



EFFECT OF TWO GENUS OF MICROORGANISMS AS BIO-FERTILIZERS AND PHOSPHATE FERTILIZATION WITH BRASSINOLIDE SPRAYING ON PLANT GROWTH, AND QUALITY AND YIELD OF POTATO TUBERS

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ABSTRACT

The experiment was carried out in southwest Baghdad- the Radwaniyah region during fall season 2021 and spring season 2022 that aimed to study the effect of biological inoculation, and phosphate fertilization and spraying with Brassinolide on the growth, productivity and quality of industrial potato (Arsenal cultivar), the experiment carried out as a three factors (4×3×3) in the order of split plot within the design of Randomized complete block design (RCBD), the biological factor was distributed into main plots and interaction between phosphorus levels and the growth regulator Brassinolide within sub plots using three replicates, Bio-fertilizers included *Aspergillus niger* alone and *Bacillus megaterium* alone and the combination between them in addition to the comparison treatment, phosphorus at three levels (0, 10, 200) kg P₂O₅ ha⁻¹ and Brassinolide spraying of three levels (0, 0.1, 0.2) mg L⁻¹. The results showed that triple combination of Bio-fertilizers together with the addition of phosphorus at the level of 200 kg P₂O₅ ha⁻¹ and spraying with Brassinolide at a concentration of 0.1 mg L⁻¹ produced significant highest leaves area (141.110 and 142.856) dm² plant⁻¹ and dry weight of plant (58.778 and 75.000) g plant⁻¹ and marketable yield (32.136 and 35.606) tons ha⁻¹ for both seasons, the combination treatment of Bio-fertilizers together with phosphorus at the level of 200 kg P₂O₅ ha⁻¹ and spraying with Brassinolide at 0.2 mg L⁻¹ in the percentage of total sugars (3.667 and 2.453)% and the specific density (1.089 and 1.084) g cm⁻² compared to the comparison treatment produced lowest value for both seasons.

Keywords: industrial potato, biological inoculation, fertilizer, p₂o₅. *Aspergillus niger*, *Bacillus megaterium*.

* This article is taken from the doctoral dissertation of the first researcher.



تأثير جنسين من الاحياء الدقيقة كأسمدة حيوية والتسميد الفوسفاتي مع الرش بالبراسينولايد في نمو النبات وجودة وحاصل درنات البطاطا

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الخلاصة

نفذت التجربة في جنوب غرب بغداد منطقة الرضوانية الشرقية للموسم الخريفي 2021 والموسم الربيعي 2022 بهدف دراسة تأثير المخصبات الحيوية والتسميد الفوسفاتي والرش بالبراسينولايد في نمو وانتاجية ونوعية البطاطا الصناعية صنف ارسنال (Arsenal)، ونفذت كتجربة عاملية بثلاث عوامل (3×3×4) بترتيب الألواح المنشقة (split plot) ضمن تصميم القطاعات العشوائية الكاملة (RCBD)، وتم توزيع العامل الاحيائي ضمن الألواح الرئيسية والتداخل بين مستويات الفسفور ومنظم النمو البراسينولايد ضمن الألواح الثانوية وبثلاث مكررات، تضمنت المخصبات الاحيائية فطر *Aspergillus niger* لوحده وبكتريا *Bacillus megaterium* لوحدها والتوليفة بينهما اضافة الى معاملة المقارنة والفسفور بثلاث مستويات هي 0، 10، 200 كغم P₂O₅ هـ¹ والرش بالبراسينولايد بثلاث مستويات 0.1، 0.2، 0.3 ملغم لتر⁻¹. اظهرت النتائج تفوق التوليفة الثلاثية بين كلا المخصبين معاً مع الفسفور عند المستوى 200 كغم P₂O₅ هـ¹ والرش بالبراسينولايد بالتركيز 0.1 ملغم لتر⁻¹ معنوياً في المساحة الورقية (141.110 و 142.856) دسم² نبات⁻¹ والوزن الجاف للنبات (58.778 و 75.000) غم نبات⁻¹ والحاصل القابل للتسويق (32.136 و 35.606) طن هـ¹ مقارنة لكلا الموسمين، ومعاملة التوليفة بين كلا المخصبين معاً مع الفسفور عند المستوى 200 كغم P₂O₅ هـ¹ والرش بالبراسينولايد بالتركيز 0.2 ملغم لتر⁻¹ في النسبة المنوية للسكريات الكلية (3.667 و 2.453) % والكثافة النوعية (1.089 و 1.084) غم سم⁻² مقارنة بمعاملة المقارنة التي أعطت أقل القيم لكلا الموسمين.

