity >97%). All solvents were bought from Merck KGaA in Germany (99.9% purity) and were of HPLC grade. England company Romil developed HPLC-grade water. A nylon filter (0.45) µm was used when applying HPLC-grade H_2O and C_2H_3N. Using a criterion solution, analytical criterion for HPLC, standardization in the 1–50 mg/L range was produced. *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2 (OP672320.1) provided from the Department of Biofertilizers by the Plant Protection Director in Baghdad.

Culture of microbe growth

According to the manufacturer's recommendations, distilled water was used to create MSM (Mineral Salt Medium) media and was autoclaved for 15 minutes at 121ºC to guarantee that the solutions was sterilized (Sigma-Aldrich, USA). Imidacloprid (25 ppm) was administered using a syringe filter. According to **(Zhao *et al*.,2018)** the mineral salt medium (MSM) was employed for biodegradation testing and contains (g/L) ammonium sulfate (2.0), potassium hydrogen phosphate (0.625), sodium dihydrogen phosphate (0.6), magnesium sulfate (0.2), and calcium chloride. Antarctictite (hexahydrate) (0.15) (pH 7.0). The medium was cleaned by autoclaving it at 121C degrees for 15 minutes.

Instruments

An HPLC (LC-2010 A HT Shimadzu Japan model) with a DAD was used for the analysis of imidacloprid. ChemStation software was used for all data collection and processing. Each sample was separated using a 250 4.6 mm Orbit C18 reversed-phase column. At 1 ml/min at a flow rate and an oven temperature of 40 °C, the mobile phase's binary composition was 60% water and 40% acetonitrile. The detector's wavelength was adjusted at 270 nm. Each time a sample was inspected, the calibration curve was verified. was developed with the use of external standards and a quantitative technique known as linear regression analysis. In order to identify imidacloprid, the retention time was employed. For measuring the optical density was employed., a spectrophotometer (Cecil/ CE 7200) **(Sabourmoghaddam *et al.*, 2015)**.

Temperature Variation Assay

Temperature Tolerance for *psychrobacter* sp. and *Rhizobium* sp. were investigated by incubating bacterial cultures in MSM medium with 25 ppm imidacloprid at 20 ºC, 24 ºC, 28 ºC, 32 ºC, and 37 ºC and after that measured optical density after 1 and 3 days.

PH Variation Assay

The ability of *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2 (OP672320.1) isolates to grow at different pH was tested separately in MSM broth with 25 ppm imidacloprid by adjusting the pH to 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 with NaOH and HCl. Growth of 1×10^7cfu/ml at different pH in optimum temperature was determined by measuring O.D. at 550 nm after 1-4-7 days. Each experiment was performed in triplicates.

Imidacloprid Concentration Variation Assay

The ability of cultures *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2(OP672320.1) to grow in different imidacloprid concentrations was tested by adding them concentrations 1×10^7 separately in MSM medium containing 25, 50, 75, 100, 150, and 200 ppm of imidacloprid with incubation at optimum temperature and pH.

Biodegradation of Pesticides in Minimal Salt Medium10^7

Sterilized 50 ml falcon tubes were used in all biodegradation experiments. Each tube received 30 ml of MSM containing 25 mg/L imidacloprid. This MSM functioned as the only source of carbon and nitrogen in a carbon-limited medium. Also used control tubes containing medium and a pesticide (without any bacteria) to prevent environmental effects such as photodegradation. for imidacloprid. 10^7 bacterial cells per ml were cultivated in the falcon tubes for three weeks at optimum temperature in the dark on a rotating shaker set to 120 rpm. For analyses of pesticide residue from imidacloprid taken subsamples from each treatment were taken after 1, 7, 15, and 21 days. A one-half milliliter of each subsample was combined with a one-half milliliter of acetonitrile in two milliliters Eppendorf tubes, and the resulting mixture was centrifuged at 12,000 rpm for five minutes to measure the concentration of each treatment. To store the supernatant used Pasteur pipettes were used to transfer it to the amber HPLC vials and kept in a refrigerator. Each sample was injected into the HPLC at a volume of 50 microliters. And also measure the growth bacteria in each falcon tubes at the same time on 550nm optical density (OD) was recorded. **(Sabourmoghaddam *et al*, 2015)**.

Statistical Analysis

The standard error of mean results for each experiment was calculated after each experiment was carried out in triplicate. It was done using one-way analysis of variance (ANOVA). based on HPLC data indicating variations in the concentration of imidacloprid in treatments. The LSD test ($p< 0.05$) in SPSS 19 was used to assess mean differences. Controls were only utilized to monitor the growth of the isolates; they were not employed in the data analysis. **(Cary., 2012)**.

RESULTS AND DISCUSSION

HPLC calibration

Imidacloprid retention time were 4.934 minutes under experiment conditions (Fig. 1). Working standard solutions of imidacloprid were produced at different dilutions (1, 5, 25, and 50 ppm) and used to calibrate the instrument before any sample analyses were injected in order to determine the sensitivity of the HPLC. The correlation coefficient for imidacloprid was 0.999, indicating a linear relationship between the amount of standard solution injected and the resulting peak area.

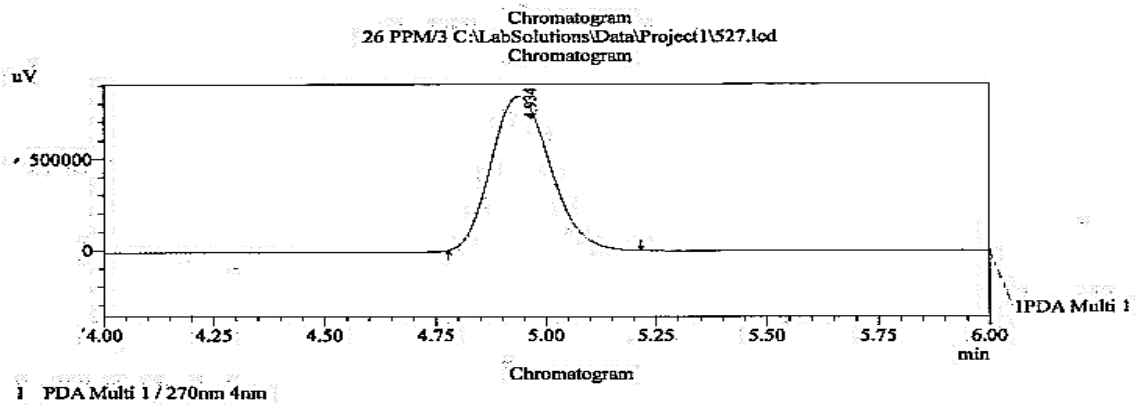

Figure (1): Separation of the imidacloprid (4.934 min) 1 ml per minute flow rate on a C18 column.

Optimum condition for Growth isolates:

Temperature effects

The optimal temperature for growth *Psychrobacter celer* strain IHB2(OP672320.1) was 28°C (Fig.2), while temperatures higher than 32°C reached 37°C reduced growth markedly. The effect of temperature on growth rate was determined for several selected temperatures (Fig. 3), and the temperature for maximal growth rate was also 28°C. while the optimal temperature for growth and maximal growth of *Rhizobium pusense* strain IHB 1 (OP218458.1) was 24°C and reduced markedly growth was determined towards 37°C which according with **(Bipte *et al.*, 2012)** when used optimum temperature for *Rhizobium* sp. to degraded imidacloprid and fipronil.

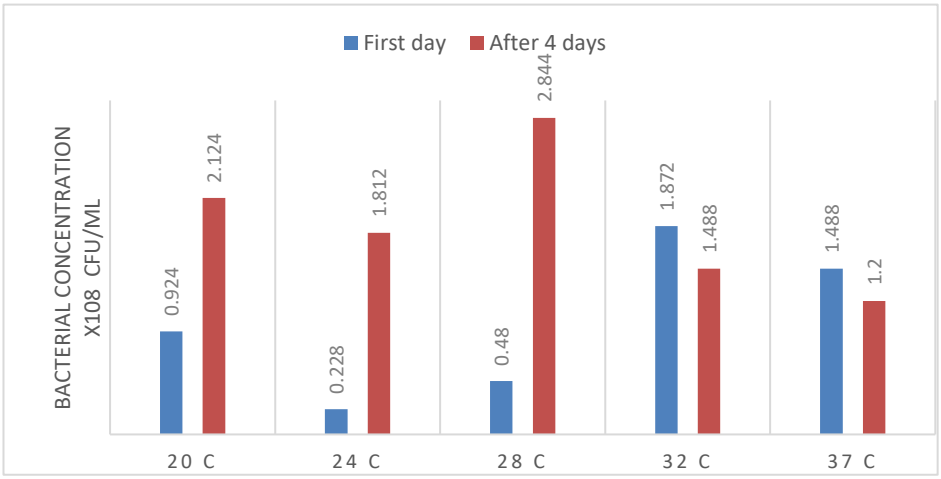

Figure (2): Tolerance of *Psychrobacter celer* strain IHB2(OP672320.1) to Temp.

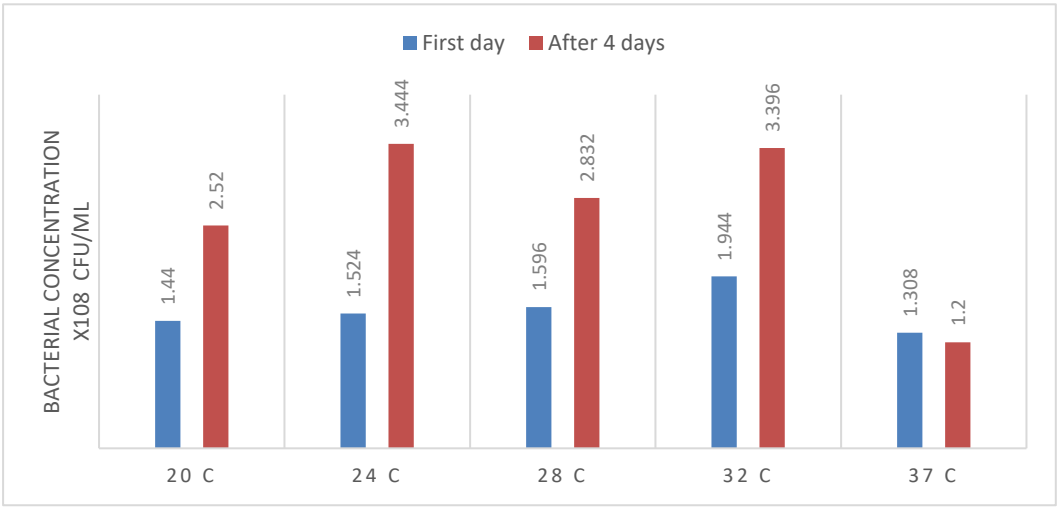

Figure (3): Tolerance of *Rhizobium pusense strain* IHB 1 (OP218458.1) to Temp.

Effect of pH

The optimum pH for *Psychrobacter celer* strain IHB2(OP672320.1) growth was 6 within the range of 4.0-9.0 with limited growth toward acid (pH 4) and normal growth toward neutral and base (pH 7 to pH9) after 7 days (Fig.4). While the optimum pH for *Rhizobium pusense* strain IHB1 (OP218458.1) growth was 7 within the same range of 4.0-9.0 after 7 days of incubation which according with **(Madariaga-Navarrete *et al.*, 2017)** when used optimum pH for bioremediation of Atrazine pesticide (Fig.5).

Figure (4): Tolerance of *Psychrobacter celer* strain IHB2(OP672320.1) to pH.

Figure (5): Tolerance of *Rhizobium pusense strain* IHB 1 (OP218458.1) to pH.

Effects of Imidacloprid Concentration

The optimal concentration of Imidacloprid for degradation by *Psychrobacter celer* strain IHB2(OP672320.1) within the range used of 25-200 ppm was (25 and 50 ppm) in optimum temperature and pH at 21 days of incubation and the degradation becomes lower toward high concentration of imidacloprid (Fig.6), while the result when used the same range concentration of imidacloprid with *Rhizobium pusense* strain IHB 1 (OP218458.1) was 75 ppm within optimal conditions from temperature and pH for it, with continues fewer growth markedly toward high concentration of imidacloprid which indicates tolerance to high concentrations of pesticides, which is compatible with **(Madariaga-Navarrete *et al.*, 2017)** when *Rhizobium* sp exposed. to 10,000 mg L^{-1} from Atrazine and it continues growth lowest which suggests that the bacteria are resistant to the Atrazine herbicide in high concentration (Fig.7).

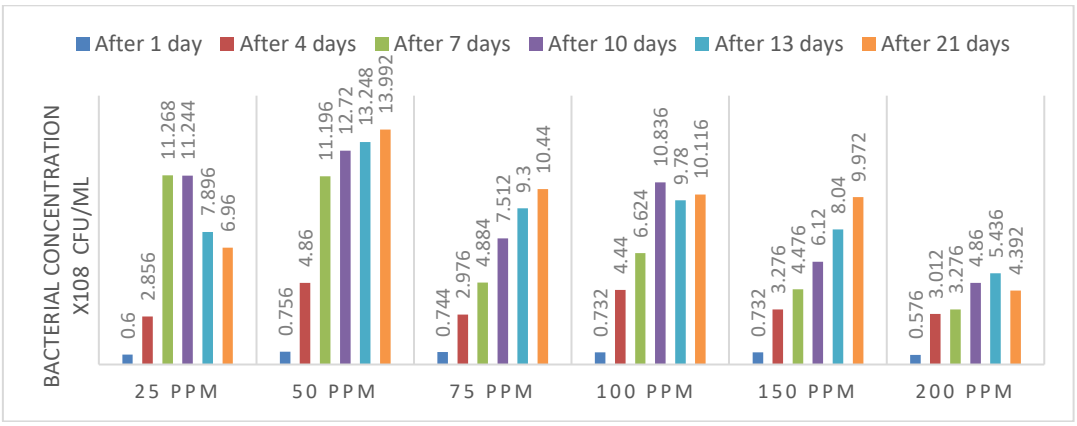

Figure (6): Tolerance of *Psychrobacter celer* strain IHB2(OP672320.1) to Imidacloprid concentrations.

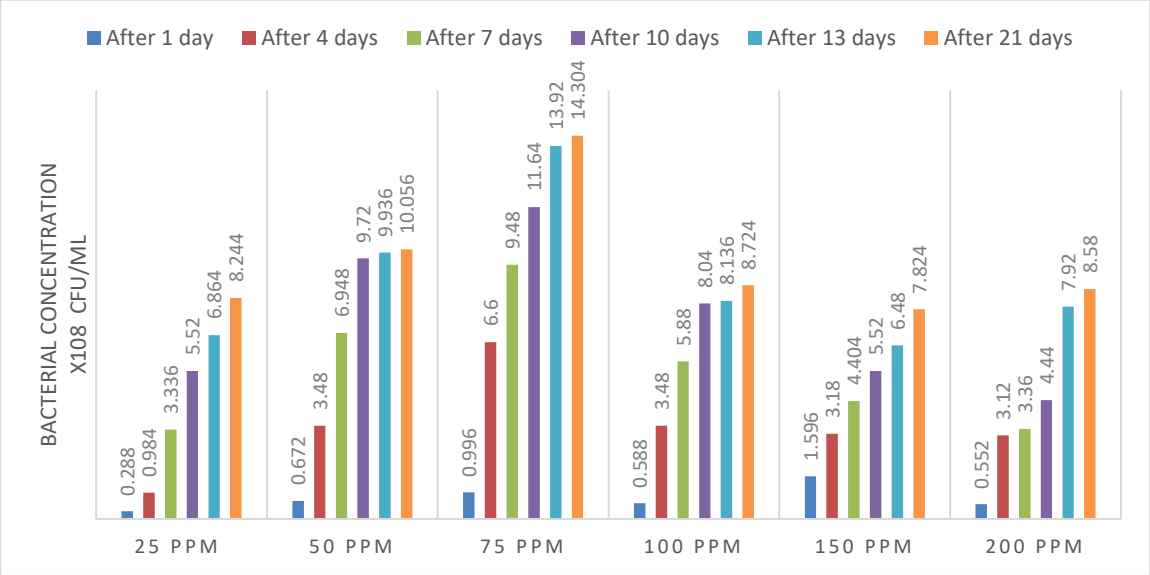

Figure (7): Tolerance of *Rhizobium pusense strain* IHB 1 (OP218458.1) to Imidacloprid concentrations.

Measured Imidacloprid Residues by Degrading Bacteria

Rhizobium pusense strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB 2(OP672320.1) bacterial strains were used to test imidacloprid biodegradation in optimum condition for 21 days and measured residual. A one-way ANOVA was employed to examine variations between the isolates taken from the MSM medium. The One-way analysis of variance (ANOVA) was used to see whether there were any statistically significant differences between the two groups. The daily concentration means (7, 15, and 21) days were shown to be substantially different from one another at a 95% confidence interval. was significant for all groups after days 7, 15, and 21 were also significant for differences between groups. (Table 1). Imidacloprid biodegradation by two isolates was between 39.52% to 50.23% after seven days and 45.07 % to 53.50% on day 15 of incubation, although it increased afterward to 59.01% and 50.20% of the spiking quantity by day 21 of incubation. Statistically, the analysis found a significant relationship between the growth of isolate and days, and, a significant correlation between bacterial population size and degradation ability which according to with study **(Sabourmoghaddam *et al.*, 2015)**.

Table (1): A one-way ANOVA test of the Significance for Days (1,7,15,21).

Days.	Groups	T	Df	Mean Square	F	Sig.
After 1 day	Between Groups	1.046	2	0.523	0.557	0.600
	Within Groups	5.637	6	0.939		
	Total	6.683	8			
After 7 days	Between Groups	137.197	2	68.599	24.864	0.001
	Within Groups	16.554	6	2.759		
	Total	153.751	8			
After 15 days	Between Groups	174.736	2	87.368	51.522	0.000
	Within Groups	10.175	6	1.696		
	Total	184.911	8			
After 21 days	Between Groups	152.716	2	76.358	33.465	0.001
	Within Groups	13.690	6	2.282		
	Total	166.407	8			

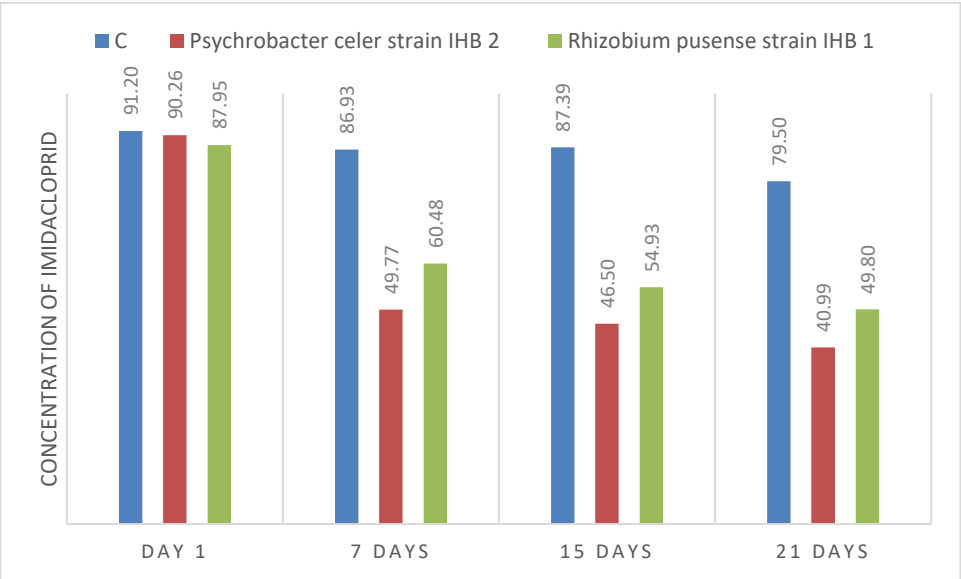

Figure (8): Degradation of Imidacloprid by isolates in 21 days.

CONCLUSIONS

The results of this research demonstrated that imidacloprid might be degraded by *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2(OP672320.1) in optimum conditions. The chosen bacterial isolates from soil were able to degrade between 50.2 and 59.01% of imidacloprid by 21 days. However, isolates bacteria (*Rhizobium and psychrobacter*) introduced in this study were the first locally isolated from a greenhouse in Iraq about the degradation of imidacloprid in MSM media. These bacterial conversions of imidacloprid open up new avenues for its chemical degradation in soil.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 149-162
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(13)
Omaish & Al-Bayati

المجلة العراقية لبحوث السوق
وحماية المستهلك

SYNTHSIS A HIGHLY SENSITIVE MOLECULARLY IMPRINTEND POLYMER AS AN ELECTROCHEMICAL SENSOR FOR THE DETERMINATION OF AMLODIPINE IN PHARMACEUTICAL SAMPLES

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Received 6/ 6/ 2023, Accepted 19/ 5/ 2024, Published 31/ 12/ 2024

ABSTRACT

This paper demonstrates the synthesizing and storage of molecularly- imprinted polymers (MIP) at room temperature using bulk polymerization of amlodipine (AD) is characterized by high sensitivity, low costs and high stability. The research used 0.99:6:20 mmol ratios of template, monomer, and cross-linking agents for the polymerization in order to ensure an appropriate adsorption capacity. A functional monomer tripolyphosphate with cross-linking ethylene glycol dimethyl acrylate was attained by creating MIP for amlodipine as AD-MIP that could be characterized using a UV-VIS spectrophotometer at 238 nm, Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Mass spectrometric (MS) detection may use tritolylephosphate to determine amlodipine levels in pharmaceutical preparations. The GC/MS methods developed in this study are accurate, sensitive, and precise and can be easily applied to (AMADAY/India, NORVASC/U.K.) tablets in pharmaceutical preparation the elution process was applied to the template (AD) from the AD-MIP developed cavities caused by using pyrogenic solutions of methanol, chloroform, and acetic acid (70:20:10, v/v). The maximum adsorption capacity of AD-MIP was 0.263 µmol/g, and the ratio of template to monomer was 1:1 in adherence to the Langmuir isotherm model. A solid-phase extraction (SPE) syringe packed with molecularly imprinted polymers (MIPs) was used to selectively separate and pre-concentrate AD. from aqueous solutions and estimations of Amlodipine.

Keywords: Molecularly imprinted polymer, Amlodipine, Isothermal process.

[*]**The article is taken from the doctoral thesis of the first researcher.**

تصنيع بوليمرات مطبوعة جزيئيًا عالي التحسس بالمستشعر الكهروكيميائي لتقدير الاملوديبين في المستحضرات الصيدلانية

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الخلاصة

يوضح هذا البحث تركيب وتخزين البوليمرات الجزيئية المطبوعة (MIP) في درجة حرارة الغرفة باستخدام البلمرة السائبة للأملوديبين (AD) الذي يتميز بحساسية عالية وتكاليف منخفضة وثبات عالي. استخدم البحث 0.99: 6: 20 مليمول نسب من القوالب والمونومر وعوامل الربط المتبادل للبلمرة من أجل ضمان قدرة امتصاص مناسبة. تم الحصول على مونومر تراي توليل فوسفيت وظيفي مع إيثيلين جلايكول ثنائي ميثيل أكريليت متقاطع من خلال إنشاء MIP للأملوديبين مثل AD-MIP الذي يمكن تمييزه باستخدام مقياس الطيف الضوئي UV-VIS عند 238 نانومتر ، مطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR) والمسح المجهري الإلكتروني (SEM). قد يستخدم الكشف عن مقياس الطيف الكتلي (MS) تراي توليل فوسفيت لتحديد مستويات الأملوديبين في المستحضرات الصيدلانية. طرق GC / MS المطورة في هذه الدراسة دقيقة وحساسة ودقيقة ويمكن تطبيقها بسهولة على أقراص (AMADAY/ India، NORVASC/ UK). في التحضير الصيدلاني تم تطبيق عملية الشطف على القالب (AD) من التجاويف المطورة لـ AD-MIP الناتجة عن استخدام محاليل مسامية من الميثانول والكلوروفورم وحمض الخليك (70:20:10، على التوالي). كانت السعة القصوى لامتصاص AD-MIP 0.263 ميكرو مول / جم ، وكانت نسبة القالب إلى المونومر 1: 1 في الالتزام بنموذج متساوي الحرارة Langmuir. تم استخدام حقنة استخلاص ذات المرحلة الصلبة (SPE) معبأة بالبوليمرات الجزيئية المطبوعة (MIPs) لفصلAD شكل انتقائي وتركيزه مسبقًا. من المحاليل المائية وتقديرات أملوديبين.

الكلمات المفتاحية: عملية الايزوثيرم ,الاملوديبين,بوليمرات الطبعة الجزيئية.

INTRODUCTION

Amlodipine is a synthetic dihydropyridine and a calcium channel blocker with antihypertensive and antianginal properties. Amlodipine inhibits the influx of extracellular calcium ions into myocardial and peripheral vascular smooth muscle cells, thereby preventing vascular and myocardial contraction. This results in a dilatation of the main coronary and systemic arteries, decreased myocardial contractility, increased blood flow, oxygen delivery to the myocardial tissue, and decreased total peripheral resistance. This agent may also modulate multi-drug resistance (MDR) activity through inhibition of the p-glycoprotein efflux pump **(Civantos *et al.*, 2004; Coseberg, *et al.*, 1997).**

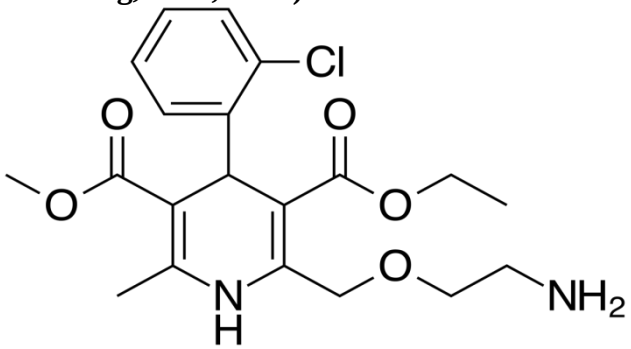


Figure (1): Structure of Amlodipine.

A molecularly imprinted solid phase (MI-SPE) preparation method is currently being developed which has so far shown a good level of selectivity. Molecular imprinting polymer is a technique for preparing polymer materials that have pre-ordered structures and specific molecular recognition capabilities. In this study, the selection of functional monomers was important in order to produce molecular-specific cavities of templates. tritolylephosphate is a functional monomer that can act as a hydrogen bond acceptor to its template. Previous research has been conducted on MISPE for amlodipine (**Bodoki *etal*., 2018 ,Y. Al-Bayati & E. Hadi.**et ***al*., 2022)**, with several GC–MS studies specifically investigating amlodipine as well **(Alsamarrai , *et al.*, 2017,Chaturvedi, *et al* ., 2005)**. Forsdahl et al. reported a method for the sensitive detection of isopropyl substituted b-blocking agents in human urine. Their sample-preparation phase involved enzymatic hydrolysis; solid-phase extraction; and derivatization with N-methyl-N-trimethylsilyl trifluoroacetamide. GC–MS was then used to detect atenolol as its bis, tris, and tetra-TMS derivatives. Angier and their co-workers **(E. A. Hadi & Y. K. Al-Bayati. 2022)**. Following this study, the use of mass selective detectors with a capillary GC coupled to MS has considerably increased **(Aljabari & Al-Bayati. 2023).** This development has led to the improvement of gas chromatographic properties of both the compounds and yield compounds, with mass spectra containing high relative intensity and high-mass fragments suitable for selected ions.

Initially, the important molecule of a molecular imprinted polymer (MIP), forms a complex of the actual monomers. Following the polymerization cycle, as is shown in Figure 2, the functional groups are kept in place by a highly cross-linking polymeric structure **(Al-Bayati,*et al.*, 2017)**. In addition, the steric configuration of all of these connections based around a given substratum and template is really an important characteristic for the formation of binding sites providing additional shape, size and flexibility to promote selective identification followed by a high target affinity. As a result, the process of recognition in MIPs can be characterized by resemblance to enzyme-proven mechanisms.The substratum- complex is formed like the (lock/ key) model **(Al-Bayati & F. I. Aljabari. 2016).**

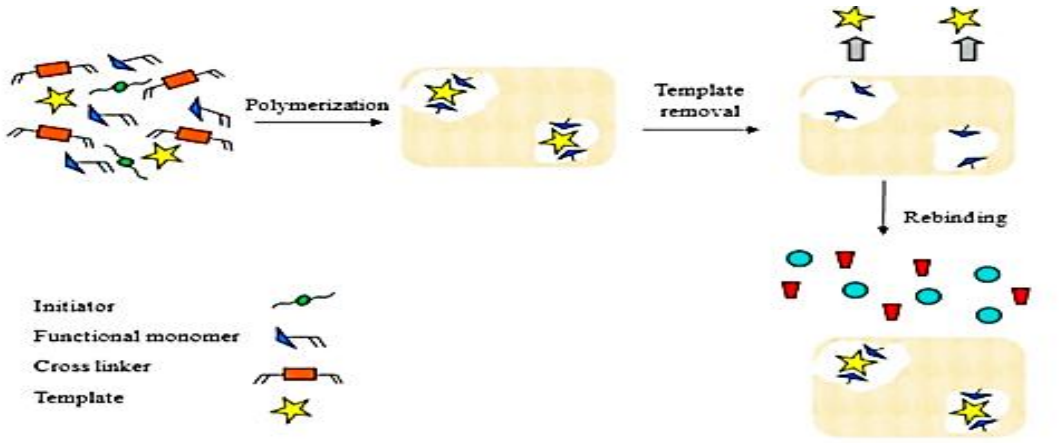


Figure (2): Molecular imprinted polymer cycle **(M. A. Sandoval Riofrio. 2017)**

Gas chromatography-mass spectroscopy (GC/MS) is a useful technique, a gas chromatograph (GC) coupled to a mass spectrometer (MS), used in the separation and quantification of complex mixtures of chemicals. It is injected into the GC inlet and, after vaporization, is forced into the column by a carrier gas (usually helium). The sample flows through the column and the constituent compounds of the respective mixture will be separated by virtue of their relative interaction with the column coating (stationary phase) and carrier gas (mobile phase). The latter passes from the column through a heated transmission line and ends at the inlet of the ion source where compounds separated from the column are converted into ions **(Karasek, *et al.*, 2012).**

EXPERIMENTAL PART

MATERIALS AND METHODS

Amlodipine from Samarra/Iraq was provided; tripolyphosphate, ethylene glycol dimethyl acrylate, and benzoyl peroxide were purchase from Sigma Aldrich (USA); methanol; and nitrogen gas (99.99) was supplied by the Al-Watan factory (Al-Nahda street/ Baghdad/Iraq); chloroform and acetic acid were purchased from Merck (Germany); and sulphuric acid of 98% purity was purchased from the CDH (***Central Drug House)***.

Preparation and Processing:

High-purity grade chemicals were used for the preparation process:
AD-MIP was prepared by stirring to dissolve 0.9977 mmol of amlodipine 0.408 g, in 4 mL of methanol; 6 mmol of tritolylephosphate, 1.473 g, was then added and was left for a few seconds at room temperature to dissolve. 20 mmol cross-linker ethylene glycol dimethyl acrylate, 4g, was then dissolved in the solution, followed by the addition of 0.3g, of benzoyl peroxide dissolved chloroform to act as an initiator. The solution was then shacked and bubbled for 20 min with pure nitrogen to remove the dissolved oxygen from the monomer solution, after which the tube was sealed with a rubber stopper. The stoppered solution was left in a water bath overnight at 60 ºC, following which the polymerization process 0.99:6:20 of AD-MIP was completed. The solution presented as a white- colored polymer with a rigid structure, and the formation of fine particles could be observed with the naked eye. The solution was, left to dry at room temperature overnight. AD- MIP was synthesized through the self-assembly (non-covalent) technique of bulk polymerization. Soxhlet solid liquid phase extraction for the template was performs to remove it from MIP by using pyrogenic solvent v/v (acetic acid, chloroform and methanol, at a ratio of 10:20:70,v/v respectively) performed successfully by repeatedly washing for 18-24 hours. The polymer was dried at room temperature, then crushed with a mortar and sieved to a particle size of 125µm.

A 3ml solid phase extraction vacuum through a plastic syringe (column) was used, and each syringe was packed with 0.1 g of AD-MIP and a flow rate of 70ml/min of standard solution amlodipine.

A series of standard solutions of amlodipine (0.1;0.08;0.06;0.04;0.02; and 0.01 mmol/ml) was prepared by dissolving 0.0368g of AD. In a methanol volumetric flask of 100 ml as a stock solution. A calibration curve between an x-axis describing the concentration of amlodipine and a y-axis describing its absorption A, was achieved using a 238 nm UV-VIS

instrument.The pharmaceutical samples were prepared by taking the average weight of the powder of amlodipine tablets (as is shown in Table 1) and dissolving it in 100 mL of methanol solution,before filtering it through cellulose filter paper of 0.07µm in order to obtain concentrations from the calibration curve 0.4×10^{-4}mmol/mL(0.4µmol/ml) of amlodipine drugs (AMADAY/India, NORVASC/U.K.) which have the lowest standard addition (SD) value. These were then used with MIP in a solid phase extraction (SPE) column,by which MIP-SPE was prepared.

Table (1): Pharmaceutical drugs prepared for treatment with AD-MIP polymer.

No. of samples	Commercial name, Country Content 100mg	Average weight for 10 of tablets (g)	Weight of sample equivalent to 0.012g (0.4×10^{-4}) mmol/mL of the active ingredient
1	AMADAY/India	1.783	0.2916
2	NORVASC/U.K.	3.99	0.6384

RESULTS AND DISCUSSION

After passing the solution of atenolol through a syringe packed with Ate-MIP, the residue with the least absorption was measured by UV-VIS.This indicated that a lower concentration during the final process had been a good expressive example of the advantages of the use of impressed polymers in SPE in the quantification of an amlodipine,as shown in Figures 4& and 5.

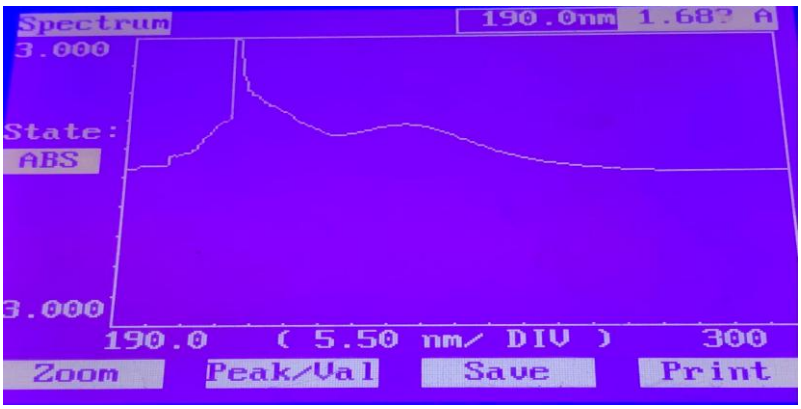


Figure (3): the absorption at 238 nm of the concentration amlodipine standard.

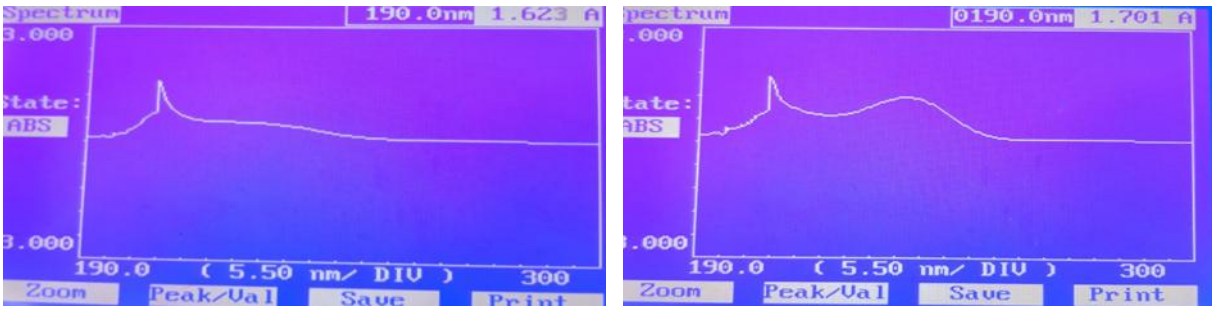


Figure (4): A, B the absorption at 238 nm of the concentration of Amlodipine drug (AMADAY/India) at 0.4×10^{-4} mmol/mL (0.4 µmol/ml) before & after passing through MIP column.

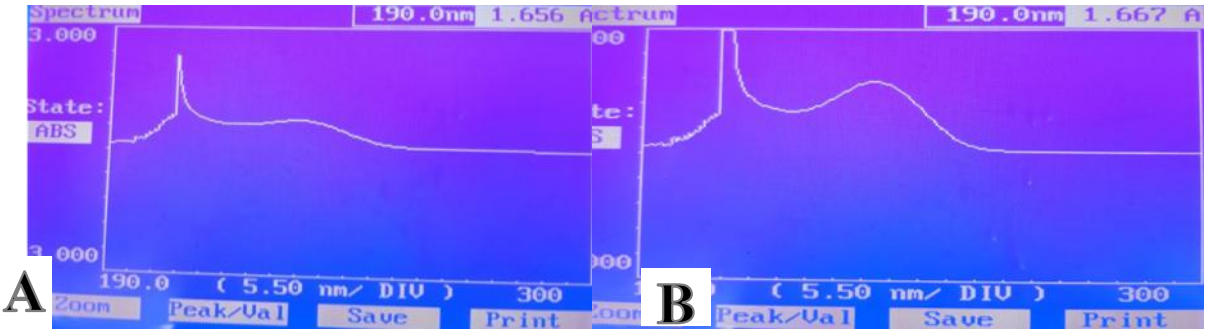


Figure (5): A, B the absorption at238 nm of the concentration of Amlodipine drug (NORVASC/U.K.) at 0.4×10^{-4} mmol/mL (0.4 µmol/ml) before & after passing through MIP column.

Transform Infrared Spectroscopy (FTIR) analysis:

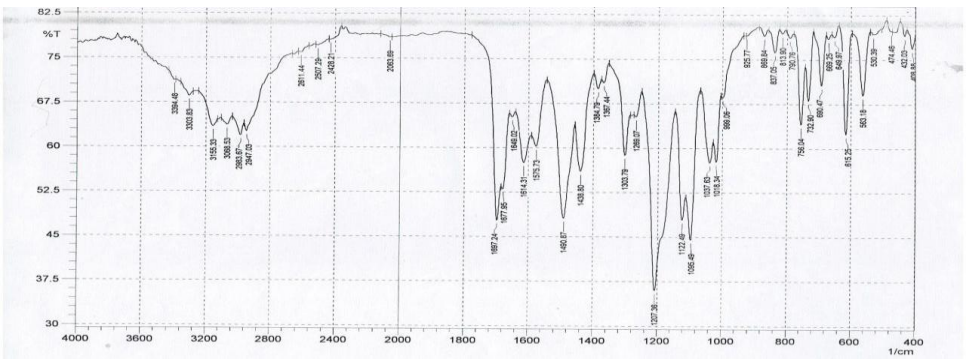


Figure (6): FTIR spectra of Amlodipine standard.

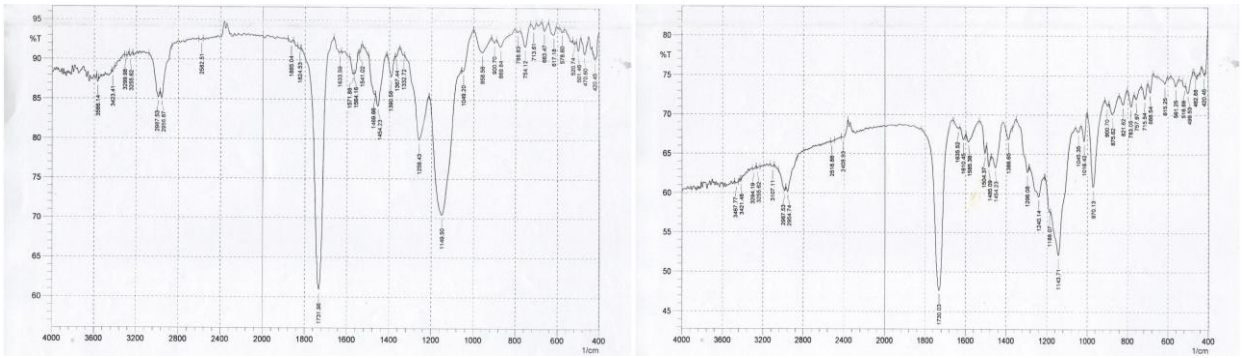


Figure (7): A, B FTIR spectrum of AD-MIP before and after extraction (after removal of the template amlodipine).

The MIP of AD was synthesized via a non-covalent bulk polymerization method. Functional monomers played an important role in studying the interactions that occur with the template. The monomer tritolylephosphate was used for the synthesization of MIP and NIP. FTIR analysis an important chemical characterization method to detect the functional groups present in a compound was also employed with the FTIR spectra found amongst different MIP_s and NIP_s shown in Table 2, and Figures 6 and 7.

Table (2): The structures of the main three compositions of AD-MIP and the bands indicate MIP before &after the removal template.

Template (Atenolol)	**Monomer (Allyl chloride)**	**Cross linker (Ethylene glycol di methacrylate)**	
Band	**Drug(Template)**	**MIP before extraction**	**MIP after extraction**
N-$H_{str.}$	3155	3294	-
$NH_{2\ str.}$	3394-3303	3921 3467	-
O-C=O	1697	1635	1633
C-$H_{str.Aromatic}$	3068	_	_
C-$H_{STR.Aliph}$	2983,2947	2987 2954	2987 2956
O-P=O	_	1240	1259
P-O	_	1143	1149
CH2=CHCOOH	_	1730	1731

The Fourier transmission infrared spectrometry spectra of the leached and unleached amlodipine (AD) imprinted polymer MIP and NIP were recorded in the range of 400–4000 cm^{-1} by the KBr pellet method (Tabl 1).Within this table, the FTIR spectrum of the AD showed the following bands:3394;3303; 3155; 3068; 2983;2947and 1697 cm^{-1} for N-H_2 stretching;N-H stretching; C-H stretching aromatic ;C-H stretching aliphatic and O-C=O respectively. The

FTIR spectrum of atenolol MIP-(AD) before template removal showed the following bands :3421; 3467;3294;2987;2954;1635;1730;1240 and 1143 for cm^{-1} for N-H_2 stretching;N-H stretching; C-H stretching aliphatic; O-C=O;CH2=CHCOOH;O-P=O and P-O, respectively. The FTIR spectrum of the MIP(AD) after template removal demonstrated the absence of N-H stretching, NH_2 stretching O-C=O and CH stretching, which excise in the template (AD) spectrum and indicate the extraction of the drug from the template. When using the tritolylephosphate as a monomer for the synthesis of other MIPs for amlodipine, an FTIR spectrum was produced for MIPs both before and after template removal and NIP, which may be found in Table.The band's values **(Huang, *et al.*, 2018).**

The process of seizing the drug in the solid phase of the prepared molecular polymer may indicate the successful formation of the molecular polymer. In order to ensure the entry of the drug and the formation of the cavity, a spectrum GS/MS was measured for the prepared molecular polymer, with the structure of amlodipine shown in Figure 8.

