Iraqi Journal of Market Research and Consumer Protection
(2026) 18(1): 125-145.
DOI: http://dx.doi.org/10.28936/jmracpc18.1.2026.(11)
Balasim & et al.,

المجلة العراقية لبحوث السوق
وحماية المستهلك

ISOLATION AND IDENTIFICATION *SERRATIA FONTICOLA* FROM SOIL FOR PREPARATION IRON OXIDE NANOPARTICLES AND USING WITH GLYPHOSATE HERBICIDE AGAINST *CYNODON DACTYLON L.* (BERMUDA GRASS)

Halah M. Balasim[1], Faiza K. Emran[2], Laith A. Yaaqoob[3]
[1] Student PhD, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. Halah.majeed@yahoo.com
[2] Assistant Professor, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. faiza101q@gmail.com
[3]Assistant Professor, Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq. Laith.yaaqoob@sc.uobaghdad.edu.iq

Received 2/ 4/ 2024, Accepted 6/ 8/ 2024, Published 31/ 3/ 2026

ABSTRACT

The mixture of Fe_2O_3 Nanoparticles prepared from *Serratia fonticola* with glyphosate herbicide were exposed against weeds (Bermuda) during the field experiment. A total of 42 soil samples were collected from various agricultural areas in Baghdad city, five species of bacteria that grew the most were selected for regarding other isolates: *Enterobacter cloacae complex, Serratia fonticola, Aeromonas hydrophila, Pantoea* spp. and *Pseudomonas mendocina*. Extracellular extracts of these bacteria were made and then used to manufacture iron oxide nanoparticles biologically. It was observed after measuring the average diameter by Atomic Force Microscopy (AFM) for each nanoparticles prepared from these bacteria were 65.14, 29.21, 63.87, 57.89 and 36.59 nm, respectively. Additionally, the synthesis conditions were precisely taken into consideration a pH of 7 and a temperature of 50°C. Based on the AFM values, *Serratia fonticola* was selected as having the smallest average diameter of 29.21 nm to complete the rest of the techniques, such as:- UV-VIS, FE-SEM and AFM. The wavelength of biosynthesis of Fe_2O_3 NPs is 378 nm, Image of Fe_2O_3 NPs displays spherical form and the average volume is 29.21 nm, respectively. Three concentrations of the prepared Iron Oxide Nanoparticles (Fe_2O_3 NPs) from *Serratia fonticola* were 0.5, 1 and 1.5 g/ml which mixed with Glyphosate herbicide that according to the concentration recommended by the manufacturer and used against Bermuda grass in a controlled experimental environment. The best treatment was the 5 treatment was concentration of Fe_2O_3 NPs 1.5 g/ml with Glyphosate showed a significant impact on the growth and health of Bermuda grass during 7 days, it was shortest period of synergistic effects with the Glyphosate, influencing the color of grass, change, wilting, and growth cessation. Controlling *Cynodon Dactylon L.* (Bermuda weed) using Glyphosate herbicide and varying concentrations of Fe_2O_3NPs and choose the treatment that combined 1.5g/ml of Fe_2O_3 NPs with glyphosate had the highest control percentage 90% against Bermuda weed.

Keywords: Bacterial species, Soil, Iron Nanoparticles, *Cynodon dactylon, Glyphosate herbicide.*

المجلة العراقية لبحوث السوق وحماية المستهلك

Iraqi Journal of Market Research and Consumer Protection

Balasim & et al.,

(2026) 18(1): 125-145.

عزل وتشخيص *Serratia fonticola* من التربة لتحضير جزيئات اكسيد الحديد النانوية واستخدامها مع مبيد الاعشاب الجليفوسات ضد عشبة البرمودا

هالة مجيد بلاسم[1] ، فائزة كاظم عمران[2]، ليث احمد يعقوب[3]

[1] لباحث طالب دكتوراة قسم علوم الحياة , كلية العلوم , جامعة بغداد, بغداد, العراق، Halah.majeed@yahoo.com

[2] الاستاذ المساعد الدكتور, قسم علوم الحياة , كلية العلوم, جامعة بغداد, بغداد , العراق، faiza101q@gmail.com

[3] الاستاذ المساعد الدكتور, قسم التقنيات الاحيائية , كلية العلوم, جامعة بغداد, بغداد , العراق، Laith.yaaqoob@sc.uobaghdad.edu.iq

الخلاصة

تم تعريض خليط جزيئات اكسيد الحديد النانوية المصنعة من بكتريا *Serratia fonticola* مع مبيد الاعشاب الجليفوسات ضد الاعشاب (برمودا) خلال التجربة الحقلية . تم جمع 42 عينة تربة من مناطق زراعية مختلفة في مدينة بغداد وتم اختيار خمسة انواع من البكتريا الاكثر نموا بالنسبة للعزلات الاخرى: *Enterobacter cloacae complex* و *Serratia fonticola* و *Aeromonas hydrophila* و *Pantoea spp.* و *Pseudomonas mendocina* . وتم تصنيع مستخلصات خارج الخلية من هذه البكتيريا ثم استخدامها لتصنيع جزيئات أكسيد الحديد النانوية بيولوجيًا. وقد لوحظ بعد قياس متوسط القطر بواسطة مجهر القوة الذرية (AFM) لكل الجسيمات النانوية المحضرة من هذه البكتريا هو 65.14 و 29.21 و 63.87 و 57.89 و 36.59 نانومتر على التوالي. بالإضافة إلى ذلك، تم أخذ ظروف التوليف بعين الاعتبار على وجه التحديد الرقم الهيدروجيني 7 ودرجة الحرارة 50 درجة مئوية. بناءً على قيم AFM، تم اختيار *Serratia fonticola* على أنها تمتلك أصغر متوسط قطر يبلغ 29.21 نانومتر لإكمال بقية التقنيات، مثل: - UV-VIS و AFM وFE-SEM. يبلغ الطول الموجي للتخليق الحيوي لـ Fe_2O_3 NPs هو 378 nm نانومتر ويبلغ متوسط الحجم 29.21 نانومتر وتعرض صورة جزيئات الحديد النانوية شكلا كرويا على التوالي . كانت ثلاث تراكيز من جزيئات أكسيد الحديد النانوية المحضرة من *Serratia fonticola* هي 0.5 و 1 و 1.5 غرام/مل والتي تم خلطها مع مبيد أعشاب الجليفوسات وفقًا للتركيز الموصى به من قبل الشركة المصنعة واستخدمت ضد عشب برمودا في بيئة تجريبية محكمة. أفضل معاملة كانت المعاملة 5 وهي تركيز 1.5 غرام/مل من جزيئات الحديد النانوية مع مبيد الاعشاب الجليفوسات وقد أظهرت تأثيراً معنوياً على نمو وصحة عشب البرمودا خلال 7 أيام، وكانت أقصر فترة تأثير تآزري مع الجليفوسات في التأثير على لون العشب و التغير والذبول وتوقف النمو . السيطرة على (عشب البرمودا) باستخدام مبيد أعشاب الجليفوسات وتراكيز مختلفة من جزيئات الحديد النانوية وتم اختيار المعاملة التي جمعت 1.5 غرام/مل من جزيئات الحديد النانوية مع الجليفوسات حصلت على أعلى نسبة سيطرة 90% ضد اعشاب برمودا.