الكلمات المفتاحية: بطاطا صناعية، مخصبات احيائية، سماد P₂O₅، *Bacillus megaterium*، *Aspergillus niger*.

INTRODUCTION

Potatoes are one of the most important starchy crops, they are used as food for more than a billion people all over the world and are also used in industrial applications (Ahmed *et al.*, 2018) it is one of the most important four agricultural crops after wheat, corn and rice and the most consumed because it has many energy sources from carbohydrates, proteins, vitamins and minerals (Hassan, 2021).

And sustainable food production in accordance with environmental health conditions and in a way that fulfills the desired purpose in terms of providing food security and with high quality to face the challenges of population increase and current obstacles to agriculture, such as the balanced use of chemical fertilizers, inappropriate climate conditions, lack of water and small distances exploited in agriculture, therefore modern technologies are necessary to achieve the goal of production and quality and this is happen only by increasing the supply of nutrients in the soil, and the efficiency of fertilizer that use is essential in sustainable potato production so bio-fertilization can be used for its importance in agriculture and this was proved by various field experiments in different environmental conditions and with different microbial species (Caradonia *et al.*, 2022).

Phosphorus is one of the major nutrients that the plant needs in high quantities for suitable plant growth as it plays an important role in many biological and physiological processes in the plant (Kalayu, 2019) and it also enters into ATP energy compounds that provide energy for biological processes in the plant from absorption and transport of elements and root growth and



development as well as has an effect on the early tuber formation, maturation and quality of tuber yield (Muthoni, 2016), it has a role in the construction of nucleic acids and it was found that the activity of microorganisms in the soil has a role in the availability of phosphorus that fixed in the soil for plant (Ali & Majeed, 2016). However, this element is not easily absorbed into the soil by the plant so it was found that phosphate dissolving bio-fertilizers are the ideal solution for dissolving organic and mineral phosphorus and converting it to the available form for absorption in economical shape (Kalayu, 2019).

The using of bio-fertilizers to dissolve phosphates is the ideal choice in sustainable agriculture as they secrete many and varied organic acids, and among the most important microorganisms that dissolve phosphates that used as bio-fertilizers are *Aspergillus niger* fungus and *Bacillus megaterium*. *Aspergillus niger* is a fungus that work on dissolves phosphates and lowers soil PH to produce organic acids and has a high ability to produce auxins and gibberellin also it has been recommended for it's using as biofertilizers in agricultural fields in many researches (Jyothi & Basaiah, 2022). it was found that it has a high ability to secrete Citric acid in addition to a variety enzymes, proteins and secondary compounds (Cairns *et al.*, 2018). and this type of microorganism is non-toxic and harmless to the plant (Mohamed & AL-Shamary, 2022).

Bacillus megaterium is one of phosphate dissolving bacteria that belong to the PGPR group and works to stimulate plant growth and it has ability to produce auxins, gibberellins (IAA and GA) and Siderophores compounds, it is considered as one of the bacteria that suitable for use as bio-fertilizers also it has the ability to prevent pathogens (Kesaulya *et al.*, 2015). The most important acids that released by this type of microorganism are citric, gluconic and proponic acids (Hassan, 2012).

Brassinolide is one of the active hormones that found in plant with effective physiological effects and, this hormone is produced in various parts of the plant and has a role in plant growth and development because of its effect in the division and elongation of plant cells, cell wall construction, differentiation of the vascular system, the growth of branches and transverse roots, increased production and resistance to biological and non-biological stresses, as well as it work to stimulating DNA and RNA building, and the most effective compounds are Brassinolide that use as a growth regulator on a commercial field from biological and productive side (AL-Khafaji, 2014).