From the injection of amlodipine as a liquid the spectrum of amlodipine with a molecular weight of 408.9 g.mol-1 begins to dip, though several peaks were confirmed using GS/MS. One such peak occurred at m/z 304 in the mass spectrum of amlodipine. The ionic fragment (m/z 97) observed in the MS/MS experiment was C_5H_8NO+, generated by the loss of the alcohol group from the precursor ions (m/z 97).

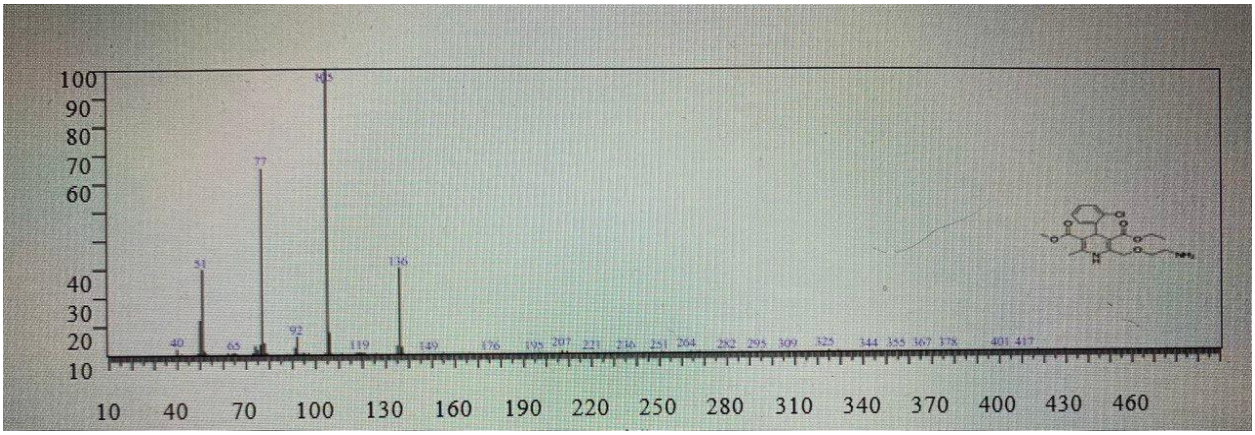


Figure (8): GC/MS structure of amlodipine.

Scanning electron microscope (SEM)

The morphological evaluation is critical to the appreciation of certain morphological traits, as well as the cavity sizes and surface configurations of MIPs both prior to and following the amlodipine template removal. SEM images were used to analyze the morphology of the AD-MIPs, as shown in Figure 8 (A, B).

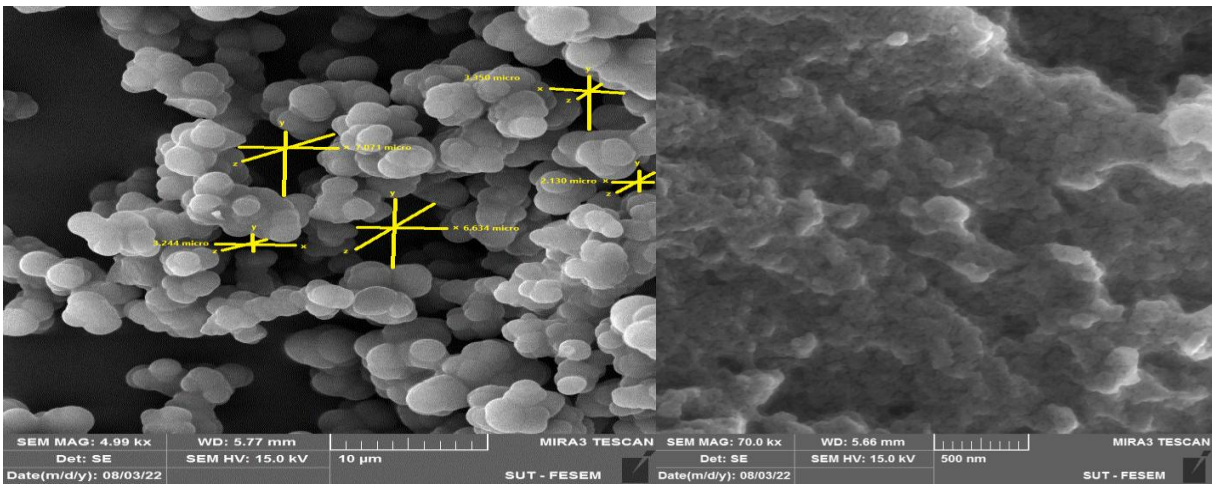


Figure (9): A, B surface morphologies of the particles before and after elution for AD- MIP respectively, and three dimensions of cavities with their areas.

show the surface morphologies of the particles before and after elution for Amlodipine–MIP and the relative cavities calculation table 3.

Table (3): Calculated mean, angle and lengths of some cavities (selected six of them) and their areas using image j program.

Area	Mean	Min- Max	Angle	Length
0.379	6488.741	1238.72-50153.79	6.911	7.071
0.356	6944.732	2445.17-23692.88	12.031	6.634
0.175	4392.59	2549-13038	0.939	3.244
0.116	2417.001	935.2-3722	2.862	2.13
0.181	2767.781	477-32673	0	3.35

Through Figure 9 and Table 3 the 3D of Cavities between min = 2417.001 (2.417001µm) to max = 6944.732nm (6.944732µm) we notice that the holes vary in diameter range between (2417.001-6944.732) nm and most of the holes are large, which leads to the retention of large quantities of the drug and this is consistent with the high value of the capacity in isotherm.

Relation between initial concentration and capacity

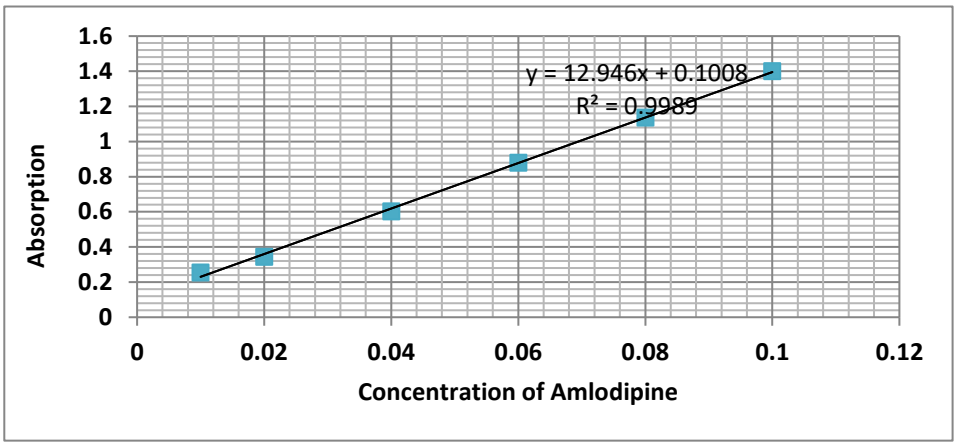


Figure (10): Calibration curve between concentrations of Amlodipine standard µmol/ml and its absorptions.

Adsorption capacity and pre-concentration:

A series of absorption achievements for different initial concentrations of AD-MIP ranging from 0.01 to 0.1 µmol/ml on adsorption capacity µmol/g was studied using the following equation **(Abass, *et al*., 2010)**

Q = (Ci - Cf) (µmol/ml)∗ the concentrations from (0.1-0.01) µmol/ml consume (3-7) ml range of volumes when using 0.1g weight of AD-MIP,Table4.

Table (4): The optimal synthesis conditions for the molecularly imprinted polymer for Amlodipine developed in

W/ MIP (g)	Ci (µmol/ml)	Cf (µmol/ml)	Vol (ml)
0.1	0.1	0.0965	3
	0.08	0.0721	3
	0.06	0.0516	3
	0.04	0.0328	3
	0.02	0.0169	5
	0.01	0.0079	7

The relation between initial concentration Ci (µmol/ml) and capacity Q (µmol/g)

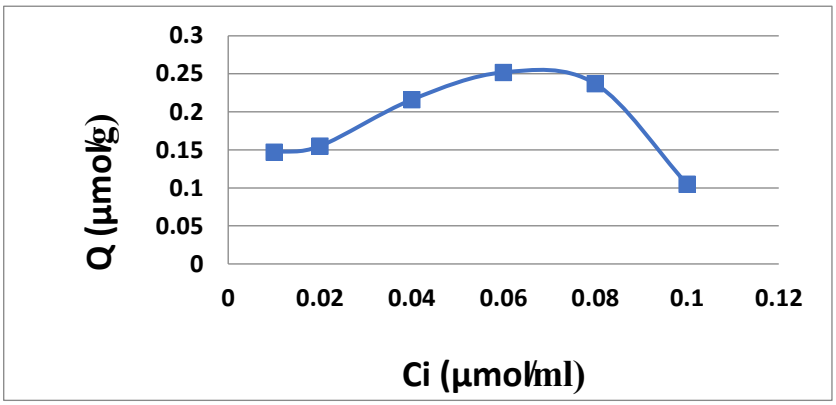
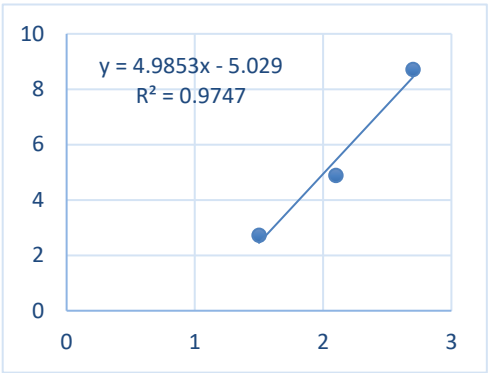
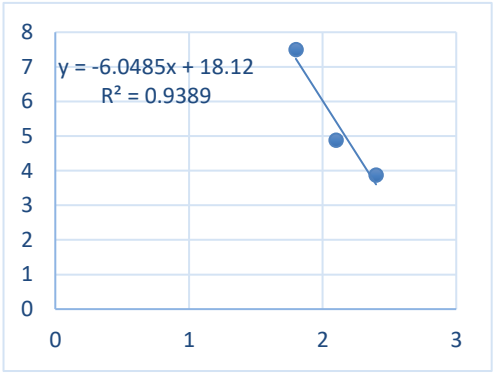


Figure (11): Illustrate the Langmuir isotherm model.

The relation between capacity Q (µmol/g) and Q/Cf (ml/g):





Slope = -1/kd
-124.36= -1/ kd Kd= 0.008
Intercept = 32.928 Intersept = Qmax/ kd
Qmax =32.928 * 0.008 = 0.263 µmol /g
That mean there was one capacities for AD-Mip equal 0.263 µmol/g it follow Langmuir isotherm model which has scattered value and one slope.

Table (5): Precision and accuracy of the analysis of pharmaceutical drugs in uv-vis spectrophotometry instrument before and after the isotherm process.

Drug name 100mg	MIP	Concentration Ci µmol/ml	Absorption before isotherm process	Absorption after isotherm process	Concentration Cf µmol/ml	Vol ml	Q µmol/g	RSD% =(δn-1/Mean) *100 Precision	Rec. % = (practical value/True value)*100 Accuracy	Re%= 100-Rec
AMADAY/India	MIP 0.1g	0.04	0.602	0.501	0.033	3	0.21	0.1661	99.717	0.16
NORVASC/U.K.		0.04	0.6036	0.511	0.0338	3	0.186	0.016	100.082	-0.0829

* For n=5 drugs were absorbed before the isotherm process (passing through MIP column). * The true value is the absorption at 0.04 µmol/ml in the calibration curve of amlodipine.
Isotherm adsorption of amlodipine MIP based on tripolyphosphate monomer was the same pattern which shows one sites connection of amlodipine with the polymer with a covalent bond. Scatchard plot showed only one equilibrium dissociation constant Kd and apparent maximum amount Qmax for the high affinity sites were calculated This behavior indicated that the adsorption was Langmuir isotherm and the binding was homogenies.

CONCLUSION

A novel bulk polymer was created by using different functional groups as monomers, with tritolylephosphate and crosslinked ethylene glycol dimethyl acrylate used to create AD-MIP.A variety of analytical approaches and experiments were used to reach selective molecular imprinted polymers by preparing and optimizing the required monomers; cross-linking through suitable solvents; applying pyrogen solvents for template removal, and adhering to optimal molar ratios of template (Amlodipine) to monomer for cross-linking. The irregularly three dimensional network structure of the polymer can be seen via SEM both before and after template removal, with FTIR, GC, and isotherm processing all improving the accuracy of this work. One slope gain when studied the capacity of adsorption of AD-MIP which follows the Langmuir isotherm model with scatter values (heterogeneous structure) and the ratio of template to monomer is 1:1. The maximum adsorption capacity of AD-MIP was 0.263 µmol/g.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 163-171
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(14)
Hussein & et al.,

المجلة العراقية لبحوث السوق
وحماية المستهلك

ASSOCIATION OF THE C2077G SNP IN GDF-9 GENE WITH THE PRODUCTIVE PERFORMANCE OF LOCAL AND SHAMI GOAT

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Received 14/ 6/ 2023, Accepted 15/ 10/ 2023, Published 31/ 12/ 2024

ABSTRACT

The study was conducted on a sample of 44 local goats and 52 Sham goat at the Ruminant Research Station of the Agricultural Research Department / Ministry of Agriculture. Nucleotide sequencing technology was used to detect the SNP (C2077G) in the studied segment of the GDF-9 gene, in order to determine the relationship of the genotypes resulting from the mentioned mutation to the growth traits, milk yield and fertility. The genetic polymorphism are wild (CC), Heterozygous (CG), and mutant (GG). The percentage of individuals carrying the wild genotype was superior to each of the individuals carrying the mutant and hybrid genotype (52.27, 45.45, 2.27)%, respectively, in the local sample, while the percentage of individuals was superior The carriers of the wild genotype over each of the individuals carrying the heterozygous and mutant genotype were (65.38, 32.69 and 1.92)%, respectively, in the Shami goat sample, and the allelic frequency of the wild allele C in both Goat was (0.53, 0.82%), compared to the mutant allele G (0.47, 0.18). The results showed that there was a significant effect of the polymorphism of the mutation (C2077G) in the characteristics of the weaning weight and the current weight in the local goat and in favor of individuals with hybrid genotype (CG) (20.70, 38.21) kg, respectively, but in the sample of the Shami goats, an effect was found significant of the genetic variation caused by the mutation (C2077G) in birth weight and weaning weight in favor of individuals carrying the hybrid genotype (3.04, 22.20) kg, respectively, The results showed a significant effect of the polymorphism of the C2077G SNP on litter size in local and Shami goats in favor of individuals carrying the hybrid genotype CG (1.49, 1.76) births per litter. The mutation (C2077G) in the GDF-9 gene was recently discovered and is not registered in NCBI, so it has no identification number(rs) .

Keywords: GDF-9 gene , local goats, Shami goats, productive traits.

علاقة تعدد المظاهر الوراثية للطفرة (C2077G) في جين GDF-9 في الاداء الانتاجي للماعز المحلي والشامي

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الخلاصة

تم اجراء الدراسة على عينة مؤلفة من 44 معزة محلية و 52 معزة شامية في محطة ابحاث المجترات العائدة لدائرة البحوث الزراعية/ وزارة الزراعة. استخدمت تقانة التتابع النيوكليوتيدي في الكشف عن الطفرة (C2077G) في القطعة المدروسة من جين (GDF-9) ليتم تحديد علاقة التراكيب الوراثية الناتجة من الطفرة المذكورة بصفات النمو وانتاج الحليب والخصب، اظهرت نتائج الدراسة ظهور الطفرة في كل من سلالتي الماعز المحلي والشامي، اذ ظهرت بثلاث مظاهر وراثية هي البري (CC) والهجين (CG) والطافر (GG) وتفوقت نسبة الافراد الحاملة للتركيب الوراثي البري على كل من الافراد الحاملة للتركيب الوراثي الطافر والهجين (52.27،45.45،2.27)% على التوالي في عينة المحلي، في حين تفوقت نسبة الافراد الحاملة للتركيب الوراثي البري على كل من الافراد الحاملة للتركيب الوراثي الهجين والطافر (65.38،32.69 و 1.92)% على التوالي في عينة الماعز الشامي، وتفوق التكرار الاليلي للاليل البري C في كلا السلالتين (0.53،0.82)% موازنة بالاليل الطافرG (0.47،0.18)%.وبينت النتائج وجود تأثير معنوي لتعدد المظاهر الوراثية للطفرة (C2077G) في صفتي وزن الفطام والوزن الحالي في عينة المحلي ولصالح الافراد الحاملة للتركيب الوراثي الهجين (CG) (20.70 ،38.21) كغم على التوالي، اما في عينة الماعز الشامي فقد وجد تأثيراً معنوياً للتغاير الوراثي الناجم عن الطفرة (C2077G) في الوزن عند الميلاد ووزن الفطام لصالح الافراد الحاملة للتركيب الوراثي الهجين (3.04 ،22.20) كغم على التوالي، وبينت النتائج تأثير معنوي لتعدد المظاهر الوراثية للطفرة (C2077G) في عدد المواليد في البطن الواحدة في الماعز المحلي والشامي لصالح الافراد الحاملة للتركيب الوراثي الهجين CG (1.49، 1.76) مولود/البطن الواحدة. لقد تم اكتشاف الطفرة (C2077G) في جين GDF-9 مؤخرًا وهي غير مسجلة في NCBI، لذا ليس لها رقم تعريف (rs).

الكلمات المفتاحية : جين GDF-9، الماعز المحلي ، الماعز الشامي، الاداء الانتاجي.

INTTRODUCTION

Goat are among the first animals that were domesticated by humans since the Neolithic era, that is, approximately more than 10,500 years ago, by humans in the Fertile Crescent region (Mesopotamia and the Levant), and its breeding spread after that to all parts of the world **(Amills *et al*., 2017; Gipson, 2019).** Goats are also considered the most adapted animals to harsh environmental conditions, which can maintain their productivity despite the lack of fodder and poor pasture compared to sheep and cattle **(Ibtisham *et al*., 2017)**, in addition to that goat are more resistant to epidemic disease and parasites compared to other farm animals **(Al-Jorani *et al*., 2020)**, its management is simple and does not require much investment **(Al-Samarai *et al*., 2016).**

Due to the lack of natural pastures and the spread of diseases and infection with diseases, including viruses, in sheep and goats **(Mohammed & Yousif ,2022)** and exposure to conditions of heat stress resulting from high or low temperature, which led to a decrease in the reproductive performance of agricultural animals **(Ishaq & Ajeel ,2013)**. The breeder always seeks to increase the herd of animals by increasing the number of births, which is linked to the high fertility rate of the herd **(Al- Khazraji *et al,* 2014)**. This requires improving reproductive performance through an extensive study of the most important genes related to reproduction, including those associated with the ovulation process and the number of births in one litter, as

it was found that there are many Among the genes that have a role in improving fertility **(Abdullah & Al-Khazraji, 2021)**, one of these genes is growth differentiation factor 9 (GDF-9), which is one of the genes that affects ovulation or sterility in agricultural animals **(Demars *et al.*, 2013; Al-Barzinji, 2022)**. **Al-Jubori *et al.* (2019)** indicated a significant effect of the GDF-9 gene on the number of births per litter in Awassi sheep. Therefore, the research aims: To identify the multiple manifestations of the C2077G mutation in the GDF-9 gene in a sample of local and Shami goats, and to determine their relationship to productive performance (growth, milk production, and fertility).

MATERNALS AND METHODS

The research was conducted on total 96 does belonging to two breeds (Local and Shami goat), with the same management and feeding conditions. Animals used were 2–5 years old, multiparous, lactating and in their first to fourth lactation.

Sampling, data collection and DNA extraction: A jugular vein blood sample was collected from each female goat using vacuum tubes with EDTA k2 as an anticoagulant. Birth weight was measured by using a small scale after birth directly. Kids were weaned at 120 days/ age, scale capacity 150 kg was used for weaning weight and body live weight. Total milk yield was calculated according to equation mentioned below.

Total milk production = (one milking / day* 2) × No. of milking days.

fecundity was calculated from equation mentioned below according to **Amnate *et al* (2016)**:

$$\text{litter size} = \frac{\text{Total no. of kids}}{\text{No. of parturition ewes}}$$

DNA extraction Genomic DNA: was isolated from blood sample according to the protocol ReliaPrep™ Blood g DNA Miniprep System, Promega.

Primers designed: The primer were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/ìl as a stock solution. A working solution of the primer was prepared by adding 10µl of primer stock solution (stored at freezer -20C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl (Table 1).

Table (1): Primer sequence.

Primer Name	Seq.	Annealing Temp. (°C)	Product size (bp)
GDF9-F	5'-CTCTCACCCTCCAAATCAAC-3'	60	987
GDF9-R	5'-TTCTTCCCTCCACCCATTA-3'		

Reaction setup and thermal cycling protocol:

Materials and PCR reaction condition showed in Tables 2 and 3.

Table (2): Master Mix components and volumes.

Master mix components		Volume
Master mix		12.5 µl
Primer	Forward	1 µl
	Reverse	1 µl
Nuclease free water		7.5 µl
DNA		3 µl
Total volume		25 µl

Table (3): Thermal cycling protocol.

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Standard sequencing: PCR product were sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea. The results were received by email then analyzed using geneious software.

Statistical analysis: The data was analyzed by used Statistical Analysis System **(SAS, 2012)** to study of GDF-9 gene polymorphism **(C2077G)** (CC, CG, GG) according to the mathematical model, significant differences was compared by used Least square means method.

$Yijk = \mu + Gi + eijk$

Yijk: Observed value, µ: Overall means, Gi: Effect of C2077G SNP polymorphism (CC, CG, GG), eijk: Random error which distributed normally with mean = 0 and variation $\sigma^2 e$. Chi-square- χ 2 test were used to compare between the percentages of GDF-9 gene polymorphisms. Calculator of allele frequency of GDF-9 gene according to Hardy Weinberger's equilibrium **(Falconer &Mackay, 1996).**

$$P_A = \frac{2 * \text{No. of Homozygous} + \text{No. of Heterozygous}}{2 * \text{Total number of sample}}$$

$p+q = 1$

$qa = 1 - PA$

RESULTS AND DISCUSSION

It appears from the results of reading the sequence of the nitrogenous bases of the studied second exon region of the GDF-9 gene (Fig 1), that there is a point mutation in the base (2077) from cytosine to guanine, and it resulted in the change of the allele amino acid to glycine. As a result of the mutation (C2077G) in the studied local goat sample, three genotype appeared Hereditary (wild CC 52.27%, hybrid CG 2.27% and mutant GG 45.45%) with a highly significant difference among. As for the allelic recurrence, the wild allele C (53%) was distinguished over the mutant allele (47%), with a highly significant difference. The results of the Shami goats are in the same direction, with the emergence of three genetic manifestations as a result of the mentioned mutation (CC 65.38%, the hybrid CG 32.69% and the GG mutant 1.92%). The allelic frequency was in favor of the wild allele with a highly significant difference (C 82%) over the mutant allele (G 18%). (Table 4).

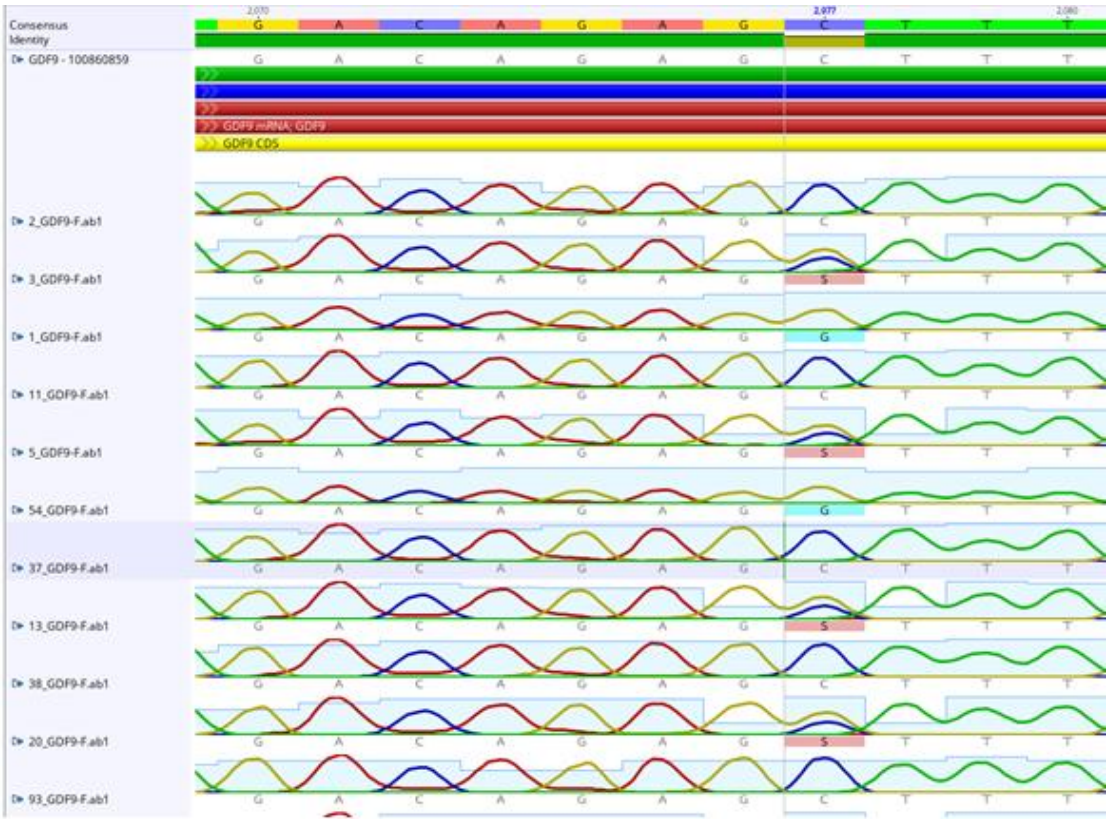

Figure (1): Sequence chromatograph of C2077G SNP of GDF-9 goat gene.

Table (4): Distribution and allele frequency for the mutation (C2077G) in the GDF-9 gene in local and Shami goat.

Genotype (Local goat)	**No.**	**(%)**	χ^2	**Allele frequency**	
CC	23	52.27	19.609**	C	G
CG	1	2.27		0.53	0.47
GG	20	45.45			
Total	44	100%	p-value **		
Genotype (Shami goat)	**No.**	**(%)**	χ^2	**Allele frequency**	
CC	34	65.38	37.230**	C	G
CG	17	32.69		0.82	0.18
GG	1	1.92			
Total	52	100%	p-value**		

** :($p \leq 0.01$)

It is noted from the results of Table (5) that there is a highly significant effect of the genotypes of the mutation (C2077G) in the local goat sample at weaning and current weight, in favor of the mutant genotype in both traits (20.70, 34.08) k**:**g. No significant effect was observed for the mentioned mutation in the birth weight trait.

In the Shami goat sample, both birth weight and weaning weight were significantly affected in the genetic variation resulting from the mentioned mutation and in favor of the hybrid genotype (3.04, 22.20) kg over individuals carrying the wild genotype (2.65, 18.12) kg, while the current weight was not affected with the genetic variation mentioned in Table (5). These results were consistent with what **Abbas (2018); Ali (2022)** mentioned in terms of the relationship and importance of the GDF-9 gene in growth traits.

Table (5): Relationship of C2077G mutation polymorphism in GDF-9 gene on the growth traits of local and Shami goat.

Genotype (Local goat)	**Mean ±SE**		
	BWT(Kg)	**WWT(Kg)**	**LBWT(Kg)**
CC	2.64±0.31	15.99±0.24 b	34.08±1.64 b
GG	2.65±0.29	20.70±0.30 a	38.21±1.13 a
p-value	N.S	**	**
Genotype (Shami goat)	Averages ±SE		
	BWT(Kg)	WWT(Kg)	LBWT(Kg)
CC	2.65±0.15 b	18.12±0.33 b	38.59±1.44
CG	3.04 ±0.19 a	22.20 ± 0.45 a	37.34±1.16
p-value	*	*	N.S

**($p \leq 0.01$) ،-*($p \leq 0.05$), (N.S) non-significant.

-Values in columns with different letters differ significantly.

It is noted from Table (6) a significant effect of the genetic variation resulting from the mutation (C2077G), as it was found that there were significant differences in litter size, as individuals carrying the mutant genotype(1.49) Kids/litter, were superior to individuals carrying the wild genotype (1.29 Kids/litter) This result is consistent with what was mentioned by **Al-Jubouri *et al* .(2019)** regarding the importance of the GDF-9 gene in fertility, while no significant effect of the aforementioned mutation appeared on the rest of the milk production traits studied (total milk production, daily milk production, season length.

As for the Shami goat sample, there were significant differences in the litter size and in favor of individuals carrying the hybrid genotype (1.76 Kids/litter) over individuals bearing the wild genotype (1.24Kids/litter), and no effect was shown. Significant in the rest of the traits (Table 6). The current results agreed with the results of the study of **Ali (2022)** that there was no significant effect on milk production traits.

Table (6): The relationship of genetic polymer phism of the mutation (C2077G) in the GDF-9 gene withe total and daily milk production, length of milk season, and Litter size in Local and Shami goats.

Genotype (Local goat)	**Averages ±SE**			
	TMP(Kg)	**DMP(Kg)**	**SL(Day)**	**Litter size (Kids/litter)**
CC	444.15± 5.19	2.14 ± 0.17	207.46± 7.14	1.29±0.07 b
GG	451.35± 5.82	2.10± 0.29	215.14±7.06	1.49±0.09 a
p-value	N.S	N.S	N.S	*
Genotype (Shami goat)	**Averages ±SE**			
	TMP(Kg)	**DMP(Kg)**	**SL(Day)**	**Litter size (Kids/litter)**
CC	488.30±14.07	1.90 ±0.19	257.00 ±9.29	1.24 ±0.17 b
CG	464.18± 19.26	1.93±0.21	240.51±8.13	1.76±0.20 a
p-value	N.S	N.S	N.S	*

-*($p \leq 0.05$), (N.S) non-significant.

-Values in columns with different letters differ significantly.

There are many genes that affect litter size in local goats, including LHX3 and CYP17 gene (**Salam *et al.*, 2022; Abdullah *et al.*, 2023)**.

CONCLUSION

By studying (C2077G) SNP in the GDF-9 gene, we can say that there are broad prospects for the future selection program as well as improvement strategies for farm animals to increase economic return and select individuals that have the best productive performance.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 172-187
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(15)
Al-Hashemi & Al-Janabi

المجلة العراقية لبحوث السوق
وحماية المستهلك

BIOCHEMICAL AND GENETIC IDENTIFICATION OF TWO LOCAL DIACETYL PRODUCER BACTERIAL ISOLATES

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Received 14/ 6/ 2023, Accepted 23/ 8/ 2023, Published 31/ 12/ 2024

ABSTRACT

Four local bacterial isolates (Q3, K7, K2 and K4) were selected that were isolated from Qargoli cheese with the ability to produce diacetyl , all isolates showed a negative result in the catalase test, they fermented glucose when grown at 20 and 37 ºC but all of them were unable to grow at 45 ºC , the isolates K2, K4 and K7 in MRS broth and Q3 in M17 broth tolerated 0.5% and 2% NaCl excepted K7 , isolates K2 and K4 were able to grow at 5% NaCl ,the tested isolates were not tolerated 10% NaCl , isolates have acidic activity in skim milk and all of them gave a white clot that ranged from soft to very soft, isolate K4 ranked first in diacetyl production 59.59, 68.36 and 179.09 µg/ml at of 0.05, 0.1 and 0.15% sodium citrate concentration. Genetic examination proved that isolate Q3 is *Lactococcus lactis strain HANM Diacetyl* and K7 is *Pediococcus pentosaceus strain Bro DiAc*.

Keywords: Diacetyl , Genetic examination, PCR , biochemical test , *Lactococcus lactis , pediococcu pentosaceus*.

الفحوصات الكيموحيوية والتشخيص الجيني لعزلتين بكتيريتين محليتين منتجتين لثنائي الاسيتيل

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الخلاصة

اختيرت أربع عزلات بكتيرية محلية (Q3, K7 , K2 و K4) تم عزلها من جبن القرغولي تميزت بقدرتها على إنتاج ثنائي الأسيتيل ، أظهرت جميع العزلات نتيجة سلبية في اختبار الكاتليز ، و استطاعت العزلات تخمير الجلوكوز عند النمو بدرجة 20 و 37 م° وغير قادرة على النمو في 45 م ، تحملت العزلات K2 و K4 و K7 في الوسط MRS السائل و Q3 في الوسط M17 السائل التركيز 0.5٪ و 2٪ كلوريد الصوديوم فيما عدا K7 ، وكانت العزلات K2 و K4 قادرة على النمو عند 5٪ كلوريد الصوديوم. وجميعها لم تتحمل 10٪ كلوريد الصوديوم ، وكان للعزلات نشاط حامضي في الحليب الفرز وجميعها أعطت خثرة ناعمة بيضاء تراوحت من طرية إلى شديدة الطراوة ، واحتلت العزلة K4 المرتبة الأولى في إنتاج ثنائي الأسيتيل 59.59 و 68.36 و 179.09 ميكروغرام / مل عند تركيز 0.05 و 0.1 و 0.15٪ سترات الصوديوم . أثبت الفحص الجيني أن العزلة Q3 هي *Lactococcus lactis strain HANM Diacetyl* والعزلة K7 *Pediococcus pentosaceus strain Bro DiAc*.

الكلمات المفتاحية: ثنائي الاسيتيل ،التشخيص الجيني ، PCR ، الاختبارات الكيموحيوية ،*Lactococcus lactis , pediococcu pentosaceus*. .

*The article is taken from a master's thesis by the first researcher.

INTRODUCTION

LAB metabolism creates other end-products beside lactic acid with organoleptic properties like improved flavor texture, and/or extended shelf-life (**Kleerebezemab *et al.*, 2000**). Several LAB under different physiological conditions are able to produce acetate, acetaldehyde, acetoin, diacetyl and 2,3-butanediol (**Hugenholtz & Starrenburg, 1992; Bintsis, 2018**). The LAB distribute products are relevant in fermentations. In minutely, diacetyl and acetaldehyde are required flavor compounds in dairy products. Diacetyl is an aromatic compound known for its buttery aroma and taste. It is found in several dairy products, fundamentally butter, margarine, sour cream and some cheeses (**Rincon-Delgadillo *et al.*, 2012; Clark & Winter, 2015**), this compound is synthesized originally by leuconostoc and some lactococci that can metabolize citrate, once a subject of controversy, the biosynthetic pathway of diacetyl is now well known, citrate is transported inside cells by a citrate permease (**Harvey, 1962**), and is then cleaved to acetate and oxaloacetate by citrate lyase. Oxaloacetate is decarboxylated by oxaloacetate decarboxylase, (**Suhad, 2001**) yielding pyruvate.a-Acetolactate synthase transforms pyruvate to acetaldehyde-thiamine pyrophosphate and condenses it with a second molecule of pyruvate to form α-acetolactate. Diacetyl originates from the chemical oxidative decarboxylation of a-acetolactate (**Hugenholtz & Starrenburg, 1992; Verhue, 1991**) and acetoin originates from the decarboxylation of α-acetolactate by a-acetolactate decarboxylase, by reduction of diacetyl by diacetyl reductase, or by nonoxidative chemical decarboxylation (**Hugenholtz, 1993**) ever after lactic acid bacteria produce relatively small quantities of diacetyl (generally less than 5 mg/liter), considerable work has been devoted to increasing its production. Factors such as pH, temperature (**Bassit *et al.*, 1995; Pack, *et al.*, 1967**) citrate concentration (**Petit *et al.*, 1989; Libudzisz &Galewska, 1991**) and oxygen (**Bassit *et al.*, 1993**) have been studied for their effects on diacetyl production. Metabolic engineering strategies have also been proposed to obtain strains producing larger quantities of diacetyl. So It has an antibacterial effect (**Al-Khafaji *et al.*, 2005; Al-Azawi & Al-Khafaji, 1988**).

Besides its natural appearance in dairy products, diacetyl has a high commercial value and it is manufactured for use as a food additive. Starter distillates are also relevant in the formulation of many food products such as cottage cheese and sour cream. The amount of diacetyl in SD ranges from 1.2 to 22,000 µg/g (0.00001–0.22 M). **(Hernandez-Valdes, *et al.*, 2020**)

MATERIALS AND METHODS

Biochemical tests:

The four highly productive isolates of the flavoring compound diacetyl(Q3, K7, K2 and K4) were selected based on the qualitative detection and quantitative estimation of it in a previous study of ours, with the four (Q3,K7,K2 and K4) isolates varying in their productivity and inferred by the culture characteristics of the colonies on the culture media (MRS and M17 at 37 ^{o}C/ 48 hr) anaerobic conditions and the microscopic characteristics, biochemical test was conducted by using inoculum(1.5 x 10^{8} cfu/ml MacFarland method) (Jawan, *et al.*, 2020) that included:

Catalase test:

Add a few drops of hydrogen peroxide (H_2O_2 3%) to the separated colony growing on the (MRS, M17) solid culture medium, the test is considered positive when bubbles are formed. According to (**Ramalingam& Karara, 2011**).

Glucose fermentation test:

Test medium was used (N.B, 1% glucose and 0.004% bromo cresol purple), then inoculated with the bacterial isolate (1.5 x 10^8) cfu/ml at (37 ^oC/ 24 hr /anaerobic conditions). The change in color of the culture medium from violet to yellow in the medium is evidence of a positive test (**Jawan *et al.*, 2020; Ahmaed, 2014**).

Growth test at different temperatures:

The three isolates (K7,K2 and K4) were inoculated on tubes of MRS broth and Q3 isolate on M17 broth incubated at (20, 37, and 45) ^oC for 24-48 hr , the appearance of growth with the change of color from violet to yellow and compared with the control tube without inoculum the medium is evidence of a positive test.

Test of growth in different concentrations of NaCl:

The tubes containing MRS and M17 broth were inoculated with 3 isolates and 1 isolate (1.5 x 10^8) cfu/ml respectively using different concentrations of NaCl (0.5, 2.0, 5.0, and 10%) w/v in the presence of bromo cresol purple (0.004%), the appearance of turbidity and a change in the color of the indicator from violet of yellow compared to the control tube (without inoculum) is the positive result (Jawan *et al.*, 2020).

Acidity activity:

Tubes containing skim milk 10% (w/v) were inoculated with (1.5 x 10^8) cfu/ml of culture. It was incubated at 37 C^O, with a change in the incubation period for each isolate (24, 48 and 72) hr. Acidity is tested by changing the pH using a pH-meter, clot, change in color, flavor, and aroma.

Standard curve of diacetyl

By adding different volumes of a standard diacetyl compound in test tubes, and completing the volume to 10 ml by distilled water, the absorbance of all tubes was measured using a spectrophotometer at 540 nm(table 1). Only Blank's solution of distilled water was used to zero the device at the same wavelength.

Table (1): Absorbance readings and corresponding concentrations for the diacetyl compound

No. tube	Conc. of standard diacetyl (µg/mL)	Absorbance (540 nm)
1	2.5	0.003
2	5	0.012
3	10	0.030
4	20	0.150
5	50	0.370
6	100	0.804
7	150	1.198
8	200	1.602

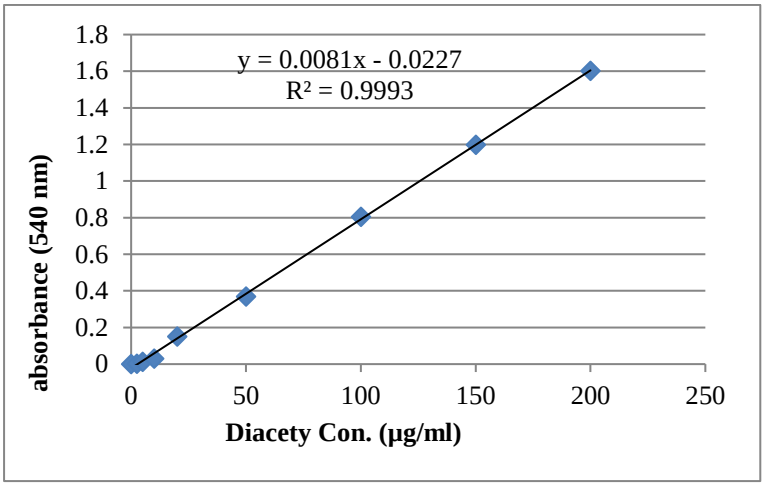

Figure (1): The standard curve of diacetyl.

THE ABILITY TO CONSUME CITRATE:

Addition of citrate at different concentrations to skim milk:

Different concentrations of sodium citrate (0.05%, 0.1%, 0.15%, 0.2% and 0.25%) were added to 100 ml of sterilized skim milk in test tubes. Each tube was inoculated with (1.5×10^8 cfu/ml) of the isolate. It was incubated at 37C°/24-48 hr , and its ability to consume citrate was tested. Then centrifugation was conducted and the absorbance was read at 540 nm (**Asmaa *et al.*, 2016**).

Addition of citrate at different concentrations to the broth medium:

Different concentrations of Sodium Citrate (0.05%, 0.1%, 0.15%, 0.2%, 0.25%) were added to 100 ml of the two broth media (M17 and MRS) according to the medium in which the isolate was grown, each tube was inoculated with (1.5×10^8) cfu/ml of culture was incubated at 37C°/24-48 hr . Then centrifugation was conducted and the absorbance was read at 540 nm.