الكلمات المفتاحية : الانواع البكترية ، التربة ، جزيئات الحديد النانوية ، عشب البرمودا ، مبيد الاعشاب الجليفوسات.

INTRODUCTION

Soil is an intricate, ever-changing, and thriving environment for a multitude of creatures. Bacteria play a crucial role in the soil micro-flora due to their high numbers, wide range of species, and diverse metabolic functions **(Bedekar *et al*., 2021)**. Additionally, they possess the capacity to mirror the previous chronicles of a specific habitat **(Al-Halfi & Al-Azzawi, 2022)**. Traditional culture-dependent methods have been employed to assess the microbial composition of soil **(He *et al*., 2008)**. However, these techniques only capture a small fraction (0.1-1%) of soil bacteria, leaving the majority of the diverse phylogenetic groups understudied **(Zhang & Xu, 2008)**.

Nanotechnology is a modern technology that plays an important part in everyday life. Nanotechnology is concerned with developing, manipulating, and applying nanometer-sized materials **(Kavitha *et al*., 2013)**. Nanotechnology is primarily concerned with 1-100 nm nanoparticles size in one dimension. Nanotechnology is receiving widespread attention due to its extensive range of applications across various industries, including textiles and medicine **(Shah *et al*., 2022)**. One particularly significant aspect is the utilization of nanostructures in mechanics, optics, electronics, biotechnology, microbiology, environmental remediation, medicine, engineering, and material sciences **(Al-Azawi *et al*., 2019)**.

Recently, the synthesis of metal nanoparticles (MtNPs) using microorganisms such as (bacteria) and plants has been recognized as an efficient and biological method for further exploitation of microorganisms as Nano factories **(Singh *et al*., 2016)**.

The utilization of ecologically friendly raw materials, such as biological extracts derived from bacteria, for the production of iron oxide nanoparticles offers numerous economic and compatible benefits **(Christian *et al*., 2008; Al-Azawi *et al*., 2019).** Microorganisms, particularly bacteria, play a crucial role as nano factories in the accumulation and detoxification of heavy metals. This is possible because they possess a variety of enzymes that can effectively convert metal salts to metal nanoparticles (Mt NP) **(Singh *et al*., 2016)**. The bacterial extracts contain components that are capable of reducing iron-containing substrates, such as ferric sulfate. These extracts can also be utilized for the reduction of iron **(Forbes *et al*., 2007)**.

Iron particles that are smaller than a micron is known as Nano scale iron particles. They have a large surface area, which makes them very reactive. They quickly oxidize to produce free iron ions when oxygen and water are present.

In 1979, *Serratia fonticola* was described as a new species of *Serratia*. The strains were isolated from freshwater and soil **(Gavini *et al*., 1979).**

Nanotechnology can enhance agricultural operations, specifically by employing nanoparticle-based fertilizers or by promoting plant development, so improving soil quality and the overall quality of agricultural goods. Furthermore, the utilization of fertilizers and insecticides employing transporters and chemicals based on nanoparticles is diminished without compromising productivity **(Duhan *et al*., 2017)**.

The Bermuda grass plant (*Cynodon dactylonis* L.) belongs to the Poaceae family. It is a warm-season weeds. Its origin is South Asia. It is widespread in the countries of the Middle East and West Africa **(Bunnell *et al*., 2003)**.

Due to its rapid growth and ability to store nutrients, the *Cynodon dactylon* is one of the most significant and challenging species of weeds in agricultural crop management **(Doroh, 2010)**. It is also difficult to control as it multiplies by rhizomes. This weeds severely damages agricultural crops, particularly horticultural crops that forming a network of rhizomes and

purlin stems both above and below the soil **(Johnson & Davis, 2012)** and increasing the cost of tillage when preparing the ground for planting **(Campbell *et al*., 2010)**.

One of the dangerous jungles that spreads over orchards, especially the recently formed ones, is called Bermuda grass plant in Iraq. Although this weed is dangerous, there aren't many research on how to combat it in Iraq, in comparison to other pesticides, glyphosate has shown to be particularly efficient in reducing their danger that compare to TCA herbicide (trichloroacetic acid) and Dalapon **(Johnson & Ware, 1978; Gerald, 2002)**. However, it is difficult to use this non-selective herbicide by spraying in orchards because. The drift of herbicide drops as a result of the pressure of the spraying machine or the force of the wind causes the death of trees.

Glyphosate herbicide has been proven to be very effective in reducing its risk compared to other systemic pesticides **(Teuton *et al*., 2005)**.

The fundamental objectives of this study are twofold: firstly, to examine the bacterial species that are often found in the studied soil and secondly to assess the biological characteristics of iron oxide nanoparticles (Fe_2O_3 NPs) that have been manufactured using an extracellular extract that derived from *Serratia fonticola*. Additionally, the synergistic effects of iron oxide nanoparticles (Fe_2O_3 NPs) and Glyphosate herbicides on *Cynodon dactylonis* management.

MATERIALS AND METHODS

Sample selection for research

This study involved the collection of a total of forty-two soil samples from several agricultural areas, spanning different time intervals.

Collection of samples

To start the process of bacterial isolation, soil samples were initially gathered. The samples were gathered in a random manner from the topmost layer of soil, specifically from a depth of 5-10cm. A sterile spatula was utilized to collect the samples, which were then placed into sterilized polyethylene bags. The samples were then subjected to air drying, sieved (2.0mm). Finally, the samples were kept at a temperature of 4°C until they were ready for use.

Preparation of samples

In order to prepare the soil sample, 5g of soil was dissolved in 5ml of sterile distilled water. The resulting mixture was then homogenized using a vortex machine. A tenfold serial dilution was performed by combining 1 ml of the homogenized material with 9 ml of D.W. Subsequent dilutions were performed, up to a magnitude of 10^{-6} **(Ahmad *et al*., 2013)**.