MATERIALS AND METHODS

The research was carried out in south-west of Baghdad- the Radwaniyah region for the fall season 2021 and spring 2022 season using industrial potato crop cultivar Arsenal. The experiment was carried out as a three-factors in the arrangement of split plot RCBD. The biological factor was within the main plots and the interaction between the composting levels of phosphorus and the Brassinolide within the sub plots 36 treatments with three replicates. The agricultural operations were carried out on the designated field for the study. The land was divided into experimental units with a length of 1.75m and a width of 2m, with area of 3.5m². Each unit includes two rows for cultivation, spaced 1m apart, with a distance of 0.25m between each plant. This results in an average of 7 plants per row, or 14 plants per experimental unit.



The biological factor included two types of fertilizers: *Aspergillus niger* and *Bacillus megaterium*, symbolizing the treatment without Bio-fertilizer (A0), *Aspergillus niger* (A1), *Bacillus megaterium* (A2), and a combination of them (A3) Microbiological isolates and mixed with peat moss (*Aspergillus niger* fungus from the Agricultural Research Department - Ministry of Science, Technology and bacteria *Bacillus megaterium* from the Agricultural Research Department - Crop Protection Department with a bio density of 1×10^8 CFU gm⁻¹ carrier material), which were added at a rate of 20 g of biomaterial for each plant with soil, adding 800 g of organic matter for each experimental unit and a fixed quantity for all treatment as a medium for bacterial activity and to improving their work. The second factor is fertilization with phosphorus and includes three levels (0, 100, 200) kg P₂O₅ ha⁻¹ and symbolized by P0, P1, and P2 respectively, the amount of phosphate fertilizer added on a single batch after five days from planting. The third factor included three levels of growth regulator Brassinolide (0, 0.1, 0.2) mg L⁻¹ and symbolized by BL0, BL1, and BL2 respectively, sprayed with three. Nitrogen and potassium fertilizer were added in the two batches and all experimental units were added equally (250 nitrogen and 300 potassium) kg ha⁻¹ as recommended (Ali, 2012). The Genstat program was used for statistical analysis and the averages for all the study indicators were compared by significant differences (L.S.D) at 5%.

FACTORS OF STUDY

1. Vegetative study indicators (leaves area dm² plant⁻¹, dry weight of plant g plant⁻¹).

Three leaves from each plant was taken (from the top, middle and bottom) from five plants and photographed using a scanner and then the images entered into the Digimizer program, where it extracts the leaves area for each leaf and multiplies by the number of leaves of one plant and then extracts the final rate leaves area rate.

The total vegetative of 10 plants was taken randomly and placed in large paper bags and dried in an oven at a temperature of 60-70 C° until the weight stabilized, then calculating the dry weight of each plant and calculating the rate.

2. Qualitative yield indicators (specific density g cm⁻², percentage of total sugars in tubers %)

Specific density = $1.0988 + \{ (\text{dry matter percentage} - 24.182) \div 211.04 \}$

Sugars were estimated by method of (Joslyn, 2012).

3. Marketable Yield. It was calculated by:

Multiplying the product of one plant for the marketing yield \times the number of plants in hectare.

RESULTS AND DISCUSSION

1. The individual effects of study factors in plant growth, qualitative traits, and marketing yield

The results of table (1) shows the significant effect of the study factors on the vegetative growth indicators and the qualitative traits of the yield and marketing yield, as the A₃ treatment was characterized by producing highest value in the leaves area and the percentage of total sugars and the marketing yield of the potato plant about 117.420, 110.360 dm² plant⁻¹ and 3.181, 1.894% and 30.554, 32.257 ton ha⁻¹ for both seasons respectively and in the dry weight