Genetic identification:

The genetic identification of the two isolates Q3 and k7, characterized by higher productivity of diacetyl and distinct appearance in the biochemical test was carried out as follow:

DNA extraction:

Genomic DNA was isolated from bacterial growth according to the protocol of ABIOpure Extraction as the following steps:

- For pellet cells, 1ml of overnight culture for 2min at 13000 rpm. The supernatant was then discarded.
- For protein digestion and cell lysis, 20µl of Proteinase K solution (20 mg/ml) was added to 200µl of Buffer BL and cell pellet, then the tube was mixed vigorously using vortex and incubated at 56˚C for 30 min.

- From absolute ethanol 200µl was added to the sample, pulse-vortex to mix the sample thoroughly.
- All of the mixtures were transferred to the mini- column carefully, then centrifuged for 1 min at 6,000 x g above (>8,000 rpm), and the collection tube was replaced with a new one.
- From Buffer BW 600µl was Added to the mini- column, then centrifuged for 1 min at 6,000 x g above (>8,000 rpm) and the collection tube was replaced with a new one.
- From Buffer TW 700µl was applied. Centrifuged for 1 min at 6,000 x g above (>8,000 rpm). The pass-through was discarded and the mini- column was reinserted back into the collection tube.
- The mini-column was Centrifuged at full speed (>13,000 x g) for 1 min to remove residual wash buffer, then the mini-column was placed into a fresh 1.5 ml tube.
- From Buffer AE 100µl was added and incubated for 1 min at room temperature, then centrifuged at 5,000 rpm for 5min.

Quantitation of DNA:

Quantus Fluorometer was used to detect the concentration of extracted DNA to detect the goodness of samples for downstream applications. For 1 µl of DNA, 200µl of diluted Quantifluor Dye was mixed. After 5min incubation at room temperature, DNA concentration values were detected for both isolates Q3 and K7. The equation below was adopted to determine the purity:

DNA purity = (photo absorption at 260 nm) / (photo absorption at 280 nm)

Primer preparation:

Table (2): Preparation of primer.

Primer Name	Vol. of nuclease free water (µl)	Concentration(pmol/µl)
27F	300	100
1492R	300	100

These primers were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution. A working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 ºC) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl.

Reaction setup and thermal cycling protocol:

Table (3): PCR Component Calculation.

No. of Reaction	2	Rxn	Annealing temperature of primers	60
Reaction Volume /run	25	µl	No. of PCR Cycles	30

Amplification 16S RRNA:

Polymerase chain reaction (PCR) technology was used to amplify the *16S rRNA* gene of bacteria in order to confirm the type of the selected isolate using the following universal primer (4) according to what was stated in (**Sacchi *et al.*, 2002**).

Table (4): Primers used to amplify the *16S rRNA* gene by PCR.

Primer Name	Sequence	Annealing Temp. (ºC)	Product Size (bp)
27F	5`-AGAGTTTGATCCTGGCTCAG-3`	60	1500 bp
1492R	5`-TACGGTTACCTTGTTACGACTT-3`		

The amplification was 25 µl, and the Master Mix prepared by the American company Promega was added according to table (5) to the Eppendorf tube as follows:

Table (5): Materials added and their quantities to the reaction tube to amplify the 16SrRNA gene in PCR technology.

Master mix Components	Stock	Unit	Final	Unit	Volume 1 Sample
Master Mix	2	X	1	X	12.5
Forward primer	10	µM	0.5	µM	1
Reverse primer	10	µM	0.5	µM	1
Nuclease Free Water					8.5
DNA		ng/µl		ng/µl	2
Total volume					25
Aliquot per single rxn	23µl of Master mix per tube and add 2µl of Template				

The tube was transferred to a PCR device (thermal cycler) and the program shown in Table (6) was used for the purpose of amplifying the gene (*16S rRNA*) according to what was stated in (**Shripama *et al.*, 2013**) with some minor modifications.

Table (6): Phases of the PCR process After the end of the time allotted for the reaction, 5 µl of the amplification products of the *16S rRNA* gene were withdrawn to be used for electrophoresis.

Steps	Temperature	m: s	Cycles
Initial Denaturation	95°C	05:00	1
Denaturation	95°C	00:30	30
Annealing	60°C	00:30	
Extension	72°C	01:00	
Final extension	72°C	07:00	1
Hold	10°C	10:00	

Agarose gel electrophoresis:

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria. Solutions :1 X TAE buffer, DNA ladder marker, Ethidium bromide (10mg / ml)).

Preparation of agarose:

100 ml of 1X TAE was taken in a flask. 1.5 gm (for 1.5%) agarose was added to the buffer. The solution was heated to boiling (using Microwave) until all the gel particles were dissolved. 1µl of Ethidium Bromide (10mg/ml) was added to the agarose. The agarose was stirred in order to get mixed and to avoid bubbles. The solution was left to cool down at 50-60°C.

Casting of the horizontal agarose gel:

The agarose solution was poured into the gel tray after both the edges were sealed with cellophane tapes and the agarose was left to solidify at room temperature for 30 minutes. The comb was carefully removed, and the gel was placed in the gel tray. The tray was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of the gel, as stated in (**Michael & Sambrook, 2012**).

DNA loading:

PCR products were loaded directly. For the PCR product, 5µl was directly loaded into the well. Electrical power was turned on at 100v/mAmp for 60min. DNA moves from Cathode to plus Anode poles. The Ethidium bromide-stained bands in gel were visualized using Gel imaging system.

Standard sequencing:

PCR products were sent for Sanger sequencing using ABI3730XL, an automated DNA sequencer, by Macrogen Corporation – Korea. in order to determine the sequences of nitrogenous bases, and these sequences were adopted in comparison with the available information about the amplified gene in the NCBI Gen Bank (National Center for

Biotechnology Information) and in light of the website according to the BLAST Nucleotide program for the purpose of identifying the type of the selected isolate.

RESULTS AND DISCUSSION

Biochemical tests: catalase test:

The test results (Table 7) showed that the catalase-negative isolates did not form bubbles. Therefore, we infer the absence of the catalase enzyme in these isolates because are unable to decompose H_2O_2 to water and oxygen as figure (2), agree with **(Khalil, 2020**).

Table (7): Catalase test.

Isolates	Result
Q3	-
K7	-
K2	-
K4	-

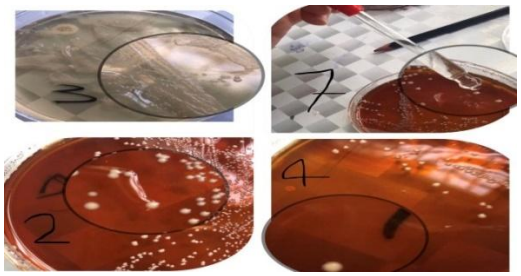

Figure (2): Add H_2O_2 (3%) to the bacterial isolate's colonies on MRS and M17 medium.

Glucose fermentation test:

The results of table (8) shows the ability of four bacterial isolates (Q3,K7,K2 and K4) glucose fermentation by changing the color of the culture medium in the presence of an indicator that gives a yellow color at the acidic pH , so the color of control did not change due to the absence of inoculum, this agree with (**Al-dosari, 2002**).

Table (8): Glucose fermentation test result

Isolates	**Result**
Q3	+
K7	+
K2	+
K4	+
Control	-

Growth test at different temperatures:

The results of table (9) show the ability growth of four bacterial isolates (Q3,K7,K2 and K4) at 20,37 and 45 ^oC with some difference between them (20 ^oC), it was found that the isolates have the ability to grow at t 20 ^oC and 37 ^oC , and they could not grow at 45 ^oC , the best result we got at 37 ^oC .

Table (9): Growth of isolates at different temperatures.

Isolates	20 ºC	37 ºC	45 ºCº
Q3	+	+	-
K7	+-	+	-
K2	+	+	-
K4	+	+	-
Control	–	–	-

(+-) poor growth , (+) growth ,(-) no growth , Control (C) without inoculum

Test of growth in different concentrations of Nacl:

That all isolates cannot grow in concentration 10% NaCl , but they can grow in 0.5% NaCl while isolate K7 grew only in 0.5%NaCl , isolates K7 and K4 were able to grow in 5% NaCl.

Table (10): The ability of bacteria to grow and tolerate different concentrations of NaCl.

Isolates	**0.5% NaCl**	**2% NaCl**	**5% NaCl**	**10% NaCl**
Q3	+	+	-	-
K7	+	-	-	-
K4	+	+	+	-
K2	+	+	+	-

Acidity activity:

The color of the clot formed (white) by the acidity caused by bacteria did not change through 24,48 and 72 hr at the same temperature (37 ºC). The control treatment was the highest in the pH during the three time periods (24, 48 and 72) hr (6.88, 6.75 and 6.60) respectively, noting that pH of all treatments decreased with the progression of the time period. As for the curd it was soft and tender, except clot of isolate K2 which was distinguished by its stuffiness at 37 ºC /72hr, with acidic flavor also clot of isolate K4 it was an acidic.

Table (11): The ability of the isolates to produce acid on the skim milk medium.

24 hr/37 Cº					**48 hr/37 Cº**				**72 hr/37 Cº**			
Iso.	color	pH	Clot	Flavor	color	pH	Clot	Flavor	color	pH	Clot	Flavor
Q3	White	4.97	Soft	Mild acidity	White	4.64	Soft	Mild acidity	White	4.16	Soft	Acidic
K7	White	6.35	-	-	White	6.00	-	-	White	5.61	Soft	Mild acidity
K2	White	5.40	Very soft	Very mild acidity	White	4.92	Soft	Mild acidity	White	4.35	Solid clot	Acidic
K4	White	5.29	Very soft	Very mild acidity	White	4.57	Soft	Mild acidity	White	4.09	Soft	Acidic
Control	White	6.88	-	-	white	6.75	-	-	White	6.60	-	-

(-): no clot and not change flavor.

The ability to consume citrate:

Addition of citrate at different concentrations to skim milk:

The isolate K4 ranked first among the tested isolates in the production of diacetyl at first and third concentrations reached 59.59, 179.09 µg/ml, with superiority of isolate K2 at conc. 0.1% and conc. 0.2 % 98.48 and 184.03 µg/ml while the productivity of the four isolates decreased at the fifth conc. 0.25%.

Table (12): The ability of isolates to consume citrate added to skim milk.

	0.05%		0.1%		0.15%		0.2 %		0.25%	
Iso.	540 nm	Conc. DA. µg/ml	540 nm	Conc. DA. µg/ml	540 nm	Conc. DA. µg/ml	540 nm	Conc. DA. µg/ml	540 nm	Conc. DA. µg/ml
Q3	0.245	33.05	0.145	20.70	0.165	23.17	0.311	41.20	0.126	18.36
K7	0.212	28.98	-	-	0.956	120.83	1.302	163.54	-	-
K2	0.279	37.25	0.775	98.48	0.317	41.94	1.468	184.03	0.403	52.56
K4	0.460	59.59	0.531	68.36	1.428	179.09	1.011	127.62	0.323	42.68

(DA.): Diacetyl ,(-) : can't produce diacetyl

Addition of citrate at different concentrations to the broth medium:

It is clear from the results of table (13) that the addition of certain concentrations of sodium citrate to different media, it was found that the ability of bacteria to consume citrate is better when the culture medium is skim milk.

Table (13): The ability of isolates to consume citrate added to (MRS and M17) medium at different concentrations.

		0.05%	0.1%		0.15%	
Isolates	Abs. 540nm	Conc. DA. µg/ml	Abs. 540nm	Conc. DA. µg/ml	Abso. 540nm	Conc. DA. µg/ml.
Q3	0.151	21.44	0.216	29.47	0.157	22.19
K7	0.163	22.93	-	-	-	-
K2	-	-	-	-	-	-
K4	0.156	22.06	-	-	-	-

	0.2%		0.25%	
Isolates	Abs. 540 nm	Conc. DA. µg/ml.	Abs. 540 nm	Conc. DA. µg/ml.
Q3	0.387	50.58	0.336	44.28
K7	0.237	32.06	-	-
K2	0.150	21.32	-	-
K4	0.206	28.23	-	-

(DA.): Diacetyl ,(-) : can't produce diacetyl ,(Q3): on M17 broth,(K7,K2 and K4): on MRS broth.

Genetic identification:

The results obtained for the two isolates after electrophoresis of the amplified gene showed that one bundle representing the *16S rRNA* gene appeared in both isolates (Q3, K7) Figure (3), this indicates the success of the binding process between the primers and the target gene, which is the *16S rRNA* gene, without being bound to parts. The rest of the DNA extracted from the isolates to be diagnosed. The molecular size of the amplification products was estimated, and the gene size was 1500 base pairs from both isolates compared to the size of the

guide (ladder) used in electrophoresis along with the test samples. (**Jebur & Auda 2020; Abbas *et al.*, 2020**) also used the genetic diagnosis of a local isolate.

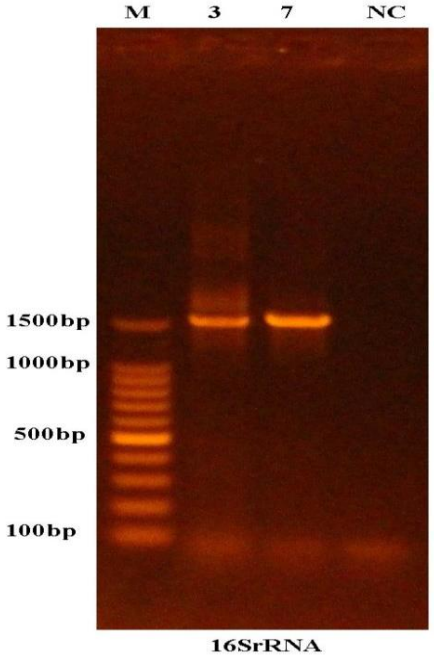

Figure (3): Results of the amplification of *16s RNA* gene of Unknown bacterial species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 3-7 resemble 1500bp PCR products.

The *16S rRNA* gene gives crucial results in diagnosis and is used to distinguish between different types of bacteria (**Naser *et al.*, 2013; Balcázar *et al.*, 2007**).

Analysis of amplification product sequences:

Table (14): FASTA of the *16S rRNA* amplified gene of isolate Q3.

Gene	The nitrogen base sequence of the 16S rRNA gene	The total number of nitrogenous bases
1_27F *16S rRNA*	AAAGTCTGACCGAGCACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTG GTAGAGAAGAACGTTGGTGAGAGTGGAAAGCTCATCCAGTGACGGTAACTACCCAGAAA GGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGA TTTATTGGGCGTAAAGCGAGCGCAGGTGGTTTATTAAGTCTGGTGTAAAAGGCAGTGGCT CAACCATTGTATGCATTGGAAACTGGTAGACTTGAGTGCAGGAGAGGAGAGTGGAATTCC ATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCT GGCCTGTAACTGACACTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG GTAGTCCACGCCGTAAACGATGAGTGCTAGATGTAGGGAGCTATAAGTTCTCTGTATCGCA GCTAACGCAATAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC CTTACCAGGTCTTGACATACTCGTGCTATTCCTAGAGATAGGAAGTTCCTTCGGGACACGG GATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC	1057

	AACGAGCGCAACCCCTATTGTTAGTTGCCATCATTAAGTTGGGCACTCTAACGAGACTGCC GGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGG CTACACACGTGCTACAATGGATGGTACAACGAGTCGCGAGACAGTGATGTTTAGCTAATCT CTTAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGC TAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACACCACGGGAGTTGGGAGTACCCGAAGTAGG	

Table (15): FASTA of the *16S rRNA* amplified gene of isolate K7.

Gene	The nitrogen base sequence of the 16S rRNA gene	The total number of nitrogenous bases
1_27F 16S rRNA	TGATTATGACGTACTTGTACTGATTGAGATTTTAACACGAAGTGAGTGGCGAACGGGTGA GTAACACGTGGGTAACCTGCCCAGAAGTAGGGGATAACACCTGGAAACAGATGCTAATA CCGTATAACAGAGAAAACCGCATGGTTTTCTTTTAAAAGATGGCTCTGCTATCACTTCTGG ATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCAGTGATACG TAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACG GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGT GAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACGTGGGTAAGAG TAACTGTTTACCCAGTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGC CGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAG GCGGTCTTTTAAGTCTAATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATTGGAAACT GGGAGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAG ATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGC TCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGA TTACTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGTAATCCGC CTGGGGAGTACGACCGCAAGGTTGAAACTCAAAAGAATTGACGGGGGCCCGCACAAGCG GTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTCT GACAGTCTAAGAGATTAGAGGTTCCCTTCGGGGACAGAATGACAGGTGGTGCATGGTTG TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTACT AGTTGCCAGCATTAAGTTGGGCAGTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGG TGGGGACGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGG ATGGTACAACGAGTCGCGAAACCGCGAGGTTAAGCTAATCTCTTAAAACCATTCTCAGTTC GGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCA TGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGT AACACCCAAAGCCGGT	1393

The results were analyzed by BLAST program to find similarity or genetic match with the NCBI Gen Bank, as the results showed that there was a match in the sequences of the nitrogenous bases of the amplified *16S rRNA* gene of the local isolate S2 with the sequences of the nitrogenous bases of the same gene for several strains of *Lactococcus lactis* and *pediococcu pentosaceus* registered in the NCBI bank. NCBI genes and *Lactococcus lactis* match rate was 99.91% with a strain belonging to this bacteria. The match rate was 99.81% with more than 80 other strains from the same isolate (Table 16), so the local isolate Q3 was considered to belong to the bacterium *Lactococcus lactis*, while the match was for the results of the nitrogen base sequences of the amplified *16S rRNA* gene of the local isolate K7 with the nitrogen base sequences of the same gene and other strains Also numerous of *pediococcu pentosaceus*

bacteria isolated from other sources and also registered in NCBI and the match rate was 99.93% (Table 17).

Table (16): Matching percentage of the sequences of the nitrogenous bases of isolate Q3 for several strains of *Lactococcus lactis* registered at the National Center for Biotechnology Information NCBI.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lactococcus lactis strain HANM Diacetyl 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1953	1953	100%	0.0	100.00%	1057	OQ712009.1
Lactococcus lactis partial 16S rRNA gene, strain L-4	Lactococcus lactis	1947	1947	100%	0.0	99.91%	1462	LT853603.1
Lactococcus lactis subsp. lactis strain LM 0404 16S ribosomal RNA gene, partial sequence	Lactococcus lactis subsp. lactis	1941	1941	100%	0.0	99.81%	1453	OQ569384.1
Lactococcus lactis subsp. lactis strain LM 0403 16S ribosomal RNA gene, partial sequence	Lactococcus lactis subsp. lactis	1941	1941	100%	0.0	99.81%	1458	OQ569383.1
Lactococcus lactis subsp. lactis strain LM 0306 16S ribosomal RNA gene, partial sequence	Lactococcus lactis subsp. lactis	1941	1941	100%	0.0	99.81%	1456	OQ569380.1
Lactococcus sp. strain L1-2CT-A-OTU36MT(U71) 16S ribosomal RNA gene, partial sequence	Lactococcus sp.	1941	1941	100%	0.0	99.81%	1412	OM164537.1
Lactococcus sp. strain P1-5CT-J-OTU36MT(U07AKAV70) 16S ribosomal RNA gene, partial ...	Lactococcus sp.	1941	1941	100%	0.0	99.81%	1412	OM164513.1
Lactococcus sp. strain P1-19CT-B-OTU36MT(R28) 16S ribosomal RNA gene, partial seque...	Lactococcus sp.	1941	1941	100%	0.0	99.81%	1413	OM164429.1
Lactococcus lactis strain M34 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1941	1941	100%	0.0	99.81%	1431	OQ225229.1
Lactococcus lactis strain M31 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1941	1941	100%	0.0	99.81%	1433	OQ225199.1
Lactococcus lactis strain M33 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1941	1941	100%	0.0	99.81%	1422	OQ225197.1
Lactococcus lactis strain M3 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1941	1941	100%	0.0	99.81%	1429	OQ224981.1
Lactococcus lactis strain M2 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1941	1941	100%	0.0	99.81%	1432	OQ224980.1
Lactococcus lactis strain M1 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1941	1941	100%	0.0	99.81%	1430	OQ216739.1
Lactococcus lactis strain VHProbi V60 chromosome, complete genome	Lactococcus lactis	1941	11640	100%	0.0	99.81%	2416269	CP110849.1
Lactococcus lactis strain LL15 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1941	1941	100%	0.0	99.81%	1454	OQ002429.1
Lactococcus lactis strain LL14 16S ribosomal RNA gene, partial sequence	Lactococcus lactis	1941	1941	100%	0.0	99.81%	1447	OQ002428.1

Table (17): Matching ratio of the sequences of nitrogenous bases of isolate K7 from strains of *pediococcu pentosaceus* registered at the National Center for Biotechnology Information NCBI

		Score	Score	Cover	value	Ident		
Pediococcus pentosaceus strain Bro DiAc 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2573	2573	100%	0.0	100.00%	1393	OQ714402.1
Pediococcus pentosaceus strain 4942 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1488	MT512069.1
Pediococcus pentosaceus strain HBUAS1-3 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1499	MT000126.1
Pediococcus pentosaceus strain MG5316 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1447	MN704679.1
Pediococcus pentosaceus strain L25 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1452	MN638797.1
Pediococcus pentosaceus strain L18 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1446	MN638790.1
Pediococcus pentosaceus strain L1 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1457	MN638773.1
Pediococcus pentosaceus strain IAH_35 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1458	MK990078.1
Pediococcus pentosaceus strain OCPP3 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1492	MK605953.1
Pediococcus pentosaceus strain 24 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1423	MH229959.1
Pediococcus pentosaceus strain HBUAS56024 16S ribosomal RNA gene, partial seque...	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1499	MK396642.1
Pediococcus pentosaceus strain S3c 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1433	MH169740.1
Pediococcus pentosaceus strain ACD43-3 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1454	MH111691.1
Pediococcus pentosaceus strain ACD43-1 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1447	MH111689.1
Pediococcus sp. strain CAU504 16S ribosomal RNA gene, partial sequence	Pediococcus sp.	2567	2567	100%	0.0	99.93%	1438	MF424971.1
Pediococcus pentosaceus strain CAU7108 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1439	MF423947.1
Pediococcus pentosaceus strain CAU 229 16S ribosomal RNA gene, partial sequence	Pediococcus pentosaceus	2567	2567	100%	0.0	99.93%	1481	MF369881.1
Pediococcus sp. strain CAU:1799 16S ribosomal RNA gene, partial sequence	Pediococcus sp.	2567	2567	100%	0.0	99.93%	1416	MF354845.1

Recording local isolates globally:

Based on the results of the analysis and matching of the aforementioned nitrogenous base sequences ,the local isolates under study were registered in the NCBI Gen Bank and named the isolate Q3 isolated from Qargoli cheese and given the symbol *Lactococcus lactis strain HANM Diacetyl*, in the National Center for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/nuccore/OQ712009 .

while the isolate K7 isolated from cheese was named Al-Qargoli K was also given the symbol *Pediococcus pentosaceus strain Bro DiAc*, the National Center for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/nuccore/OQ714402 . (**Khalil, 2020**) also, two local isolates of lactic acid bacteria were recorded in the GenBank.

CONCLUSION

The isolates isolated from Qarghouli cheese Q3and K7 new isolates that produce a diacetyl compound and are safe for their application in food and medicine,it was showed good productivity of the diacetyl compound used in the food industry as a flavoring agent and a preservatives agent because it has antimicrobial properties, It is also known that lactic acid bacteria produce compounds with many benefits of *lactobacillus* bacteria and applied them in the production of therapeutic milk products. The PCR technique was used to identify the isolates, and it is considered the accurate method in the identification of genes.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 188-199
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(16)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

ANTIOXIDANTS ACTIVITY IN STEVIA PLANT AS EFFECTED BY SHADING AND FOLIAR SPRAYING OF MORINGA AND LIQUORICE EXTRACT

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Received 14/ 6/ 2023, Accepted 2/ 10/ 2023, Published 31/ 12/ 2024

ABSTRACT

A field experiment was conducted in the fields of the College of Agricultural Engineering Sciences/University of Baghdad for the year 2021-2022 to study the effect of shading and the foliar application of some plant extracts on the growth, yield and natural antioxidants activity in the Stevia plant leaves. The experiment was conducted using a randomized complete block design (RCBD) using a split-plot arrangement with three replicates. The results showed that S_1 significantly outperformed the leaves' dry weight by 41.49 g plant^{-1} and leaves' dry weight per hectare by 6.88 tons ha^{-1}. On the other hand, S_2 showed significant superiority in the total antioxidant activity by 11000.20 mg L^{-1}, total phenols by 9.947 mg L$^{-1,}$ and leaves the chlorophyll b content by 76.16 mg 100 g^{-1}. Furthermore, M_2 significantly outperformed in the dry weight of the leaves with 38.21 g plant^{-1}, followed by G_2 with 36.30 g plant^{-1}.

Similarly, the dry weight of the leaves per hectare reached 6.33 tons ha^{-1} in the M_2, followed by G_2 with 6.13 tons ha^{-1}. G_2 also excelled in total antioxidant activity with 12852.50 mg L^{-1}, while M_1 showed significant superiority in total phenols with 16135 mg L^{-1}. M_1 also showed a higher chlorophyll concentration with 189.45 mg 100 g^{-1}, followed by G_1 with 188.55 mg 100 g^{-1} and M2 with 180.50 mg 100 g^{-1}. The liquorice extract (G_2) exhibited the highest overall antioxidant activity and chlorophyll b when interacting with shaded plants. Additionally, the Moringa extract (M_1) contributed to a significant increase in total phenols in shady Stevia plants.

Keywords: Stevia plant, Shading, Antioxidants, Plant extracts.

نشاط مضادات الأكسدة في نبات الستيفيا بتأثير التظليل والرش الورقي لمستخلص المورينجا وعرق السوس

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الخلاصة

زرعت شتلات نبات ورق السكر (الستيفيا) والناتجة من الزراعة النسيجية للصنف الاسباني Spanti في منطقة الجادرية– بغداد بتاريخ 15-3- 2021 بهدف دراسة تأثير دور التظليل وبعض المستخلصات النباتية في نمو وحاصل الاوراق ومحتواها من مضادات الاكسدة الطبيعية في نبات ورق السكر (الستيفيا). نفذت تجربة حقلية في حقول كلية علوم

*The article is taken from a master's thesis by the first researcher.

الهندسة الزراعية / جامعة بغداد للعام 2021-2022، وفقاً لتصميم القطاعات الكاملة المعشاة (RCBD) ضمن ترتيب الألواح المنشقة بثلاثة مكررات. اظهرت النتائج تفوق S_1 معنويا في الوزن الجاف للأوراق 41.49 غم نبات$^{-1}$ والوزن الجاف بالهكتار 6.88 طن ه$^{-1}$، أما S_2 فقد تفوق معنويا في الفعالية الكلية لمضادات الأكسدة 11000.20 ملغم لتر$^{-1}$ والفينولات الكلية 9947 ملغم لتر$^{-1}$ ومحتوى الأوراق من كلوروفيل b الذي بلغ 76.16 ملغم 100 غم$^{-1}$ وزن رطب. كما تفوق M_2 معنويا في الوزن الجاف للأوراق بلغ 38.21 غم نبات$^{-1}$ يليه G_2 بلغ 36.30 غم نبات$^{-1}$ وحاصل الوزن الجاف للأوراق بلغ 6.33 طن ه$^{-1}$ يليه G_2 بلغ 6.13 طن ه$^{-1}$، وتفوق G_2 في الفعالية الكلية لمضادات الاكسدة 12852.50 ملغم لتر$^{-1}$، اما الفينولات الكلية فقد تفوق مستخلص M_1 وبلغ 16135 ملغم لتر$^{-1}$. كذلك ظهر تفوق M_1 في محتوى الأوراق من كلوروفيل a الذي بلغ 189.45 ملغم 100 غم$^{-1}$ وزن رطب يليه G_1 بلغ 188.55 ملغم 100غم$^{-1}$ وزن رطب وM_2 بلغ 180.50 ملغم 100 غم$^{-1}$ وزن رطب. حقق رش مستخلص عرق السوس G_2 أعلى فعالية كلية لمضادات الاكسدة ومحتوى الأوراق من كلوروفيل b بالتداخل مع النباتات المظللة، وساهم مستخلص المورينجا M_1 في اعطاء على نسبة للفينولات الكلية لنباتات الستيفيا المظللة.

الكلمات المفتاحية: نبات الستيفيا ، التظليل ،مضادات الاكسدة ،المستخلصات النباتية.

INTRODUCTION

Sugar leaf (*Stevia rebaudiana* bertoni) is a perennial herbaceous plant that can reach a height of 60-70 cm and sometimes up to 1 m. Stevia belongs to the Asteraceae family. The genus Stevia includes more than 230 species, but only two of them, *Phlebophylla* and *Rebaudiana*, contain the steviol glycosides compound **(Lemus-Mondaca *et al.*, 2012).** The native habitat of the sugar leaf plant is South America. It was first discovered in Paraguay in 1887 and later spread as a major cash crop to China, Japan, Pakistan, India, and the rest of the world, including Spain and America (**Barroso, 2018).** Sugar leaf (Stevia) was recently introduced to Iraq and planted for research purposes in the College of Agricultural Engineering fields in 2017, both in shaded plastic houses and in the open field **(Abdul-Qader *et al.*, 2022).** In the 1960s, a crystalline white compound was extracted from this plant, and it was found that the crystalline compound is 300 times sweeter than sugar without having a negative effect on blood sugar levels **(Kondak *et al.*, 2018).** Stevia extract can be used in various food industries, including beverages **(Al-Hamdani, 2020)**, making it ideal for use by diabetic patients, individuals with chronic diseases, and healthy individuals in dietary programs to lower blood sugar, cholesterol, and total fat levels **(Al-Hamdani, 2019).** The medical importance of the sugar leaf plant lies in its containing several natural antioxidants and its medicinal role in patients with high blood pressure, cancer, and its antimicrobial and antifungal properties **(Al-Hamdani *et al.*, 2019**). Stevia leaves are a source of diterpene glycosides, with stevioside being the main sweetening compound present in Stevia leaves at a percentage of 5-15%, followed by rebaudioside at 3-6%, and other Stevia glycosides exhibit high chemical stability due to their three-dimensional chemical structure, which provides resistance to acid and enzymatic degradation, ensuring their stability in terms of biochemical and physiological aspects **(Mishra *et al.*, 2010).** Stevia leaves also contain other compounds such as phenols, flavonoids, terpenes, coumarins, saponins, quinones, and other oils (**Al-Hamdani, 2020; Al-Taweel *et al.*, 2022)**. Due to its sweetness, nutritional value, and the medicinal compounds present in Stevia plant, it has become an important and essential crop soon **(Al-Taweel *et al.*, 2021)**, and the total global trade of Stevia sweeteners exceeded $771 million in 2022 **(Schieber, 2017).** Plant extracts have been successfully used as organic fertilizers due to their containing biologically active compounds that stimulate plant growth and improve nutritional

status without harmful effects on many crops **(Hoque *et al*., 2021).** Moreover, **Al-Temimi & Al-Hilfy (2022)** indicated that plant extracts contain natural growth regulators such as auxins, gibberellins, and cytokinins. The study aims to investigate the role of shading as a primary factor and adding aqueous extracts of Moringa leaves and Licorice root extract as a secondary factor in improving growth, yield of dry leaves, and their content of natural antioxidants.

MATERIALS AND METHODS

A field experiment was conducted in the fields of the College of Agricultural Engineering Sciences / University of Baghdad in the spring season of 2021. Randomized Complete Bock Design (RCBD) with three replications was used using split plot arrangement. The experiment included two factors, the main plots included two levels of shading, the first without shading (exposed) and the second 50% shading and they are denoted S_1 and S_2, respectively, while the s sub-plots included the spraying of aqueous plant extracts of Moringa at a rate 10 and 15% **(Yasmeen *et al*., 2013),** denoted M_1 and M_2 respectively, and licorice extract at a rate 3 and 6% **(Hussein, 2002),** denoted G_1 and G_2 respectively with an interval of 15 days between the time of each spray, in addition to the M_0G_0 as control treatment which sprayed with distilled water in three sprays.

The experimental units were distributed with a total of 30 experimental units resulting from the experimental factors (2 × 5 × 3), with 10 experimental units for each replication. Each experimental unit consisted of 18 plants. The plants were cultivated with a spacing of 20 cm between each plant and 30 cm between each row **(Abdul-Qader *et al*., 2022).** When the plant reached a height of approximately 60 cm after 60 days of cultivation, the plant was stuffed and the chlorophyll rate (a, b and total) was calculated from the fresh leaves. Also, the leaf yield was collected for drying. The leaves were placed on a table at room temperature ranging from 25-30°C. and the leaves were stirred daily until they reached a stable weight and completely dried **(Al Amrani *et al*., 2018)**

CHARACTERS UNDER STUDY

1. Dry weight of plant leaves (g plant^{-1}): Selected plants were naturally dried in a special ventilated room with daily turning until reaching a constant weight and complete leaf dryness, then the leaves were weighed and the means was calculated.

2. Dry leaf yield (ton ha^{-1}): It was calculated from the following equation:

Dry leaf yield (ton ha^{-1}) = Dry leaf weight (g plant^{-1}) x Plant density (plants ha^{-1}) / 10^6

3. Total antioxidant activity (mg L^{-1}): The antioxidant capacity of the leaves was estimated based on the equivalent of ascorbic acid using the spectrophotometric method according to the method of (**Prieto, 1999).**

4. Total phenols (mg L^{-1}): The total phenols in Stevia plant leaves were estimated based on the gallic acid equivalent using a spectrophotometer according to the method described by **(Singleton *et al*., 1999).**

5. Chlorophyll content in leaves (mg 100 g^{-1} fresh weight): Chlorophyll (a, b and total) was calculated using the developed method by **Goodwin (1976)** and the following equations: Chlorophyll a (mg L^{-1}) = 12.7 D(663) - 2.69 D(645)

Chlorophyll b (mg L^{-1}) = 22.9 D (645) - 4.68 D (663)
Total chlorophyll (mg L^{-1}) = 20.2 D (645) + 8.02 D (663)
The results were converted to mg $100g^{-1}$ fresh weight by the following equation:
mg 100 g^{-1} fresh weight = mg L^{-1} x final volume of extract (L) x 100 / sample weight (g)
The data were statistically analyzed using the Genstat program, and the least significant difference (LSD) test at 0.05 probability level was used to compare between means **(Steel & Torrie, 1980).**

RESULTS AND DISCUSSION

Leaf dry weight (g $plant^{-1}$)

The results reveal significant differences between the studied factors and significant interaction between two factors in the dry leaf weight of the plant (Table 1). The results demonstrate a significant decrease in the mean of S_2 compared to S_1, with a percentage decrease of 37.18%. Furthermore, results also indicate a significant superiority of M_2 treatment (38.21 g $plant^{-1}$) over the means of M_1, G_2 and control treatment with percentages of 13.24, 15.12 and 39.19%, respectively, then following by G_2 treatment (36.30 g $plant^{-1}$).

Table (1): Effect of shading and some plant extracts on the leaves' dry weight (g $plant^{-1}$)**.**

Plant extracts	Shading		Mean
	S_1	S_2	
M_0G_0	32.67	22.23	27.45
M_1G_0	42.17	25.31	33.74
M_2G_0	47.47	28.94	38.21
M_0G_1	40.61	25.77	33.19
M_0G_2	44.54	28.05	36.30
$LSD_{0.05}$	3.08**		2.18*
Mean	41.49	26.06	
$LSD_{0.05}$	**2.57****		

The results of the Table 1 showed a significant interaction, as the highest combination was recorded between S_1 and M_2, amounted to 47.47 g $plant^{-1}$, followed by the S_1 and G_2 which amounted to 44.54 g $plant^{-1}$, with non-significant difference between them, and the lowest leaf dry weight was recorded between S_2 and the control treatment (22.23 g $plant^{-1}$).

Stevia plant stress resulting from low light affects the biomass in the plant due to a decrease in the plant's photosynthetic capacity and changes in the function of stomatal cells on the leaf surface, affecting gas exchange and carbon fixation. Therefore, high levels of shading should be avoided, and cultivation with a shade level below 50% is recommended to achieve high dry weight **(Kumar *et al.*, 2012).** Under intense light radiation, which affects gene expression, there is an improvement in photosynthetic activity and an increase in carbohydrate production and accumulation in the leaves, leading to an overall biomass increase in the plant **(Bote *et al.*, 2018**). Moringa leaf extract is rich in plant hormones such as indole-3-acetic acid (IAA), gibberellins, and zeatin cytokinin, which contribute to improving plant growth and the accumulation of carbohydrates and salts **(Al-Taweel & Al-Anbari, 2019**), which leads to increased vegetative and root growth in Stevia, thereby enhancing water and nutrient uptake

from the soil, which is then transferred to the leaves to improve crop growth, ultimately resulting in increased fresh and dry weight of the plant **(Casanova *et al.*, 2009).** Furthermore, the presence of sugars in the extract of Liquorice roots increases the osmotic pressure of cells, thereby enhancing the absorption of water and other nutrients, positively impacting overall yield. Licqurice root extract is rich in potassium, which is known to regulate and stimulate physiological processes in plants, including its effect on photosynthesis and the transfer of its products, which stimulate ATP synthesis needed by the plant for various physiological activities, to storage sites in the plant.

Additionally, potassium plays a role in the accumulation of sugars, amino acids, protein formation, and carbohydrate accumulation **(Musa *et al.*, 2002)**. required for vegetative growth and increasing economically important leaf yield. Additionally, the availability of nutrients is crucial **(Yoneda *et al.*, 2017)**. Analyses conducted on Moringa leaf extract revealed the presence of macro and micro essential elements such as Ca, Mg, K, P, Fe, Mn, Cu, and Zn, which contribute to the development and improvement of vegetative growth in Stevia plants **(Jain *et al.*, 2020**). Foliar application of Licorice root extract, rich in active and nutritious components and containing gibberellin, played a significant role in stimulating cellular activities, resulting in cell enlargement and division, leading to increased leaf growth and branching in the plant **(Babilie *et al.*, 2015).**

Leaves dry weight yield (ton ha^{-1})

The results indicate a significant difference between the studied factors and significant interaction in the dry weight yield of leaves per hectare (Table 2). The results indicate that the mean of S_2 by 37.20% compared to S_1. The results also demonstrate a significant superiority of M_2 treatment, which reached 6.33ton ha^{-1}, over the M_1, G_1 and M_0G_0 treatments by 13.23, 17.43 and 39.12% respectively, followed by G_2 treatment, which reached 6.13 ton ha^{-1}.

Table (2): Effect of shading and some plant extracts on the leaves dry weight yield (ton ha^{-1}).

Plant extracts	Shading		Mean
	S_1	S_2	
M_0G_0	5.42	3.68	4.55
M_1G_0	6.99	4.20	5.59
M_2G_0	7.87	4.80	6.33
M_0G_1	6.51	4.27	5.39
M_0G_2	7.61	4.65	6.13
$LSD_{0.05}$	0.64*		0.45**
Mean	6.88	4.32	
$LSD_{0.05}$	**0.24****		

The results in Table 2 indicate that there was a significant interaction. The highest combination of interaction between S_1 and M_2 was recorded at 7.87ton ha^{-1}, followed by the combination of interaction between S_1 and G_2, amounted to 7.61ton ha^{-1} with non-significant difference between them, whereas the lowest interaction between S_2 and control treatment (M_0G_0) was 3.68 ton ha^{-1}.

The results in Table 2 illustrates that obtaining dry biomass under sunlight is significantly higher for the dry leaf yield compared to shaded plants. Increased shading levels

resulted in delayed growth and increased time required to reach various stages of plant development in Stevia. It is recommended to reduce shading levels to achieve a high dry weight yield per unit area **(Kumar *et al*., 2012).** Additionally, the extract of Moringa leaves, rich in cytokinins, plays an effective role in maintaining leaf area, leaf count per plant, and maximizing photosynthesis. This leads to increased plant size in the field and the preservation of leaves from senescence **(Jain *et al.*, 2020). Babilie *et al.* (2015)** indicated that the sugar compounds, organic compounds, and major nutrients such as potassium, phosphorus, magnesium, and trace elements present in Liquorice root extract, which contains the gibberellin precursor hormone, contributed to increasing the accumulation of dry matter in the plant by maximizing carbohydrate accumulation. Consequently, the results were consistent and aligned with an increase in leaf count, length, and area per plant and an increase in fresh weight of the total vegetative mass, ultimately resulting in a higher percentage of dry weight in cultivated lands.

Total Antioxidant Activity (mg L^{-1}) (Ascorbic acid equivalent)

The results in Table 3 demonstrate a significant difference between the factors and significant interaction between the studied factors in the total antioxidant activity. The results showed that the S_2 significantly surpassed S_1 by 71.95%. The results also indicate that the means of M_2, G_1, M_1 and control treatment decreased considerably compared to the mean of G_2 by 66.95, 54.81, 22.87 and 16.95%, respectively.

Table (3): Effect of shading and some plant extracts on the total antioxidant activity (mg L^{-1}).

Plant extracts	Shading		Mean
	S_1	S_2	
M_0G_0	9319.00	12029.00	10674.00
M_1G_0	7853.00	11971.00	9912.00
M_2G_0	2755.00	5739.00	4247.00
M_0G_1	3876.00	7740.00	5808.00
M_0G_2	8183.00	17522.00	12852.50
$LSD_{0.05}$	311.90**		220.50**
Mean	6397.20	11000.20	
$LSD_{0.05}$	**123.90****		

The results in Table 3 indicated a significant interaction between the studied factors. The highest combination recorded between S_2 and G_2, amounted to 17522.00 mg L^{-1}, and the lowest combination was between S_1 and M_2 at 2755.00 mg L^{-1}.

Decreases light optimum conditions by shading may increase the total antioxidant activity in Stevia during the studied crop growth period. This increase is likely due to reduced photosynthesis efficiency under shading. Shading can potentially enhance the activities of antioxidant compounds in Stevia against less light stress, as antioxidants are produced as secondary metabolites in cells not exposed to environmental stress and through respiratory processes in the leaf **(Rady *et al*., 2019).**

Moreover, the biostimulants, such as Liquorice root extract, impact the contents of phenols, flavonoids, and pyruvic acid, as well as the overall capacity of antioxidants and their increased production in the plant. This is attributed to the presence of various chemical

elements in the Liquorice root extract, such as Mg_2 and Fe_2, along with other elements and stimulants that arise from natural metabolic pathways, stimulating plant growth, development and stress tolerance when applied at specific concentrations **(Rouphael & Colla, 2018)**.