Isolation of bacteria

The research used agar medium as the nutrient agar medium. A quantity of 28g of agar

powder was measured and afterwards dissolved in 1000ml of distilled water. The solution was forcefully agitated and afterwards dissolved using a hot plate. Following this, it underwent sterilization in an autoclave at 121°C 15 pounds/inche² for 15 min. Then the substance was let to undergo the cooling process, following which it was distributed onto Petri plates and thereafter allowed to harden.

Sample inoculations

A fraction of the suspension was placed onto the nutrient agar using the streaking method, followed by incubation at a temperature of 28°C for a duration of 24 hours **(Thrupp *et al*., 1992)**, subsequently, the colonies were subjected to observation.

Identification of isolated bacteria

The identification of every individual bacteria was conducted based on the utilization of biochemical tests and the Vitek2 system.

Gram Staining

Gram staining is a prevalent laboratory technique in microbiology that enables the categorization of bacteria into two primary groups: Gram-positive (+) and Gram-negative (-) bacteria can be differentiated using a differential staining technique known as Gram staining. When seen under a light microscope, Gram-positive cells exhibited a purple coloration, whereas Gram-negative cells had a pink or red color. Cell morphology was examined and recorded as well **(Gomashe *et al*., 2013)**.

Biochemical tests

1. Catalase test

The experiment involved the application of a small quantity of hydrogen peroxide onto a sterile microscope slide. Using the edge of a separate slide, a sample of the organism colony was selected and placed in contact with hydrogen peroxide. The presence of bubbles is indicative of a good reaction, whereas the lack of bubbles suggests a negative reaction **(Reiner, 2010)**.

2. Oxidase test

This test was the utilization to ascertain the bacterial capacity for oxidase production oxidase was done by saturating a filter paper with oxidase reagent, the colony was rubbed on the filter paper with a sterile wooden stick. A purple color develops in 10 seconds indicates a positive reaction **(MacFaddin, 2000)**.

Identification of bacterial isolate by Vitek-2 compact System

The VITEK® 2 Compact system is an advanced technology used for fast and accurate microbial identification in laboratories. It's designed to optimize laboratory workflow and increase productivity, thanks to its extensive and robust identification database **(Alabi *et al*., 2023)**.

Bacterial suspension preparation

Few colonies of pure bacterial culture were collected by a sterile cotton swab, suspended into 3 ml distilled water. The turbidity was measured by using a turbidity meter densi-check and adjusted to 0.5 McFarland turbidity **(Pincus, 2006)**.

Preparation of standard turbidity McFarland (0.5)

The preparation of standard McFarland solution No. 0.5 was carried out in accordance with the procedure outlined in reference **(Forbes *et al.*, 2007)**. Solution (A) was made by dissolving 1.175g of barium chloride in 90ml of distilled water (D.W) and thereafter adjusting the volume to 100ml.

Solution (B) was made by adding 1ml of concentrated sulfuric acid to 90ml of distilled water (D.W.), and then bringing the total volume to 100 ml. The two solutions were combined by the addition of 0.5ml of solution (A) to 99.5ml of solution (B). To get a mean cellular density of 1.5 x 10^8 colony-forming units per milliliter (CFU/ml), the prepared solution was employed to assess the turbidity of the bacterial suspension.

Extracellular production

The bacterium was cultured on nutrient broth media at 28˚C for 48 hours, then centrifuged, the bacterial supernatant is an extracellular extract visible through the broth as a light yellow solution **(Moore *et al.*, 2010)**.

Precreation of iron oxide nanoparticles Fe_2O_3 by using extracellular extract of *Serratia fonticola*

The present study focuses on the synthesis of iron oxide nanoparticles by a biological technique approach, utilizing Ferric sulfate $Fe_2 (SO_4)_3$ (Indian) as the precursor with some modifications **(Yaaqoob, 2022)**, *S. fonticola* extracellular components were employed for the creation of Fe_2O_3 nanoparticles. A solution containing 5 gm of Ferric Sulfate $Fe_2 (SO_4)_3$ was prepared by dissolving it in 50 ml of the extracellular product solution obtained from *S. fonticola*. The resulting mixture was subjected to ultra-sonication in a bath for a duration of 10 min to enhance component dispersion. And kept in a dark condition overnight on the shaker. The resulting solution was centrifuged at 8000 rpm for 10 minutes and washed twice with deionized distilled water to remove residual extracellular cells. It was then dried in an oven at 40 °C overnight to obtain a brown powder and stored in a dark vial for later use.

Application of parameters (treatments) in studied area

Studied area: An area of grand land containing Bermuda herbs was taken with an area of 3.375 m^2, which was then split into 6 equal squares that depending on the number of treatments used in the studied experiment. It was a square with 75 cm sides and left the space among these squares 10 cm.

First parameter: Iron oxide nanoparticles (Fe_2O_3 NPs) this parameter includes four levels were

a- Without iron oxide nanoparticles Fe_2O_3 NPs
b- with iron oxide nanoparticles (Fe_2O_3 NPs) Concentrations (0.5, 1, 1.5) g/ml.
Secondary parameter: The rate of herbicide spraying this parameter includes two levels were
a- Without herbicide
b- With herbicide in the recommended dose of company.
Through the combinations between the levels of the above parameters, we have (6) treatments were shown in Table 1.

- Without herbicide - without nanoparticles (negative control)
- Herbicide without nanoparticles (positive control)
- Herbicide with nanoparticles (0.5) g/ml
- Herbicide with nanoparticles (1) g/ml
- Herbicide with nanoparticles (1.5) g/ml
- Nanoparticles (1) g/ml - without herbicide

Table (1): Treatments by adding glyphosate pesticide and iron nanoparticles by spraying.

No.	Treatment	Amount of spray	Symbol code
	Without (Herbicide + Fe_2O_3 NPs)	-	Control negative (white)
	Herbicide	8 ml	Control positive (yallow)
	Herbicide with Fe_2O_3 NPs con. (0.5)	8ml+0.4g	Three (pink)
	Herbicide with Fe_2O_3 NPs con. (1)	8ml+0.8g	Four (baby pink)
	Herbicide with Fe_2O_3 NPs con. (1.5)	8ml+1.2g	Five (orange)
	DDW with Fe_2O_3 NPs con. (1)	10ml+1g	Six (green)

*The amount recommended by Syngenta, the manufacturer of glyphosate pesticide, % concentration.

The experiment was conducted according to Randomized Complete Block Design (R.C.B.D.) methodology, with three replications, and apply the specified parameters shown in Table 2.

Table (2): Common and trade name, percentage of active ingredient, and usage rate of glyphosate pesticide.