of plant in the spring season and amounted to $66.370 \text{ g plant}^{-1}$ and the specific density in the fall season and amounted to 1.086 g cm^{-2} compared with A_0 treatment which produced lowest value for each of them, The P_2 treatment was characterized by producing highest value in leaves area, dry weight of plant, total sugars, specific density and marketing yield and about 118.641, 111.098 $\text{dm}^2 \text{ plant}^{-1}$, 52.102, 69.861 g plant^{-1} , 3.056, 1.669 %, 1.086, 1.078 g cm^{-2} , 29.780, 30.941 ton ha^{-1} for both seasons respectively compared to the P_0 treatment that produced lowest value for each of them. The BL_1 treatment was characterized by producing highest value in the leaves area and dry weight of plant and the marketing yield and amounted 112.200, 96.247 $\text{dm}^2 \text{ Plant}^{-1}$, 51.731, 65.046 g plant^{-1} and 29.254, 30.327 ton ha^{-1} for both seasons respectively, while the BL_0 treatment produced lowest value for each of them and for both seasons, the BL_2 treatment was characterized by producing highest value in total sugars and specific density of 2.914, 1.672% and 1.086, 1.078 g cm^{-2} for both seasons compared to the BL_0 treatment, which produced lowest value for each of them for both seasons.

Table (1): Effect of Bio-fertilization, Brassinolide and Phosphate Fertilizer in the Leaves area($\text{dm}^2 \text{ Plant}^{-1}$), dry weight of plant (g plant^{-1}), Percentage of total sugars (%), specific density (g cm^{-2}) and Marketable yield (ton ha^{-1}) for industrial potato plant for the Fall and Spring Seasons.

T	fall season 2021					spring season 2022				
	Leaves area	dry weight plant	total sugars	specific density	Marketable yield	Leaves area	dry weight plant	total sugars	specific density	Marketable yield
A_0	79.772	47.691	1.944	1.0830	26.078	59.827	55.778	1.149	1.0760	24.180
A_1	111.942	51.247	2.856	1.0850	29.270	96.459	65.593	1.569	1.0770	31.888
A_2	108.150	48.642	2.904	1.0850	28.435	93.589	63.778	1.358	1.0760	30.138
A_3	117.420	51.840	3.181	1.0860	30.554	110.360	66.370	1.894	1.0780	32.257
$L.S.D_{0.05}$	6.640	N.S	0.062	0.0022	1.886	5.570	4.084	0.112	N.S	1.464
P_0	87.221	47.500	2.317	1.0830	26.766	68.004	56.157	1.335	1.0750	28.024
P_1	107.101	49.963	2.792	1.0860	29.207	91.075	62.620	1.474	1.0770	29.882
P_3	118.641	52.102	3.056	1.0860	29.780	111.098	69.861	1.669	1.0780	30.941
$L.S.D_{0.05}$	5.873	2.152	0.036	0.0015	1.100	4.088	2.877	0.028	0.0014	0.942
BL_0	96.429	48.843	2.506	1.0840	27.846	80.122	60.981	1.303	1.0750	29.041
BL_1	112.200	51.731	2.744	1.0850	29.254	96.247	65.046	1.504	1.0770	30.327
BL_2	104.334	48.991	2.914	1.0860	28.652	93.807	62.611	1.672	1.0780	29.479
$L.S.D_{0.05}$	5.873	2.152	0.036	0.0015	1.1001	4.088	2.877	0.028	0.0014	0.942

2. Effect of bilateral interaction of study factors in vegetative growth, qualitative qualities, and marketing yield

Table (2) shows the bilateral interaction between the study factors where the A_3P_2 treatment was characterized by producing highest value in leaves area, dry weight of plant, total sugars, specific density and marketing yield and amounted to 127.558, 133.442 dm^{-2}