Total phenols (mg L^{-1}) (Gallic acid equivalent)

The results in Table 4 demonstrate a significant difference between the studied factors in the total phenolic concentration in the leaves and the presence of a significant interaction between the studied factors in this trait. The results reveal a significant superiority of the S_2 mean over S_1, with a percentage difference of 14.49%. Furthermore, all means of M_2, G_1, G_2 and M_0G_0 show a decrease compared to the mean of M_1, with percentages of 85.54, 70.98, 24.47 and 30.25, respectively.

The results in Table and Figure 4 indicate a significant interaction. The highest combination recorded between S_2 and M_1, amounted to 20288 mg L^{-1}, and the lowest interaction combination was between S_2 and M_2, which amounted to 1764 mg L^{-1}.

Table (4): Effect of shading and some plant extracts on the total phenol (mg L^{-1}).

Plant extracts	Shading		Mean
	S_1	S_2	
M_0G_0	12021.00	10487.00	11254.00
M_1G_0	11982.00	20288.00	16135.00
M_2G_0	2902.00	1764.00	2333.00
M_0G_1	4683.00	4681.00	4682.00
M_0G_2	11854.00	12517.00	12185.50
$LSD_{0.05}$	563.80**		398.70**
Mean	8688.40	9947.40	
$LSD_{0.05}$	**321.70****		

Sugars, aromatic amines, sulfur dioxide, ascorbic acid, organic acids, and others can react to create high phenolic concentrations by increasing photosynthesis while reducing environmental stress through shading **(Prior *et al.*, 2005)**. The potential of moringa extract, which is an important bio-stimulant for enhancing plant growth and biological activities, lies in its ability to increase the concentration of hormonal contents and mineral nutrients, as well as its role in providing zeatin, ascorbic acid, calcium, and potassium, all of which contribute to increasing phenolic concentrations and numerous antioxidants. Foliar spraying enhances the plant's ability to increase the uptake of essential elements, thereby increasing the nutritional value of Stevia plant leaves. The antioxidant activity in Moringa is due to its high content of phenolic compounds, which are essential for the growth and defense of Stevia plants against diseases **(Al Taweel & Al-Anbari, 2019)**. Bio-stimulants primarily enhance crop productivity by improving root system structure, enhancing plant water and nutrient uptake, and optimizing photosynthesis by improving chlorophyll content and its role in maximizing growth and the antioxidant defense system while reducing oxidative stress in plants **(Shah *et al.*, 2019).**

Leaves content of chlorophyll a (mg 100 g^{-1} fresh weight)

The results in Table 5 reveal that there were no significant differences between shading treatments (S_1 and S_2) in the leaves content of chlorophyll a, while there was a significant difference between plant extract treatments in this trait, as M_1 was significantly superior and gave the highest mean at 189.45 mg 100 g^{-1} fresh weigh, followed by G_1 and M_2 which gave 188.55 and 180.55 mg 100 g^{-1} fresh weigh respectively, compared to G_2 and control treatment.
The results in Table 5 and Figure 5 indicate that there was a significant interaction between studied factors in this trait, as the highest interaction recorded between S_1 and M_2, reached 212.00 mg 100 g^{-1} fresh weight with a non-significant difference with the interaction between S_2 and M_1, which reached 205.30 mg 100 g^{-1} fresh weight. In contrast, the lowest value observed between S_1 and G_2, reached 134.90 mg 100 g^{-1} fresh weight.

Table (5): Effect of shading and some plant extracts on the leaves content of chlorophyll (mg 100 g^{-1} fresh weight).

Plant extracts	Shading		Mean
	S_1	S_2	
M_0G_0	171.50	147.80	159.65
M_1G_0	173.60	205.30	189.45
M_2G_0	212.00	149.10	180.55
M_0G_1	189.30	187.80	188.55
M_0G_2	134.90	191.10	163.00
$LSD_{0.05}$	20.65**		14.60**
Mean	176.26	176.22	
$LSD_{0.05}$	**N.S**		

Foliar spraying with Moringa leaf extract enhances chlorophyll content and the rate of photosynthesis **(Hoque *et al*., 2020),** attributed to the role of Moringa extract in increasing gas exchange and stomatal conductance, along with its content of growth hormones such as cytokinins, gibberellins, and auxins, which promote plant growth and increase its green surface area, thereby enhancing photosynthesis **(Abdalla, 2013).** Furthermore, the Liquorice root extract containing gibberellin precursors plays a positive role in the plant's bio-building and activates chlorophyll pigmentation **(Al-Abdali, 2002)**.

fresh leaves content of chlorophyll b (mg 100 g^{-1} fresh weight)

The results indicate a significant difference between shading treatments and a significant interaction between the two studied factors in the leaves' chlorophyll b content (Table 6). The mean of S_2 was significantly superior by 8.70% compared to S_1. There were no significant differences between the means of the plant extract treatments.

The results in Table 6 and Figure 6 show that there was a significant interaction between the two studied factors in this trait, as the highest combination recorded between S_2 and G_2 (91.10 mg 100 g^{-1} fresh weight) with non-significant difference with the combinations of S_2G_1 and S_1M_2 which recorded 81.30 and 81.00 mg 100 g^{-1} fresh weight respectively, while the lowest value was observed between S_1 and G_2 which was 52.70 mg 100 g^{-1} fresh weight.

Table (6): Effect of shading and some plant extracts on the leaves content of chlorophyll b (mg 100 g^{-1} fresh weight).

Plant extracts	Shading		Mean
	S_1	S_2	
M_0G_0	70.00	64.50	67.25
M_1G_0	70.70	76.30	73.50
M_2G_0	81.00	67.60	74.30
M_0G_1	75.90	81.30	78.60
M_0G_2	52.70	91.10	71.90
$LSD_{0.05}$	10.95**		N.S
Mean	70.06	76.16	
$LSD_{0.05}$	**2.73***		

Increasing shading reduces photosynthesis and plant growth and increases crop plants' stomatal and mesophyll resistance to gas exchange. Additionally, shading reduces leaf thickness due to the formation of a thin layer of barriers that allows the plant to suffice with a minimal amount of light for photosynthesis and gas exchange **(Nygren & Killomaki, 1993).** However, under shading conditions and due to low plant density, the reduced spacing between plants may diminish the shading effect and decrease competition among plants in the Stevia cultivation ecosystem, thereby enhancing photosynthetic efficiency and delaying leaf senescence **(Ramesh *et al*., 2007).**

Leaves content of total chlorophyll (mg 100 g^{-1} fresh weight)

The results indicate there were non-significant differences between the studied factors. In contrast, the interaction between them significantly affected the leaves content of total chlorophyll, as the results in Table 7 and Figure 7 show that the S_1M_1 combination recorded a highest value (292.90 mg 100 g^{-1} fresh weight). In contrast, the S_1G_2 combination recorded the lowest value (187.50 mg 100 g^{-1} fresh weight).

Table (7): Effect of shading and some plant extracts on the leaves content of total chlorophyll (mg 100 g^{-1} fresh weight).

Plant extracts	Shading		Mean
	S_1	S_2	
M_0G_0	241.40	205.60	223.50
M_1G_0	244.20	281.60	262.90
M_2G_0	292.90	216.70	254.80
M_0G_1	265.20	269.10	267.15
M_0G_2	187.50	282.20	234.85
$LSD_{0.05}$	54.12**		NS
Mean	246.24	251.04	
$LSD_{0.05}$	**N.S**		

CONCLUSION

Based on the results concluded that Stevia plants grown in the open field (without shading) produced the highest leaves dry weight, leaves content of chlorophyll b, and leaves

yield per unit area, while the Stevia plants grown in the 50% shading had the highest values of total antioxidant activity, total phenols and leaves content of chlorophyll a. Also, spraying moringa extracts at a concentration of 15% made the highest leaves dry weight, leaves content of chlorophyll b, and leaves yield per unit area while spraying it at 10% produced the highest leaves content of chlorophyll a. However, the spraying of Liquorice root extract at a concentration of 6% had the highest values of total antioxidant activity and total phenols. Therefore, it is recommended to plant Stevia plants in the 50% shading and spraying of Liquorice root extract at the concentration of 6% to increase the total antioxidant activity and phenols.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 200-210
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(17)
Fodeal & Shaher

المجلة العراقية لبحوث السوق
وحماية المستهلك

MOLECULAR CONFIRMATION OF THE MITE *Varroa destructor* ISOLATED FROM HONEY BEES IN 15 IRAQI PROVINCES

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Received 14/ 6/ 2023, Accepted 20/ 9/ 2023, Published 31/ 12/ 2024

ABSTRACT

***Varroa destructor* was identified based on the cytochrome C oxidase subunit I (COI) gene. DNA was extracted from 15 Varroa mite samples collected from 15 provinces in Iraq. Polymerase chain reaction (PCR) was performed using primer set targeting COI gene. Nucleotide sequence analyses DNA amplified confirmed that all Varroa mite isolates belong to the species *V. destructor*. Neighbor-Joining tree grouped the mite isolates into different clades indicating they are variable. Sequence comparison showed Varroa isolates 1 and 10 were highly diverged from the equivalent GenBank isolates suggesting they are new, To the best of knowledge, *V. destructor* is the dominant species in Iraq.**

Key words: Honey bee parasite, Varroa isolates, Colony collapse disorder.

التأكيد الجزيئي لحلم *Varroa destructor* المعزول من نحل العسل في 15 محافظة عراقية

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الخلاصة:

تم التعرف على *Varroa destructor* على أساس جين السيتوكروم cytochrome C oxidase subunit I (COI) I. تم استخراج الحمض النووي DNA من 15 عينة من الفاروا تم جمعها من 15 محافظة في العراق. تم إجراء تفاعل البلمرة المتسلسل (PCR) باستخدام مجموعة بادئات تستهدف جين COI. أكد تحليل تسلسل النيوكليوتيدات المضخمDNA أن جميع عزلات الفاروا تنتمي إلى النوع *V. destructor* . أظهرت شجرة التحليل الوراثي تجمع عزلات الفاروا في فروع مختلفة مما يشير إلى أنها متغيرة. أظهرت مقارنة التسلسل أن عزلات الفاروا 1 و10 كانت متباعدة بشكل كبير عن عزلات البنك الجيني المكافئة مما يشير إلى أنها جديدة. وعلى حد علمنا، فإن *V. destructor* هو النوع السائد في العراق.

الكلمات المفتاحية: طفيل نحل العسل، عزلات الفاروا، اضطراب انهيار الطائفة.

INTRODUCTION

The Varroa mite is a honeybee pest, native to the *Apis cerana* species in Asia **(FAO,2015; Al-Jourani *et al.*, 2004).** It attacks honeybee worldwide causing serious losses. The major injury of varroa infection is weakening honeybee colonies and resulting in a significant reduced of honey production **(Abdulhay & Yonius,2020; Al-Badri, 2017)**. The Varroa mites:*Varroa destruction* Anderson and Trueman (Arachnida: Acari: Varroidae) can causes huge economic losses to the beekeeping sector because it is prevalent and high tolerated to treatments. This mite feeds on hemolymph both in broods and adults bees making them

susceptible to other diseases, mainly viral diseases. Stronger colonies with the largest brood number are the most targeted due to the high probability of mite breeding at the brood level **(Hamed *et al.*, 2016; Mohammed & Nawar, 2020; Hadi *et al.*, 2019)**. There are four types of Varroa mites: *Varroa jacobsoni*, *Varroa underwoodi*, *Varroa rindereri*, and *V.destruction*, the most dangerous species for honeybee colonies **(Oldroyd, 1999; Loeza-Concha *et al.*, 2018; Tantillo *et al.*, 2015)** . *V.destruction* has crossed from its original host *A.cerana* to *A.mellifera* in the mid-20th century, and has become the most devastating parasite on *A.mellifera* since then **(Lanzi *et al.*, 2006; Shen *et al.*, 2005; Yang & Cox-Foster, 2007).** The Varroa can transmit a number of viruses including the deformed wing virus DWV through feeding on the internal tissues of both honeybees and pupae, and in the process can cause an extra damage **(Yang & Cox-Foster, 2005; Awad, 2023; Martin, 2001).** Research showed that the Varroa parasite was a major factor in the phenomenon of bee collapse (CCD Colony Collapse Disorder) in the United States, Canada and Iran **(Ongus *et al.*, 2004; Yang, 2004).**

The Varroa parasite can be affected by the geographical location and natural climatic conditions, especially temperature, humidity, and others. This may resulted in a genetic variation that can change some morphological, physiological, and behavioral characters of mite **(Hou *et al.*, 2016).** Therefore, the aim of our study was to identify the species present in Iraq and the different isolates based on morphology and molecular approaches**.**

V. destructor are relatively large external parasites that feed on the body fluids of adult and developing honey bees. *V. destructor* cause physical damage, weaken bees and transmit a variety of pathogens, particularly viruses. In almost all cases, when varroa infestations are not effectively managed they will eventually result in the death of the entire honey bee colony. It is crucial that beekeepers manage the health of their honey bees by suppressing the population of varroa in all of their honey bee colonies throughout the beekeeping season **(Shegaw *et al.*, 2022). Mahdi *et al.* (2020)** were able to diagnose 17 different isolates of the Varroa collected from different regions of southern, central and northern Iraq and some Middle Eastern countries such as Iran, Turkey, Syria, Egypt and Jordan using polymerase chain reaction (PCR) technology, determine the sequence of nitrogen bases of the cytochrome oxidase 1 gene zone in mitochondria (mtCOI) and diagnose two isolates of Varroa (*V. destructor*) (MK482687 and MK509767) are genetically different from other isolates registered at the National Center for Biotechnology Information (NCBI), **Muntaabski *et al.* (2020)** also showed, by evaluating the genetic diversity of *V. destructor* isolates in Argentina the existence of a difference between isolates is closely related to geographical latitude.

MATERIAL AND METHODS

Varroa sampling:

Varroa samples were collected from 15 provinces in Iraq (Erbil, Diyala, Maysan, Anbar, Nineveh, Baghdad, Najaf, Basra, Dhi Qar, Al-Qadisiyah, Sulaymaniyah, Babylon, Karbala, Kirkuk, Salah al-Din) (Table1and figure1). Each sample contained 15 -20 Varroa individuals and were preserved in collection tubes containing ethyl alcohol with the collection place and date.

Table (1): Varroa samples collection in this study.

Sample number	Location	Collection date
1	Erbil	12/3/2021
2	Diyala	20/4/2021
3	Maysan	15/3/2021
4	Anbar	12/5/2021
5	Nineveh	21/5/2021
6	Baghdad	18/3/2021
7	Najaf	19/3/2021
8	Basra	1/3/2021
9	Dhi Qar	3/4/2021
10	Qadisiyah	21/3/2021
11	Sulaymaniyah	26/4/2021
12	Babylon	24/3/2021
13	Karbala	20/3/2021
14	Kirkuk	19/4/2021
15	Salahaddin	12/3/2021

Morphology identification

Random samples were taken from Varroa samples collected from the provinces in Iraq (Table 1) and examined using optical and electron microscopy to identify varroa species. Optical microscopy was performed in the Graduate, under magnification (×20) using the LOMO LABOROSCOPE™ AL-2000 Binocular Microscope, BF. Field Emission Scanning Electron Microscopy (FESEM) electron microscopy was performed in the Al-Khora Laboratory for Nanoscale Research, using the Inspect F-50 electron microscope, of Dutch origin, produced by the FEI Company. The varroa sample is placed on a glass slide, then the slide is placed on a metal base containing an adhesive substance that fixes the slide on it ،The metal base is then transferred with the slide to Plasma sputter Coatings for coating by depositing gold nanoparticles on the sample, as gold provides high-quality microscopic images due to its high reflectivity under a microscope, and then transferred for examination under an electron microscope . The diagnosis (optical and electron microscopy) was based on the following characteristics (size, colour, shape of the shield, number of hairs on both sides of the shield, rhombic shape of the body inside the shield, shape of the iliac crest of the leg, length of the leg) following the classification key established by **(Zhang, 2000).**

Molecular diagnosis of Varroa parasite

Varroa samples were transferred to the Plant Virus Laboratory / College of Agriculture/ University of Karbala for molecular diagnosis. DNA was extracted from the samples using the G-spin Total DNA Extraction Kit (iNtRON Biotechnology, South Korea) supplied by Intron biotechnology/Korea and following the steps described by the manufacturer. Polymerase chain reaction (PCR) was carried out using the kit (Maxime PCR PreMix (i-Taq)) supplied by iNtRoN (South Korea).

PCR was carried out in a volume of 25 µL containing 17 µL of sterile distilled water (Nuclease-free water) and 1 µL each of forward (LCO 14905'-GGTAACAAATCATAAAGATATTGG-3') and the reverse (HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA- 3') primer **(Mahdi *et al*., 2020).** 1 µL of DNA template were add to 0.2 the tube containing PCR pre supplied **(Folmer *et al*., 1994).**The PCR amplification was carried out using 1 initial denaturation for five minutes at of 98 °C, followed by 35 cycles of denaturation for 40 seconds at 94 °C, primer annealing for 40 seconds at 58 °C, for one minute at 72 °C, and final extension for 10-min at 72 °C **(Zhang *et al*., 2012).**

Electrophoresis

The agarose gel layer was prepared by dissolving 1 gram of agarose powder in 100 ml of 1×TBE buffer solution (Tris-boric acid EDTA buffer). After the mixture turned into a clear solution and its temperature decreased, 3 micro A liter of Ethidium tincture and mix well.Pour the melted agarose into an Agarose gel tray that has a comb at one end for the purpose of making holes inside the gel layer. After the agarose layer had hardened at room temperature, the comb was carefully lifted, the mold was placed in place in the electrophoresis tank, and the 1×TBE buffer solution was added to cover the agarose gel layer by approximately 70 mm. Approximately 5 microliters of agarose gel was added to each hole. DNA multiplied by polymerase reaction (PCR products), and 5 microliters of DNA size marker (Molecular-weight size marker) was placed in the hole located on the left side of the added samples. Then I connected the electrodes of the power supply to the electric current and ran it for one hour at 150 mA After completing the transillumination process for the samples, I examined the agarose gel layer containing the duplicated DNA products under ultraviolet (UV transillumination) and took pictures of it.

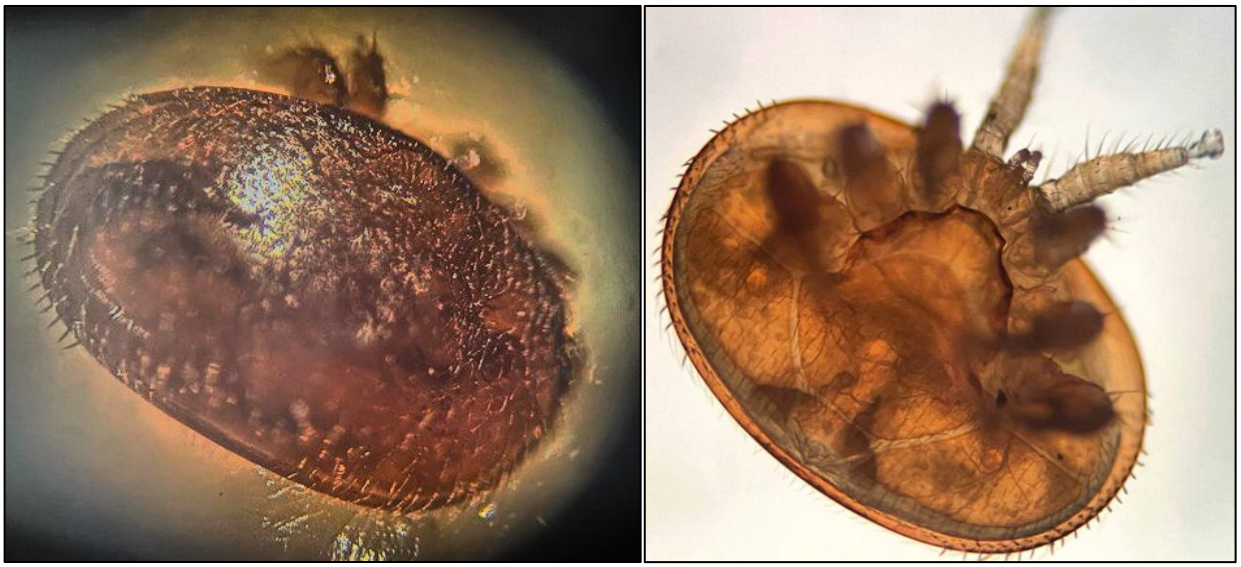
Sequence analyses.

DNA fragment amplified from Varroa isolates were sequenced in Macrogen (South Korea). Sequence analyses were performed using MEGA-X software **(Kumar *et al*.,2018).**

RESULTS AND DISCUSSION

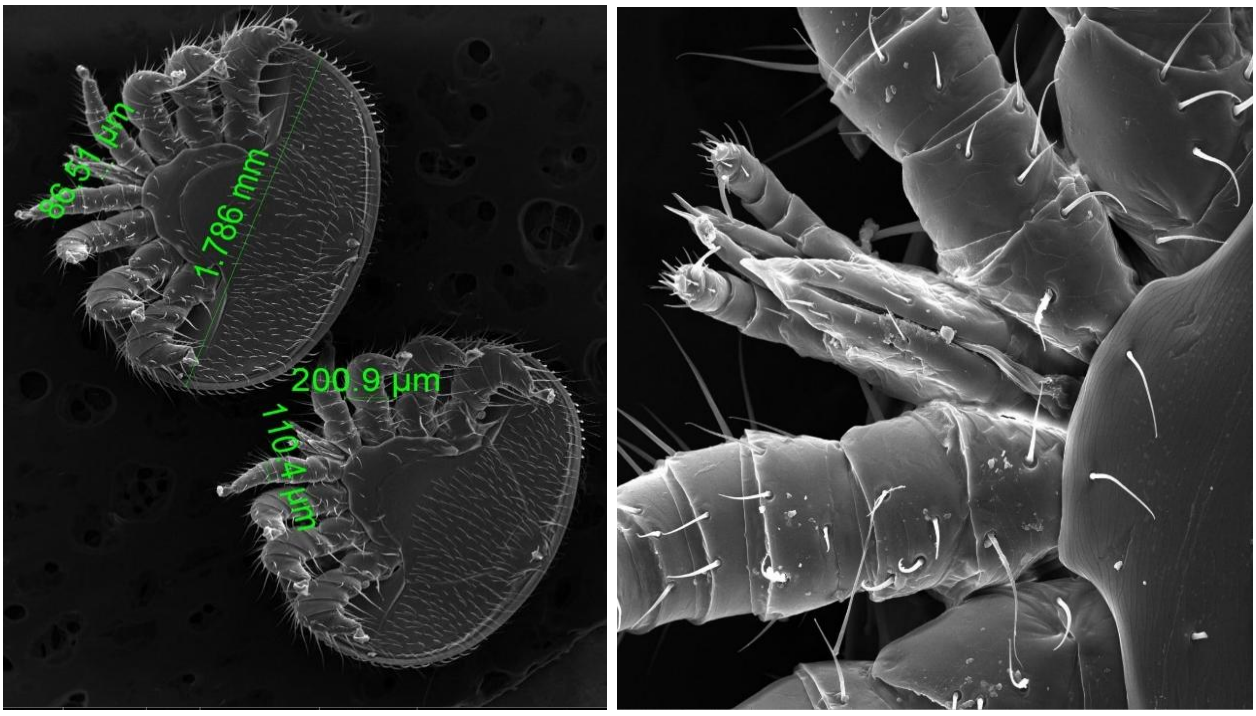
Varroa morphology identification

Microscopic examination of the Varroa samples showed that the females were 1.5 mm long and 1.7 mm wide, with flattened, oval shapes, and they appeared to be transverse rather than longitudinal, and they had eight short legs, and were brown to reddish brown in color. Their bodies, mouth parts, and legs were covered with hairs. On both sides of the body or shield, there were hard hairs ranged 19-22, resembling a sickle. The abdominal plates bore fine hairs (Fig.1and2). The males, were distinguished through their spherical body, has smaller size than the females, and with a pale-yellow color.



B A

Figure (1): Varroa female at 20x magnification. A: Dorsal view, B: Ventral view.



A B

C

Figure (2): Field Emission Scanning Electron Microscopy (FESEM) ahowing Varroa female A: Ventral view (70x). B: Mouth parts (500x). C: Ventral view (130x).

Molecular confirmation of the mite *Varroa destructor* isolates in Iraq

Gel electrophoresis of PCR products confirmed PCR using LCO1490 and HCO2198 primer set could amplify 720 bp. DNA fragments from all 15 samples tested Figure (3).

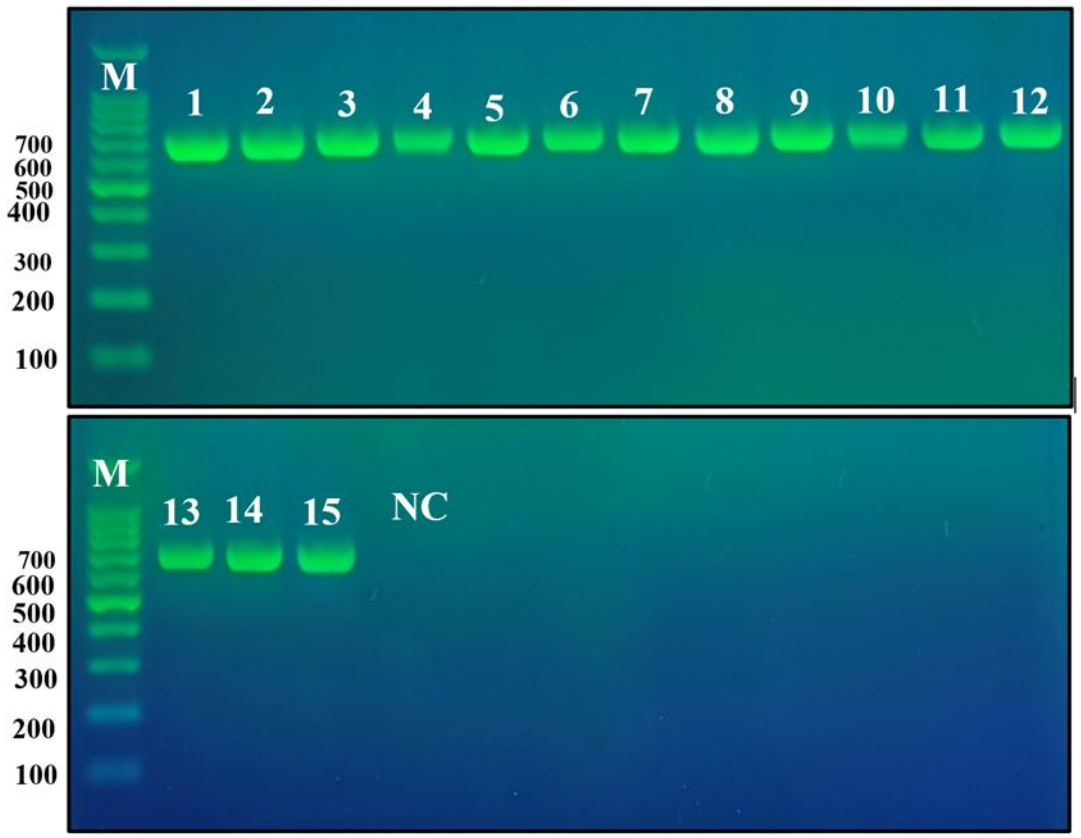


Figure (3): Gel electrophoresis patterns showing 720 bp DNA fragments amplified using polymerase chain reaction (PCR) and LCO1490 and HCO2198 primer set from Varroa samples (*V. destructor*) (lanes 1-15) isolated in this study. M = 100 bp DNA marher ,NC;negative control.

Nucleotide sequence analysis of the DNA fragments amplified from Varroa samples confirmed all 15 isolates belong to *V. destructor* when shared 100% highest nucleotide sequence identity with the equivalent sequences,retrieved from the NCBI. Nucleotide sequences from Varroa parasite isolates 7, 8, 9, 13, 14 and 15 shared the highest similarity up to 100% with those inform Canada (MN360198), South Korea (MW725321) and America (AY163547) suggesting common origin. These isolates were collected from Najaf, Basra, Dhi Qar, Karbala, Kirkuk, Salah al-Din, respectively, indicating a homologues population of *V. destructor* infecting honeybees in these locations. Whereas Varroa isolates 1, 2, 3, 4, 5, 6 and 11 shared 100% similarity when compared to each other them and 99% maximum identify with equivalent from NCBI, including those reported in Taiwan (MW599147), Argentina (MT462468) and Taiwan (MW599128), while It was most similar (98%) to that isolate from Taiwan (MW599122) and Germany (KR528383). Based on these results, isolate (1) was chosen to be representative of the other isolates (2, 3, 4, 5, 6 and 11) for the purpose of completing the comparison and analysis with isolates registered globally in the National Center for Biotechnology Information (NCBI) (Fig.4and 5).

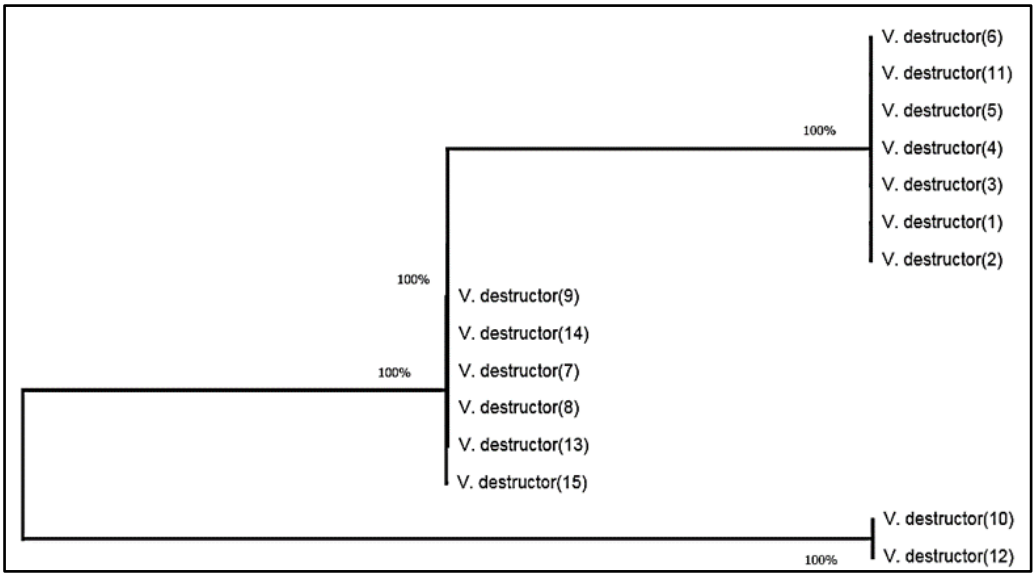


Figure (4) phylogenetic tree shows the relationship between the sequences of the nitrogenous bases of the doubled DNA products of Varroa parasite (*V. destructor*) (1-15) isolated in this study.

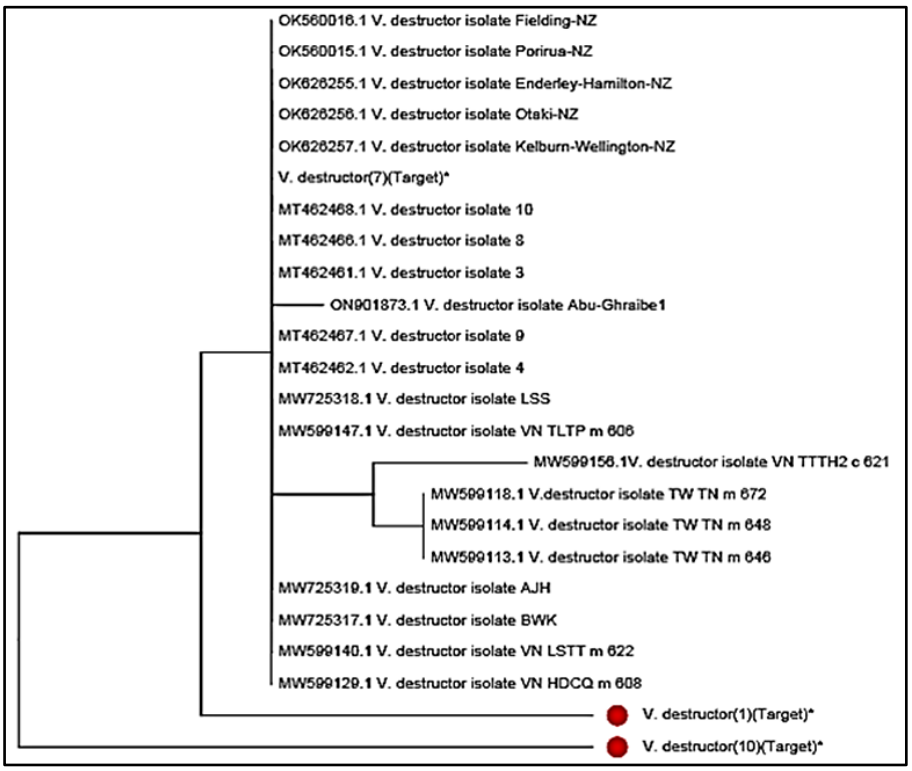


Figure (5) Neighbor-Joining tree shows the genetic relationship between Varroa parasite (V. destructor) isolates 1, 7 and 10 isolated in this study and other isolates previously registered in the National Center for Biotechnology Information (NCBI).

The results of this study show that there is genetic diversity among varroa samples collected from different regions in Iraq, and it is believed that one of the reasons for this difference indicates the entry of varroa-infected beehives or the occurrence of genetic mutations due to changing geographical locations, pressures or climatic changes, especially temperature and humidity, which contribute to morphological and behavioral changes that allow the adaptation, growth and natural reproduction of varroa **(Maggi *et al*.,2009; Dadgostar & Nozari,2018).** It is concluded from the molecular diagnosis of Varroa parasite isolates isolated in this study that isolates 1 and 10 are new isolates previously unknown in the world and as shown in Figure (5), so they were registered in the US National Center for Biotechnology Information (NCBI) under entry numbers OP984055. 1 and P984078.1, respectively.

CONCLUSION

The molecular diagnostic and base sequence determination of the DNA products from 15 isolates of Varroa collected from different provinces of Iraq (Erbil, Diyala, Maysan, Anbar, Nineveh, Baghdad, Najaf, Basra, Dhi Qar, Qadissiya, Sulaymaniyah, Babil, Karbala, Kirkuk, and Salahaddin) showed that isolates 1 and 10 (Erbil and Qadissiya) were not previously identified in the world, so they were registered in the National Center for Biotechnology Information (NCBI) under deposit numbers OP984055.1 and OP984078.1, respectively

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 211-223
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(18)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

EFFECT OF SPRAY PRESSURE AND VERTICAL BOOM POSITION ON THE SPRAY QUALITY

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Received 14/ 6/ 2023, Accepted 15/ 8/ 2023, Published 31/ 12/ 2024

ABSTRACT

The research to focuses on spraying the plant leaves from the bottom and top by developing a sprayer. The sprayer was making a sprayer made of aluminum with a movable arm containing a nozzle holder divided into three pieces. Each piece contains a nozzle of the type Flat Fan 120-C3. The tasting of the sprayer was done in one of the greenhouses of the Department of Horticulture and Forestry. The study has two factors, the first is changing eff changing the positions of the nozzle holder tube to two levels A1, A2. The second factors were the spray pressure with three levels P1=2, P2=3, P3=4 bar. The spraying speed was 3.12 km/h. The effect of the previous factors on the following characteristics namely: spray losses, spray penetration, and spraying homogeneity. The research began in 2021.The experimental design was Randomized Complete Block Design (RCBD) according to the SPLIT-PLOT system and with three replications using a significant difference LSD with a probability level of 0.05. The results showed that the study factors at the level of A1 position and pressure 4 bar were superior to the losses and penetration. The level of A2 and P3 pressure achieved the highest result with the uniformity of the spray.

Key words: Agricultural nozzles, eggplant plant, greenhouses, nozzle holder positions, spray pressure.

تأثير ضغط الرش وموضع حامل الفوهات الرأسي على جودة الرش

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الخلاصة

يهدف البحث إلى التركيز على رش أوراق النبات من الأسفل والأعلى عن طريق عمل مرشة مصنوعة من الألمنيوم بذراع متحرك تحتوي على حامل فوهة مقسم إلى ثلاث قطع وتحتوي كل قطعة على فوهة من نوع Flat Fan 120-C3 وتجربة الآلة في أحد البيوت المحمية التابعة لقسم البستنة والغابات ودراسة تأثير تغيير مواضع أنبوب حامل الفوهة إلى لمستويان A1, A2 وتأثير تغيير الضغط على ثلاثة مستويات P1=2،P2=3،P3=4 بار بمتوسط سرعة سحب 3.12 كم\ساعة عند دراسة تأثير العوامل السابقة على بعض خصائص الدراسة وهي خسائر الرش واختراقه الغطاء النباتي مع تجانس الرش. بدأ البحث في عام 2021. والتصميم التجريبي هو RCBD وفقا لنظام SPLIT-PLOT ولثلاث مكررات باستخدام فرق معنوي LSD بمستو ى احتمالية 0.05 أظهرت النتائج أن عوامل الدراسة عند

*The article is taken from a master's thesis by the first researcher.

مستوى وضعية A1 وضغط 4 بار كانت متفوقة الضائعات و الاختراق كما حقق مستوى A2 وضغط 4 بار أعلى نتيجة مع تجانس الرش واختراقه الغطاء النباتي مع تجانس الرش.

الكلمات المفتاحية: البيوت المحمية ، ضغط الرش ، الفوهات الزراعية ، نبات الباذنجان ، وضعيات حامل الفوهات.

INTRODUCTION

Growing vegetable crops is essential in human life because it is part of daily food. Each crop has a certain service process; the essential process for plants is the addition of chemical liquids of all kinds (pesticides, fertilizers or growth regulators) to it **(Sanaa, *et al*, 2020; Sura &Al-Hilfy, 2022; Alrawi *et al.*, 2023; Al-Karawi & Al-Jumaily, 2022; Shaymaa,*et al*, 2022)**.

Saving spray while continuing its quality leads to a reduction in the material costs of service operations **(Yarpuz-Bozdogan *et al.*, 2011; Subr, *et al*, 2020).** The sprinkler irrigation method instead of surface irrigation has overcome increasing production and reduce water consumption **(Hassan, *et al*, 2021).**

Eggplant is an agricultural crop which is grown in the off-season in greenhouses. Eggplant is called *Solanum melongena* L and is the Solanaceae family. The eggplant crop is grown in the Mediterranean region in Central Asia and reserves, while in the northern regions, it is not grown because it needs daylight hours within (14-12) hours of sunlight, and the optimum temperatures are (30-21) degrees Celsius as for the temperature (35<temperature<15), the temperature above (35) degrees Celsius reduces the flowering contract and thus reduces the number of fruits on one plant, while the temperature below (15) degrees Celsius negatively affects growth and production **(Koller *et al*., 2016) & (Baudoin *et al*., 2017)**.

The spraying in Iraq does not follow international standards, especially in terms of calibration and type of nozzle **(Subr,*et al*, 2019).** The methods used to serve crops are still evolving. Considering their pattern, whether it is exposed or protected, as they affect the machines and manage the crop. The eggplant crop is important in the requirements of daily per capita consumption. Because its leaves are wide and drooping, they do not allow spraying from below, which makes the frequency of spraying increase the consumption of liquids with an increase in the costs of the spraying process with the survival of toxic deposits on plants and the ground and their impact on human and animal health alike **(Nansen *et al*., 2015)**.

In Iraq, three types of sprayers are used in greenhouses plastic sprayers which were shoulder bag, drawn and hanging on the tractor. The common between the spray holder is manual, which makes the spray ununiformed. The loss of volatile spray and its penetration is into the vegetation system with the amount of homogeneity of the spray. All of these are characteristics of the study associated with their overlapping factors, namely the pressure and the position taken by the nozzle holder and directly affected spraying processes.

They worked **Foque & Nuyttens, (2011)** On increaseing spraying on crops in greenhouses to rise penetration and strengthen spraying on the underside of the leaf using three types of nozzles. They use of two pressures (6,3) bar, but due to the density of vegetation cover, there is a discrepancy in the results, especially with penetration and spraying of the underside of the plant leaf, and more experiments were recommended.

They did **Derksen *et al.*, (2009)** Spraying ornamental crops in greenhouses with a nozzle holder horizontal from the top of ornamental plants to cover their leaves from the bottom and top. It has increased coverage on the top side of the leaves but not significantly on the lower side of the leaves.

Similarly, crops are threatened by pests that feed on the lower surface of the leaves, such as whitefly and aphids **(Derksen *et al.*, 2010 a)**.

The experiment was conducted on ornamental plants of one of the greenhouses by nozzles that operate using air induction of five different companies and a normal flat nozzle. The result was the deposition of the spray on the upper surface increased by (500%) while the lower surface of the leaves results showed a difference in the result. The researcher recommended further research in this area in to cover the lower surface of the leaves of the plants with increased penetration of the spray to the vegetative parts. The operator's skill to deposit with manual spraying devices is the most reliable on him to reduce fluctuations in spraying **(Derksen *et al.*, 2010 a; Derksen *et al.*, 2010 b).**

In order to prove the concept of spraying in new ways, the coverage of ornamental plants from the three sides to preserve the environment with increased sedimentation and penetration and to reduce the sprayed material, the three nozzles were placed for spraying, with different spray angles. The first and second are on both sides of the plant, and the third is on the top. The result was more coverage on the upper and lower surface of the leaf than if traditional methods, such as manual spraying, were used to perform **(Zwertvaegher *et al.*, 2017)**.

Illing, (1997) stated that the exposure of workers in greenhouses increases to volatile and dangerous residues of chemicals and pesticides sprayed with an unclear indication caused by the fluctuation of the number of workers, workers in greenhouses with other causes, so it is not possible to limit the number of real workers in greenhouses and for this it is not possible to predict the real percentage of the number of injured, although it is found at least for every 10,000 workers two in greenhouses.

Vertical patterns were used to determine and adjust the most appropriate form of spraying, adapt the faucet openings settings in terms of direction and position of the nozzle on the nozzle holder and create a training system for plant spraying personnel and adapt to the stages of plant growth grown by the Plant Protection Preparation Program (PPP) Plant Protection Products **(Garcera *et al*., 2022; Dereń *et al*., 2017)**.