Spraying rate	Percentage of active ingredient	Common name	Trade Name
800ml / 30ml/ 1000m^3	36%	Glyphosate	Touchdown S4®

Ultra sonication bath: involves using ultrasonic waves to mix herbicides with nanoparticles. This method can enhance the dispersion of nanoparticles in the herbicide solution. Prepare a mixture of herbicide and nanoparticles in a solvent. Use an ultrasonic bath to apply ultrasonic waves to the mixture for a specified duration (5-10) min. The ultrasonic energy helps to break up agglomerates and disperse the nanoparticles evenly.

RESULTS AND DISCUSSION

Samples collection and bacterial isolation

This study collected 42 samples from eight different agriculture regions of Baghdad city to isolate and identification of a group bacteria spread in soil samples. These samples, a total of 62 bacterial isolates were isolated, different genus belongs to *Enterobacteria, Serritia, Aeromonas, Pantoea, Pseudomonas, Koucuria, Staphylococcus, Leclercia, Leuconostoc, Streptococcus, Citrobacter, Esherichia, and Bascillus*.

Identification of the isolates

The cultural properties of the isolated colonies were determined using the Vitek-2 compact system and biochemical identification.

Microscopic observation of the isolates

The isolates were subjected to microscopic examination following Gram staining in order to ascertain their Gram-negative or Gram-positive nature, as well as to observe their respective arrangements. Figure 1 displays the distribution of Gram-negative and Gram-positive bacteria as a fraction of the total isolated bacterial population.

The examination of the isolates under a microscope indicated that the majority of them exhibited a rod-shaped morphology and displayed motility. The morphological properties of several colony types in each sample were documented using Gram staining. The identification of isolates was accomplished by the utilization of microscopic inspection and analysis of their biochemical reactions.

Biochemical identification

In the present study, a total of 62 isolates were identified from a sample pool consisting of 42 samples. The identification of all the isolates was conducted by the implementation of

several biochemical assays, as outlined in Table 3.

Table (3): Biochemical test results of isolated bacteria.

Bacteria	No. of Isolates	percentage% of isolates	Catalase test	Oxidase test	Gram staining	Growth on	
						Mannitol	MacConky
***Bacillus* spp.**	4	6.45	+	+	+	+	N
Staphylococcus hominis	2	3.22	+	-	+	+	N
Streptococcus thorltensis	2	3.22	-	-	+	+	N
Leuconostoc pseudomesenteroides	2	3.22	-	-	+	+	N
Kocuria kristinae	2	3.22	+	+	+	+	N
Staphylococcus lentus	2	3.22	+	+	+	+	N
Pseudomonas oryzihabitants	3	4.83	+	-	-	N	+
Enterobacter cloacae complex	10	16.12	+	-	-	N	+
Serratia fonticola	6	9.67	+	-	-	N	+
Aeromonas hydrophila	4	6.45	+	+	-	N	+
Leclercia adecarboxylate	1	1.61	+	-	-	N	+
***Pantoea* spp.**	8	12.9	+	-	-	N	+
Pseudomonas stutzeri	3	4.83	+	+	-	N	+
Pseudomonas mendocina	6	9.67	+	+	-	N	+
Citrobacter freundii	1	1.61	+	-	-	N	+
Escherichia coli	2	3.22	+	-	-	N	+
Pseudomonas aeruginosa	4	6.45	+	+	-	N	+
Total	62						

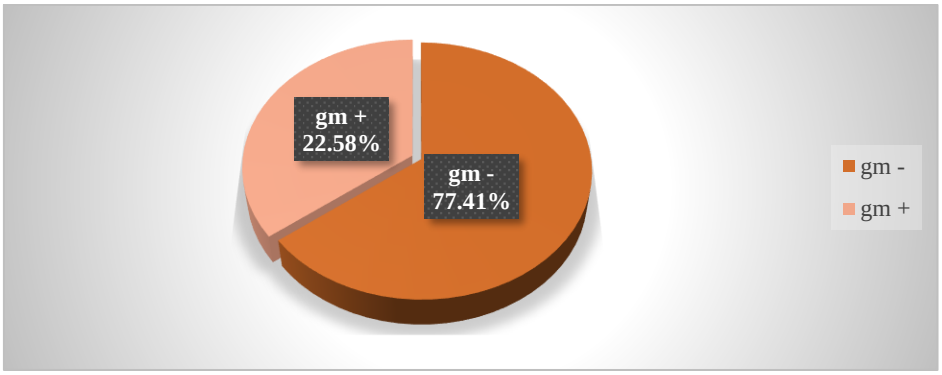

Figure (1): Percentage of Gram-negative and Gram-positive bacteria isolated

Vitek-2 compact system

Based on their physical and biochemical characteristics, and by Vitek-2 compact System these total sample of 62 isolates were confirmed. The percentage of bacterial isolates from the samples collected from soil after calculating isolate, showed according to Figure 2.

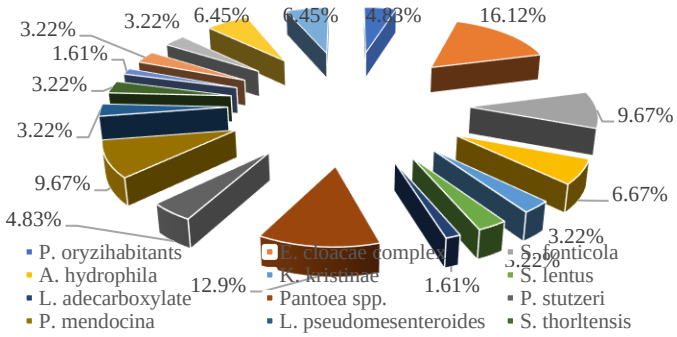

Figure (2): Percentage distribution of isolated bacteria in Baghdad soil.

Characterization of extracellular extract of *Serratia fonticola*

Ultraviolet-visible light (UV-VIS) Scanning Spectrum of extracellular extract of *Serratia fonticola*

Explain the absorption spectra of the used extracellular extract component. The absorption peak was a wavelength of 378 nm. As shown in Figure 3.