plant⁻¹, 55.704, 72.593 g plant⁻¹, 3.486, 2.223%, 1.088, 1.083 g cm⁻², 31.586, 1.203%, 1.088, 1.083 g cm⁻², 31.586, 1.586, 2.223%, 1.088, 1.083 g cm⁻², 31.586, 34.111 ton ha⁻¹ for both seasons respectively compared to the A₀P₀ treatment which produced lowest value for each of them for both seasons, the A₃BL₁ treatment also producing highest value in the leaves area, dry weight of plant and marketing yield that amounted to 124.802, 117.363 dm⁻² Plant⁻¹ and 54.370, 69.815 g plant⁻¹ and 31.076, 33.048 ton ha⁻¹ for both seasons respectively compared to A₀BL₀ treatment which produced lowest value for each of leaves area, marketing yield and dry weight in spring season, while A₀BL₂ treatment produced lowest value of the dry weight in fall season, and the interaction treatment A₃BL₂ was characterized by producing highest value for both seasons in the percentage of total sugars and specific density and amounted to 3.378, 2.128% and 1.087, 1.078 g cm⁻² respectively compared to A₀BL₀ treatment which produced lowest value for each of them and for both seasons, the P₂BL₁ treatment was characterized by producing highest value in the leaves area, dry weight of plant and marketing yield and amounted to 126.827, 117.769 dm⁻² plant⁻¹ and 54.222, 72.111 g plant⁻¹ 30.428, 31.830 ton ha⁻¹ for both seasons respectively compared to P₀BL₀ treatment which produced lowest value for each of them for both seasons, while P₂BL₂ treatment was characterized by producing highest value in the percentage of total sugars and specific density and amounted to 3.208, 1.851% and 1.087, 1.079 g cm⁻² for both seasons respectively compared to P₀BL₀ treatment which produced lowest value for each of them for both seasons.

Table (2): Effect of Bilateral interaction of Bio-fertilization, Brassinolide and Phosphate Fertilizer in the Leaves area (dm² Plant⁻¹), dry weight of plant (g plant⁻¹), Percentage of total sugars (%), specific density (g cm⁻²) and Marketable yield (ton ha⁻¹) for industrial potato plant for the Fall and Spring Seasons.

T		fall season 2021					spring season 2022				
		Leaves area	dry weight plant	total sugars	specific density	Marketable yield	Leaves area	dry weight plant	total sugars	specific density	Marketable yield
A ₀	P ₀	59.755	44.556	1.489	1.0800	23.906	45.517	48.667	0.952	1.0740	22.576
	P ₁	77.986	48.148	1.989	1.0850	26.504	57.446	52.074	1.172	1.0760	24.400
	P ₂	101.576	50.370	2.356	1.0850	27.825	76.518	66.593	1.322	1.0770	25.564
A ₁	P ₀	97.674	49.333	2.556	1.0850	27.421	71.176	59.556	1.489	1.0750	30.225
	P ₁	114.495	52.593	2.900	1.0850	29.991	97.945	65.667	1.570	1.0780	32.351
	P ₂	123.657	51.815	3.111	1.0860	30.399	120.257	71.556	1.649	1.0770	33.089
A ₂	P ₀	89.520	47.519	2.456	1.0840	27.065	70.631	57.074	1.254	1.0770	28.967
	P ₁	113.156	47.889	2.989	1.0860	28.929	95.962	65.556	1.339	1.0760	30.448
	P ₂	121.774	50.519	3.267	1.0860	29.310	114.175	68.704	1.481	1.0760	30.998
A ₃	P ₀	101.936	48.593	2.767	1.0840	28.672	84.693	59.333	1.646	1.0750	30.328
	P ₁	122.767	51.222	3.289	1.0860	31.403	112.946	67.185	1.814	1.0770	32.331
	P ₂	127.558	55.704	3.489	1.0880	31.586	133.442	72.593	2.223	1.0830	34.111
L.S.D _{0.05}		11.746	4.304	0.073	0.0030	2.200	8.175	5.755	0.056	0.0028	1.884
A ₀	BL ₀	73.768	47.407	1.733	1.0810	25.602	26.608	26.024	1.008	1.0730	23.636
	BL ₁	86.103	49.000	1.978	1.0830	26.608	65.817	57.111	1.161	1.0770	24.628
	BL ₂	79.445	46.667	2.122	1.0850	26.024	62.557	56.296	1.278	1.0780	24.276
A ₁	BL ₀	98.950	49.852	2.600	1.0860	28.528	86.534	63.185	1.338	1.0770	31.067