Spraying eggplant plants was carried out in one of the greenhouses to study three working pressures which were (5,3,2) bars with four spray patterns. Moreover studies, the properties of these drops using a program to analyze the smudges ImageJ has shown that the pattern of spraying and pressure directly affect the amount of spray reaching the parts of the vegetative system and the extent of homogeneity and penetration with its distribution to most plant parts **(Braheem & Alheidary, 2022)**.

Grisso *et al*.,(1988); Alheidary, (2018) have explained. The nozzle holder pattern used during spraying (the shape of the nozzle holder) significantly reduce loss and completing work efficiently.

In a study conducted by **Hanafi, *et al*., (2016)** to prove that when placing pairs of three twin nozzles, Type the flat fan type at an angle of (110) degrees and inclined at an angle to the nozzle holder in the upward direction (45) degrees, and placing water-sensitive leaves on pea plants on the lower surface of the leaves of plants, the results of sedimentation, coverage, and elimination of the red spider showed one of the pests spread on the aforementioned plant. The results were better when compared to some of the results of previous experiments with the current study.

Whit the increase in operating pressure caused increases with the coverage, and the penetration of the plant canopy increases with the use of the appropriate speed **(Marwan & Subr, 2022)**

In a study by **Coelho *et al.*, (2021)**, the result was more droplets at the highest pressure, resulting in better coverage of the plant canopy. This resulted in the coverage of vegetation at the highest rate. To increase the pressure from (approximately one bar) 15psi to (approximately three bars) 45psi, both the percentage of coverage and the spray rate (l/ha) with the number of drops almost doubled.

Gavali & Kalashetty, (2018); Liu *et al*., (2020) The causes of off-target spraying losses are primarily due to large differences in vegetative density, plant sizes, arrangement and spacing of planting sites, the rate of delivery of pesticides with their continuity for spraying, as well as the spraying machine, used when spraying, as most of the work is related to the type of traditional sprayers, which in turn cause many losses during spraying. In order to increase the efficiency of spraying physically, practically, environmentally, and economically, the above points must take order achieve sustainable environmental development.

The penetration is one of the important characteristics of the spraying process as it gives us an indication of the amount of spray penetrating to most plant parts, especially in the center of the vegetative system of the plant. And that the closer the target is to be sprayed, the greater the penetration and sedimentation and vice versa

Failla & Romano, (2020); Oliveira *et al*., (2014) pointed out that the skill of the spraying person requires him to take Guidance regulations on the spraying machine, including getting as close as possible to the target to be sprayed to increase penetration and deposit on the plant canopy.

When using a spray cart with a four-nozzle spray arm was vertical. The distance between one nozzle and its adjacent (0.5 m) at an angle of inclination (15 °) of the nozzles is directed upward from the horizon level with a pressure of (20 bar = 2000 kPa and 12 bar = 1200 kPa) and a spray rate of (1500 l / ha) increased the total sedimentation and its percentage is about (33.9%) and (40%) for the same efficiency, pressures. Homogeneity of spraying and minimization of loss on the soil. Furthermore, manual spraying produced lower values than the towed cart **(Sanchez-Hermosilla *et al.*, 2012)**.

Llop *et al.*, (2015) have confirmed Knapsack sprayer that when used in greenhouses, the spraying is heterogeneous, and cost-effective with a longer spraying time, and the spraying operator is exposed to more risk of sprayed chemicals. The researchers showed that using a vertical spray arm with a spray trolley is the opposite of what was mentioned above, with an increase in the homogeneity of the spray. Spraying inside greenhouses in Iraq still faces problems in delivering spray to some areas in the vegetation system (the surface of the lower face of the leaves). As the work of the second person is to extend the spray hose at the time, while the first person works to complete the spraying process by the manual spray arm, which in turn leads to irregular spraying of the plants to be sprayed. As this leads to the failure to cover the lower surface of the leaf. With spraying the worker to his body. As well as not using clean energy to complete the spraying process, and using more than one equipment for spraying according to the stages of plant life.

The spraying of liquid agriculture (Pesticides and liquid fertilizers) is common in many agricultural experiments in Iraq. That necessary to study the increased efficiency of these sprays and less than costs to raise recommendations for agricultural sprayers **(Al-Karawi &Al-Jumaily, 2022; Sara & Mohammed, 2022**)

The research aims to design and implement a sprayer for controlled spraying in greenhouses, with the sprayer -mentioned above testing and estimating the efficiency of its

spraying. And study the effect of the study factors on the characteristics to be measured, as well as finding the best combination between the levels of the studied factors.

MATERIALS AND METHODS

1 The sprayer machine - its main components

The weight of the sprayer machine without the battery box and the tank is empty (36 kg), and the weight of the battery box alone is (11 kg), where as the total weight of the sprayer machine with the battery box and its tank is empty (47 kg), while its weight and the tank are full (77 kg). The sprinkler system consists of the following main parts: (four pumps, three spray nozzles, a line strainer, a tank, a manual-controlled valve, a nozzle tube, a splitter, pressure gauge, pressure control valve, a connecting pipe and a flexible high-pressure hoses). The pressure can be adjusted by the control panel switches and the regulation of the manual drain control valve to ensure constant pressure and discharge when spraying through the nozzles mounted on the nozzle tube. There are three imported nozzles produced by agroplast. Type (AP120-03C). They are called flat fan nozzles. The distance between one nozzle and another adjacent to it is (50cm), which is the same distance between the sprayed plants and the spray nozzles, according to the manufacturer's recommendations above. The spray angle is (120°) and (03C) it means the Spray nozzle discharge rate in gallon/minute. It operates with a pressure range ranging from tow bars to six bars, and the discharge is (0.98-1.70) l/m. The hydraulic system has three split pieces. Its usefulness is to connect and connect parts with each other when going out for pumps. It can also be used to connect the end parts of the wet stand without involving the middle part with them. The electrical system contains the battery box that is the source of energy supply to the equipment. The battery box can be charged from the solar charging kit supplied with the equipment. The stomach is equipped with two battery boxes that feed the stomach with electrical energy, the two battery sets can be charged together when the drop reaches half to preserve and extend battery life. Attached to the sprayer is a solar charging kit and consists of a solar panel with a capacity of (150W) installed on an iron structure and a plastic box is installed on it, inside the plastic case is a charging controller to control the charging process and disconnect the battery when charging is complete.

2. The Boom shape

The first factor consists of two positions, the first position resembles the shape of the letter (C) in English and its symbol A1 and the nozzles to the inside, while the second position is similar to the shape of the letter (I) in English and its symbol is 2A. The position affects the amount of substance the sprayed plants and the spray waste.

3. Operating pressure

The second factor was the pressure in the spraying process, which was consists of three pressures. The first pressure (P1=2bar), the second pressure (P2=3bar), and the third pressure (P3=4bar). Thus, it improves the reception of the sprayed substance by the plants because it is within the medium and minimum pressure limits of the nozzle used.

4. Carry out the experiment - and analyze the test papers

The experiment was conducted in one of the greenhouses of the Horticulture Research Department within the National Program for the Propagation of Seeds, Varieties and Hybrid

Strains of Local Vegetable Crops of the Department of Horticulture and Forestry of the Ministry of Agriculture and located in the district of Abu Ghraib. To checking and testing the sprayer machine operating inside the greenhouses. The greenhouse, was dimensions were (56 m), width (9 m) and height (3 m) and contains two gates, the first from the front and the second from the back. As in to analyze the distributions of spraying on glossy white sheets test papers. With known dimensions, then the samples are scanned with a scanner, i.e., the Scanner device, and with a resolution of up to (600dip), the previously taken image is converted to (8bit) images with a grayscale. Place the square on the sprayed white paper to take the exact measurement.

5. Studied qualities

5.1. Spray loss (amount of spray falling on the ground between plant lines)

It is a trait that can be calculated through test sheets placed on the planting line between plants of one experimental unit. This is shown by the percentage (%) of the number of stains per unit area of the test paper when sprayed with blue dye reaching the ground during the movement of the machine from in front of the plant of the experimental unit. The image processor software calculates this percentage. This adjective is denoted by the symbol (L).

5.2. Spray penetration of the vegetative canopy

It is a trait that shows the amount of penetration and penetration of droplets within the vegetative system **(Grisso,*et al*, 2019)**. This characteristic can be calculated by comparing the average percentage (%) of the coverage values on the face of the plant leaf, calculated by placing the test sheet at a height of (80 cm and 40 cm), which is denoted by the symbol (μ (f)) and the coverage values in the core of the plant by placing the test paper in the heart and on the upper side of the plant and symbolized by the symbol (H) at a height of (50 cm). The calculation of the spray for the vegetative total and the labeled Spray Penetration is the percentage of the quotient of the coverage (μ (f)) and for the same treatment on the coverage value (H) multiplied by (100%) for the same previous treatment to extract the penetration rate.
Whereas:

$$P\,n\,\% = \frac{H}{\mu\,(f\,)} \times 100 \dots\dots\dots\dots\dots (1)$$

P n: percentage of spray penetration of vegetative total, %.
H: the coverage value of the test paper placed in the heart of the vegetative system of the plant.
μ (f): Percentage of height test sheets (80cm and 40cm) covered for experimental unit plant

5.3. uniformity of spraying

The homogeneity can be calculated vertically on the vegetative total of the plant by knowing the percentage of the spray coverage value of the test paper at the height of (80cm or 40cm) with a lower value after comparing it to the higher value of the same treatment at the height of (80cm or 40cm) multiplied by (100%) and as in the following equation:

$$\boldsymbol{Un}\ \% = \frac{(UPf)or\ (Mf)Highest\ Value}{(Mf)\ or(\ UPf)Minimum\ Value} \times 100 \dots\dots\dots\dots\dots\dots (2)$$

Whereas:
Un % Spray Uniformity percentage (%).
UPf: percentage coverage at altitude (80cm) (%).
M f: percentage coverage at height (40cm) (%).

6. Statistical analysis

In the experiment, the design of the Randomized Complete Block Design (RCBD) working with the split plot design system was used to experiment with its two factors consisting of two pressure factors and (3) levels and the position of the nozzle holder with (2) positions, so the number of factors becomes (6) factors. Using the program (Origin lab 2018).

RESULTS AND DISCUSSION

1. Amount of loss between plant lines

It is clear from Figure 1. That the effect of the nozzle holder positions is high significant by (LSD=6.07) at a significant level (0.05) on the characteristic of the amount of losses between plant lines at the level of (A1), as it gave the highest average percentage of losses between plant lines (44.3%) and this may be due to the fact that the droplets coming out of the nozzles due to the proximity of the upper and lower part of the nozzle holder from the route of the sprayed plants and thus increasing the concentration of spraying leads to the fusion of free droplets and their fall faster Between plant lines and this is consistent with **(Alheidary, 2018**; **Braheem & Alheidary, 2022)** and the effect of pressure was not significant at a significant level (0.05) on the characteristic of the amount of losses between plant lines at the pressure level (P3) as it gave the highest percentage of coverage (43.1%) and this may be due to the fact that the speed used is the same for all cases or the reason may be that the difference between the pressures used is small and this is consistent with (Cerruto et al., 2021).and (Gandolfo & Moraes, 2014). As for the bilateral interference, its effect is not significant at a significant level (0.05) in the aforementioned characteristic, it resulted in the interference of the position (A1) and pressure (P3) the highest percentage of losses between the lines of plants (45.7%) and the reason may be due to the position (A1), which gave the highest amount of spray and fusion of the droplets sprayed from the position (A2) and this is consistent with **(Cerruto et al., 2021; Gandolfo & Moraes, 2014).**

It is clear from Figure 1. That the effect of the nozzle holder positions is high significant by (LSD=6.07) at a significant level (0.05) on the characteristic of the amount of losses between plant lines at the level of (A1), as it gave the highest average percentage of losses between plant lines (44.3%) and this may be due to the fact that the droplets coming out of the nozzles due to the proximity of the upper and lower part of the nozzle holder from the route of the sprayed plants and thus increasing the concentration of spraying leads to the fusion of free droplets and their fall faster Between plant lines and this is consistent with **(Alheidary, 2018**; **Braheem & Alheidary, 2022)** and the effect of pressure was not significant at a significant level (0.05) on the characteristic of the amount of losses between plant lines at the pressure level (P3) as it gave the highest percentage of coverage (43.1%) and this may be due to the fact that the speed used is the same for all cases or the reason may be that the difference between the pressures used is small and this is consistent with **(Cerruto *et al.*, 2021.; Gandolfo & Moraes, 2014)**. As for the bilateral interference, its effect is not significant at a significant level (0.05) in the aforementioned characteristic, it resulted in the interference of the position (A1) and pressure (P3) the highest percentage of losses between the lines of plants (45.7%) and the reason may be due to the position (A1), which gave the highest amount of spray and fusion of the droplets sprayed from the position (A2) and this is consistent with **(Cerruto et al., 2021; Gandolfo & Moraes, 2014)**.

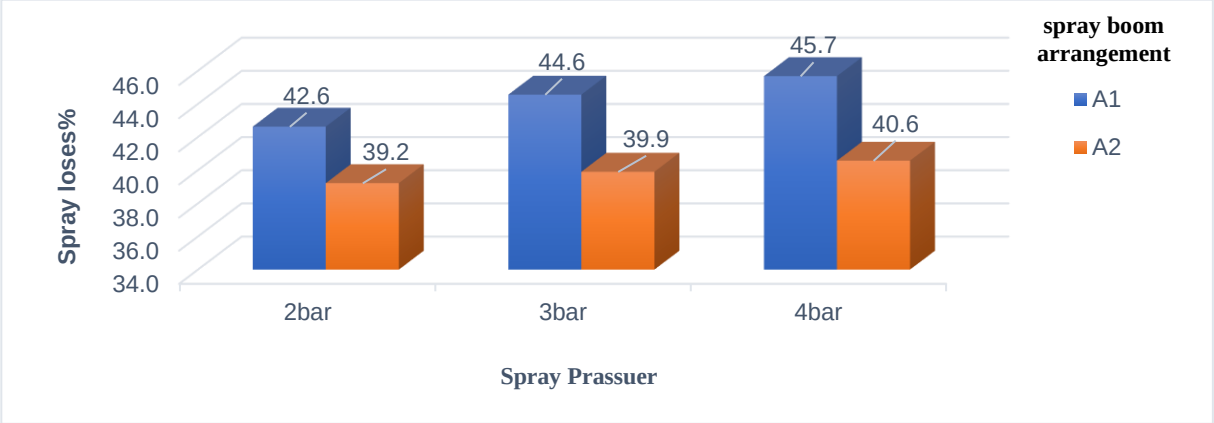

Figure (1): effect of spray boom arrangement and spray pressure on spray loses.

2. Spray penetration of the green group It is clear from Figure 2. at a significant level (0.05) that the effect of placing the nozzle holder is not significant with the penetrating characteristic of the spray of the vegetation system at the level of (A1), as it gave the highest percentage of penetration (71.9%) and this may be due to the inclination of the lower and upper part of the nozzle holder towards the inside and its approach to the sprayed plants, and this corresponds to **(Oliveira *et al*., 2014)** as well as the effect of operating pressure is not significant with the aforementioned characteristic with the level (P3) as it gave the highest percentage of penetration (76.4%) This may be due to the fact that the more pressure the more the amount of the sprayed substance increases, the smaller the volume of droplets and the more volatile of the sprayed material towards the sprayed target, and this is consistent with (**Alheidary, 2018; Braheem & Alheidary, 2022)**. As for bilateral interference, its effect is also not significant with the aforementioned characteristic. The interference of position (A1) and pressure (P3) resulted in the highest percentage penetration (79.3%) and this may be due to the tilt of the bottom and upper part of the nozzle holder towards the inside and its approach to the sprayed plants with increasing pressure, and this corresponds to **(Oliveira et al., 2014; Alheidary, 2018)** The reason for the convergence of the results is the probability of the speed used, which is 3.12 km/h, which may cause fluctuation in penetration with the above experiment factors. **(Braheem & Alheidary, 2022)**.

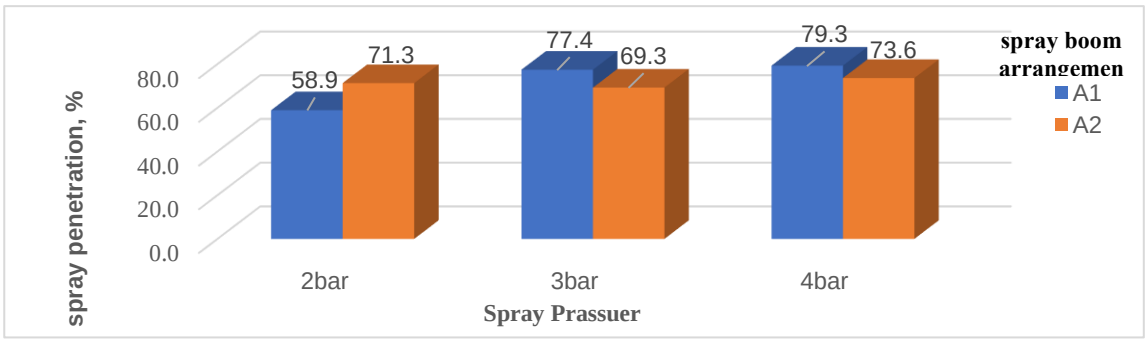

Figure (2): effect of spray boom arrangement and spray pressure on spray penetration.

7.3. Spray uniformity:

It is clear from Figure 3. Shows the effect of the position of the nozzle holder is not significant with the characteristic of homogeneity of spraying, but at the level of (A1) gave the highest percentage of homogeneity in spraying (83.9%) and this may be due to the approach of the bottom and upper part of the nozzle holder and the inclination of the spray nozzles towards the sprayed plants and this corresponds to **(Grisso *et al*., 1988; Alheidary, 2018)** As well as the pressure did not have a significant effect with the aforementioned characteristic at the pressure level (P3) if it gave the highest percentage of homogeneity in the spray (85.3%) and this may be due to the fact that the high pressure gives more discharge and water and the most distance by which the sprayed material flies towards the sprayed target and this is consistent with **(Grisso *et al*., 1988; Alheidary, 2018)** As for the inside of the duo, its effect is also not significant, as it resulted from the interference of the position (A1) and pressure (P2) On the sprinkled plant and this agrees with **(Hanafi, *et al*., 2016; Braheem & Alheidary, 2022).**

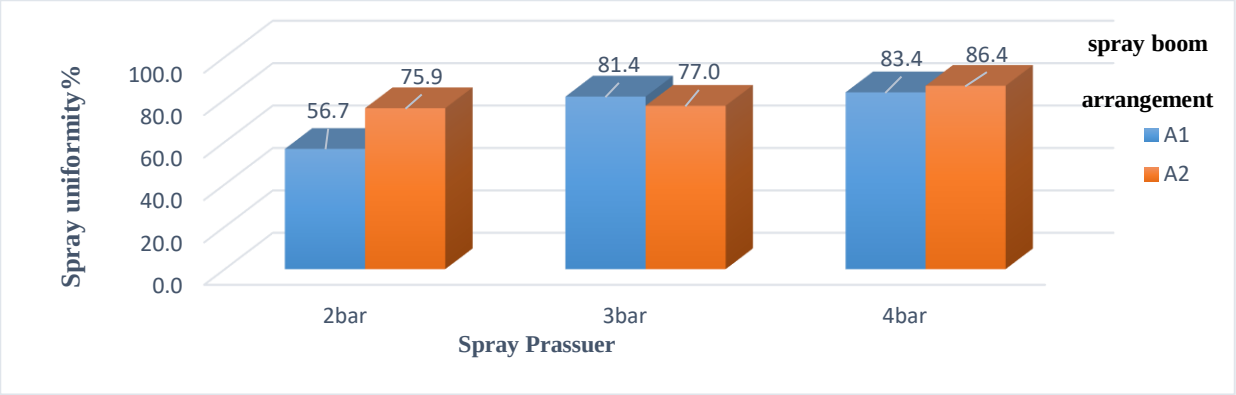

Figure (3): effect of spray boom arrangement and spray pressure on spray uniformity.

8. Efficient of use the sprayer machine

Table (1): Comparison of the costs of using the traditional spraying methods and the developed sprinkler spraying methods for five greenhouses per season.

Seq	Paragraph	Traditional spraying methods	Developed sprayer
1	The cost of buying the sprayer	Spraying (withdrawn + backpack) 350.000 IQD.	One sprayer machine 450,000 IQD
2	Spray operators' fees for (20) spraying times	600,000 IQD	300,000 Iraqi dinars
3	Fuel and maintenance for (20) times spraying	(50,000 fuel + 50,000 maintenance) IQD per season	(75,000 batteries and solar panels + 15,000 maintenance) IQD per season
4	The average price of pesticides and fertilizers for (5) plastic houses per season	About 1,500,000 Iraqi dinars.	About 750,000 Iraqi dinars.
Grand Total Cost		2,550,000 IQD/Season	1,590,000 IQD/season

Table (2): Comparison between the efficiency of using the traditional spraying methods

and the developed sprayer to the five of greenhouses in one season.

Seq	Paragraph	Traditional spraying methods	Developed sprayer
1	Type of energy used	One of the oil derivatives	Solar Energy
2	Its relationship to the environment	Harmful to the environment	Eco-friendly
3	The time Completion for the one line	2 min	1 min
4	It is estimated that one house needs pesticides and fertilizers diluted with water in one spray.	(100-120) liters / house.	(33-45.5) liters/house.
5	The effect of spraying on the operator.	The operator affects the middle and lower part of his body	They affect less on the operator's body because they pull.
6	Spray type.	Heterogeneous	Relatively homogeneous
7	The Stages of using	The dorsal sprayer is used at the beginning of the plant's life after its growth, the sprinkler with a push cart or carried behind the puller is used	Used with all stages of growing plant
8	Number of operators	You need 2 operators for the cart-mounted sprayer, while mounted the sprayer on the tractor needs 3 operators.	Its only need one operator.

THE CONCLUSIONS

1. The sprayer machine is designed successfully, and the sprayer machine is tested using study factors.
2. Treatment A1 and compression P3 showed superiority over the rest of the study levels in the characteristics of penetration and loss. The A1 level with pressure P2 achieved the highest result with the spray homogeneity characteristic. I recommend using the sprinkler with smallholder greenhouse farmers who suffer from water scarcity.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 224-237
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(19)
Al-Mishyyikh& Jarallah

المجلة العراقية لبحوث السوق
وحماية المستهلك

THE INTERACTION EFFECT OF BIOCHAR AND BINTONITE ON SOME PHYSICAL PROPERTIES OF DESERT SOIL AND BROAD BEAN PRODUCTIVITY

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Received 14/ 6/ 2023, Accepted 4/ 9/ 2023, Published 31/ 12/ 2024

ABSTRACT

A field experiment was carried out to evaluate effect of biochar and bentointe and their interaction on some physical properties of desert soil and broad bean productivity. The study included four levels of biochar (Seek) (0, 10, 20 and 30 Mg ha^{-1} (S_0, S_1, S_2 and S_3) and four levels of bentonite (0, 10,20 and 30 Mg ha^{-1}) (B_0, B_1, B_2 and B_3). The experiment was carried out according to randomized complete block design (RCBD) with three replicates. The results indicated that the application of biochar and bentonite significantly affected on physical properties. The fourth level of biochar and bentonite gave the highest values of moisture content at field capacity, wilting point and available water reached 16.38, 7.00 and 9.39%, respectively for biochar and reached 16.29, 6.75 and 9.54%, respectively for bentonite. While the fourth level of both two amendments gave lowest value of bulk density which reached 1.34 and 1.55 Mg m^{-3} for biochar and bentonite respectively. The results showed also that the fourth level of biochar and bentonite gave the highest values of green pods and seeds yield which reached 17.540 and 3.039 Mg ha^{-1}, respectively for biochar and 28.990 and 3.353 Mg ha^{-1}, respectively for bentonite, The interaction between two amendments, the treatment (S_3B_3) was the most efficient in giving the lowest value for bulb density and highest values for the other physical soil properties and plant productivity.

Keyword: Available water, Bulk density, Broad bean, Desertification, Field capacity.

[*] **The article is taken from a master's thesis by the first researcher.**

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Iraqi Journal of Market Research and Consumer Protection

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(2024) 16(2): 224-237

التأثير المتداخل للفحم الحيوي والبنتونايت في بعض الصفات الفيزيائية لتربة صحراوية وانتاجية الباقلاء

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الخلاصة

اجريت تجربة حقلية بهدف معرفة تأثير بعض مصلحات التربة (الفحم الحيوي والبنتونايت) في بعض الصفات الفيزيائية لتربة صحراوية وانتاجية الباقلاء. تضمنت التجربة اربعة مستويات من الفحم الحيوي نوع (Seek) هي 0 و5 و10 و 15 ميكاغرام ه-1 ورمز لها (S_0 و S_1 و S_2 و S_3) واربعة مستويات من البنتونايت هي 0 و 10 و 20 و 30 ميكاغرام هـ-1 و رمز لها (B_0 و B_1 و B_2 و B_3). نفذت تجربة عاملية باستعمال تصميم القطاعات العشوائية الكاملة وبثلاثة مكررات. أظهرت النتائج أن زيادة مستوى إضافة كل من الفحم الحيوي و البنتونايت اثرت تأثيراً معنوياً في الصفات الفيزيائية للتربة لقد حقق المستوى الرابع من الفحم الحيوي والبنتونايت أعلى القيم اذ بلغت 16.38 و 7.00 و 9.39% للمحتوى الرطوبي عند السعة الحقلية ونقطة الذبول وكمية الماء الجاهز على التتابع للفحم الحيوي و 16.29 و 6.75 و 9.54% للصفات على التتابع للبنتونايت بينما حقق المستوى الرابع من اضافة كلا المصلحين اقل قيمة للكثافة الظاهرية بلغت 1.34 و 1.55 ميكاغرام م-3 لكل من الفحم الحيوي والبنتونايت على التتابع. كما أوضحت النتائج ايضا بان المستوى الرابع من الفحم الحيوي والبنتونايت اعطى اعلى القيم في حاصل القرنات الخضراء وحاصل البذور بلغت 17.540 و 3.039 ميكاغرام هـ-1على التتابع للفحم الحيوي اما بالنسبة للبنتونايت فقد بلغت القيم 18.990 و 3.353 ميكاغرام هـ-1 على التتابع. أما بالنسبة للتداخل بين اضافة المصلحين فقد تفوقت المعاملة S_3B_3 (15 ميكاغرام هـ-1 فحم حيوي و 30 ميكاغرام هـ-1 بنتونايت) في تحقيق اقل قيمة للكثافة الظاهرية واعلى القيم للمحتوى الرطوبي في التربة واعلى انتاجية لنبات الباقلاء.

الكلمات المفتاحية: الماء الجاهز، الكثافة الظاهرية، السعة الحقلية، التصحر ، الباقلاء.

INTRODUCTION

Desertification threatens at the present time vast areas in Iraq, and that most regions of Iraq are affected by desertification in all its forms to one degree or another, and the areas of desertified lands threatened with desertification and sand dunes occupied the majority of the area of Iraq, and there are other areas threatened with desertification annually if measures are not taken in sufficient ways prevention and minimizing the effects of this phenomenon. The environmental statistics report for the year 2009 indicated that the percentage of lands threatened with desertification amounts to 92% of the total lands of Iraq **(Hammadi, 2009; Al-Timimi,2021).**

Desert soils are often coarse textured soils, which makes them suffer from many constraints related to the poor quality of some of their physical and chemical properties and low nutrients availability, which makes them have low productivity for the crops grown in them, which requires taking several measures to reduce their constraints in order to optimally exploit them agriculturally **(Al-Janabi, 2016)**. The means in this field, including the use of some compounds, which are called soil amendments. Many soil amendments have been used in coarse-textured soils with the aim of improving their physical, chemical and biological properties and improving their fertility and productivity for various agricultural crops, including bentonite clay **(Al-Kinani & Jarallah, 2021).** vermiculite and kaolinite **(Wyszkowski *et al.*, 2009).** Organic amendments were also used in different animal and plant

sources, compost, and waste from cities and factories **(Al-Kinani & Jarallah, 2022; Abdulridha & Essa, 2023).**

Biochar was used as an improver in 1998 by the American Chemical Society and described as a solid product from biomass burning of plant waste **(Bapat & Monahan, 1998)**. **Lehmann *et al.* (2006)** suggested that the addition of biochar is one of the modern technologies to improve the properties of different soils. The different properties of the soil, including the physical ones, by increasing the ability of the soil to retain water and surface area, improving soil structure and reducing the bulk density, which contribute to increasing the productivity of agricultural crops in those soils **(Karhu *et al.*, 2011, Al-Tameemi & Jaber, 2019). Sudhir (2006)** noted that the addition of biochar at a level of 10% to two soils (sandy loam and sandy) led to an increase in the amount of available water by 52 and 124% for the two soils, respectively, compared to the control treatment.

Biochar has a high ability to improve the ability of sandy soil to retain water to a greater degree compared to clay and silty soils, in addition to its role in reducing water infiltration and increasing available water within the root zone of the plant, which increases the efficiency of irrigation water rationing and treatment of desertification, since its addition to the soil improves their structure, porosity, and decrease in bulk density **(Liu *et al.*, 2012; Alkheero *et al.*, 2019).** Many studies have indicated the positive effect of adding biochar on plant growth and productivity in various agricultural soils, especially degraded ones, for its role in improving the different soil properties and increasing nutrient availability **(Kayama *et al.*, 2016; Japakumar *et al.*, 2021; Li *et al.*, 2021).**

Several studies that used bentonite as an improver for sandy soils indicated an improvement in the physical properties of the soil such as soil structure, increased stability of soil aggregates, increased ability to retain water and bulk density, and decreased water conductivity **(Kayama *et al.*, 2016; Al-Hayani *et al.*, 2022). Semalulu *et al.* (2015)** indicated that increasing the level of bentonite addition from 0 to 5, 10, 15, and 20% to sandy soil led to an increase in the moisture content in the soil, and they obtained a positive correlation of the second degree between the level of bentonite addition and the moisture content of the soil ($R^2 = 0.996^{**}$). This study was aimed to evaluate the effect of two types of soil conditioners (biochar and bentonite) and their interaction on some physical properties of desert soil and broad bean productivity.

MATERIALS AND METHODS

A field experiment was conducted at desert soil with sandy loam texture in Al-Karma district of Al-Anbar Governorate. The experiment was divided into 3 blocks, each one includes 15 experimental unit and the distance between them was 1 m while the distance between blocks was 2 m. Soil samples were collected from the layer (0 – 0.3 m) before planting. Some physical and chemical properties of the soils were measured by **(Page *et al.*,1982)** Table 1.

Table (1): Some physical and chemical properties of soil before planting.

Property		Value	Unit
EC $_{1:1}$		3.20	dS m^{-1}
pH $_{1:1}$		7.40	
O.M		7.60	g kg^{-1} soil
Carbonate minerals		263.15	
CEC		4.11	c $mol_{+}kg^{-1}$ soil
Soluble ions	Ca^{++}	19.32	m mole L^{-1}
	Mg^{++}	9.24	
	Na^{+}	3.65	
	K^{+}	0.88	
	$CO_3^{=}$	-	
	HCO_3^{-}	1.00	
	$SO_4^{=}$	0.91	
	Cl^{-}	22.65	
Available nutrients	NH_4^{+}	11.20	mg kg^{-1} soil
	NO_3^{-}	18.80	
	P	4.23	
	K	116.14	
Bulk density		1.64	Mg m^{-3}
Field capacity		9.38	%
Wilting point		2.50	
Available water		6.88	
Particle size analysis			
Clay		124.0	g kg^{-1} soil
Silt		116.0	
Sand		760.0	
Texture		Sandy loam	

The experiment included four levels of biochar (Seek Chinese origin: it is an organic charcoal produced from bamboo cane) which are 0, 5, 10, and 15 Mg ha^{-1} and their symbols (S_0, S_1, S_2 and S_3) Table 2 shows some characteristics of the biochar. Four levels of bentonite were used which 0, 10, 20 and 30 Mg ha^{-1} and their symbols (B_0, B_1, B_2 and B_3) some characteristics of the bentonite clay (Table 3). Both conditioners were added after mixing them with the soil. Faba bean (*Vicia faba* L.)

Table (2): Some characteristics of biochar (Seek).

Characteristic	Value	Unit
EC $_{1:5}$	3.18	dS m^{-1}
pH $_{1:5}$	7.15	-
N	1.31	%
P	0.65	
K	1.65	
O.C	25.32	
O.M	43.65	
C / N	19.33	-

Spain cultivar (Luz-de-otono variety) was sown in 17/10/2021 for winter season, N was added at level 100 kg N ha^{-1} as urea (46% N) and P at level 120 kg P_2O_5 ha^{-1} was added as TSP. (46% P_2O_5) and K at a level of 40 kg K_2O ha^{-1} in the form of K_2SO_4 (50% K_2O) according to the fertilizers recommendation of board bean **(Ali *et al.*, 2014).** N was added in two doses, As for P and K fertilizers, they were added once. At the end of the experiment the crop was harvested in 26/3/2022.

Table (3): Some characteristics of bentonite**.**

Characteristic		Value	Unit
EC $_{1:1}$		3.14	dS m^{-1}
pH $_{1:1}$		7.00	
$CaCO_3$		130.10	g kg^{-1}
Gypsum		2.40	
CEC		60.1	c mol_+kg^{-1} clay
Oxides content	P_2O_5	0.65	%
	K_2O	0.50	
	CaO	0.58	
	MgO	0.18	
	Fe_2O_3	1.52	
	Al_2O_3	34.58	
	Na_2O	0.18	
	SO_3	0.23	
	SiO_2	51.96	
	Cl	0.05	
Particle size analysis			
Clay		899.0	g kg^{-1} clay
Silt		99.0	
Sand		2.0	
Texture		Clay	

A factorial experiment was carried out within randomized complete block design (RCBD) with three replications. Least significant differences test (LSD 0.05) was used to compare the means of the treatments **(Steel &Torrie, 1980).**

RESULTS AND DISCUSSION

Bulk density

The results shown in Table 4 showed that there were significant differences for the effect of biochar on the bulk density of the soil after harvest, as the a mean bulk density was 1.65, 1.56, 1.46 and 1.34 Mg m^{-3}, with a decrease of 5.45, 11.52 and 18.79% for the soil levels. Biochar added 5, 10 and 15 Mg ha^{-1}, respectively compared to the control treatment, as the bulk density of soil decreased with increasing the level of biochar addition and the fourth level of addition achieved the highest percentage of decrease in bulk density. Increasing the level of bentonite addition from 0 to 10, 20 and 30 Mg ha^{-1} led to an increase in the bulk density reaching an a mean of 1.45, 1.49, 1.52 and 1.55 Mg m^{-3}, with an increase rate of 2.76, 4.83 and 6.90% for bentonite levels. Sequentially compared to the control treatment, the fourth level of bentonite addition achieved the highest increase in bulk density.

The results shown in Table 4 showed that the interaction between the two study factors led to a decrease in the bulk density of the soil, as it reached its lowest value of 1.25 Mg m^{-3} in the interaction treatment S_3B_0 , with a decrease of 22.36% compared to the control treatment for the overlap S_0B_0 of 1.61. Mg m^{-3}. The results showed that the behavior of the two study factors were opposite in their effect on the bulk density, but the biochar was more effective through the interaction of adding it with bentonite.

Table (4): Effect of biochar and bentonite on soil bulk density after harvest (Mg m^{-3}).

Biochar (S)	Bentonite (B)				B Mean
	B_0	B_1	B_2	B_3	
S_0	1.61	1.62	1.66	1.70	1.65
S_1	1.51	1.56	1.57	1.61	1.56
S_2	1.41	1.46	1.48	1.49	1.46
S_3	1.25	1.33	1.37	1.39	1.34
S Mean	1.45	1.49	1.52	1.55	
LSD 0.05	Biochar	Bentonite	S x B		
	.001	0.01	0.02		

Water content at field capacity

The results showed in Table 5 that increasing the level of biochar addition from 0 to 5, 10 and 15 Mg ha^{-1} led to an increase in soil moisture content at field capacity by 18.44, 31.08 and 43.81%, respectively compared to the control treatment, the results also showed a significant increase For the addition of bentonite in the soil moisture content at the field capacity, it increased by 9.49, 25.52, and 36.78% for bentonite levels 10, 20, and 30 Mg ha^{-1}, respectively compared to the control treatment (0 Mg ha^{-1}). The fourth level of addition excelled in achieving the highest value of moisture content at field capacity and the highest increase percentage. Biochar was superior to bentonite in achieving the highest rates of increase in moisture content at the field capacity. The results showed that there was a significant effect of the interaction between the two study factors (biochar and bentonite) on the soil moisture content at the field capacity. The interaction treatment S_3B_3 gave the highest value, reaching 18.98%, with an increase of 103.21% compared to the comparison treatment

S_0B_0 which amounted to 9.34% , the interaction between the two study factors had a positive effect on the soil moisture content at the field capacity was better than if each factor added alone to the soil.

Table (5): Effect of biochar and bentonite on water content at field capacity of soil after harvest (%).

Biochar (S)	Bentonite (B)				B Mean
	B_0	B_1	B_2	B_3	
S_0	9.34	10.93	12.15	13.13	11.39
S_1	11.23	12.45	14.46	15.80	13.49
S_2	12.81	13.73	15.94	17.24	14.93
S_3	14.26	15.04	17.26	18.98	16.38
S Mean	11.91	13.04	14.95	16.29	
LSD 0.05	Biochar	Bentonite	S x B		
	.004	0.04	0.07		

Water content at wilting point

Increasing the level of biochar addition from 0 to 5, 10, and 15 Mg ha^{-1} increased the moisture content at the wilting point of the soil by 26.20, 43.99, and 68.27% for the levels of biochar addition, respectively compared to the control treatment, and the fourth level (15 Mg ha^{-1}) showed the highest value and increase in soil moisture content at wilting point compared to other levels Table 6. The addition of bentonite increased the moisture content by 3.36, 35.41 and 50.33% for the bentonite addition levels 10, 20 and 30 Mg ha^{-1}, respectively compared to the comparison treatment. An increase compared to other addition levels. Biochar was more effective than bentonite in increasing the moisture content of the soil to give it the highest values and rates of increase.

Table (6): Effect of biochar and bentonite on water content at wilting point of soil after harvest (%).

Biochar (S)	Bentonite (B)				B Mean
	B_0	B_1	B_2	B_3	
S_0	2.49	3.90	4.91	5.35	4.16
S_1	4.11	4.76	5.67	6.47	5.25
S_2	5.13	5.23	6.48	7.14	5.99
S_3	6.22	6.48	7.25	8.04	7.00
S Mean	4.49	5.09	6.08	6.75	
LSD 0.05	Biochar	Bentonite	S x B		
	.003	0.03	0.05		

As for the interaction between the addition of biochar and bentonite, the interaction treatment S_3B_3 achieved the highest value of 8.04%, an increase of 222.89% over the comparison treatment of the overlap S_0B_0 , which gave the lowest value of 2.49%. The results showed a positive effect of the interaction between the addition of biochar and bentonite.

Clearly, through the high rate of increase in moisture content at wilting point, it is clear that adding these two conditioners together to the soil is better than adding each conditioner separately.

Available water

Increasing the level of biochar addition from 0 to 5, 10, and 15 Mg ha^{-1} led to an increase in the available water in the soil by 13.83, 23.65, and 29.88% for the levels, respectively, compared to the comparison treatment Table 7. The fourth level of addition achieved the highest value and percentage increase in the available water. in the soil. The results also showed that there was a significant effect of the level of bentonite addition in the available water (bentonite 10, 20 and 30 Mg ha^{-1}). The highest value of available water and increase percentage was at the fourth level of bentonite addition. The results showed that there was a significant effect of the interaction between the level of adding biochar and bentonite in the available water in the soil. The comparison for the interference S_0B_0 59.71% gave the lowest value for available water 6.85%.

Table (7): Effect of biochar and bentonite on available water of soil after harvest (%).

Biochar (S)	Bentonite (B)				B Mean
	B_0	B_1	B_2	B_3	
S_0	7.78	7.24	7.03	6.85	7.23
S_1	9.33	8.79	7.69	7.12	8.23
S_2	10.10	9.47	8.61	7.59	8.94
S_3	10.94	10.01	8.82	7.79	9.39
S Mean	9.54	8.88	8.04	7.34	
LSD 0.05	Biochar	Bentonite	S x B		
	.004	0.04	0.07		

The addition of biochar to the soil led to a significant effect on the physical properties under study, as the increase in the level of biochar addition led to a decrease in bulk density and an increase in soil moisture content at field capacity, wilting point, and available water. Biochar has a bulk density of less than 0.6 Mg m^{-3}, which is much lower than the bulk density of soil. Adding it to the soil leads to a decrease in bulk density by mixing and thinning **(Omondi *et al.*, 2016)**. Also, biochar is an organic material that works to increase soil agglomerations and increase its volume and stability depending on the raw materials from which it is produced. In addition, biochar has a large proportion of micropores, which increase the surface area, which contributed to reducing the bulk density of soil **(Jien, 2019)**.

These results are consistent with what many researchers have obtained **(Al-Tamemi & Jaber, 2019; Simansky *et al.*, 2022).**

As for the increase in soil moisture content at field capacity, wilting point, and available water after adding biochar, this may be attributed to the fact that biochar works to improve soil structure, redistribute pores, and increase the specific surface area of soil, in addition to that it has the ability to hold large amounts of water because it possesses High porosity, which contributed to increasing the moisture content in the soil **(Kang *et al.*, 2018)**.