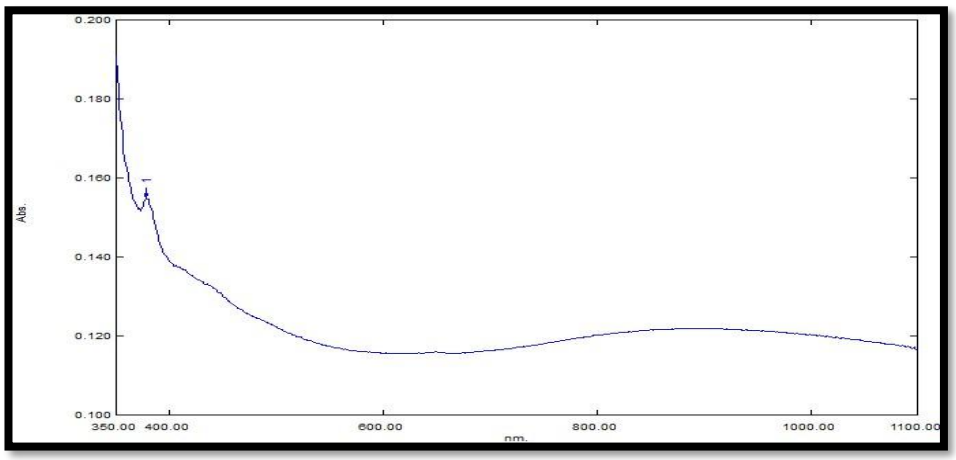

Figure (3): UV-VIS images of extracellular extract of *S. fonticola.*

Fourier transform - infrared (FTIR) of extracellular extract of *Serratia fonticola*

The spectrum IR spectrum of the extracellular extract of *Serratia fonticola* lyophilized sample analyzes the spectrum of the six major peaks at 3415.70-3371.34, 3001.03-2935.46, 1573.81, 1415.65, 1124.42-1012.56, and 649.97-430.10 cm^{-1}, as shown in Figure 4. A method for determining the bond vibration frequencies of a molecule is FTIR spectrographic analysis. In the extracellular extract of *Serratia fonticola,* the region between 3415.70 to 3371.34 cm^{-1} has stretching mode of O-H Alcohol, absorption of N-H stretching of Amine salt group is (3001.03-2935.46cm^{-1}), N-H bonds of amine group is (1573.81cm^{-1}), O-H bond of Alcohol group is 1415.65, the region between (1124.42-1012.56 cm^{-1}) has group of C-N stretch Amine, and the absorption from 649.97 to 430.10 is due to the represent of the Fe-O band and Fe-O- Fe skeletal frequency, as shown in Table 4.

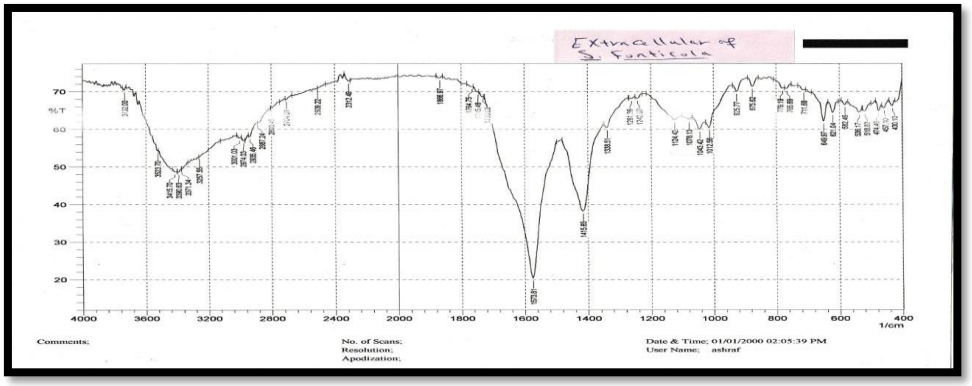

Figure (4): Fourier transforms-infrared FTIR in the extracellular extract of *Serratia fonticola***.**

Table (4): Fourier transform infrared (FT-IR) spectroscopy measurement of extracellular extract of *Serratia fonticola.*

	Frequency of Absorption (cm^{-1})	**Bonds**	**Compound class of Functional Groups**
Extracellular Extract	3415.70-3371.34	O-H stretch	alcohol
	3001.03-2935.46	N-H stretch	Amine salt
	1573.81	N-H bond	amine
	1415.65	O-H bond	alcohol
	1124.42-1012.56	C-N stretch	amine
	649.97-430.10	Metal oxygen	Metal oxygen

Characterization of Iron oxide Nanoparticles (Fe_2O_3 NPs) from extracellular extract of *Serratia fonticola*

Atomic Force Microscopy (AFM) of Fe_2O_3 NPs

Atomic force microscopy analysis of Fe_2O_3 NPs surfaces revealed that both 2D and 3D shapes may form (Figure 5). Fe_2O_3 NPs AFM pictures demonstrate the spherical shape of the biosynthesized Fe_2O_3 NPs. The average diameter determined by AFM was 29.21 nm (Table 5) and (Figure 5).

Table (5): The average diameter of Fe_2O_3 nanoparticles that were synthesized from *S. fonticola*

Avg. Diameter:29.21 nm	**<=10% Diameter:16.00 nm**
<=50% Diameter:26.00 nm	**<=90% Diameter:44.00 nm**

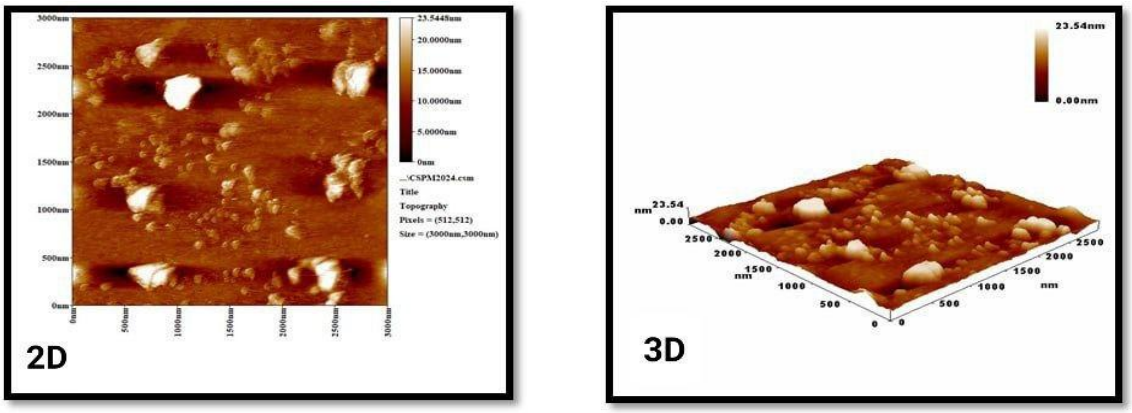

Figure (5): Atomic force microscopy (AFM) of Fe2O3 NPs synthesized using intracellular illustrate 2D and 3D topological.

The examination of ultraviolet-visible light (UV-VIS) spectra the presence of Fe2O3 inside the extracellular

The optical properties of the nanoparticles were analyzed with a UV-Visible spectrophotometer. The graphical representation illustrates the absorbance of the sample within the nanometer range at standard room temperature that to detect the maximum absorption. Absorbance is measured at a wavelength of 359 nm. As shown in Figure 6 .