	BL ₁	122.563	52.519	2.889	1.0850	29.559	102.671	67.296	1.604	1.0750	32.800
	BL ₂	114.313	51.370	3.078	1.0850	29.724	100.172	66.296	1.766	1.0780	31.797
A ₂	BL ₀	100.964	47.704	2.722	1.0850	27.701	80.784	62.407	1.198	1.0740	29.671
	BL ₁	115.332	51.037	2.911	1.0850	29.773	99.138	65.963	1.360	1.0770	30.830
	BL ₂	108.154	47.185	3.078	1.0870	27.830	100.845	62.963	1.517	1.0780	29.911
A ₃	BL ₀	112.034	50.407	2.967	1.0860	29.554	102.062	64.407	1.667	1.0780	31.791
	BL ₁	124.802	54.370	3.200	1.0850	31.076	117.363	69.815	1.889	1.0780	33.048
	BL ₂	115.425	50.741	3.378	1.0870	31.030	111.656	64.889	2.128	1.0780	31.931
L.S.D _{0.05}		11.746	4.304	0.073	0.0030	2.200	8.175	5.755	0.056	0.0028	1.884
P ₀	BL ₀	71.334	45.028	2.042	1.0820	25.218	54.621	55.000	1.149	1.0740	27.391
	BL ₁	97.596	50.083	2.358	1.0820	27.537	74.483	57.806	1.364	1.0750	28.570
	BL ₂	92.734	47.389	2.550	1.0850	27.542	74.907	55.667	1.493	1.0770	28.110
P ₁	BL ₀	100.660	49.722	2.600	1.0850	28.755	82.371	59.639	1.276	1.0750	29.568
	BL ₁	112.177	50.889	2.792	1.0860	29.796	96.490	65.222	1.473	1.0780	30.580
	BL ₂	108.466	49.278	2.983	1.0860	29.068	94.362	63.000	1.673	1.0780	29.500
P ₂	BL ₀	117.294	51.778	2.875	1.0860	29.566	103.372	68.306	1.483	1.0780	30.165
	BL ₁	126.827	54.222	3.083	1.0860	30.428	117.769	72.111	1.673	1.0780	31.830
	BL ₂	111.803	50.306	3.208	1.0870	29.346	112.153	69.167	1.851	1.0790	30.826
L.S.D _{0.05}		10.172	3.728	0.063	0.0026	1.906	7.080	4.984	0.048	0.0024	1.631



Table 3. Effect of triple interaction of Bio-fertilization, Brassinolide and Phosphate Fertilizer in the Leaves area ($\text{dm}^2 \text{ Plant}^{-1}$), dry weight of plant (g plant^{-1}), Percentage of total sugars (%), specific density (g cm^{-2}) and Marketable yield (ton ha^{-1}) for industrial potato plant for the Fall and Spring Seasons.