The increase in soil moisture content after adding biochar is also attributed to the fact that it has the ability to hold water at a rate of six times its dry weight **(Robb & Joseph, 2019).** Many researchers have emphasized an increase in soil moisture content, especially coarse-texture soil, after adding biochar to it **(Sudhir, 2006; Alkheero *et al.*, 2019; Al-Halfi & Al-Azzawi,2022; Jasim & Hamid,2023).**

As for the effect of bentonite on the physical properties of the soil under study, increasing the level of its addition led to an increase in the bulk density and moisture content at the field capacity and wilting point and the available water for the soil after harvesting. soil micropores, Hence, reducing soil volume and increasing its mass, as well as its contribution to reducing the total porosity of the soil **(Al- Ani, 2009).** The results obtained are consistent with what was found by **(Kayama *et al.*, 2016).**

As for the increase in soil moisture content at field capacity, wilting point, and available water after adding bentonite, it may be attributed to the fact that the bentonite mineral particles are soft because of their small size. Which leads to an increase in the ability of the soil to hold water and reduce infiltration, evaporation and the total water conductivity of the soil, in addition to that it has the ability to hold water at a rate of more than 10% than it is in sandy soils **(Salih, 2000; Kayama *et al.*, 2016).** It was also found that bentonite clay has the ability to hold water in a range ranging from 1-5 times its weight, which contributes to increasing the moisture content in the soil, especially the coarse texture of it. The results are consistent with what was also found by **(Al-Kinani & Jarallah, 2021).**

As for the interaction between the two factors of the study (biochar and bentonite), it had a significant positive effect on the moisture content of the soil at field capacity, wilting point, and available water. In bulk density, they had opposite effects, as biochar contributed to reducing it, while bentonite contributed to increasing it. Biochar outperformed bentonite in its effect in reducing bulk density of soil.

Adding the two conditioners together is better than if they were added alone to the soil, and this was found by a number of studies that used soil conditioners jointly **(Jabakumar *et al.*, 2021; Al-Mishyyikh & Jarallah, 2024).**

Green pods yield

The results shown in Table 8 showed an increase in the yield of green pods with an increase in the level of biochar addition with an increase rate of 11.59, 21.98 and 29.36% for the levels of biochar addition. 5, 10 and 15 Mg ha^{-1}, respectively compared to the control treatment. The results shown in Table 8 showed an increase in the yield of green pods with an increase rate of 20.67, 38.51 and 57.61% for the levels of bentonite addition 10, 20 and 30 Mg ha^{-1}, respectively compared to the control treatment. The results showed a significant effect of the interaction between biochar and bentonite on the yield of green pods. The interaction treatment S_3B_3 achieved the highest value of 20.543 Mg ha^{-1}, an increase of 107.36% over the comparison treatment S_0B_0 , which gave the lowest value for the yield of green pods, which reached to 9.907 Mg ha^{-1}.

Table (8): Effect of biochar and bentonite on green pods yield (Mg ha^{-1}).

Biochar (S)	Bentonite (B)				B Mean
	B_0	B_1	B_2	B_3	
S_0	9.907	12.807	14.280	17.240	13.559
S_1	11.447	13.750	16.676	18.650	15.131
S_2	13.790	15.517	17.320	19.527	16.539
S_3	15.050	16.087	18.480	20.543	17.540
S Mean	12.049	14.540	16.689	18.990	
LSD 0.05	Biochar	Bentonite	S x B		
	.0438	0.438	0.875		

Seeds yield

The results showed in Table 9 an increase in seed yield with an increase in the level of biochar addition, as it reached an average of 2.083, 2.398, 2.665, and 3.039 Mg ha^{-1}, with an increase rate of 15.12, 27.94 and 45.90% for the levels of addition 5, 10 and 15 Mg ha^{-1}, respectively compared to the control treatment (0 Mg ha^{-1}). As for the addition of bentonite, the results showed in Table 9 that increasing the level of bentonite addition from 0 to 10, 20 and 30 Mg ha^{-1} led to an increase in the seed yield, as it reached an average 1.744, 2.379, 2.709, and 3.353 Mg ha^{-1}, with an increase of 36.41, 55.33 and 92.26% for the addition levels, respectively, compared to the comparison treatment (0 Mg ha^{-1}). As for the interaction between the two factors of the study (biochar and bentonite), the results showed that the interaction treatment S_3B_3 was superior in achieving the highest value of seed yield amounted to 4.120 Mg ha^{-1} with an increase of 224.67% over the comparison treatment of interference S_0B_0 , which gave the lowest value of seed yield reached to 1.269 Mg ha^{-1}.

Table (9): Effect of biochar and bentonite on seeds yield (Mg ha^{-1}) .

Biochar (S)	Bentonite (B)				B Mean
	B_0	B_1	B_2	B_3	
S_0	1.269	2.028	2.352	2.681	2.083
S_1	1.651	2.247	2.574	3.120	2.398
S_2	1.844	2.518	2.808	3.491	2.665
S_3	2.211	2.722	3.103	4.120	3.039
S Mean	1.744	2.379	2.709	3.353	
LSD 0.05	Biochar	Bentonite	S x B		
	.0160	0.160	0.320		

The results showed the positive overlapping effect of biochar and bentonite in achieving a significant increase in seed yield was better than if each was added separately, and the increase in seed yield with an increase in the level of addition of biochar and bentonite was incremental, which indicates that the soil under study still needs to higher levels of these two reformers and did not reach the optimal levels of them in increasing plant indicators. This may be attributed to the role of biochar in improving the properties of the soil under study (sand mixture) (Table 1) through its contribution to improving soil structure, increasing soil agglomerations, decreasing bulk density, increasing the ability of soil to retain water and

available water in the root zone, as well as reducing water loss. Irrigation, reducing the degree of soil interaction and electrical conductivity, and increasing the readiness of nutrients by increasing the exchange capacity of positive ions and the mineralization of nitrogen and phosphorus, as it is a source for them, as well as increasing the organic carbon in the soil, which contributed to increasing the biological activity in the soil and biological interactions. All of these roles that biochar contributes to improve most of the different soil characteristics (physical, chemical, and biological) and improve soil fertility, which contributes to increasing plant nutrient absorption, increasing vital and physiological processes, processing food and storing it in seeds, and increasing yield indicators and its components under study (**Kayama *et al.*, 2016; Al-Tamemi & Jaber, 2019).** The results are consistent with the results of a number of studies that obtained an increase in the yield and its components for multiple crops (legumes, maize, and rice) when they added biochar to coarse-textured soils **(Armanto,2019; Lee *et al.*, 2021).**

The results also indicated that the addition of bentonite had a significant effect on the yield indicators and its components under study, and this may be due to the positive effect of bentonite in the study soil in terms of increasing the ability of the soil to retain water, increasing its readiness for plants, and increasing the readiness of some nutrients, including (nitrogen, phosphorus and potassium) through its role in Increasing the exchange capacity of positive ions and preserving nutrients from loss, which contributed to increasing their readiness and absorption by the bean plant, and this was reflected in the increase in yield indicators and its components under study **(Dhary & Al-Baldawi,2017; Minhal *et al.*, 2020).**

Many studies using bentonite clay as an improver for coarse-textured soils have confirmed an increase in the yield and yield components of different crops (yellow and white corn, barley and potatoes) (**Semalulu *et al.*, 2015; Al-Kinani & Jarallah,2021)**. As for the interaction between the level of adding biochar and bentonite, it had a significant positive effect on increasing the yield indicators and its components under study. These two reformers excelled in achieving the highest value of the indicators under study within the fourth level for each of them. Most of the physical, chemical and fertility characteristics of the soil combined, and the interfering effect was superior in increasing yield indicators compared to if each reformer was added individually, and biochar was more efficient in increasing the indicators under study compared to bentonite **(Abbas *et al.*, 2018; Hasin *et al.*, 2021).**

CONCLUSION

It can be concluded the addition of soil conditioners (seek biochar and bentonite) significantly affects the soil physical properties (bulk density, water content at field capacity and wilting point and available water). The addition of biochar (15 Mg ha^{-1}) and bentonite (30 Mg ha^{-1}) with achieved a great response to the broad bean plant represented by increasing the parameters of vegetative growth and improving soil physical properties. The effect of the two conditioners to the soil together was more efficient under study compared to adding each of them separately.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 238-245
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(20)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

A STUDY OF THE EFFECT OF AN EIGHT-PURPOSE COMPOUND MACHINE ON SOME PHYSICAL SOIL PROPERTIES

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Received 14/ 6/ 2023, Accepted 31/ 7/ 2023, Published 31/ 12/ 2024

ABSTRACT

A field experiment was conducted to evaluate the effect of a locally developed and assembled eight-purpose compound equipment on some physical soil properties in a silty clay loam soil in one of the fields of the College of Agricultural Engineering Sciences/University of Baghdad/Al-Jadriyah for the spring growing season of 2023. The experiment used, the MF-650 Tractor. The study included two factors: the first factor had three seed rates 3, 4, and 5 kg donum^{-1}, and the second factor had three tillage depths of 15, 20, and 25 cm, have been studied the bulk density of the soil, soil moisture content, germination rate, number of fallen seeds, and percentage of broken grains. The experiment used a randomized complete block design (RCBD) with split-plot arrangement and three replications. The least significant difference (LSD) at a significance level of 0.05 was used to compare the means of the treatments. The results indicate significant differences in the physical soil properties due to plowing depth and seeding rate. A plowing depth of 15 cm resulted in the soil's lowest bulk density, reaching 1.02 µg m^{-3}. It also resulted in the lowest soil moisture content (12.69%), germination rate (68.83%), and percentage of broken seeds (3.77%). The results indicated no significant differences in the physical soil properties due to the seeding rate and no significant interaction in the physical soil properties between plowing depth and seeding rate.

Keywords: Bulk density, soil moisture content, germination rate, broken grains.

دراسة تأثير الة مركبة ثمانية الغرض في بعض صفات التربة الفيزيائية

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الخلاصة

نفذت تجربة حقلية لدراسة تاثير الة مركبة ثمانية الغرض مطورة ومجمعة محليا في بعض صفات التربة الفيزيائية في تربة مزيجة طينية غرينية في احد حقول كلية علوم الهندسة الزراعية /جامعة بغداد/الجادرية للموسم الزراعي الربيعي 2023. استخدم في التجربة جرار من نوع MF-650 وتضمن البحث دراسة عاملين الاول فيه ثلاث كميات للبذار(التقاوي) وهي 3و 4 و5 كغم. دونم$^{-1}$ والعامل الثاني اعماق الحراثة وهي15 و20 و25 سم، تم دراسة الكثافة الظاهرية للتربة، المحتوى الرطوبي للتربة، نسبة الانبات، عدد البذور النازلة و نسبة عدد الحبوب المتكسرة. تم استخدام ترتيب الالواح المنشقة Split-Plot تحت تصميم القطاعات العشوائية الكاملة المعشاة(RCBD) وبثلاثة مكررات واستخدم اقل فرق معنوي (LSD) عند مستوى 0.05 لمقارنة متوسطات المعاملات وأشارت النتائج الى وجود فروق معنوية لصفات التربة الفيزيائية جراء عمق الحراثة وكمية البذار(التقاوي) اذ تفوق العمق 15 سم في اعطائه اقل

كثافة ظاهرية للتربة بلغت(1.02) ميكا غرام. م$^{-3}$ واقل محتوى رطوبي للتربة بلغ (12.69 %) واقل نسبة انبات بلغت (68.83 %) واقل نسبة بذور متكسرة بلغت (3.77 %). وثبتت النتائج عدم وجود فروق معنوية في صفات التربة الفيزيائية جراء كمية البذار(التقاوي) وعدم وجود فروق معنوية في صفات التربة الفيزيائية جراء التداخل بين عمق الحراثة وكمية البذار(التقاوي).

الكلمات المفتاحية: الكثافة الظاهرية، المحتوى الرطوبي للتربة، نسبة الانبات، الحبوب المتضررة.

INTRODUCTION

Agricultural machinery is considered a fundamental component of modern agriculture due to its ability to control various factors affecting productivity, increase production, improve its quality, reduce costs, and minimize working hours by efficiently performing agricultural operations quickly. It also enables the cultivation of larger areas with different crops. Agricultural machinery is a key indicator of transitioning from traditional to modern agriculture **(Al-Tahan *et al*., 1991).**

The combined machine reduces soil compaction, improves soil physical properties, reduces time and operating costs, and prepares a good bed for seeds in a short time **(Frank *et al*., 2012).** It is necessary to develop methods and techniques that do not negatively affect soil properties but rather improve the physical soil characteristics and reduce the negative impact of machinery and agricultural equipment, and the use of mechanical assembly, which involves using compound machines that perform all means of production in one, while reducing the entry of machinery and agricultural equipment into the field. **(Ahmed & Amran, 2004).**

The compound machine accomplishes multiple agricultural operations in one, and the compound machines contain primary equipment and secondary equipment, and crop servicing machinery. The compound d machine requires significant power, which must be available in the agricultural tractor to complete the agricultural operations. Plowing and seeding machines are the basis for the productivity of the crop to be cultivated **(Jasim, 2019; Al-Shukraji *et al*., 2006).**

The plow sole or hard pan layer in the soil is considered one of the problems and obstacles that agricultural fields face in general, and crop productivity in particular, due to its direct negative impact on the physical properties of the soil. **(Gill & Vanden, 1968; Jasim & Abd, 1996).**

Maize (*Zea mays* L.) is the third most important cereal crop in terms of cultivated area and production, following wheat and rice, globally. It is considered a strategic crop that plays a significant role in the livelihood of the Iraqi people and contributes to the development of agro-industries. It also plays a crucial role in animal production by providing green and concentrated fodder **(Al-janabi *et al*., 2023).** Many international organizations have emphasized the need to double production by the year 2050. This study was conducted in view of the importance of knowing the effect of the eight-purpose compound machine on some physical soil properties.

Maize is a versatile crop, allowing for its cultivation in a wide range of agricultural environments **(Al-Aridhee & Mahdi, 2022),** especially considering the current world population of 7.2 billion, expected to increase to approximately 9.2 billion by 2050 **(Tawfic, 2019).**

MATERIALS AND METHODS

A field experiment was conducted at one of the research stations of the College of Agriculture, University of Baghdad, Al-Jadriyah, in the year 2023 to evaluate the effect of a locally developed and assembled eight-purpose compound machine on some physical soil properties. Random samples were taken from the experimental field at depths of 0-30 cm, and the analyses were performed at the central laboratory of with the College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriya. The samples were analyzed to determine some field soil properties, as shown in Table (1).

Table (1): Physical and chemical properties of field soil.

Parameter	Unit		Value
Soil separates	Clay	gm kg^{-1}	390
	Sand	gm kg^{-1}	110
	Silt	gm kg^{-1}	500
Soil texture			Silty clay loam
pH			7.4
EC	ds.m^{-1}		3.7
Soil bulk density	Mg m^{-3}		
Soil moisture content At field capacity	%		42.62
Soil moisture content At wilting pint	%		20.62
Soil moisture content	%		2.62

The compound machine was used, which consists of several interconnected tools in one structure. It includes eight agricultural machines, namely the primary tillage machine, which utilizes a subsoil plow, the smoothing machine equipped with a rotary harrow, the furrow opener machine, the planting machine (seeder), the fertilizing machine, the first irrigation machine, the weed control machine, and the boundary leveling machine (Figure 1).

Rear view **Side view**

Figure (1): Rear view and front view of the compound machine.

The experiment used a randomized complete block design (RCBD) with a split-plot arrangement and three replications. Seed rates (3, 4, 5 kg donum^{-1}) were allocated to the main plot, while the tillage depth treatments (15, 20, 25 cm) were allocated to the sub-plot. The total number of experimental units was 27, with an area of 30 m^2 (10 x 3 m) each, was left a distance of 10 meters before each replicate, until the tractor gains a constant speed. The area of the experimental field was 350 m^2.

In the implementation of the experiment, a tractor of the type (MASSEY FERGUSON) of Brazilian origin (MF-650) was used to tow the compound machine, so that the tractor with the compound machine would be one unit.

Measurement of Studied Characteristics:

Bulk Density of Soil (Mg/m^3):

The bulk density was calculated using the Core Sampler Method based on the equation proposed by **Blake and Hartge (1986).**

Pp = MS/Vt ----------1

Where:

Pp = Bulk density (Mg m^{-3})

MS = Mass of the dry sample (1 Mg)

Vt = Volume of the sample (m^3)

Moisture Content of Soil (%):

The moisture content was measured using the following equation proposed by **Gardner (1965).**

$$Mc = \frac{Msw-Ms}{MS} \times 100$$----------2

Where:

Mc = Moisture content of the soil (%)

Msw = Mass of the wet soil (g)

Ms = Mass of the dry soil (g)

Germination Ratio (%):

Several factors, such as seeds type, size, and planting depth affect germination ratio. The field germination ratio was measured after 20 days of planting by calculating the number of seeds required for one theoretical hectare and monitoring the actual seeds planted. The following equation, proposed by **Baqer (2011),** was used to determine the field germination ratio.

$$GR = \frac{\text{Number of germinated seeds in the experimental unit}}{\text{Number of seeds planted in that unit}} \; x\; 100$$---------- 3

Where:

GR = Germination Ratio (%)

Percentage of Broken Seeds (%):

It was calculated according to the method proposed by **Mohammed (2019)** and **Al-Banna (1990).** Samples were collected after passing through the feeding mechanism and through bags placed at the end of four randomly selected seed tubes from the planting lines. At

the end of each experimental unit, and with different studied factors (seeding rate and tillage depth), the number of seeds that fell from the tubes was manually counted for four bags per experimental unit. Then, the number of damaged seeds was manually counted for each bag, and the percentage of broken seeds was calculated using the following equation:

$$DG = \frac{ND}{Nt} X\ 100 \quad ---------4$$

Where:
DG: Percentage of broken seeds (%)
Nd: Total number of broken seeds
Nt: Total number of seeds

RESULTS AND DISCUSSION

Bulk Density (Bb) (μg m^{-3}).

The results indicate significant differences between tillage depths on the bulk density of soil. Increasing the depth leads to an increase in the bulk density of the soil. The bulk density at the first depth (15 cm) had the lowest value of 1.02 Mg m^{-3}. The second depth (20 cm) recorded an average bulk density of 1.04 Mg m^{-3}, while the third depth (25 cm) had the highest average bulk density of 1.15 Mg m^{-3}. The reason for this is that increasing the average depth increases the pressure on the soil due to the load applied by the machine and the weight of the soil layers on top of each other, resulting in an increase in soil compaction due to the subsoil layers and consequently an increase in bulk density. These results are consistent with the findings of **Menkhi and Jebur (2022) and Aridhee *et al.* (2020).**

It was also clear from the results a significant interaction between seeding rate and tillage depth. The lowest value of soil bulk density was recorded at a first seeding rate (3 kg donum) and tillage depth of 15 cm at 1.02 Mg m^{-3}, while the highest soil bulk density was recorded for third seeding rate of 5 kg donum^{-1} and tillage depth of 25 cm at 1.19 Mg m^{-3}.

Table (2): Effect of plowing depth and seeding amount on soil bulk density (Mg m^{-3})

Seeding rate kg donum^{-1}	Tillage depth (cm)			Mean seeding rate
	15	20	25	
3	1.02	1.04	1.15	1.07
4	1.05	1.08	1.11	1.08
5	1.10	1.14	1.19	1.14
LSD $_{0.05}$	0.081			N.S
Mean tillage depth	1.06	1.08	1.15	
LSD $_{0.05}$	0.073			

2- Soil Water Content (%)

The results revealed a significant effect of tillage depth on soil water content (Table 3). It is observed that an increase in average depth leads to an increase in soil water content. The first average depth recorded a moisture content rate of 14.10%, while the second average depth recorded a moisture content rate of 15.18%. The third average depth recorded the highest

moisture content value at 16.52%. Additionally, the result indicates no significant effect of seeding rate on soil water content. It was also clear from the results that there were significant differences in the moisture content of the soil due to the interaction between the first seeding rate (3 kg donum^{-1}). and the depth of the first plowing of 15 cm, as they recorded the lowest soil moisture content of 12.69%, and the third seeding rate (5 kg. Donum^{-1}) and the third plowing depth of 25 cm, as they recorded the highest soil moisture content of 17.24%.

Table (3): Effect of plowing depth and seeding amount on Soil water content (%).

Seeding rate kg donum^{-1}	Tillage depth (cm)			Mean seeding rate
	15	20	25	
3	12.69	13.85	15.89	14.14
4	13.74	15.70	16.46	15.30
5	15.90	16.02	17.24	16.39
LSD $_{0.05}$	1.01			N.S
Mean tillage depth	14.11	15.19	16.53	
LSD $_{0.05}$	0.73			

3 - Germination Percentage (%)

The result shows a significant effect of the seeding rate on germination percentage. It is observed that the first seeding rate (3 kg ha^{-1}), recorded the lowest average germination percentage at 71.58%. The second seeding rate (4 kg ha^{-1}), recorded an average germination percentage of 80.58%. The third seeding rate (5 kg ha^{-1}) recorded the highest average germination percentage at 88.89%. The reason behind this is that the germination percentage increases with an increase in seeding rate and to an increase in the forward speed of the tractor **(Omar *et al.*, 2021)**. Additionally, the result indicates no significant effect of tillage depth. The results also indicate that there was a significant difference in the percentage of germination due to the interaction between the first seeding rate (3 kg. Donum^{-1}) and the first plowing depth of 15 cm, as they recorded the lowest percentage of germination amounting to 68.83%, and the third seeding rate of 5 kg. Donum^{-1} and the third plowing depth of 25 cm, as they recorded the highest percentage of germination, at 93.75%.

Table (4): Effect of plowing depth and seeding amount on germination (%).

Seeding rate kg donum^{-1}	Tillage depth (cm)			Mean seeding rate
	15	20	25	
3	12.69	13.85	15.89	14.14
4	13.74	15.70	16.46	15.30
5	15.90	16.02	17.24	16.39
LSD $_{0.05}$	1.01			N.S
Mean tillage depth	14.11	15.19	16.53	
LSD $_{0.05}$	0.73			

4- Broken Seed Percentage (%)

Table 5 demonstrates the effect of tillage depth and seeding rate on the percentage of fallen, broken seeds. The result reveals significant differences in the percentage of fallen

broken seeds, and this is due to an increase in seeding rate (kg donum^{-1}) per unit area. The average number of broken seeds in the first seeding rate of 3 kg dunum-^{1} was 4.84% , and for the second seeding rate of 4 kg dunum^{-1} was 6.04%. While for the third seeding rate of 5 kg dunum-^{1} resulted in a higher percentage of fallen broken seeds at 12.68%. The result indicates no significant effect of tillage depth on the seeding rate (kg/ha). The results also indicate significant differences in the percentage of broken seeds due to the interaction between the first seeding rate of 3 kg donum^{-1}and the depth of the first plowing of 15 cm, with the lowest percentage of broken seeds at 3.77%. Furthermore, between the third seeding rate of 5 kg per donum^{-1} and the depth of the third plowing of 25 cm, with the highest percentage of broken seeds was at 14.78%.

Table (5): Effect of plowing depth and seeding amount on Broken Seed Percentage (%).

Seeding rate kg donum^{-1}	Tillage depth (cm)			Mean seeding rate
	15	20	25	
3	3.77	4.72	6.02	4.84
4	4.26	6.23	7.65	6.04
5	10.02	13.25	14.78	12.68
LSD $_{0.05}$	3.913			4.854
Mean tillage depth	6.02	8.06	9.48	
LSD $_{0.05}$	N.S			

CONCLUSIONS AND RECOMMENDATIONS:

Based on the above results, the following conclusions can be drawn. The successful use of the compound machine in executing eight agricultural tasks at the same time, included primary plowing, cultivation, furrow opening, planting, fertilization, initial irrigation, weed control, leveling, and achieving a maximum depth of 15 cm with lower bulk density, moisture content, germination rate, and broken seed percentage. There was no significant effect of the seeding rate on the physical soil properties. Therefore, we recommend the use of the combined machine to execute eight agricultural tasks simultaneously, including primary plowing, secondary plowing (cultivation), furrow opening, planting, fertilization, initial irrigation, weed control, and leveling, for its ability to reduce effort, optimize time, and minimize fuel consumption. We also recommend measuring other characteristics of the machine in different soil textures.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 246-253
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(21)
Obaed & Ahmaed

المجلة العراقية لبحوث السوق
وحماية المستهلك

INHIBITORY ACTIVITY OF POMEGRANATE PEEL EXTRACT AGAINST BACTERIA *CRONOBACTER SAKAZAKII* ISOLATED FROM DIFFERENT FOOD

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Received 15/ 6/ 2023, Accepted 30/ 10/ 2023, Published 31/ 12/ 2024

ABSTRACT

The present study was conducted to demonstrate the effectiveness of the pomegranate peel aqueous extract against *Cronobacter sakazakii* (*C. sakazakii*) isolated from different food sample, *C. sakazakii* was isolated and identified with biochemical tests. On the other hand, active compounds were extracted from pomegranate peel and a concentration of (0.8,1.6,3.2,6.4,12.8,25.6,51.2,100) mg/ml was prepared. The effectiveness of the pomegranate extract was then evaluated against the growth of the isolates under study using Minimum inhibitory concentration method, the results showed a sensitivity of *C. sakazakii* to all concentrations. The results showed a difference in the inhibitory effect according to the concentration used. The bacteria were more sensitive to the concentration of 100 mg/ml and less sensitive to the concentration of 25 mg/ml by measuring the diameters of the bacterial inhibition zones of the different concentrations of pomegranate peel extract, the largest diameter inhibition was 37 mm at 100 mg/ml, while the 25 mg/ml concentration showed the lowest diameter was 15 mm. The results of the study suggest that pomegranate peel extracts have an inhibitory effect on *C. sakazakii* and increase this activity by increasing the concentration used.

Keywords: Food sample Pomegranate peel extracts, Minimum inhibitory concentration.

الفعالية التثبيطية لمستخلص قشر الرمان ضد بكتريا *Cronobacter sakazakii* المعزولة من أغذية مختلفة

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الخلاصة

أجريت الدراسة الحالية لبيان فعالية المستخلص المائي لقشر الرمان ضد *C. sakazakii* المعزولة من عينات غذائية مختلفة، وتم عزل *C. sakazakii* وتشخيصها بالاختبارات الكيموحيوية. ومن ناحية أخرى، تم استخلاص المركبات الفعالة من قشر الرمان وتم تحضير تركيز (51.2،25.6،12.8،6.4،3.2،1.6،0.8 و100 ميكروجرام/مل). تم بعد ذلك تقييم فعالية مستخلص الرمان مقابل نمو العزلات قيد الدراسة باستخدام طريقة التركيز المثبط الأدنى، أظهرت النتائج حساسية C. sakazakii لجميع التراكيز. أظهرت النتائج اختلافاً في التأثير المثبط تبعاً للتركيز المستخدم. وكانت البكتيريا أكثر حساسية لتركيز 100 ملغم/مل وأقل حساسية لتركيز 25 ملغم/مل وذلك بقياس أقطار مناطق التثبيط البكتيري للتراكيز المختلفة لمستخلص قشر الرمان، وكان أكبر قطر تثبيط 37 ملم عند 100 ملغم/مل، بينما التركيز 25 ملغم/مل أظهر أقل قطر 15 ملم. وتشير نتائج الدراسة إلى أن مستخلصات قشر الرمان لها تأثير مثبط لبكتيريا Cronobacter sakazakii وتزيد من هذا النشاط عن طريق زيادة التركيز المستخدم.

الكلمات المفتاحية: عينات غذائية، مستخلص قشور الرمان، التركيز المثبط الأدنى.

INTRODUCTION

Cronobacter spp (previously known as *Enterobacter sakazakii*) are Gram-negative, catalase positive, oxidase negative, motile by peritrichous flagellae, rod shaped, non sporforming bacteria belonging to the *Enterobacteriaceae* family. This ubiquitous microorganism has been associated with severe neonatal infections; these include meningitis, meningoencephalitis, sepsis, and necrotizing enterocolitis. It is also associated with serious sequelae including brain abscess and impaired sight and hearing (**Farmer *et al*., 1980**). The bacterium was first implicated in a case of neonatal meningitis in 1958 when an outbreak resulted in the death of two infants in England (**Maclean *et al*.,2008**). Although the frequency of infection generally tends to be low, the prognosis is poor with case-mortality rates varying from 33-80% among infected infants (**Lai *et al*.,2001**). Consequently, *Cronobacter* spp. have been ranked as a 'severe hazard for restricted populations, life threatening or substantial chronic sequelae or long duration' by the International Commission for Microbiological Specifications for Food (ICMSF), which places this pathogen in the same group as *Listeria monocytogenes, Cryptosporidium parvum* and *Clostridium botulinum* types A and B (**ICMSF, 2002**). , the species of E. sakazakii was defined to include 5 genomogroups, which were differentiated according to the division of the 15 *E. sakazakii* biogroups. Accordingly, in 2007 the Cronobacter genus was first defined, and this definition was subjected to more revisions in 2008 and 2012. Currently, Cronobacter genus consist of 7 species; *C. sakazakii, C. malonaticus, C. turicensis, C. muytjensii, C. dublinensis, C. universalis and C. ondimenti* (**Iversen *et al*., 2007a; Joseph *et al*., 2011**). It is known that *C. sakazakii, C. malonaticus* and C. turicensis are the only species associated with clinical incidence and particularly with neonatal infections so far (**Stephan *et al*., 2010; Hariri *et al*., 2013; Asato *et al*., 2013; Holý *et al*., 2014**) ,*Punica granatum* L. (pomegranate) fruit belongs to the Punicaceae family and is distinguished by its high nutritive benefits assignable to their bioactive components of phenolic acids, flavonoids and tannins (**Coronado *et al*., 2021**) In this regard, pomegranate plant has been assayed for possible healing effects recording antiradical, antimicrobial, anti-inflammatory, hypolipidemic, antiproliferative and hypoglycemic properties (**Di sotto A. *et al*., 2019**) .The potential antiradical and antitumor efficiency of pomegranate peel extracts has been directly assigned to their phytoactive constituents of polyphenolic compound (**MasciA ,*et al*,. 2016**). Punica granatum L. peels were previously described to exhibit antimicrobial efficiency against food borne pathogens involving *Escherichia coli*, *Bacillus subtilis, Penicillium italicum* and *Fusarium sambucinum* (**Hunter *et al*., 2008**) .Moreover, pomegranate exhibited strong antioxidant activity due to the prevalence of several active phytochemicals as polyphenols, flavones, flavonoids, anthocyanins and catechins in seeds, fruits and peels of pomegranate (**Kothary *et al*., 2009**).Furthermore, pomegranate has been reported as a potential source of anti-tumor agents owing to the prevalence of many active phytochemicals as polyphenols and flavonoids (**Al-Lami *et al*., 2015**). The aim of this study to demonstrate to demonstrate the effectiveness of the pomegranate peel aqueous extract against *Cronobacter sakazakii* isolated from different food sample.

MATERIAL AND METHODS

Sample Collection

After recording the labeling information name of company, commercial name of product, contents, origin, date of expired and production, batch number, 50 samples were collected from various food sources form local market in Baghdad during period January 2021, to December 2022. The samples included 10 gm from powdered infant formula, Hash meat, spices, and dairy products and 5 gm from Cereals and vegetable as show in table (1).

Table (1): Food samples used for isolation of *Cronobacter spp*.

Food sample	Type of sample	Amount (gm)
Powdered Infants formula	almudhish, dialac, Nido, Similac novolack,rainbow	10
Spices, herbs and dried food	thyme, cumin,cinnamon, ginger,tea,chamomile semolina,sesame	10
Cereals and flour	flour, rice adass, homs, corn,	5
Meats and meat products	hash spiced meat, chicken meat, sausage, processed meat	10
Dairy Products	cheese, yogurt, cream	10
Vegetables	cucumber Vegetables carrot, green olive,	5
Total Number		50

These samples were collected in packages and bags (Plastic or paper) sterilize. After completing the sampling, the following information was documented before being sent to the laboratory and then transported to the laboratory. Food samples were kept at 4 °C until use.

Culture Media

The following culture media were present in this study for isolate *C. sakazakii* include Violet red bile glucose agar, Brain heart infusion broth ,Enrichment Enterobacteraceae broth ,Tryptic soy agar and chromogenic agar all this media were prepare according to the manufacturer's instructions and use medium EEbroth for activation ,TSA and chromogenic agar used as differential medium ,All culture media was prepared according to the manufacturing company instructions pH was adjusted with 0.1 N NaOH or 0.1N HCl , The culture media used in this study was sterilized, which needs to be sterilized by autoclaving at 121ºC (15 pound/Inch $_2$ pressure) for 15min , except Violet Red Bile Glucose Agar (VRBGA) sterilized by boiling(**kim *etal*., 2008**).

Isolation from food sample

The isolation of *Cronobacter spp* from different food samples was done according to the Food and Drug Administration (**2012**) with modification (**kim *et al.*, 2008**). food samplewere cultured on VRBGA media and incubated for a while 24 h at 37°C, after which all samples were streaking on Tryptic soy agar and incubated with temperature25 °C for 72 h, and finally it was cultured on chromogenic agar It was incubated at 37 °C for 24 h.

Identification

Bacterial isolates were identified according to microscopical and morphological features by viewing colonies developing on TSA and chromogenic agar, biochemical tests, and molecular analysis (**Barron *et al.*, 2007; Iversen *et al.*, 2007**)

Pomegranates peel

Pomegranates fruits were collected from different places in the local markets in Baghdad-Iraq, using plastic bags and sent to the laboratory. The pomegranate seeds were removed and separated from the peels, then they were dried using a convection oven, then it was first grounded using a ceramic mortar and then blender using an electric mixer for fine powder. The powder was kept in clean plastic bags and marked with the name of the plant and the date of collection, then kept in a dark sterile place away from moisture at room temperature until it was used later.

Extraction technique:

Aqueous Extract (AE)

The water was extracted from the plant according to the method described by Pin-Der and Gow-Chin,(1997), where 10 g of each sample of the dried plants was taken with 150 ml of distilled water at boiling point and left for 2 h on the magnetic mixture was filtered by funnel through filter paper (What man No.1) and then the concentrated extract was poured into a petri dish and placed in an electric oven a temperature ranging from 45-55ºC until the extract dried ,then the dried powder was scraped and collected in dry sterile containers and kept until use .

Ethanolic Extract (EE)

The extraction was carried out according to the method described by (**Zhou *et al.*, 2005**), as 10 gm of each sample of the mentioned dried plants prepared was taken with 150 ml of 70% ethanol alcohol and left for 2 h on the magnetic stirrer. Filter by funnel through a filter paper (Whatman NO.1), then pour the extract into petri dishes and put in the electric oven at a temperature of 45-55ºC until dry, then the dried powder was scarped and collected in dry test tubes and kept until use.

Preparing the stock solution of the extraction

Weigh 1 g of the obtained extract powder was used, dissolved in 10 ml of solvent distilled water in a sterile glass container and left for 3 d in the laboratory until the extract dissolved well. The concentration of the stock solution of the extract becomes 100 mg /ml.

Preparing the extract concentrations used in the present study

The stock solution of plant extracts (aqueous and alcoholic) was prepared by dissolving 100 mg of dry extract/10 ml of distilled water and the following concentrations were prepared from it: 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2 and 100 mg/ml **(Teramoto *et al.*, 2010).**

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of pomegranate peel aqueous extract against *C. sakazakii* were determined as described previously (**Shi *et al.*, 2018**), with some modifications. Briefly, overnight bacterial culture was diluted 400× in TSB medium (approximately1×10^8 cfu/ml) before 125 ml of the

diluted culture were added to individual wells of a 96-well Microtiter plate. Equal volumes of pomegranate peel aqueous extract solution were gently added to each well to achieve final extract concentrations of 0 (control), 10, 25, 50 and 100 mg/ml. TSB medium containing 0.1% DMSO was used as the negative control. Plates were incubated at 37°C for 24 h, and cell growth was monitored at 600 nm at 1-h intervals using a microplate reader (Model 680; Bio-Rad, Hercules, CA, USA). The MIC of extract was defined as the lowest concentration at which there was no visible growth of *C.sakazakii* (**Jasim *et al.*, 2015**).

Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration is explained as the least concentration of the pomegranate peel extract showing bactericidal activity. After 24 h incubation, 100 µL from the well of the micro-broth assay plates was cultured onto MHA plates then the plates were further incubated at 37 °C for 24h.The minimum concentration of extracts exhibiting no visible bacterial growth was recorded as MBC (**Saleh *et al.*, 2023).**

RESULTS & DISCUSSION

Through the results of isolation, it was found that 36 bacterial isolates (72%) that were isolated from 50 food samples gave yellow colonies and turbidity (**Mossawi *et al.*, 2015**).All positive samples that were cultured on Violet Red Bile Glucose Agar (VRBGA) the results showed that 10 bacterial isolates (27.8%) grew on VRBGA medium and gave typical colonies of Enterobacter spp. (Cronobacter), which appeared in the form of pink or purple colonies, because this medium contains crystal violet color and bile salts (**Al-joubori *et al.*, 2014**). This is why the color of the colony is pink (FDA, 2002) as shown in Figure (1).

Figure (1): (A)*Cronobacter spp* on VRBGA (B) On Tryptic soy agar (C) On Chromogenic agar.

MIC and MBC of *C. sakazakii* by Pomegranate peel extracted.

The Minimum Inhibitory Concentrations (MICs) for Pomegranate peel extraction against *C. sakazakii* isolates were determined using the micro dilution method in Mueller-Hinton broth, and the results were interpreted after 24 hours of incubation at 37 °C according to the Clinical Laboratories Standards Institute CLSI (2020) (Table 1). The results of MIC confirmed the previous results of disc diffusion method, where the current study showed the high level sensitive of most isolates to the different concentration of pomegranate peel aqueous extract **(Mahindroo *et al.*, 2016).** The result showed strong antibacterial activity against *C. sakazakii*, with an observed MIC of 100 mg/ml for both strains, the effects of

pomegranate peel aqueous extract on the growth of *C. sakazakii* strains are shown in table(2) concentration of 51.2 µg/mL, the lag phase of both *C. sakazakii* cultures was longer than that of the control culture grown in the absence of aqueous extract However, the growth weak of *C. sakazakii* treated with 12.8,6.4, 3.2, 1.6, or 0.8 mg/ml aqueous extract were not different from that of the untreated control.**(Jaber *et al.*, 2015).**

Table (2): MIC and MBC of Pomegranate peel toward ten isolate of *C.sakazakii*.

No	Strain	Origin	MIC (µL/mL)	MBC (µL/mL)
1	*C.sakazakii* ATCC (CFSAN068773)	Infant formula	25.6	51.2
2	A1	Infant formula	25.6	51.2
3	A2	Infant formula	51.2	102.4
4	A3	Hash Meat	51.2	102.4
5	A4	Hash Meat	25.6	51.2
6	A5	Spices	25.6	51.2
7	B1	Blood	51.2	40.2
8	B2	Blood	6.4	12.8
9	CSF1	Cerebrospinal fluid	25.6	51.2
10	CSF2	Cerebrospinal fluid	51.2	102.4
11	CSF3	Cerebrospinal fluid	51.2	102.4

CONCLUSION

Through studying the effect of aqueous extract of pomegranate peels on the growth of *C. sakazakii* bacteria, it was found that growth is affected, and this effect increases with increasing concentration of the extract. We note that it was more sensitive to the concentration of 100 mg and less sensitive to the concentration of 0.8 mg. Therefore, this extract can be used and adopted as one of the methods to control the growth of bacteria. *C. sakazakii*.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 254-266
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(22)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

SATISFACTION OF CORN FARMERS WITH THE PERFORMANCE OF THE AGRICULTURAL INNOVATION SYSTEM IN BABYLON GOVERNORATE

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Received 15/ 6/ 2023, Accepted 13/ 9/ 2023, Published 31/ 12/ 2024

ABSTRACT

The research aimed to identify the extent of satisfaction of corn farmers on the performance of the agricultural innovation system in the Governorate of Babylon and in each of its fields, and to achieve the objectives of the research, a questionnaire was prepared in the light of literature and previous studies related to the subject and the opinions of specialized experts and their approval of them, consisting of (19) items distributed over (4) fields, , and the research population included all corn farmers numbering (13065) farmers registered within the agricultural divisions of the Directorate of Agriculture of Babylon and the number (15) agricultural division, and a random sample was selected stratified proportional from farmers by (2%) and by (155) farmers distributed over (50%) of the agricultural divisions, has been collected Data using the questionnaire and the personal interview method, The results of the research showed that the general arithmetic average of the level of satisfaction of corn farmers with the performance of the agricultural innovation system amounted to (2.3) degrees, and a standard deviation of (0.53) degrees, and a relative weight of (14.29%) degree, and that the highest percentage (91.61%) of the respondents is within the level of satisfied with a means degree to dissatisfied, and the results also showed that the mean of the fields of satisfaction of corn farmers with the performance of the agricultural innovation system, which numbered (7) fields ranged between (1.30 – 2.69) degrees, and standard deviations It ranged between (0.27 – 0.61) degrees, and relative weights ranged between (9.73% – 20.13%) degrees. Accordingly, the researcher recommends the need for the agricultural innovation system in the Governorate of Babylon to adopt the results of the research with regard to the fields and items that formed a case of dissatisfaction and little satisfaction with the performance of the agricultural innovation system and try to address them.

Keywords: farmer satisfaction, agricultural innovation system, corn crop.

[*]**The article is taken from the doctoral thesis of the first researcher.**

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Iraqi Journal of Market Research and Consumer Protection

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(2024) 16(2): 254-266

رضا زراع الذرة الصفراء عن أداء نظام الابتكار الزراعي في محافظة بابل

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الخلاصة

استهدف البحث التعرف على مدى رضا زراع الذرة الصفراء عن أداء نظام الابتكار الزراعي في محافظة بابل وفي كل مجال من مجالاته ، ولتحقيق أهداف البحث أعدت إستبانة في ضوء الادبيات والدراسات السابقة ذات العلاقة بالموضوع وأراء الخبراء الأختصاصيين وموافقتهم عليها والمتكونة من (19) فقرة توزعت على (4) مجالات ، وشمل مجتمع البحث جميع زراع الذرة الصفراء البالغ عددهم (13065) زارعاً المسجلين ضمن الشعب الزراعية التابعة لمديرية زراعة بابل والبالغ عددها (15) شعبة زراعية ، وأختيرت عينة عشوائية طبقية تناسبية من الزراع بنسبة (% 2) وبواقع (155) زارعا موزعين على (% 50) ، وقد جمعت البيانات باستخدام الأستبانة وبطريقة المقابلة الشخصية ، واظهرت نتائج البحث أن المتوسط الحسابي العام لمستوى رضا زراع الذرة الصفراء عن اداء نظام الابتكار الزراعي بلغ (2.3) درجة ، وأنحراف معياري بلغ (0.53) درجة ، ووزن نسبي (14.29%) درجة ،وأن اعلى نسبة (91.61%) من المبحوثين تقع ضمن مستوى راضي بدرجة قليلة الى غير راضي ، كما اظهرت النتائج ان المتوسطات الحسابية لمجالات رضا زراع الذرة الصفراء عن اداء نظام الابتكار الزراعي البالغ عددها (4) مجالات تراوحت بين (1.30 – 2.69) درجة ، وبأنحرافات معيارية تراوحت بين (0.27 – 0.61) درجة ، وبأوزان نسبية تراوحت بين (9.73% –20.13 %) درجة . وعليه يوصي الباحث ضرورة قيام نظام الابتكار الزراعي في محافظة بابل بتبني نتائج البحث فيما يتعلق بالمجالات والفقرات التي شكلت حالة غير راضي وراضي بدرجة قليلة عن أداء نظام الابتكار الزراعي ومحاولة معالجتها.