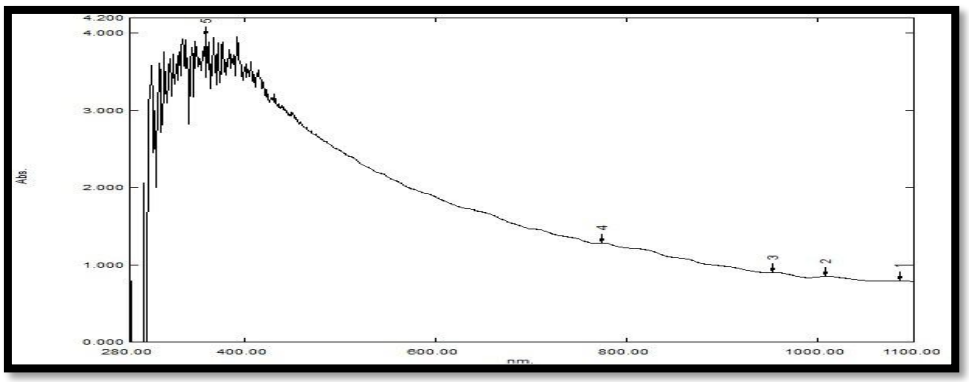

Figure (6): UV-VIS images of Fe2O3 NPs synthesized using extracellular extract of S. fonticola.

Fourier transform infrared (FTIR) analysis of Fe_2O_3 from extracellular

The functional groups of nanoparticles have been identified using FTIR spectrum (Figure 7). Illustrates the absorption spectra of nanoparticles that are produced through biological means, as observed using Fourier Transform Infrared Spectroscopy (FTIR). An intense peak at 3434.98cm^{-1} was visible due to OH stretching mode. The occurrence of the peak properties at 1639.38cm^{-1} suggested the presence of conjugated acid C=O stretch. The peak at 1552.59cm^{-1} due to nitro compound N=O starch. In the Fe_2O_3 NPs absorption S=O stretch of sulfate compound is (1413.72 cm^{-1}). Present of aliphatic ether compound C-O stretch in 1126.35 cm^{-1}. In 802.33 cm^{-1} has C-H bond of 1, 4 disubstituted or 1,2,3,4 tetra substituted. The wide peak at 640.32 to 518.82cm^{-1} indicated the Fe-O bond and the frequency of the Fe-O-Fe skeletal structure. (Table 6).

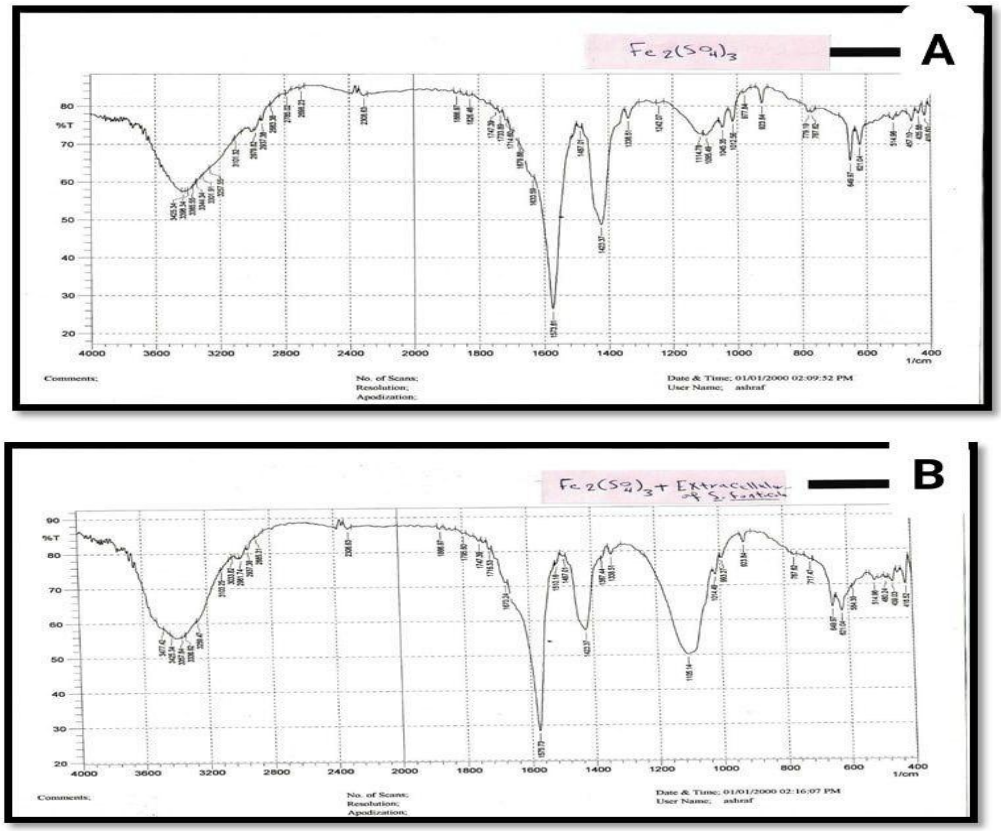

Figure (7): Fourier transform infrared (FTIR) spectroscopy measurement Fe2O3NPs using exracelluler, (A) Fe2(SO4)3, (B) Fe2(SO4)3 +Extracellular, (C) Fe2O3NPs

Table (6): Fourier transform infrared (FT-IR) spectroscopy measurement of extracellular extract of S. fonticola, Fe2 (SO4)3, extracellular+ Fe2 (SO4)3, and Fe2O3 NPs.

	Frequency of Absorption (cm^{-1})	Bonds	Compound class of Functional Groups
Ferric Sulfate	3425.34-3257.55	O-H stretch	Alcohol
	1573.81	N-H bond	amine
	1423.37	O-H bond	Carboxylic acid
	1114.78-1012.56	C-N stretch	amine
	649.97-621.04	Metal Oxygen	Fe_2O_3
Extracellular+ Ferric Sulfate	3477.42-3259.47	O-H stretch	Alcohol
	1575.73	N-H bond	amine
	1423.37	O-H bond	Carboxylic acid
	1105.14	C-O stretch	Secondary alcohol
	649.97-621.04	Metal Oxygen	Fe_2O_3
Fe_2O_3 NPs	3434.98	O-H stretch	Alcohol
	1699.17	C=O stretch	Conjugated acid
	1552.59	C=C stretching	Cyclic alkene
	1413.72	S=O stretch	sulfate
	1126.35	C-O stretch	Aliphatic ether
	802.33	C-H bond	1,4 disubstituted or 1,2,3,4 tetra substituted
	640.32-518.82	Metal Oxygen	Fe_2O_3

Field emission scanning electron microscopy analysis (FE-SEM) of Fe_2O_3 from extracellular

FE-SEM was utilized to capture images of the sample at a magnification of 50,000 times. Focused on (Figure 8). The entire sample exhibits smooth surfaces and a consistent spherical shape composed of Fe_2O_3 nano cluster centers. As shown in **Khansaa *et al* (2022).**

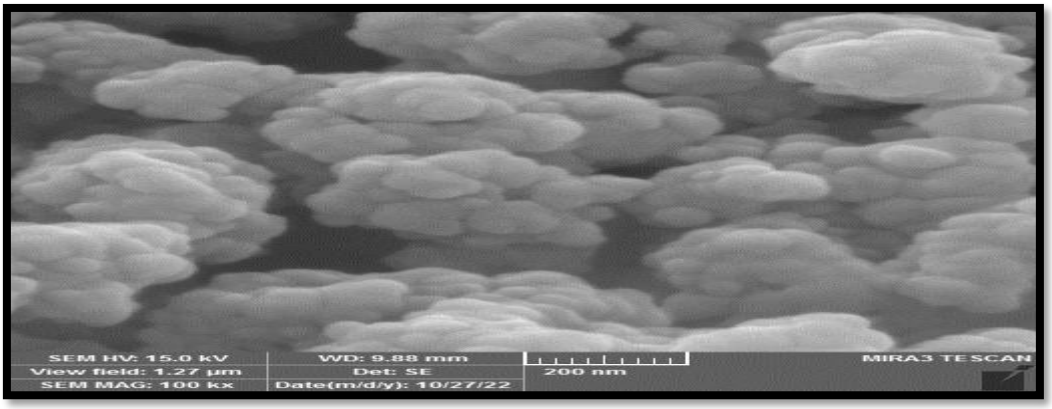

Figure (8): FE-SEM Image of Fe2O3 NPs synthesized using extracellular.

Application of studied parameters (treatments)

The study investigated the effects of six different treatments on the growth of Bermuda grass. These treatments included variations in the application of herbicide (Glyphosate) and iron oxide nanoparticles (Fe_2O_3). The six treatments were applied in a Randomized Complete Block Design (R.C.B.D.) with three replications, and recorded during 14 days post-treatment.

Treatment 1 without Glyphosate herbicide and Fe_2O_3 NPs: this treatment involved no application of herbicide or nanoparticles, this serves as the negative control. The observations noted during the experiment

- The growth and a vertical rise of grass continued with good health.
- In the negative control group, the height of Bermuda grass and rate number of leaves was higher 45cm and 48.4, respectively (Table 7).

Treatment 2 with Glyphosate herbicide: this treatment involved application of only Glyphosate at the recommended dosage, specifying it as the positive control. The observations noted during the experiment

- After more than two weeks of treatment, there was no change in the color or health of the grass.
- Notably, the average height and overall health of Bermuda grass in both treatments were remarkably similar, indicating a negligible difference in growth patterns between the herbicide-treated and untreated lands that lead to resistance the Bermuda grass to Glyphosate**.**

Treatment 3 Glyphosate herbicide with Fe_2O_3 NPs con. 0.5 g/ml: this treatment involved application of Glyphosate at the recommended dosage with Fe_2O_3 NPs con. (0.5) g/ml after 14 days from monitoring

- That showed a synergistic effect between Glyphosate and 0.5 g/ml of nanoparticles, as it was observed that the color of the grass changed from green to yellow.
- As well as wilting and weakness, which indicates that nutrients do not reach them.

Treatment 4 Glyphosate herbicide with Fe_2O_3 NPs con. One g/ml: this treatment involved application of Glyphosate at the recommended dosage with one g/ml of Fe_2O_3 NPs. It was observed after a period of 10 days of monitoring that showed

- A synergistic effect between the herbicide and 1 g/ml of prepared nanoparticles, as it was observed that the color of the grass changed from green to yellow.
- As well as wilting and weakness, which indicates that nutrients do not reach them.

Treatment 5 Glyphosate herbicide with Fe_2O_3 NPs con. (1.5) g/ml: this treatment involved application of Glyphosate at the recommended dosage with (1.5) g/ml of Fe_2O_3 NPs. It was observed after a short period of 7 days of monitoring that showed

- a synergistic effect that observed the color of the grass changed from green to yellow also
- wilting and weakness
- lack of growth that show the grass stop growing while the plants around them continue to grow
- Eventually, the grass may show signs of rotting or decay

Treatment 6 Fe_2O_3 NPs con. One g/ml without Glyphosate herbicide: this treatment involved application of iron oxide nanoparticles (Fe_2O_3) in 1 g/ml, after 14 days of treatment

- The grass that underwent nano-treatment only exhibited continued development, suggesting underway availability of nutrients (Table 7).

Table (7): The effect of treatments on some vegetative traits of Cynodon dactylon plant.

Control percentage%	Number of plants/cm^2	Number of leaves/plant	Treatments
0	12.6	72.6	**1**
39.68	7.6	44	**2**
47.14	6.66	38.66	**3**
80.63	2.44	14	**4**
90	1.26	5.33	**5**
2.38	12.3	69.66	**6**

CONCLUSIONS

Study successfully bio-synthesized iron oxide nanoparticles using an extracellular extract from *Serratia fonticola*. This green synthesis approach is a significant contribution to the field of nanotechnology, particularly for agricultural applications. This study demonstrated the effectiveness of these nanoparticles in conjunction with Glyphosate herbicide on the management of Bermuda grass. This could potentially offer a new method for controlling this invasive species. The research findings indicate a synergistic effect between the iron oxide nanoparticles and Glyphosate herbicide. This synergy led to enhanced herbicidal activity, as evidenced by changes in grass color and reduced growth. The best concentration of Fe_2O_3 was 1.5 g/ml with Glyphosate, and the wilting result appeared after the shortest period among pervious treatments, which was 7 days. For environmental implications: The use of biologically synthesized nanoparticles for herbicidal purposes could be a more environmentally friendly alternative to traditional chemical methods. This could have significant implications for sustainable agricultural practices.

REFERENCE

1. Ahmad, B., Nigar, S., Shah, S.S.A., Bashir, S., Ali, J., Yousaf, S., et al. (2013). Isolation and identification of cellulose degrading bacteria from municipal waste and their screening for potential antimicrobial activity. *World Appl Scince Journle*,27(11), 6-1420.