الكلمات المفتاحية : رضا الزراع ، نظام الابتكار الزراعي، محصول الذرة الصفراء .

INTRODUCTION

The agricultural sector performs a prominent activity in the economies of countries as the main source in providing foodstuffs to all developed and developing countries of the world alike **(Al-Jubouri & Ridan, 2021)**, and that one of the most important sources of food is cereal crops due to the basic and important elements of life for humans and animals alike, and that one of the most important of these crops is the corn crop, which constitutes the first crop in obtaining proteins compared to other crops **(Okab & Abed, 2022)**, as it constitutes the third place after wheat and barley in terms of cultivated area, productivity and nutritional value **(Khalaf & Hassan, 2022).** It used its residues to improve soil properties and improve the productivity of crops planted afterwards in the agricultural cycle, which enhances the sustainability of the farm system of the rural family **(Mutar *et al*, 2022)**, as well as its use in animal feed **(Abdulla *et al*, 2022)**. Despite the efforts made by the state and the relevant authorities, the production and productivity of the corn crop are still below the required level, and this decrease is confirmed by statistics indicating that the cultivated fields during the years 2019 and 2022 were (478), (325) don respectively, and production (633) and (374) tons respectively, while the productivity for the same years reached (134),(114)kg/ don , respectively **(Central Organization for Statistics, 2019- 2022)**. This decline in productivity can be attributed to several factors, including the agricultural innovation system, which is the main driver of sustainable agriculture and food security in the future of developing countries,

including Iraq **(Al-Taye *et al,* 2021)**. It is represented in improving and increasing the productivity and production of the farm system and developing the knowledge, skills and capabilities of farmers, as well as preserving natural resources and reflecting positively in improving their economic resources and their social and living level appropriately and in accordance with international specifications for agricultural products, as it is one of the important aspects in the development, survival, growth and continuation of its constituent organizations in providing the best services **(Saadia, 2021)**. The performance of the agricultural innovation system is affected by several factors, including the satisfaction of farmers with the services it provides to them, as the satisfaction of the targeted people is not achieved unless there is a strong and distinct relationship between the management of the organization and the relevant authority that provides them with the service, and the satisfaction of farmers is one of the indicators that determine the level of good performance of the organization's management of its activities and the achievement of its goals, and satisfaction is an indicator of the level of performance of workers in any system and the extent of their needs for training programs, which is reflected in Improving the level of their performance in providing services to the targeted, In addition, satisfaction enhances the loyalty of those targeted in the survival of dealing with governmental organizations **(Taiser & Al-Zubaidi, 2022)**. That is, satisfaction is a tool that enables the system to measure the appropriateness of the services it provides to the target (their sense of satisfaction or dissatisfaction) with those services and to make them respond to their needs and expectations **(Salih & Khan, 2021)**. Based on the above, and given the importance of knowing the satisfaction of corn farmers as an important indicator of the level of performance of the agricultural innovation system, this research came to raise the following question: What is the satisfaction of farmers with the performance of the agricultural innovation system for the corn crop in Babylon Governorate? What are the priorities for farmers' satisfaction with the performance of the agricultural innovation system for the corn crop in Babylon Governorate?

Research Goals:

1- Identify the level of farmers' satisfaction with the performance of the agricultural innovation system for the yellow corn crop in Babylon Governorate.

2- Determining the priorities of farmers' satisfaction with the performance of the agricultural innovation system for the yellow corn crop in Babylon Governorate. Research hypothesis: The existence of high satisfaction among farmers with the performance of the agricultural innovation system for the yellow corn crop in Babylon Governorate.

Research Methods and Tools Used

Research methodology: In order to achieve the objectives of the research, I use the descriptive approach, which is one of the research methods that deal with events, phenomena and practices that exist and are available for research and measurement as they are without the intervention of the researcher in their events.

Research Population and Sample: The research population included all corn farmers numbering (13065) farmers registered within the agricultural divisions of the Directorate of Agriculture of Babylon and the number (15) Agricultural Division, and a random stratified proportional sample of farmers was selected by (2%) and by (155) farmers distributed over

50% of the agricultural divisions and by (8) agricultural divisions, namely (Al-Siddha, Hillah, Al-Kifl, Al Escandariya , Abi Gharaq , Al- Al Muhaweel , Al Showmali , Al Mashrooa ').
Data Collection Tool: A questionnaire was prepared in the personal interview method as a tool for collecting data from corn farmers.

The preparation of the questionnaire went through a series of stages:
1- Preparation of the questionnaire in its initial form: In the light of the literature and previous studies related to the subject and the opinions of specialized experts, a measure of corn farmers' satisfaction with the performance of the agricultural innovation system has been prepared, consisting of (17) items distributed over (4) fields.
2- Development of the questionnaire: The questionnaire was presented to a group of experts in the field of agricultural extension, numbering (17) experts to indicate the degree of their approval on the fields and items of the questionnaire in the light of the scale of approval consisting of three levels: agree, agree with the amendment, and disagree.
3- Determining the criterion of expert approval (threshold cut) on the components of the questionnaire by (75%) or more than the degree of approval of experts for the survival of any of the components of the questionnaire (fields and items) proposed in its final form.
4- Calculating the averages of the scores of expert approval on the components of the questionnaire (fields and items): Estimated weights (numerical values) were given to the levels of approval scales as follows: (agree = 2), (agree with amendment = 1), (Disagree = zero), and thus the degree of the scale ranged between (0-2) degrees, and the averages were calculated by calculating the total scores obtained on the number of experts.
5- Preparation of the questionnaire in its final form: In light of comparing the average scores of expert approval on the components of the questionnaire (fields and items) with the threshold of cutting to prepare the questionnaire in its final form, all fields and items have achieved the threshold of cutting or more, as the threshold of cutting for the measure of satisfaction of corn farmers with the performance of the agricultural innovation system in Babylon Governorate reached (94.12%). As one item was added to each of the following fields: satisfaction with workers and satisfaction with agricultural financing. Thus, the number of items on the scale became (19) items distributed over (4) fields. Thus, the number of items of the scale became (19) items distributed over (4) fields.
Reliability: It was used to measure the reliability of the satisfaction of corn farmers with the performance of the agricultural innovation system Alpha Cronbach coefficient, which amounted to (0.84).
Data collection: The research data was collected using the questionnaire by personal interview method from the sample of (155) corn farmers during the period 5/10/2022 – 19/12/2022. Tabulation and analysis of data: The weights (1, 2, 3, 4) were given to the levels of the corn farmers' satisfaction scale on the performance of the agricultural innovation system (highly satisfied, moderately satisfied, slightly satisfied, dissatisfied) respectively, and to arrange the fields and items according to their importance from the farmers' point of view, they were arranged in descending order according to the mean, standard deviation and relative weight.
Statistical methods: The statistical methods used in analyzing research data are frequencies, percentages, Alpha Cronbach coefficient, mean, standard deviation and relative weight

RESULTS AND DISCUSSION

1- Identifying the satisfaction of corn farmers with the performance of the agricultural innovation system The results of the research showed that the highest percentage (79.35%) of the satisfaction of corn farmers with the performance of the agricultural innovation system is within the level of satisfied with a means degree, with an arithmetic average of (1.91) degrees, a standard deviation of (0.48) degrees, a relative weight of (20.76%) degrees, and (12.26%) of them falls within the level of dissatisfied, with an arithmetic average of (1.33) degrees, a standard deviation of (0.56) degrees, and a relative weight of (14.46%) degree, followed by the percentage (5.16%) within the level of satisfied with a Moderately satisfied degree, with an mean of (2.59) degrees, a standard deviation of (0.52) degrees, a relative weight of (28.15%) degrees, and the lowest percentage was (3.23%) within a level of high satisfaction, with an arithmetic average of (3.37) degrees, a standard deviation of (0.55), and a relative weight of (36.63) degrees, as shown in Table (1).

Table (1): Distribution of respondents according to their satisfaction with the performance of the agricultural innovation system.

levels of satisfaction	degrees of satisfaction	The number of farmers	%	means	standard deviation	relative weight
not satisfied	1 – 1.75	19	12.26	1.33	0.56	14.46
Slightly satisfied	1.76 – 2.51	123	79.35	1.91	0.48	20.76
Moderately satisfied	2.52 – 3.27	8	5.16	2.59	0.52	28.15
Highly satisfied	3.28–4	5	3.23	3.37	0.55	36.63
total sum		155	100			100
overall mean				2.3	0.53	25

from Table (1) that (91.61%) of the respondents had a level of satisfaction with the performance of the agricultural innovation system between dissatisfied to a means degree, and this may be attributed to several reasons, including that most of the technologies developed are unable to address the problems they face, and that the activities implemented for them did not meet their actual needs, and that workers in the agricultural innovation system do not communicate with them except within official working hours, as well as the high costs of agricultural inputs and the continuous delay in disbursement of their dues from the marketer's quotient.

2– Determine the priorities of the fields of satisfaction of maize farmers on the performance of the agricultural innovation system The results showed that the mean of the fields of satisfaction of maize farmers on the performance of the agricultural innovation system ranged between (1.3 – 2.69) degrees, standard deviations ranged between (0.27 – 0.61) degrees, and relative weights ranged between (20.13% - 9.73%) degrees, as shown in Table (2).

Table (2): Distribution of respondents according to their fields of satisfaction with the performance of the agricultural innovation system.

fields number in the questionnaire	fields	means	standard deviation	relative weight	arrangement	level of satisfaction
6	Satisfaction with agricultural extension services.	2.69	0.27	20.13	1	Moderately satisfied
1	Satisfaction with workers in the agricultural innovation system.	2.53	0.47	18.94	2	Moderately satisfied
7	Satisfaction with marketing the obtainer.	1.55	0.59	11.6	6	Not satisfied
4	Satisfaction with agricultural financing.	1.3	0.61	9.73	7	Not satisfied
the total sum				100		
The overall average of the fields		2.02	0.49	15.1		

from Table (2) that the field related to (satisfaction with agricultural extension services) came in the first place with an mean (2.69) degrees, a standard deviation (0.27) degrees, and a relative weight (20.13%) degrees, followed by the field related to (satisfaction with workers in the agricultural innovation system) came in second place with an arithmetic average of (2.53) degrees, a standard deviation of (0.47) degrees, and a relative weight (18.94%) degrees, both of which fall within the level of satisfaction with an average degree, and the reason for this may be attributed to the nature of The diversity and multiplicity of topics and extension activities provided to them by the guiding organization, which enhances the level of their knowledge and skill in applying the correct practices in the integrated management of the yellow corn crop, as well as the positive treatment of workers in the agricultural innovation system in rapid response in solving the productive and agricultural problems facing corn farmers and finding alternatives to solve them.

While the fields related to (satisfaction with the marketing of the obtainer), (satisfaction with agricultural financing) ranked third, fourth, respectively with mean of (1.55) degrees, (1.3) degrees, and standard deviations (0.59) degrees, (0.61) degrees, and relative weights (11.6%) degrees, (9.73%) degrees on respectively, All these fields mentioned above fall within the level of dissatisfied, and this may be due to several reasons, including the means quantities received from the yield of yellow corn marketed by farmers, waiting and delaying for long hours in queues of transport vehicles in front of centers. Receipt of the yield, and the continuous delay in the payment of farmers' financial dues for the marketed yield, as well as the lack of amounts allocated to support the production of the crop and the timing of obtaining it.

To arrange the items of each area of the satisfaction of corn farmers with the performance of the agricultural innovation system, the results were as follows:

1- Satisfaction with workers in the agricultural innovation system

The items related to the field of (satisfaction with workers in the agricultural innovation system) of (5) items obtained mean ranging between (1.1 - 3.81) degrees, standard deviations ranging between (0.31 - 0.56) degrees, and relative weights ranging between (8.7% - 30.12%) degrees, as shown in Table (3).

Table (3): Distribution of respondents according to the items of the field of satisfaction with workers in the agricultural innovation system.

The item number in the questionnaire	items	MEANS	standard deviation	relative weight	arrangement	level of satisfaction
1	Satisfaction with the speed in responding and solving production and agricultural problems.	3.81	0.52	30.12	1	Highly satisfied
2	Satisfaction with benefiting from the information provided by employees in the agricultural innovation system.	2.89	0.53	22.84	2	Moderately satisfied
3	Satisfaction with workers' willingness to hear and discuss farmers' opinions.	2.84	0.56	22.45	3	Moderately satisfied
5	Satisfaction with workers' respect for the expertise and skills that farmers possess.	2.01	0.31	15.89	4	Slightly satisfied
4	Satisfaction with employees' communication with them at different times.	1.1	0.43	8.7	5	not satisfied
total sum				100		
overall mean for items		2.53	0.47	18		

from Table (3) that the item related to (satisfaction with the speed of response and solving productive and agricultural problems) came in the first place with an arithmetic average of (3.81) degrees, a standard deviation of (0.52) degrees, and a relative weight (30.12%) degrees, and it is located within the level of satisfied with a high degree, and the reason for this may be attributed to the interaction and understanding of workers in the agricultural innovation system to the need for rapid response in solving productive and agricultural problems at the beginning of their emergence and the possibility of solving them in light of the possibilities available to them before Its breadth and difficulty in solving it or finding alternatives to solve it.

The item related to (satisfaction with the benefit of information provided by workers in the agricultural innovation system) came in second place with an arithmetic average of (2.89) degrees, a standard deviation of (0.53) degrees, and a relative weight of (22.84) degrees, followed by the item related to (satisfaction with the willingness of workers to hear and discuss

the opinions of farmers), which came in third place with an arithmetic average of (2.84) degrees, a standard deviation of (0.56) degrees, and a relative weight of (22.45%) degrees, both of which fall within the level of satisfaction with an average degree, and may be attributed The reason for this is that the information provided by the workers in the agricultural innovation system expresses their agricultural needs and problems, as well as the positive interaction of workers in exchanging views with farmers.

As for the item related to (satisfaction with the respect of workers for the experiences and skills possessed by farmers), it came in fourth place with an arithmetic average of (2.01) degrees, a standard deviation of (0.31) degrees, and a relative weight of (15.89%) degrees, and within the level of satisfaction to a means degree, and the reason for this may be attributed to the prevailing belief among workers in the agricultural innovation system that farmers have traditional agricultural experiences and practices inherited for the yellow corn crop, which are not suitable for the nature of modern technologies. While the item related to (satisfaction with the communication of workers with them at different times) came in the last order with an arithmetic average of (1.1) degrees, a standard deviation of (0.43) degrees and a relative weight of (8.7%) degrees, and within the level of dissatisfaction, and the reason for this may be attributed to the association of workers in the agricultural innovation system with official job timings in which working hours are specified and the large number of farmers who are difficult for them to contact them all, as well as the belief of workers in the agricultural innovation system of poor experiences and skills Which farmers own, which means that it is useless to continue communicating with them at different times, but only official times.

2- Satisfaction with agricultural financing

The (6) items related to the field of (satisfaction with agricultural financing) obtained mean ranging between (1.08-1.95) degrees, standard deviations ranging between (0.35-0.39) degrees, and relative weights ranging between (11.72% - 25.1%) degrees, as shown in Table (4).

Table (4): Distribution of respondents according to the items of the field of satisfaction with agricultural financing.

The item number in the questionnaire	items	MEANS	standard deviation	relative weight	arrangement	level of satisfaction
4	Satisfaction with the fair distribution of agricultural financing among farmers.	1.95	0.35	25.1	1	Slightly satisfied
2	Satisfaction with the amount or size of the amount given to the farmer.	1.25	0.76	16.09	2	not satisfied
6	Satisfaction with interest on agricultural financing.	1.19	0.59	15.31	3	not satisfied
1	Satisfaction with the mechanism of granting agricultural financing	1.16	0.6	14.93	4	not satisfied

The item number in the questionnaire	items	MEANS	standard deviation	relative weight	arrangement	level of satisfaction
	to farmers.					
3	Satisfaction with the timing of agricultural financing delivery.	1.14	0.55	14.67	5	not satisfied
5	Satisfaction with the speedy disbursement of financial dues to farmers when marketing the crop.	1.08	0.39	13.90	6	not satisfied
total sum				100		
overall mean for items		1.3	0.54	16.67		

from Table (4) that the item related to (satisfaction with the fairness of the distribution of agricultural finance among farmers) came in the first place with an arithmetic average of (1.95) degrees, a standard deviation of (0.35) degrees, and a relative weight of (25.1%) degrees, and it is located within the level of satisfaction with a means degree, and the reason for this may be attributed to the feeling of some respondents inequality in the distribution of funding due to favoritism and favoritism in these amounts.

As for the items related to (satisfaction with the amount or size of the amount granted to farmers), (satisfaction with the interest on agricultural financing), (satisfaction with the mechanism of granting agricultural financing to farmers), (satisfaction with the timing of delivery of agricultural financing), (satisfaction with the speed of disbursement of financial dues to farmers when marketing the obtained), they came in the second, third, fourth, fifth and sixth place respectively with mean(1.25) degrees, (1.19) degrees, (1.16) degrees, (1.14) degrees, (1.08) degrees, and standard deviations (0.76). Degree, (0.59) degree, (0.6) degree, (0.55) degree, (0.39) degree, and relative weights (16.09%) degree, (15.31%) degree, (14.93%) degree, (14.67%) degree, (13.90%) degree, and all these items mentioned above fall within the level of dissatisfied, and this may be due to several reasons, including the means amount of amounts granted to them, and the high interest on agricultural advances and loans and the long waiting period to obtain them, as well as the continuous delay and several agricultural seasons in The process of disbursing financial dues, which reflected negatively on their satisfaction with agricultural financing.

3- Satisfaction with agricultural extension services

The (4) items related to the field of (satisfaction with agricultural extension services) obtained mean ranging between (1.09 – 3.86) degrees, standard deviations ranging between (0.37 – 0.5) degrees, and relative weights ranging between (10.13% - 35.87%) degrees, as shown in Table (5).

Table (5): Distribution of respondents according to the items of the field of satisfaction with agricultural extension services.

The item number in the questionnaire	items	means	standard deviation	relative weight	arrangement	level of satisfaction
1	Satisfaction with the extension service topics related to the yellow corn crop.	3.86	0.5	35.87	1	Highly satisfied
2	Satisfaction with the guiding methods and means provided by the guiding organization (training courses, explanatory fields, guiding leaflets, etc.).	3.73	0.63	34.67	2	Highly satisfied
4	Satisfaction with the role of the media in promoting and persuading the application of elicited technologies in farmers' fields.	2.08	0.55	19.33	3	Slightly satisfied
3	Satisfaction with the timing of providing extension services.	1.09	0.37	10.13	4	not satisfied
total sum				100		
overall mean for items		2.69	0.51	25		

from Table (5) that the item related to (satisfaction with the topics of the extension service related to the yellow corn crop) came in the first place with an arithmetic average of (3.86) degrees, a standard deviation of (0.5) degrees, and a relative weight of (35.87%) degrees, followed by the item related to (satisfaction with the methods and means of guidance provided by the guiding organization (training courses, demonstration fields, guidance bulletins, etc.), which came in second place with an mean (3.73) degrees, a standard deviation (0.63) degrees, and a relative weight (34.67%). degree, and both fall within a highly satisfied level, and the reason for this may be due to the fact that the topics of the extension service related to the yellow corn crop express their needs and real problems that they suffer from and keep pace with these topics to the recent developments in the cultivation of yellow corn, as well as diversity in the use of methods and extension means that enhance and consolidate agricultural information among farmers, which helps them to apply it in their fields.

The item related to (satisfaction with the media role in promotion and persuasion in the application of technologies developed in the fields of farmers) came in third place with an arithmetic average of (2.08) degrees, standard deviation (0.55) degrees, and relative weight (19.33%) degrees, and is located within the level of satisfaction with a means degree, and the reason for this may be attributed to the lack of specialized media staff, as well as the weak investment of digital media in the delivery of ideas and modern developments to yellow corn growers.

While the item related to (satisfaction with the timing of providing extension services) came in the last trip with an arithmetic average of (1.09) degrees, a standard deviation of (0.37) degrees, and a relative weight of (10.13%) degrees, and it is located within the level of dissatisfied, and the reason for this may be attributed to the fact that workers in agricultural extension provide their extension services to farmers within the timings of their official work only and not solve most of the problems of farmers in those deaths, as well as the inappropriateness of providing extension services with Farmers' rest times for their agricultural work.

4- Satisfaction with the marketing of the obtainer.

The (4) items related to the field of (satisfaction with the marketing of the obtainer) obtained mean ranging between (1.1-1.99) degrees, standard deviations ranging between (0.3-0.39) degrees and relative weights ranging between (10.13% - 35.87%) degrees, as shown in Table (6).

Table (6): Distribution of respondents according to the items of the field of satisfaction with the marketing of the quotient.

The item number in the questionnaire	items	means	standard deviation	relative weight	arrangement	level of satisfaction
2	Satisfaction with the quantity received.	1.99	0.3	32.1	1	Slightly satisfied
4	Satisfaction with marketed prices.	1.92	0.4	30.97	2	Slightly satisfied
1	Satisfaction with the mechanism of receiving the marketed crop by the yellow corn growers.	1.19	0.58	19.19	3	not satisfied
3	Satisfaction with the timing of receipt of the harvest.	1.1	0.39	17.74	4	not satisfied
total summation				100		
The overall average of the vertebrae		1.55	0.42	25		

from Table (6) that the item related to (satisfaction with the quantity received) came in the first place with an arithmetic average of (1.99) degrees, a standard deviation (0.3) degrees, and a relative weight (32.1%) degrees, followed by the item related to (satisfaction with the prices of the marketer), which came in second place with an arithmetic average of (1.92) degrees, a standard deviation of (0.4) degrees, and a relative weight of (30.97%) degrees, and both of them fall within the level of satisfied with a means degree, and this may be attributed to several reasons, including the lack of quantities Received by the authority responsible for receiving the crop marketed by the farmers due to the limited yards designated for receiving the yield and the foot of its laboratories.

The item related to (satisfaction with the mechanism of receiving the yield marketed by corn farmers) came in first place with an arithmetic average (1.19) degrees, standard deviation (0.58) degrees, and relative weight (19.19%) degrees, followed by the item related to (satisfaction with the timing of receiving the yield), which came in second place with an arithmetic average of (1.1) degrees, standard deviation (0.39) degrees, and relative weight (17.74%) degrees, both of which fall within the level of dissatisfied, and this may be attributed to several reasons, including the delay And waiting marketers (farmers) for long hours in queues in front of the centers to receive the yield, as well as the link to the process of receipt of the functional timings of workers in those centers and their means number compared to the receipt of quantities marketed of the yield of yellow corn.

In the light of the above values of the mean of the satisfaction of maize farmers with the performance of the agricultural innovation system, we reject the hypothesis that states that there is high satisfaction among farmers with the performance of the agricultural innovation system for the yellow corn crop in the Governorate of Babylon.

CONCLUSION

In light of the results of the research, the following is concluded:

1- Satisfaction is one of the important indicators that reflect the performance of the agricultural innovation system in providing its services to corn farmers in Babylon Governorate.
2- The satisfaction of maize farmers with the performance of the agricultural innovation system is not at the required level, and here indicates that there are some gaps between farmers and the agricultural innovation system in Babylon Governorate.

RECOMMENDATIONS

In light of the results and conclusions the researcher recommends the following:

1- Developing awareness among workers in the agricultural innovation system of the importance of the satisfaction of corn farmers with the performance of the agricultural innovation system, considering that satisfaction represents the positive interface for the survival of the targeted people's dealings with the constituents of the agricultural innovation system, as well as it represents one of the important conditions in achieving the overall quality of services provided to the targeted. 2- The need for the agricultural innovation system in the Governorate of Babylon to adopt the results of the research with regard to the fields and items that formed a state of dissatisfaction and a little dissatisfied with the performance of the agricultural innovation system and try to address them to ensure the continuity of the relationship between the government agencies that make up the agricultural innovation system and the corn farmers in the Governorate of Babylon.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 267-276
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(23)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

GERMINATION EFFECT ON GLIADIN DEGRADATION IN SOUR DOUGH BREAD.

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Received 31/ 7/ 2023, Accepted 15/ 10/ 2023, Published 31/ 12/ 2024

ABSTRACT

The effect of the germination process on the decomposition of gliadins in Sour dough bread was studied. The use of sprouted wheat flour and sprouted Triticale flour in the manufacture of sour bread was tested and compared to unsprouted wheat and Triticale. Also, *Lactobacillus plantarum* starter was used in each of the four treatments, and the percentage of gliadins in each treatment was estimated using Elisa method so the percentage of gliadins in wheat flour before adding the starter culture was 17.83%, after adding the starter culture at a rate of 1%, there was a decrease in gliadins by 25%. However, when using sprouted wheat flour and when adding the starter culture, a 100% decomposition of the gliadins occurred. In the treatment of the Triticale flour, the gliadins percentage before adding the starter was 12.16%, while the gliadins decreased by 42.10% after adding the starter 85.32%. on the other side, the percentages of Vit A, Vit C, Vit K, Vit E and Vit B2 have raised with minerals, and a decrease in phytic acid was observed in sprouted wheat and triticale.

Key words: Gliadin, Germination, Celiac disease, Sour dough bread.

تأثير الإنبات في تحلل الكليادين في الخبز الحامضي

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الخلاصة

درس تأثير عملية الانبات في تحلل الكليادين في الخبز الحامضي، إذ تم تجربة استعمال طحين الحنطة المنبتة وطحين الترتيكيلي المنبتة في تصنيع الخبز الحامضي ومقارنتها بالحنطة والترتيكيلي غير المنبتين، كذلك استخدم باديء *Lactobacillus plantarum* في كل معاملة من المعاملات الأربعة وتم تقدير نسبة الكليادين في كل معاملة بطريقة الايليزا فكانت نسبة الكليادين في طحين الحنطة قبل اضافة البادئ 17.83%، بعد اضافة البادئ بنسبة 1% حصل انخفاض في الكليادين بنسبة 25% اما عند استعمال طحين الحنطة المنبتة وعند اضافة البادئ حصل تحلل للكليادين بنسبة 100% وفي المعاملة الخاصة بطحين الترتيكلي حيث كانت نسبة الكليادين قبل اضافة البادئ 12.16% بينما انخفض الكليادين بنسبة 42.10% بعد اضافة البادئ وعند اضافة البادئ الى طحين الترتيكيلي المنبتة حصل الانخفاض في الكليادين بنسبة 85.32%. ومن ناحية اخرى فقد ارتفعت نسب الفيتامينات(A,C,K,E,B2) والمعادن وانخفض حامض الفايتيك في طحين الحنطة والترتيكيلي المنبتين.

الكلمات المفتاحية: الكليادين، الانبات، حساسية الحنطة، الخبز الحامضي.

INTRODUCTION

Wheat and many other cereals include a class of proteins known as gliadin, which is a kind of prolamin. Gluten contains gliadin, which is required for bread to rise correctly during baking. The two primary parts of the gluten portion of wheat seeds are gliadin and glutenin. Wheat flour is one product that contains this gluten. Gliadin and glutenin, which make up an equal portion of gluten, are both insoluble in water; however, gliadins are soluble in 70% aqueous ethanol **(Ribeiro *et al*.,2013).**

Alpha, beta, and gamma are the three main kinds of gliadin that the body cannot handle well when celiac disease develops. **(Bethune *et al.*, 2008).**

A chronic immune-mediated intestinal illness called celiac disease occurs when the body develops an intolerance to gliadin, a protein found in gluten. Wheat, barley, and rye, which all contain prolamin, cause lifelong intolerance in people with celiac disease. In people with a hereditary predisposition, gliadin proteins may cause autoimmune enteropathy, which is brought on by an aberrant immune response. This action is caused by specific amino acid sequences in gliadin proteins. (**McGough *et al* ., 2005).**

Patients with gluten sensitivity may struggle to maintain a lifelong gluten-free diet because no wheat or related variety is safe to ingest. So, to lessen celiac disease and wheat gluten content, numerous molecular biology, genetic engineering, breeding, microbiological, enzymatic, and pharmacological treatments have been developed. (**Cohen *et al.*, 2019**).

According to (**Rico *et al.*, 2020),** germination is a promising food engineering technique to improve the nutritional value of grains, with prospective applications in functional foods and pharmaceuticals, as well as their health advantages for the prevention of chronic diseases including heart disease, diabetes, and cancer.

According to (**Shao *et al.*, 2019),** the goal of the germination process is to create and activate the enzymes that transform a significant portion of complex substances like starch into maltose, glucose into dextrin, and protein into peptones, peptides, amino acids, and other compounds. until germination, these complex chemicals maintain their most complex molecular form until being absorbed by the seed embryo after their disintegration **(Guardianelli *et al*., 2019),** through the action of lipolytic enzymes, fat degradation also takes place during germination to make up for energy needs during metabolic activities. Due to the removal of starch during germination, (**George ,2015),** observed that crude fibers—a significant component of cell walls—increase in terms of percentage and perceived value in seeds. According to (**Yang *et al*.,2011),** the germination procedure is an effective way to raise the vitamin content. The seeds, which can be germinated grains, may also synthesize vitamin C. excellent natural vitamin sources in diet foods.

Nkhata *et al*.(2018) claimed that variations in the phytate concentration of grains and legumes, as well as variations in the enzyme activity, may account for variations in the bioavailability of mineral elements after germination and over various periods. **Montemurro *et al.* (2019)** indicated that germinated wheat grains are more prone to microbiological damage, which restricts the expansion of the use of germinated grains in the manufacture of baked goods. As a result, controlling germination from enough humidity is important, a germination requires a specific temperature and the presence of oxygen. appropriate and controlled germination Steeping, germination, and drying are the three main steps in the germination process.

MATERIALS AND METHODS

Germination process

The Ministry of Science and Technology provided local wheat types (Tigris Al-Khair variety), which were harvested in 2022, while a farmer provided the Triticale variety. Before the study was done, wheat grains were cleaned, freed of contaminants, and stored in polyethylene bags at 4°C. As modified the procedure described by (**Yaqoob *et al*., 2018),** was to remove empty and broken seeds and foreign materials before washing the grains several times with distilled water at room temperature. The grains were then soaked for 24 h using the recommended water to grain ratio. To express the seeds' 40–44% moisture content, wheat is 1:3, or 3 times the weight of the seeds.

To encourage germination, the seeds were wrapped in a piece of muslin cloth. The seeds were then spread out in a single layer of steel trays after the trays had been covered with two layers of cotton cloth. Germination took place in an incubator at 25°C in complete darkness, the grains were dried with the sprouting sections at a temperature of 40°C to increase germination. This resulted in wheat grains with a moisture content of 10–11% and a good scent. Given that the length of the bud did not surpass the length of the grain, the germination process went on for 24 h **(American Association of Cereal Chemists. 2008).**

Measurement of phytic acid

Utilizing a method from 2015, Phytic acid determination for flour samples at the Ministry of Science and Technology/Department of Environment and Water/Food Research Center, using an HPLC device according to the method mentioned by **Lehrfeld. (1989).**

Sour dough processing.

The flour, salt, yeast, and starter were weighed before being put into the Japanese-made Topmatic kneading machine. To aerate the flour, aid in the growth and multiplication of the yeast, and make sure that the components are spread equally and uniformly, the ingredients were combined for one minute. Water was then added based on the absorbance data seen in the farinograph. After the appearance of evidence of the conclusion of the kneading stage, represented by the cleanliness of the kneading bowl walls, the ingredients were mixed for 3 min to produce a cohesive dough. the dough's pliability, elasticity, and lack of lumps after the gluten network construction. All the kneaders' pH and acidity levels were measured for all samples and after being coated with a small amount of fat, the dough is placed in standard Bowels and allowed to ferment for 30 min. After that, the dough is baked for 20 min at 220 C, followed by a sensory evaluation. **(Gül *et al*.,2005)**

The Tigris Al-Khair and Al-Triticale wheat flour was used according to the proportions in the following table:

The first treatment

Table (1): the proportions of the ingredients used in the production of sour bread.

The components	Quantity/gm
Wheat flour	100 gm
Sodium chloride	1.2gm
Yeast	1.5gm
Water according to the Farinograph records	65 ml

The second treatment

Using the Tigris Al-Khair wheat flour with the previously mentioned ingredients, with the addition of *Lactobacillus plantarum* starter at a rate of 1% (1×10^8).

Third treatment

Using the flour of the Tigris Al-Khair germinated wheat with the same ingredients mentioned in the table above, with the use of a starter culture of *Lactobacillus plantarum* 1%.(1×10^8).

Fourth treatment

Use whole-grain Triticale flour with the ingredients mentioned in the table above.

Fifth treatment

Use the whole grain Triticale flour with the ingredients mentioned in the above table and add 1%(1×10^8) *Lactobacillus Plantarum* starter.

Sixth treatment

Using sprouted Triticale flour with the ingredients mentioned in the table above, with the addition of *Lactobacillus Plantarum* at a rate of 1%(1×10^8)

Estimation of gliadin by Elisa gliadin test

The Gluten-Gliadin Elisa Kit was used to estimate the percentage of gliadin in the manufactured bread, as 1 gm of Sour dough bread was weighed in a test tube and 9 ml of ethyl alcohol solution at a concentration of 40% was added, and they were mixed with the homogenizer to obtain a homogeneous solution free of bread clumps, and then a procedure was carried out. Centrifuge at 3000rpm for 5 min to obtain a precipitate and a clear liquid. The sediment was thrown away, the liquid was taken, and ELISA assays were performed for it. The sample is withdrawn and placed in the ELISA pit, and then washed three times using 300 microliters of diluted washing solution in each well. After the last wash, the washing water is discarded, then 100 microliters of conjugated (anti_gliadin_peroxidase) is added for each pit, then incubation for 20 min at room temperature, the above-mentioned washing step is repeated, then 100 microliters of the substrate solution is added to each pit, the plate is left in a dark place for 20 min at room temperature, the reaction is stopped by adding 100 ml of stop solution 0.5M H_2SO_4 for all pits, color development is observed, then mixed quietly, and color stability is noted within only 30 min, using an ELISA reader at a wavelength of 450 nm to read results**. (Lacorn *et al*., 2022)**

$$\text{The rate of decrease in glaidin} = \frac{\text{control} - \text{sample}}{\text{control}} \times 100$$

RESULTS AND DISSCUSSION

Chemical Composition of Wheat and Triticale:

Table (2) shows the effect of germination on the percentage of protein in flour, where the percentage of protein in sprouted wheat flour increased to 13.0%, while it was in the non-germinated whole wheat flour 11.7%. Likewise, the percentage of protein in the sprouted Triticale flour increased to 13.5%, while it was 12.5% in the non-sprouted whole grain flour.

Table (2): The chemical composition of sprouted and unsprouted wheat and Triticale flour.

Components	Whole grain wheat flour (%)	Sprouted wheat flour (%)	Whole Grain Triticale Flour (%)	Sprouted triticale flour (%)
Protein	11.7	13.0	12.5	13.5
Fat	1.89	1.4	1.74	1.48
Fiber	1.47	2.14	1.50	2.33
Ash	1.6	1.8	1.7	1.9
Moisture	14.0	11.7	13.7	11.4

This was supported by research by **(Muñoz -Llandes *et al.*, 2019**), who found that germination is the only conventional method that increases protein content, as opposed to roasting and sterilization, which decreases it. The increase in protein content is attributed to a rise in protease activity. However, seed proteins are broken down into amino acids during germination, and storage protein breakdown is required for the production of amino acids and peptides to stimulate embryonic growth. The table also shows the effect of germination on the percentage of fat in the flour, as the percentage of fat in the germinated wheat flour decreased to 1.40%, while it was 1.89% in the whole grain wheat flour. Likewise, it decreased to 1.48% in the germinated Triticale flour, while it was in the whole grain Triticale flour 1.74%.

This was supported by the studies demonstrated that the production of energy to support metabolic processes such as the synthesis of structural proteins, enzymes, DNA, and RNA, as well as being used as a major source of carbon for seed growth, is responsible for the decrease in the percentage of fats during germination **(Megat Rusydi *et al.*, 2011; Al-Haidari *et al.*, 2019).**

The table also showed the effect of germination on the percentage of fiber in flour, as it increased to 2.14%, while its percentage was 1.47% in non-germinated whole grain wheat flour, as well as in germinated triticale flour, it increased to 2.33%, while its percentage in non-germinated Triticale flour was 1.50%.

According to researcher (**George, 2015).** germination increases crude fibers in germinated grains because starch disappears. Crude fibers, a significant component of cell walls, also rise in terms of proportion and perceived value. Like whole wheat flour, whole grain flour is a healthy and abundant source of soluble and insoluble fiber, which has a favorable and helpful impact on the health of the person (**Hammood & Nasir, 2018),** It also showed an increase in the percentage of ash in the germinated wheat flour, as it rose to 1.8%, while it was 1.6% before

germination in the whole grain wheat flour, as well as in the germinated triticale flour, where the percentage increased to 1.9%, while it was 1.7% before germination, and this was confirmed by the researchers (**Uduwerella *et al*., 2021),** where they indicated as a result of the germination process, which enhances the bioavailability of minerals like iron, zinc, and calcium in the germinated seeds, demonstrated that the reason for the increase in ash is due to the increase in the percentage of mineral elements in the germinated wheat flour. As mentioned the internal enzyme of complex organic substances is hydrolyzed, which is why the amount of ash has increased. It causes the antifoods to seep into the germination media and the phytase enzyme to be active during germination, which causes the disintegration of the bonds between protein and minerals to become free. This results in the release of more nutrients.

The table also shows a decrease in the percentage of moisture in the germinated wheat flour, as the moisture percentage reached 11.7%, while it was 14% before germination. The same applies to the germinated triticale flour, where the moisture percentage reached 11.4%, while it was 13.7% before germination. This was confirmed by the researcher (**Ersedo, 2019),** who explained that the drying of the grains serves to halt the microbial activity of the grains and extends the shelf life of food products made from flour and wheat by reducing the humidity after germination in order to stop the enzyme action.

Table (3) Percentage of vitamins in sprouted and non-sprouted flour.

Name	Vit A (IU)	Vit C (ppm)	Vit E (ppm)	Vit K (ppm)	Vit B 2 (ppm)
Whole grain wheat flour	9.22	0.47	8.25	1.11	2.55
Sprouted wheat flour	17.14	1.10	13.22	2.47	4.11
Whole grain Triticale flour	11.58	0.88	10.24	1.52	2.98
Sprouted Triticale flour	21.56	1.98	15.44	3.58	6.00

Table (3) shows that germination leads to an increase in the flour content of some vitamins, as the percentage of vitamin A in germinated wheat flour increased to 17.14[IU], where it was before germination 9.22[IU]. As for the germinated Triticale flour, the percentage of vitamin A increased to 21.56[IU], while it was 11.58[IU] before germination. The same applies to vitamins (C, E, K, and B2), where the increase occurred clearly in the sprouted wheat flour compared to what it was in other flours. Ungerminated the whole wheat This was confirmed by researchers (**Zilic *et al*., 2015; Abas**,**2012),** that it's probable that the biosynthesis carried out by the newly formed seedling as a result of the germination process is what led to the buildup of specific vitamins.

Table (4) Percentage of minerals and phytic acid in sprouted and unsprouted wheat and Triticale flour.

Name	Whole grain wheat flour	Sprouted wheat flour	Whole Triticale grain flour	Sprouted Triticale flour
Fe (µg / gm)	33.6	40.5	36.9	39.8
Zn (µg / gm)	29.3	36.5	31.5	39.5
Cu (µg / gm)	3.5	4.8	4.0	5.6
Mn (µg / gm)	34.5	41.2	37.9	44.6
Na (µg / gm)	50.1	56.9	53.6	60.3
Ca (µg / gm)	310.5	345.8	325.9	355.9
Phytic acid	5022	77.7	395	49.6

Table (4) shows that germination increases the percentage of minerals in the germinated flour, where it was observed that the percentage of (Fe, Zn, Cu, Mn, Na, and, Ca) increased clearly than it was in the whole grain ungerminated wheat flour, and this was also confirmed by the researchers (**Luo *et al*., 2014),** where they stated that the germination process is a way to improve the nutritional value of some crops like wheat by improving the bioavailability of mineral elements and increasing their percentage. Germinated seeds contain a number of mineral elements, including iron, zinc, calcium, magnesium, copper, and sodium.

The table also shows decrease in the percentage of phytic acid in the sprouted wheat flour, where the acid percentage in the whole-grain wheat flour was 5022ppm, while it decreased in the sprouted wheat flour to 77.7ppm and also in the whole-grain Triticale flour was 395ppm, while it decreased in the sprouted Triticale flour to 49.6 ppm, and this was also confirmed by the researchers (**Samia *et al*., 2013; Al-Timimi *et al*., 2006) ,** they said the soaking and emergence of the seedling improved the nutritional value of the chosen seeds in terms of the high concentration of nutrients and the reduction of phytic acid contents during germination, as demonstrated by the decrease in phytic acid contents due to the enzymatic changes during the duration of soaking and germination in the seeds, and the lack of effect of the presence or absence of light during the germination process on the achieved results. Additionally, the endogenous phytase enzyme's activity during the process of reducing phytic acid is primarily responsible for that.

Baking results

Table (5) shows the bread treatments before and after adding the starter and the extent of the effect of the starter on the percentage of gliadins, which is one of the components of gluten and is considered one of the causes of gluten sensitivity disease, where the percentage of gliadins in the first treatment before adding the starter was 17.83%, while in the second treatment after adding the starter, There was a decrease in the gliadins by 25%, but in the third treatment when using sprouted wheat flour and when adding the starter culture there was a decomposition of the gliadins by 100%. In the fourth treatment of Triticale flour, where the percentage of gliadins in the whole-grain Triticale flour before adding the starter culture was 12.16%, while in the fifth treatment, after adding the starter, there was a decrease in gliadins by 42.10%, while in the sixth treatment, when the starter was added to the sprouted Triticale flour, there was a decrease in gliadins by 42.1%. 85.32, and this was confirmed by the researchers (**Gerez*, et al*., 2009),** that the protease enzyme secreted by the lactic acid bacteria *Lactobacillus plantarum* was used to assess gliadin units. Protease enzymes are also activated during the germination phase, and this also leds to an increase in the rate of gliadin degradation (**Ohm *et al*., 2016).**

Table (5) includes the six treatments with the ratio of gliadins.