2. Alabi, E. D., Bindawa, B. L., Mzungu, I. & Adesoji, A. T. (2023). Characterization of selected multidrug-resistant bacteria from clinical and hospital environmental sources using Vitek 2 compact system.
3. Al-Azawi, M. T., Hadi, S. M. & Mohammed, C. H. (2019). Synthesis of silica nanoparticles via green approach by using hot aqueous extract of Thuja orientalis leaf and their effect on biofilm formation. *The Iraqi Journal of Agricultural Sciences*, 50, 245-255.
4. Al-Halfi, D. A., & Al-Azzawi, S. S. (2022). Effect of organic fertilization sources and chemical fertilization on some soil physical traits and yield of summer squash *Cucurbta Pepo L.*: Effect of organic fertilization sources and chemical fertilization on some soil physical traits and yield of summer squash (*Cucurbta Pepo* L.). *Iraqi Journal of Market Research and Consumer Protection*, *14*(2), 74-81.
5. Bedekar, V. V., Kolet, M., Mulye, K., & Pawar, J. (2021). Microbiome-The story untold.
6. Bunnell, B.T., McCarty, L.B., Cox, C.J. & Estes, A.G. (2003). Post emergence Poa annua control in dormant Bermuda grass. *Southern Weed Science Symposium,* 56, 241-247.

7. Campbell, P. L. W., Giffespie, E., Rhodes, R., Leslie, G.W., Phewa, F. & Smit, M. (2010). Economic control of *Cynodon dactylon*, A Theoritical strategy for emerging sugarcane farmers. *South African Sugar Technologists' Association*, 83, 147-151.
8. Christian, P., Von der Kammer, F., Baalousha, M. and Hofmann, T. (2008). Nanoparticles: structure, properties, preparation and behaviour in environmental media. *Ecotoxicology*, 17(5), 326-343.
9. Doroh, M. C. 2010 .Evaluation of best management practices for Bermudagrass (*Cynodon dactylon*) control in Zoysiagrass (Zoysia spp.) turkf. M.Sc. Thesis Auburn Universit, Alabama.
10. Duhan, J. S., Kumar, R., Kumar, N., Kaur, P., Nehra, K. & Duhan, S. (2017). Nanotechnology: The new perspective in precision agriculture. *Biotechnology Reports*, 15, 11-23.
11. He, J. Z., Zheng, Y., Chen, C. R., He, Y. Q., & Zhang, L. M. (2008). Microbial composition and diversity of an upland red soil under long-term fertilization treatments as revealed by culture-dependent and culture-independent approaches. *Journal of Soils and Sediments*, 8, 349-358.
12. Forbes, B. A., Sahm, D. F. & Weissfeld, A. S. (2007). Bailey and Scotts' Diagnostic microbiology 12th. ed. St Louis, Mosby.
13. Forbes, B.A., Sahm, D.F. & Weissfeld, A.S. (2007). Diagnostic microbiology: Mosby St Louis.
14. Gavini, F., Ferragut, C., Izard, D., Trinel, P. A., Leclerc, H., Lefebvre, B. & Mossel, D. A. A. (1979). *Serratia fonticola*, a new species from water. *International Journal of Systematic and Evolutionary Microbiology*, 29(2), 92-101.
15. Gerald, W. Evers. (2002). Herbicides for Desiccating Dallis grass (Paspalum dilatatum)-Bermuda grass (*Cynodon dactylon*) Pasture Sod Prior to Overseeing with Annual Ryegrass, (*Lolium multiflorum*) *Weed Technology*, 16, 235-238.
16. Gomashe, A., Gulhane, P. & Bezalwar, P. (2013). Isolation and screening of cellulose degrading microbes from nagpur region soil. *International Journal of Life Sciences*,1(4), 3-291.
17. Johnson B. J. & G. O. Ware.1978. Dates of Glyphosate Treatments on Weeds and Bermudagrass (*Cynodon dactylon*) *Weed Science*, 26(6), 523-526.
18. Johnson, W. C. & Davis, J. W. (2012) .Techniques for Cynodon dactylon (L.) Pers. Control suitable for use in fallow organic transition in the southeastern U.S. costal plain.*Crop Protection*, 39, 63-65.
19. Kavitha, K. S., Baker, S., Rakshith, D., Kavitha, H. U., Yashwantha Rao, H. C., Harini, B. P. & Satish, S. (2013). Plants as green source towards synthesis of nanoparticles. *Int Res J Biol Sci*, 2(6), 66-76.

20. Khansaa, A. S., Hadeel, H., Firas, S., Sundus, H., & Hamdia, M. S. (2023). In vitro evaluation of the effect of a botanical combination on cancer cell control. *iraq journal of market research and consumer protection*, *15*(2).
21. MacFaddin, J. (2000) . Individual biochemical tests. Biochemical tests for identification of medical bacteria, 3, 27-439.
22. Moore, J.C., DeVries, J.W., Lipp, M., Griffiths, J.C. and Abernethy, D.R. (2010). Total protein methods and their potential utility to reduce the risk of food protein adulteration. Comprehensive reviews in food science and food safety, 9(4), 330-357.
23. Pincus, D.H. (2006). Microbial identification using the bioMérieux Vitek® 2 system. Encyclopedia of Rapid Microbiological Methods Bethesda, MD: Parenteral Drug Association, 1-32.
24. Reiner, K. (2010). Catalase test protocol. *American society for microbiology*, 1-6.
25. Shah, M. A., Pirzada, B. M., Price, G., Shibiru, A. L., & Qurashi, A. (2022). Applications of nanotechnology in smart textile industry: A critical review. *Journal of Advanced Research*, *38*, 55-75.
26. Singh, P., Kim, Y. J., Zhang, D. & Yang, D. C. (2016). Biological synthesis of nanoparticles from plants and microorganisms. *Trends in biotechnology*, *34*(7), 588-599.
27. Teuton, T. C., Unruh, J. B., Brecke, B. J., Miller, G. L. & Mueller, T. C. (2005). Hybrid bermudagrass (Cynodon dactylon (L.) Pers. C transvaalensis Burtt -Davy) control with Glyphosate and Fluazifop. online. Applied Turfgrass Science. Plant Management Network.
28. Thrupp, L. D., Cleeland, R., Jones, R. N., Novick Jr, W. J., Reller, L. B., Thornsberry, C. L. and Washington, J. A. (1992). General guidelines for clinical bacteriology. *Clinical infectious diseases*, 15(Supplement-1): S339-S346.
29. Yaaqoob, L.A. (2022). Evaluation of the Biological Effect Synthesized Iron Oxide Nanoparticles on Enterococcus Faecalis. *Iraqi Journal of Agricultural Sciences*,53(2),440-452.
30. Zhang, L. and Xu, Z. (2008). Assessing bacterial diversity in soil: a brief review. *Journal of soils and sediments*, *8*, 379-388.