Name	Concentration of Gliadins, ppm	Percentage decrease in gliadin %	pH	Acidity%
Wheat control	17.83	_	5.6	0.63
Wheat Control+1% *Lactobacillus Plantarum*	13.33	25	4.3	1.08
Sprouted wheat flour + 1% *Lactobacillus plantarum*	Zero	100	4.6	0.81
Triticale control	12.16	_	5.4	0.72
Triticale control+1% *Lactobacillus plantarum*	7.04	42.10	4.4	0.99
Sprouted Triticale flour+1% *Lactobacillus plantarum*	1.51	85.32	4.5	0.90

CONCLUSION

The germination process leads to an increase in the percentage of protein, fiber, ash, mineral elements (iron, zinc, copper, manganese, sodium, calcium) and vitamins (A, C, E, K, B2), and a decrease in fats, moisture, carbohydrates, as well as phytic acid for the two types of Degla Khair wheat. It is also possible to produce the glutinase enzyme from the local bacteria Bacillus subtilis IHB3, which decomposes gliadin, gliadin, and gliadin, in addition to eliminating the gliadin protein by germination, as its percentage decreased by 100% when using the gliadin measurement by ELISA test.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 277-284
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(24)
AL Hamawandi & et al.,

المجلة العراقية لبحوث السوق
وحماية المستهلك

(Article Review)

A LIFE STUDY OF THE LEAF MINER *Liriomyza sativae* (DIPTERA: AGROMYZIDAE)

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Received 23/ 10/ 2023, Accepted 13/ 5/ 2024, Published 31/ 12/ 2024

ABSTRACT

The leaf miner *Liriomyza sativae* (Diptera: Agromyzidae) is a dangerous pest, causes damage to many plants in Iraq, and in results some economic losses. More than 370 species make up the genus *Liriomyza* is most common in all of the world's cucumber-cultivation areas. *L. sativae* is a very harmful pest that affects melons, tomatoes, cucumbers, beans, peas, and pumpkins. The majority of parasitoid insects that feed on leaves belong to the order Hymenoptera, which is comprised of species that fall under the taxonomic categories of the two families Ichneumonidae and Chalcidoidea.

Keywords: Leaf miner, *Liriomyza sativae*, Life cycle.

دراسة حياتية لصانعة أنفاق الأوراق *Liriomyza sativae* (Diptera : Agromyzidae)

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الخلاصة

تعد حشرة حفار الاوراق *Liriomyza sativae* (Diptera: Agromyzidae) من الآفات الخطيرة التي تسبب اضرارا للعديد من النباتات في العراق، وتؤدي إلى خسائر اقتصادية. ويضم جنس *Liriomyza* أكثر من 370 نوعًا ينتشر على نطاق واسع في جميع مناطق زراعة الخيار في العالم. *L. sativae* هي آفة ضارة جدًا تصيب البطيخ والطماطم والخيار والفاصوليا والبازلاء والقرع. يتطفل على حشرة حفار الاوراق *L. sativae* العديد من الحشرات الطفيلية التي تعود إلى رتبة غشائية الأجنحة وبالتحديد العوائل Chalcidoidea Ichneumonidae .

الكلمات المفتاحية: صانعة انفاق الاوراق، *Liriomyza sativae*، دورة حياة.

INTRODUCTION

According to Hering (1951), leaf miners are classified into four insect orders: Lepidoptera, Coleoptera, Hymenoptera, and Diptera. The majority of leaf miners are members of the Diptera order. According to Asadi *et. al.,* (2006), the genus *Liriomyza* included about 370 species that grows widely over all of the world's cucumber-cultivation areas. Dursun *et. al.,* (2010) indicated that agromyzidae is one of the largest families of the Diptera order. It included more than 3,000 species belonged to 30 genera, most of them are significant to economy. Mik's record *Liriomyza* genus in 1894. *Liriomyza trifolii* was discovered for the first time in Iraq, according to Al-Jurani *et. al.,* (2016). The classification according to EPPO/CABI (2006) is the following:

Phylum: Arthropoda
Class: Insecta
Order: Diptera
Sub-order: Brachycera
Super-Family: Opomyzoidea
Family: Agromyzidae
Subfamily: Phytomyzinae
Genus: *Liriomyza*
Species: *sativae, citrigata, bryonia*

SPREAD

L. sativae damaged leaf miners and quickly spread over western Japan. It is a major pest that reduces the productivity of vegetables, particularly tomatoes and cucumbers. It has been noticed by several researches in Vietnam and according to Anderson *et al.,* (2002) that the vegetable leaf miners *L. sativae* is a serious pest of melons, tomatoes, cucumbers, french beans, peas, and domestic beans. Rauf *et. al.,* (2000) discovered that the tunnel builders of the genus *Liriomyza* originated on the continent of South America and spread around the world, effecting harming greenhouse and open-field vegetable crops alike. Several unique species, including in *Liriomyza* as *L. sativae*, *L. bryoniae,* and *L. trifolii* have developed a resistance to most common chemical pesticides (Pape, 2001). In Indonesia, losses in cucumber crop might exceed 60% due to the leaf miner *L. sativae.* Insects are a serious pest on ornamental plants and vegetable crops in Iran, according to a research by A. Parrella (1983) and Reitz and Trunble (2002). Tokumaru *et al.* (2005) discovered that, depending on the crop type and degree of infection, the infection rate on vegetable crops in Kenya varied from 80–100% with regard to vegetative mass damage (Olaye-Chabi *et. al.,* 2008).

PLANT HOST AND INFECTION RATE

According to Anderson *et al.* (2002), *L. sativae,* the leaf miner, is a highly harmful pest that affects cucumbers, beans, peas, tomatoes, pumpkins, and melons. Depending on the crop type and degree of infection, the pest can cause large losses to vegetable harvests of 80–100% (Olaye-Chabi *et al.*, 2008). According to Rauf *et al.,* (2000), more than 60% of the cucumber harvest was lost due to plant tunnel by *L. huidobrensis.* According to Chavez and Raman (1987), Rauf *et. al.,* (2000), Milla and Reitz (2005), the plant tunnel in Peru lost up to 30% of the potato crop, 60–70% of the vegetables in Indonesia, and all of the vegetables in Western

Europe. While EPPO and CABI (2006) reported that both males and females are leaf miners (*L. sativae)*. Shehpard *et. al.,* (1998) stated that in some places of Indonesia, the leaf miner *L. huidobrensis* produced an infection rate of more than 70% on potatoes, beans, and tomatoes. During the egg-laying or feeding stages, it could be a carrier of viral infections.

DAMAGE AND ECONOMIC IMPORTANCE

In addition to causing sun blight in the fruits and the mortality of recently grown seedlings, the cucumber leaf miner also reduces yield and distorts the morphology of the fruits, which results in subpar crop selling **(Complete & Room, 2011).** The larvae and adults of the leaf miner produce the harm, according to **W.A. (2003**) mentioning it, since they burrow into the mesophyll layer while residing inside the vegetative tissue mining for leaves. *Leafy sativae* this insect deposits its eggs beneath the plant's leaf's epidermis. When the eggs hatch, the first larval instar emerges and feeds on the tissue of the leaf's middle layer, which lies between the upper and lower surfaces of the leaf. This results in the formation of the characteristic winding tunnels on the leaf. The adult additionally feeds by using the ovipositor machine to scrape the leaf's tissue and then eating on the area it has made. The *L. sativae* infection was regarding the existence of other species, but **Al-Jassani *et. al*., (2016)** discussed the relative existence of the species, insects were present on 20% of the cucumber plants. The autumn 2016 season's *L. sativae* showed a 1.4% leaf infection percentage and a 16.0 infection severity. The proportion of infection grew steadily until it reached 3.93% in the first week of September 2016, the infection rate was 7.44% in leaves, and the infection severity was 45.2. Put differently, six to ten petioles are present on around 70% of leaf plants when prudence is used. In the second week of October 2016, the fruits weighed 505 g/10 plants; the next week, they weighed 180 g/10 plants. The number of shoots increased from 5.9 bunches/leaf to 6.11 tunnels/leaf **(Abbas and Al-Jourani, 2017).** According to **Abbas (2017),** the pest infection rate for vegetable leaves (*Liriomyza sativae*) (Diptera:Agromyzidae) in cucumbers during the fall of 2016 was 20%, and for leaves in the first week of September, it was 4.1%, with a 0.16% severity level. The proportion and severity of infection grew steadily during the third week of October 2016, reaching 93.3% and 44.7% for the plants and leaves, respectively. The infestation severity was 2.45 during the same week. Hence, six to ten miners or leaves are present in 70% of the leaves.

DESCRIPTION OF THE LIFE CYCLE

The Egg

The image (1) illustrates the oval form and white hue of the *Liriomyza sativae* leaf miner egg. Maximum length of 0.2 mm. The egg size of the tunnel leaf *L. sativae* was discovered to be yellowish-white in color, with measurements ranging from 0.15 to 0.1 mm and 0.2 to 0.3 mm in width and length, respectively **(CABI, 2006).**

The Larvae

The image (1) depicts the colorless first larval instar. (After hatching, the larva has two breathing apertures in a triangular cone that are useful for differentiating between the

three caterpillar instars). The subsequent instars are yellow-orange, (Petite, 1990) (used in the illustration).

The oral hooks, which vary depending on the larva's stage, are used to distinguish between various instars. In the initial stage, the larva's length is roughly 0.57 mm, and the hook mouth's length is 95 microns; in the subsequent stage, the larva's length is roughly 1.55 mm, and the hook mouth's length is 188 microns; and in the final stage, the caterpillar's length is roughly 2.5 mm, and the mouth's length is 323 microns. According to (**CABI, 2006)**, the older larvae have a white back and a yellowish-white back.

The Pupa

Liriomyza coarctate pupae, which are young leaves of the leaf miner, according to (**Tawfiq, 1993),** it is golden yellow to black. (as seen in Image 1).

The Adult

An adult turn into a leaf miner *Plantae L.* The area between the eyes is yellow, and the upper breast region is glossy black **(Doudin, 2007),** as shown in Image (1). When in an adult *L. trifolii*, it was discovered that the upper chest region and the area behind the black eyes are gray from coarse hair, while the area behind the eyes is primarily yellow. Brazil salad its look is very similar to that of *L. sativae*. According to (**Spencer 1972; 1973)**, the male measures 1.5 mm in length, while the female is less than 2.3 mm. The male's wingspan falls between 1.75 to 2.10 mm, suggesting that the female is an adult **(CABI, 2006).** *Citrigata L*. somewhat comparable to grownups *L. andbryoniae* and *L. huidobrensis.* a mature miner extracts leaves. *Sativae L*., the female lives longer than the male, ranging from 15 to 30 days **(EPPO/OEPP, 1984).** According to (**Capinera2001),** eggs are placed singly in the pores in the leaf's epidermis, with no preference for the top or bottom surfaces, in the adult leaf miner, *L. sativae* deposit. The recently deposited eggs are elongated elliptical in form and creamy white in hue. They hatch in two to four days. The female may produce between thirty and forty eggs a day, but as she matures, she stops laying as many eggs. The female may deposit 600–700 eggs in her lifetime, depending on her age.

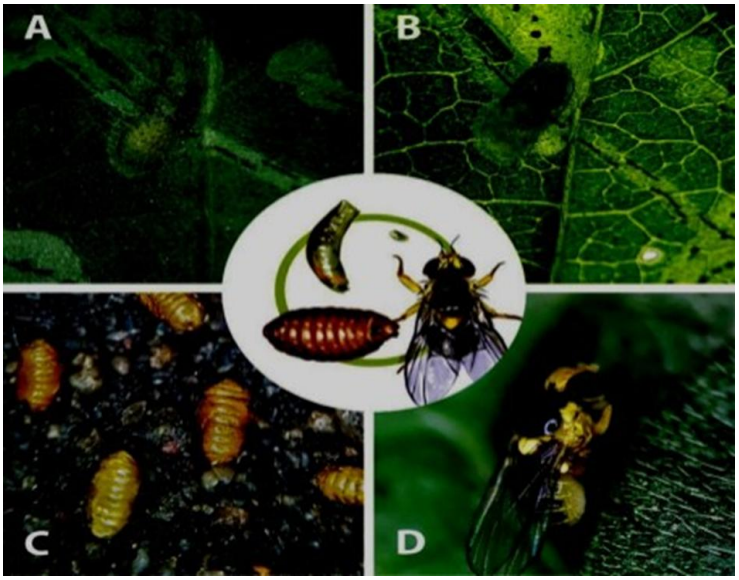

Image (1): The stage of *L. sativae* where A: Egg, B: Larvae, C: Pupa, D: Adult.

Natural Enemies

The majority of parasites that cause leafmineng are found in the two families Chalcidoideae and Ichneumonoideae of the order Hymenoptera. Several of these parasitoids, according to **(Al-Azawi, 1967)** are members of the Agromyzideae family, including the genus *Liriomyza*. In the context of Iraq, citing *O. Birio, Opius Filicorne, D. Cassinervis, and Diglyphus isaea et. al.,* (2006) as examples of these parasitoids, it can be shown that the majority of Agromyzidae parasitoids are members of the Eucoilidae, Eulophidae, and Braconidae families. Protozoan parasite *Diglyphus isaea* (Eulophidae: Hymenoptera) feeds on minnows. Liriomyza leaf miners are controlled using it (Sha *et. al.*, 2006). An experiment was carried out in Kenya to investigate the degree of *Phaedrotoma scabriventris* spam preference among three species of leaf miner producers on the parasite: *Linus huidobrensis*, *L. trifolii* and *L. sativae.* According to the findings, the preference was *L. huidobrensis* (95%), *L. sativae* (58%), and *L. trefoils* (20%). Over 140 known parasitoids have been reported to be utilized against Liriomyza leaf miners **(Chabi-Olaye *et. al*., 2009; Liu *et. al.,* 2009)**. Several of these parasitoids, such as *Dacnusa sibirica* and *Diglyphus isaea*, have been used in biological control. The principal parasites that prey on the genus *Liriomyza sp*p. Are six female Agromyzidae parasites were found by **(Fleih & Hamzaa, 2009**) on leaf miners. According to **(Rauf *et. al.,* 2000)**, these were Hemiptarsenus varicornis, Asecodes, Cirrospilus vittatus, and Neochrysocharis formosa. Surveillance research on eight distinct leaf-miner parasites whose presence fluctuated over a two-year period was conducted by **(Falih, 2009).** Study subjects in 2007 included *Cirrospilus vittatus, Diglyphus isaea, Diaeretiella rapae, Ratzeburgiola incomplete, Halticopter circle Neochrysocharis formosa, Opius sp., and Pediobius acantha*. In six governorates (Baghdad, Karbala, Najaf, Diwaniyah, Salah al-Din, and Dohuk), several species of a metallic foot, *D. crassinervis, Diglyphus isaea spammers, Halticopter circle, Cirrospilus vittatus*, and *Neochrysocharis formosa* were found by (**Abdulrassoul & Hanaa,**

2014). During the survey and identification of Hymenoptera parasitoids on larvae and pupae of leaf miners belonging to the genus *Liriomyza* on various vegetable crops. Rozeburgiola not fully completed. Complete Ratzeburgiola, a member of the Pteromalidae and Hymenoptera families, was found on *Liriomyza sativae* in Baghdad **(Faleh et. al., 2019).**

CONCLUSION

The *L. Sativae* (Agromyzidae: Diptera) is insect with a wide family range, and in an attempt to reduce or move away from traditional (chemical) control to natural control using parasites (natural enemies) is a serious and successful attempt that can keep pace with this trend of clean control. Therefore, it is necessary to continue this research to collect the largest possible amount of information in order to move to the stage of breeding and releasing these parasites within a long-term program.

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Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 285-297
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(25)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

(Article Review)

VITAMINS AND THEIR EFFECTS ON PLANT

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Received 2/ 11/ 2023, Accepted 28/ 5/ 2024, Published 31/ 12/ 2024

ABSTRACT

Vitamins are safe substances that do not harm humans or animals. These vitamins are used to improve plant growth and flower formation; they are in fact organic substances that exist in several forms, and considered as micro essential nutritional supplements for proper growth and production. In addition, they perform various functions such as forming, building new tissues as well as participating in the physicochemical processes. Not only this, but they also have a rapid and tangible effect on the functions and growth of plants. As well as its important role in the synthesis of natural hormones within the plant.

The study aims at identifying the types of vitamins, the places in which they exist, their role and effects on growth and crops.

Key words: vitamins, plant, flower, growth, yield

الفيتامينات وآثرها في النبات

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الخلاصة:

تعد الفيتامينات من المواد الآمنة التي ليس لها ضرر على الانسان والحيوان وتستخدم من اجل تحسين نمو النبات وتكوين الازهار وهي عبارة عن مواد عضوية توجد بعدة صور وتعد من المكملات الغذائية الدقيقة والأساسية للنمو والانتاج السليم تقوم بوظائف مختلفة من خلال تكوين وبناء انسجة جديدة، وتشارك في العمليات الفسيوكيميائية وتكون ذات تأثير سريع وملموس لقيام النبات بوظائفه ونموه، فضلا عن اهميتها في تكوين الهرمونات الطبيعية داخل النبات. ونظراً لأهمية هذا الموضوع فقد هدفت هذه الدراسة إلى التعرف على انواع الفيتامينات واماكن تواجدها ودورها وآثرها في نمو وحاصل النبات.

الكلمات المفتاحية: الفيتامينات، النبات، الازهار، النمو، الحاصل.

INTRODUCTION

The term 'vitamins' is derived from the word *vitamin* which is coined by the Polish biochemist Casimir Funk in 1912 who isolated a group of micronutrients essential for life. The word *vita* means 'life', while the word *amine* refers to the compounds that contain Nitrogen. Later, it was discovered that not all vitamins contain Nitrogen, but the name has not changed due to its widespread use. Vitamins are organic substances that exist in many forms; they are essential and complementary to healthy growth that helps in building tissues. Therefore, they help the tissues to carry out their proper functions through their participation in the physiological reactions to the continuation of the various functions of the plant as well as the structure of new tissues.

Vitamins are vital organic compounds available in small quantities in order to maintain their normal state, and are essential to the proper plant growth and development. These compounds act as coenzyme regulators and are involved in increasing the utilization of metabolites (**Hassanein *et al.*, 2009**). Some vitamins can be considered as antioxidants as they are one of the new ways to help the plant withstand any environmental conditions and increase the plant growth and cell cycle. They can also protect the plant from any reactive oxygen seasoning (ROS) and increase the rubiscosubunit as well photosynthetic pigments. As a result, there can an increase in the content of chlorophyll, photosynthesis rate, and plant productivity (**Chen & Gallie, 2006**). It has been proved that spraying or applying vitamins on the plant from the outside is successful in mitigating the negative effects of the stresses which plants are exposed to (**Khan *et al.*, 2006**).

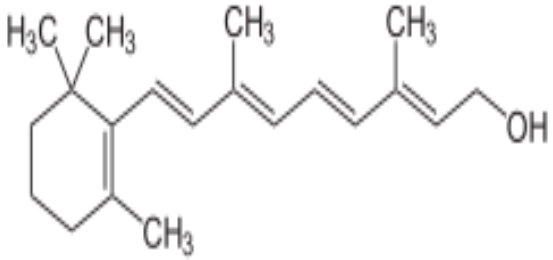
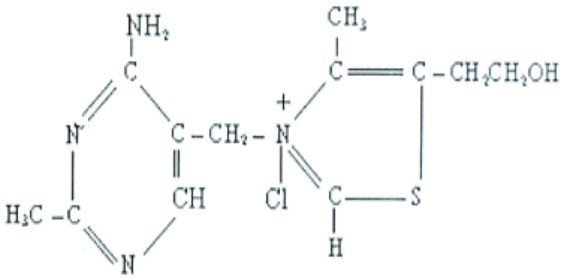
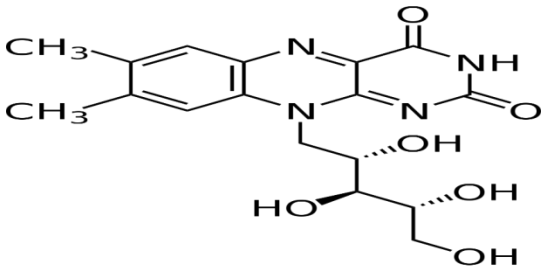
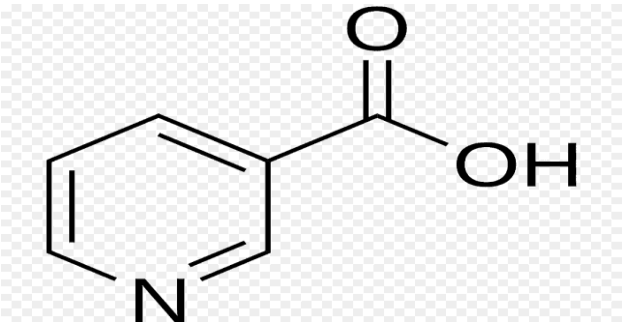
One of the approved strategies is the use of antioxidants to overcome the damage resulted from the interaction of active oxygen species (**Munir & Aftab, 2009**). This strategy is also useful for its ability to catch free radicals or active oxygen produced during the process of photosynthesis and respiration (**Foyer *et al.*, 991**).

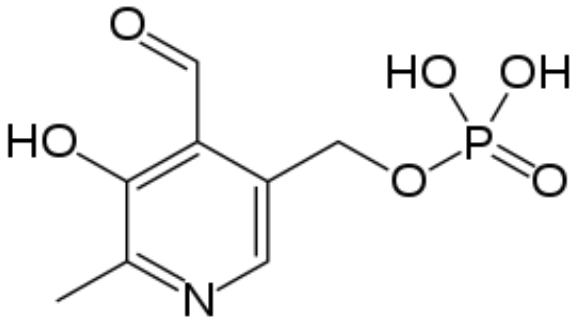
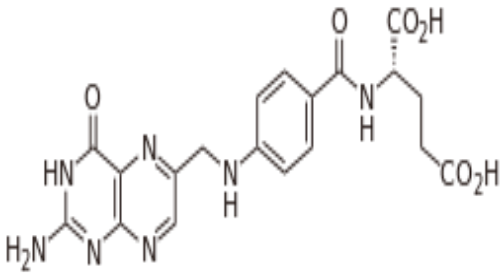
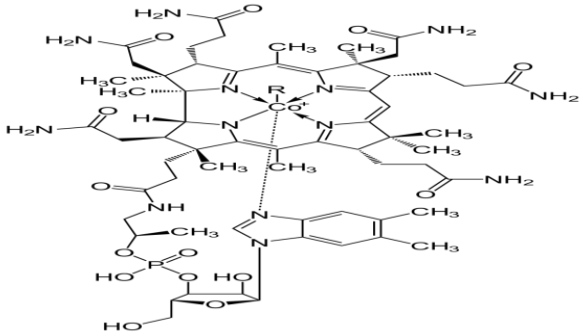
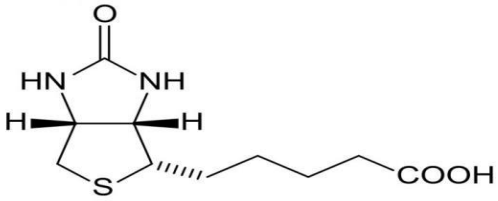
Therefore, the application of the use of vitamins externally gained great attention, as it works to resist the effects of salinity stress on the plant, improve growth, and increase yield, quantity and type (**El-Bassiouny *et al.*, 2005**).

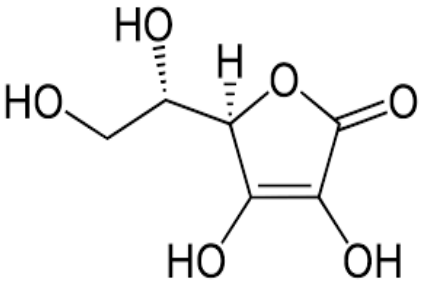
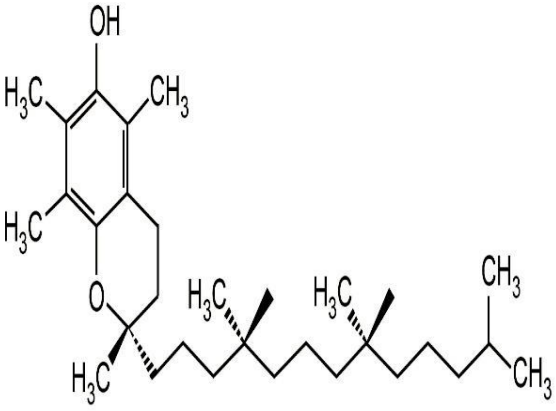
Tocopherols (Vitamin E) are powerful antioxidants involved in many physiological processes such as plant growth and development, aging, stress tolerance, prevention of lipid oxidation, and plant protection from rot (**Kumar *et al.*, 2012**). A vitamin can be considered as a group of molecules associated with nutrients that an organism needs in small quantities for proper metabolism. way. It is not possible to obtain the essential nutrients in the living organism through Biosynthesis. Even if this happens, it will not be in sufficient quantities. Thus, it must be obtained through nutrition. Some organisms can synthesize vitamin C, but many other species cannot. The term vitamin does not include the other three nutrient groups such as minerals, essential fatty acids or amino acids. Most vitamins are not single molecules; the groups of related molecules are called quasi Vitamins (Vitamir). The World Health Organization stated that there are thirteen vitaminsknown as vitamin A (retinol), vitamin A Vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_5 (pantothenic acid), vitamin B_6 (pyridoxine), vitamin B_7 (biotin), vitamin B_9 (folic acid), vitamin B_{12} and vitamin C (ascorbic acid), vitamin D, vitamin H (biotin) and vitamin E (tocopherols and tocotrienols).

This study aims to shed light on the importance of vitamins in the formation of natural hormones within the plant as well as the roles they play within the plant. Some of these vitamins are of great effect according to the role they play inside the plant. The below table (1) illustrates this idea.

Table (1): Vitamins, their formula, chemical composition and the role of vitamins inside the plant.

No	Vitamin	Chemical formula	Chemical composition	The role of vitamins inside the plant
1	Retinol vitamin A	C21H18ON3SCl2		acts as a regulator of the growth and differentiation of cells and tissues
2	Thiamine vitamin B1	C21H18ON3SCl2		has a major role in stimulating growth and regulating the growth process within the planet
3	Riboflavin Vitamin B2	C17H20N4O6		is involved in photosynthesis and participates in the transfer of electrons (oxidation and reduction processes), the activation of photosynthesis and the formation of natural auxins that stimulate growth within the plant.
4	Nicotinic Acid Vitamin B3	$C_6H_5NO_2$		works as an enzymatic assistant in the transmission of hydrogen and tryptophan, which are considered the raw material for nicotinic and auxin acids. Therefore, this acid has a major role with the help of auxin acid in the formation of roots.

5	Pyridoxine, pyridoxal, and pyroxamine Complex Vitamin B6	$C_8H_{11}NO_3$		this acid is present in all parts of the plant (stem, leaves, roots and seeds) This indicates its importance within the plant. - it plays an important role in stimulating root growth - it acts as a catalyst for biochemical changes -it acts as ammonia and carbon dioxide removal reactions - it participates in the formation of amino acid tryptophan and thus affects the formation of auxin which has an important role in the growth of plants (vegetative, flowering and root) - it has an important role in the representation of carbohydrates and fats (**Al-Daoudi, 1990**)
6	Folic acid Vitamin B9	C6O7N19H19		-it is important in amino acid metabolism, regulates cell division and elongation, and is a coenzyme in many metabolic pathways as well as DNA formation and free radical scavenging (**Andrew *et al.*, 2000; Fardet *et al.*, 2008; Naheif & Mohamed, 2013**). -By stimulating the biosynthesis of clicin, which works on the synthesis of porphyrins and chlorophyll in chloroplasts.
7	Cobalamin Vitamin B12	$C_{63}H_{88}CoN_{14}O_{14}P$		-it acts as a cofactor for enzymes (coenzymes) or as natural compounds
8	Biotin Vitamin H	S3O2N16H10C		-it has a role in regulating the processes of construction and demolition within the plant "essential in the Krebs cycle"

9	Ascorbic Acid Vitamin C	C6O8H6		-it activates the processes of photosynthesis. -it is an important regulator of the oxidation and reduction states of protoplasm. - it affects the oxidation and activity of enzymes inside the plant. - It is involved in the transition of hydrogen from NADPH to oxygen "oxidation and elimination by botanicals."
10	Tocopherol Vitamin E	C2O50H29		-it is available in all parts of the plant, but its greatest presence is in the chloroplast membranes. - it is mainly concentrated in plastids and acts as an - amphiphilic lipid antioxidant. α-tocopherol plays a protective role to membrane system in the cell of higher - plants (**Fryer, 1992; Wang & Quinn, 2000**); -it assists in maintaining membrane stability (**Munné-Bosch & Falk, 2004**) and regulates the transport of electrons in the photosystem-II system (Munné-Bosch & Alegre, 2002).. - Tocopherols play a regulatory role in a range of different physiological phenomena including plant growth and development, senescence, preventing lipid peroxidation and interact with the signal cascade that convey abiotic and biotic signals (**Sattler *et al.*, 2004; Baffel & Ibrahim, 2008; Soltani *et al.*, 2012**). -vitamin E fulfills at least two different functions in chloroplasts at the two major sites of singlet oxygen production: it preserves PSII from photoinactivation and protects membrane lipids frophotooxidation (**Havaux *etal.*, 2005**). α tocopherol levels change differentially in response environmental constraints, depending on the magnitude of the stress and the species' sensitivity to stress. -this vitamin is considered a natural antioxidant and a fat-soluble type -it consists of 8 units divided into two groups, the first group is distinguished by its saturated side chain, while the second group is distinguished by its unsaturated side chain. Each group includes alpha, beta, and sigma, both groups act as antioxidants by giving hydrogen to free radicals (**Smirnoff *et al.*, 2005**)

Locations of vitamins in plants:

Thiamine (Vitamin B_1)

Thiamine is found in high concentrations in the active growth areas of the plant. There are indications that the formation of thiamine occurs in leaves that mostly depends on the presence of light. Thiamine is transmitted from the leaves to the roots in the bark. Therefore, its role lies in stimulating and regulating growth in plants.

Riboflavin (Vitamin B_2)

Riboflavin is generally found in plants in a bound form. Riboflavin is a part of the coenzymes flavin mononucleotide (FMN) and flavin adenine nucleotide (FAD), which are involved in biological oxidation. There is a belief that flavin mononucleotide (FMN) is involved in photosynthesis; therefore, its role lies in the activation of photosynthesis and the formation of auxins along with its participation in the transmission of electrons. Riboflavin is produced in quantities in all parts of the plant. As a result, the signs of its deficiency are invisible on the plant clearly. Yet, Riboflavin can be involved in the auxin loading mechanism.

Nicotinic acid (Niacn)

The biological importance of nicotinic acid is known when it exists generally in the form of NAD and NADP as an enzyme coenzyme in many processes of hydrogen transfer. Also, it has a role in the formation of roots and the simulation of their growth. Nicotinic acid is abundant in the plants. It is also available with riboflavin in unusually high concentrations in wheat grains. In fact, tryptophan has been suggested as a precursor to nicotinic acid and auxin. This notion has led to the study of the possibility of an interaction between auxin and nicotinic acid. It was found that nicotinic acid has an auxiliary effect with auxin in the formation of roots.

Pantothenic acid (Vitamin B_5)

Pantothenic acid is found in most parts of the plant; yet, the highest concentrations of this acid were found in the aliron layer of wheat grains. pantothenic acid rarely exists in a separate form; rather, is found in the form of coenzyme A. Coenzyme A is involved in the transition reactions from one part to another. As such, it is of great importance in the biological changes of carbohydrates and lipids. Therefore, this acid can be involved in the photosynthesis of plants.

Pyridoxine, pyridoxal, and pryidoxamine

(Vitamin B6 complex)

Vitamin B6 is present in all parts of the plant such as stems, leaves, roots, seeds and fruits. However, there are indications that most of the vitamin available in the roots is transferred from the leaves. Vitamin B6 is an indispensable factor to the growth in root cultures, it was noted that the deficiency in cell division in separated roots is an outcome of the lack of vitamin B6. The competition of dexosperdixin with perdixin causes a deficiency in the growth of tomato root culture that is ignored by increasing pyridoxine. This indicates its effective role in stimulating root growth.

The most important physiological role of vitamin B6 lies in its participation in pyridoxal phosphate as a coenzyme in biological amino changes. The reactions of the removal of ammonia and carbon dioxide are among the most important functions of this vitamin. It has

been suggested that vitamin B6 may be involved in the formation of tryptophan and nicotinic acid.

Biotin (Vitamin H)

Biotin is found in all parts of higher plants. This vitamin is active in the vital changes of aspartic acid and carbon dioxide removal reactions of intermediate materials of the Krebs cycle, it has an important role in this cycle in terms of regulating the construction and demolition processes and the formation of oleic acid.

Ascorbic acid (Vitamin C)

Ascorbic acid is found in all parts of the plant. The highest concentrations are found in green leaves and in most fruits and vegetables, the most of it is found in the form of ascorbic acid, but there are small amounts found in an oxidized form. Ascorbic acid is rapidly oxidized to dehydroascorbic acid which in turn can be reduced again by copper-containing enzymes. There is an enzyme ascorbic acid oxidase in the plant- and, due to the enzyme ability to oxidize- its role lies in the fact that it is a cofactor in the phosphorous processes of photosynthesis as well as an important regulator in the oxidation and reduction states of the protoplasm. Also, it is an influencer in the state of oxidation and the activity of some enzymes important within the plant.

Types of vitamins

Vitamins can be divided into two types:

A. Group of fat-soluble vitamins (A, D, E, and K)

B.Group of water-soluble vitamins, including B and C

The effect of some vitamins on root growth and vegetative part of field crop plants:

The results of the study conducted by **Dawood *et al* (2014); Zeboon & Baqir (2023**) to evaluate the process of spraying or applying vitamin E with different concentrations ranging between (400, 800, 1000 and 1200 mg L^{-1}) indicated that there is an effect on growth and grain quality of two varieties of wheat (sids12 and sids13) for two seasons of cultivation. The process of spraying or applying vitamin E during the stages (120 DAS and 100 DAS) at a concentration of 1000 mg L^{-1} showed a significant increase in all growth traits as well as the proportion of carbohydrates in leaves and grains in addition to an increase in protein content especially in grains.

Results indicated (**Abdullatif *et al.*, 2016**) indicated that spraying vitamin E at a concentration of 100 mg L^{-1} led to the improvement of all the characteristics of vegetative, flowering and root growth of Eustoma plant of the Croma variety.

Sadak & Dawood (2014) indicated that the foliar application of α-tocopherol had a prospective effect in reducing the negative effect of salinity on flax plants. Ascorbic acid (C6 H8 O6) is found in all living organisms and plant cells, the greatest amounts of this acid exist in the leaves and flowers, especially in the parts that are actively growing (Smirnoff et al, 2001, Ebrahim, 2005).

Recently, at the level of international research, the use of vitamins including vitamin C, has increased in order to increase its resistance to various stresses. The plant naturally produces free radicals in the cell, and when exposed to these stresses, the free radicals are produced in

large quantities that affect growth. Hence, the plant uses certain mechanisms to get rid of them. One of these mechanisms isthe use of enzymatic and non-enzymatic antioxidants (Ascorbic acid) which are the first lines of defense against these radicals. These antioxidants are of great importance in plant growth and development, their presence ensures the survival of cells in the best condition in addition to the role of vitamin C in many metabolic activities it performs, including regulating plant growth (although it does not apply to it the conditions for considering any substance as a growth regulator), especially improving the growth of reproduction (**Ali & Musallam, 2008; Barth *et al.*, 2006, Ahmed & Abdel, 2017; EL- Delfi & Safi, 2023**).

The results of the study conducted by (**Mohamed, 2013**) showed that the combined individual applications of vitamins like B_{12} at 50 ppm, C at 500 ppm and folic acid at 50 ppm stimulated plant height significantly compared to the treatment comparison.

The maximum effect is noticed when spraying wheat plants with vitamin C, folic acid and vitamin B_{12} in a double or triple manner compared the manner in which these vitamins are sprayed individually. One of the main roles played by these vitamins is the promotion of cell division as well as the biosynthesis of organic foods, natural hormones, and plant dyes, which was confirmed by (**Robinson, 1973; Ortli, 1987; Samiullah *et al.*, 1988; Tzeng & Devay, 1989**). The obtained results were identical to those made by (**Gamal & Reda, 2003; Alttallah *et al.*, 2004; Abd El-Baky, 2009; Al- Qubaie, 2012**)

(**Al-Janabi & Hammadi, 2016**) concluded that when spraying bean plants with three concentrations of vitamin B_6 which include (0, 100, and 200) mg L^{-1}, the plants treated with a concentration of 200 mg L^{-1}gave them the highest average weight of 100 seeds, the biological and seed yields reached 141.07g, 6.025mg ha^{-1}, and 10.613 mg ha^{-1} as compared to the treatment comparison which gave the lowest mean of 124.09 gm, 4.181 mg ha^{-1}, and 8.532 mg ha^{-1}, respectively.

The results of the study conducted by (**Al-Dulaimi *et al.*, 2017**) showed that when an experiment was made to activate sunflower seeds with four concentrations of vitamin B_6 (0, 2, 4, 6) mg L^{-1}, the concentration of 4 mg L^{-1} exceeded by giving the highest average of leaf area estimated at 4756 cm^2 and 3176 cm^2 for Spring and Autumn seasons according to treatment comparison that gave the lowest average which amounted to 4209 cm^2 and 2884 cm^2 for both seasons respectively.

The results of the study conducted by (**Al-Saadi, 2022**) indicated the superiority of vitamin B_6 when sprayed at a concentration of 200 mg L^{-1} over the mung bean plant in the characteristics of vegetative and flowering growth including the number of branches, leaves, leaf area and leaf chlorophyll content with an average of 13.87 plant $branches^{-1}$, 110.64 plant $branches^{-1}$, 1992.67 cm^2 of plant, and 1.98 mg g^{-1} of leaves fresh weight as compared to the treatment comparison giving the lowest $^{-1}$ mean of 10.35 plant $branches^{-1}$, 83.48 plant $leaves^{-1}$, 1760.83 cm^2 of $plants^{-1}$, and 1.27 mg g^{-1} of leaves fresh weight for the characteristics respectively. The results are consisting with (**Baqir & Zeboon, 2020; Mahmood &Al- Hassan, 2017a & Mahmood &Al- Hassan, 2023**).

Effect of some vitamins on quality and yield of field crop plants:

Ascorbic acid (vitamin C) is one of the main antioxidants in the plant to increase productivity. Also, alpha-tocopherol (vitamin E) is a major antioxidant that plays a role in plant protection in environmental stress conditions.

The results of the study carried out by (**Dawood *et al.*, 2014**) indicated that spraying vitamin C at a concentration of 1000 mg L^{-1} leads to a significant increase in grain production and its protein content.

(**El- Wadi *et al.*, 2016**) confirmed that when conducting an experiment to find out the effect of foliar spraying on maize with three concentrations of vitamin B_6 (50, 75 and 100) mg L^{-1} in addition to the treatment comparison (without spraying) proved that the concentration of 50 mg L^{-1} by giving the highest average of grain yield, the number of corncobs. Also, the total average of yield reached 14.4 gm of $plant^{-1}$, 23.6 $corncob^{-1}$ and 6.3310 mg ha^{-1}, respectively, as compared to the treatment comparison which gave the lowest average of 2.3 gm of $plant^{-1}$, 23.6 $corncobs^{-1}$, and 308 mg ha^{-1} respectively.

The results of the study conducted by (**AL-Hilfy & Zeboon, 2016; AL-Hilfy & Zeboon, 2018**) showed that spraying the plant with vitamin C at a concentration of 4 gm L^{-1} had a significant effect on the yield of wheat grains and its components. This process gave the highest average of yield estimated 6,820 and 6,879 mg L^{-1} as compared to the no-spray treatment estimated 4,882 & 4,906 mg L^{-1}.

The results of the study performed by (**Al-Tamimi, 2017**) showed that the spaying of vitamin C on the mung bean plant at a concentration of 152 mg L^{-1} proved its effectiveness in the weight of one thousand seeds, the number of pods and the yield of one plant, the total average of yield estimated 51.05 gm, 21.96 $pods^{-1}$, 10.29 gm and 1.37 ton ha^{-1}.

(**Younis *et al.*, 2020**) showed that when conducting an experiment to find out the effect of foliar spraying on groundnut plants with three concentrations of B_6 (50, 100 and 150) mg L^{-1} in addition to the treatment comparison (without spraying) that plants treated with a concentration of 100 mg L^{-1} gave a high average number of pods and weight of 100 seeds, the total seed yield estimated 15.00 plant $pods^{-1}$, 87.38 g and 404.06 mg ha^{-1}.

(**Hantoush, 2021**) noticed that in his experiment to find out the effect of spraying vitamin B_6 on broad bean plants at different stages (vegetative growth stage, flowering stage, vegetative growth and flowering stage together, especially the vegetative growth and flowering stage that the plant gave the highest percentage of protein which amounted 21.90. % as compared with the treatment comparison that gave the lowest average of 20.04%. These results consist with (**Jaddoa *et al.*, 2017; Baqir & Al-Naqeeb,2019; SAFI *et al.*, 2022; Al-ziady & Hussain, 2023**).

The results of the study carried out by (**Al-Saadi, 2022**) showed that spraying vitamin B_6 at a concentration of 200 mg L^{-1} on the mung bean plant led to a significant increase in the number of flowers, the fertility rate and the number of pods. This led to- in the yield of mung been seeds- the total average of yield to reach 138.33 of $plant\ flower^{-1}$, 66.63%, 92.68 of $plant\ pods^{-1}$, 1.97 mg $ha^{-1}$and 10.26 mg ha^{-1}, respectively, as compared to the treatment comparison giving the lowest mean of 114.13 of $plant\ flowers^{-1}$, 52.37%, 61.57 pods, 1.77 mg ha^{-1} and 9.34 mg ha^{-1} for the characteristics sequentially.

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