T			fall season 2021					spring season 2022				
			Leaves area	dry weight plant	total sugars	specific density	Marketable yield	Leaves area	dry weight plant	total sugars	specific density	Marketable yield
A ₀	P ₀	BL ₀	49.876	42.556	1.233	1.0760	22.235	33.348	45.556	0.740	1.0700	21.840
		BL ₁	66.972	45.778	1.533	1.0790	25.276	51.772	50.667	1.000	1.0750	23.236
		BL ₂	62.417	45.333	1.700	1.0840	24.206	51.431	49.778	1.117	1.0790	22.651
	P ₁	BL ₀	71.166	48.667	1.800	1.0840	26.808	53.329	50.222	1.087	1.0720	23.493
		BL ₁	83.457	49.333	2.000	1.0850	26.538	60.616	53.111	1.167	1.0780	24.878
		BL ₂	79.334	46.444	2.167	1.0860	26.164	58.391	52.889	1.263	1.0780	24.829
	P ₃	BL ₀	100.261	51.000	2.167	1.0840	27.762	66.643	66.000	1.197	1.0760	25.573
		BL ₁	107.880	51.889	2.400	1.0850	28.011	85.063	67.556	1.317	1.0770	25.770
		BL ₂	96.585	48.222	2.500	1.0860	27.703	77.849	66.222	1.453	1.0770	25.349
A ₁	P ₀	BL ₀	74.848	46.444	2.267	1.0840	26.025	58.252	59.000	1.297	1.0750	29.274
		BL ₁	112.894	51.111	2.600	1.0840	27.090	77.991	59.889	1.547	1.0750	31.265
		BL ₂	105.280	50.444	2.800	1.0850	29.147	77.283	59.778	1.623	1.0760	30.135
	P ₁	BL ₀	107.769	51.333	2.633	1.0850	29.619	84.584	61.000	1.303	1.0800	31.869
		BL ₁	121.300	53.111	2.900	1.0850	30.566	106.083	68.889	1.573	1.0760	33.210
		BL ₂	114.417	53.333	3.167	1.0850	29.787	103.168	67.111	1.833	1.0770	31.975
	P ₃	BL ₀	114.233	51.778	2.900	1.0880	29.940	116.765	69.556	1.413	1.0750	32.059
		BL ₁	133.496	53.333	3.167	1.0850	31.021	123.940	73.111	1.693	1.0750	33.926
		BL ₂	123.243	50.333	3.267	1.0860	30.237	120.065	72.000	1.840	1.0800	33.282
A ₂	P ₀	BL ₀	69.076	44.444	2.167	1.0830	25.915	55.875	56.556	1.120	1.0750	28.253
		BL ₁	106.804	51.778	2.500	1.0830	28.337	77.258	59.111	1.257	1.0770	29.345
		BL ₂	92.681	46.333	2.700	1.0850	26.943	78.759	55.556	1.387	1.0780	29.303
	P ₁	BL ₀	109.895	47.333	2.867	1.0850	27.616	83.898	65.778	1.160	1.0700	30.444
		BL ₁	114.370	48.444	2.967	1.0860	30.437	100.941	66.000	1.350	1.0790	31.127
		BL ₂	115.203	47.889	3.133	1.0880	28.736	103.047	64.889	1.507	1.0800	29.771
	P ₃	BL ₀	123.922	51.333	3.133	1.0860	29.572	102.579	64.889	1.313	1.0760	30.316
		BL ₁	124.821	52.889	3.267	1.0860	30.545	119.216	72.778	1.473	1.0760	32.019
		BL ₂	116.578	47.333	3.400	1.0870	27.812	120.729	68.444	1.657	1.0760	30.659
A ₃	P ₀	BL ₀	91.535	46.667	2.500	1.0860	26.697	71.009	58.889	1.440	1.0750	30.195
		BL ₁	103.714	51.667	2.800	1.0810	29.447	90.912	61.556	1.653	1.0740	30.435
		BL ₂	110.559	47.444	3.000	1.0860	29.872	92.156	57.556	1.843	1.0750	30.352
	P ₁	BL ₀	113.808	51.556	3.100	1.0850	30.978	107.675	61.556	1.553	1.0760	32.465
		BL ₁	129.581	52.667	3.300	1.0860	31.645	118.321	72.889	1.803	1.0790	33.103
		BL ₂	124.911	49.444	3.467	1.0870	31.585	112.842	67.111	2.087	1.0760	31.426
	P ₃	BL ₀	130.759	53.000	3.300	1.0870	30.989	127.502	72.778	2.007	1.0820	32.712
		BL ₁	141.110	58.778	3.500	1.0880	32.136	142.856	75.000	2.210	1.0820	35.606
		BL ₂	110.804	55.333	3.667	1.0890	31.634	129.969	70.000	2.453	1.0840	34.015
L.S.D _{0.05}			20.345	7.456	0.126	0.0052	3.811	14.160	9.968	0.096	0.0049	3.262



3. Effect of triple interaction of study factors in vegetative growth, qualitative qualities, and marketing yield.

Table (3) shows the triple interaction between the study factors that had a significant effect on vegetative growth indicators, qualitative traits and yield, as the $A_3P_2BL_1$ treatment was characterized by producing highest value in the leaves area, dry weight of plant and marketing yield and amounted to 141.110, 142.856 $dm^2 Plant^{-1}$ and 58.778, 75.000 $g plant^{-1}$ 32.136, 35.606 $ton ha^{-1}$ for both seasons respectively, while $A_0P_0BL_0$ treatment produced lowest value of 49.876, 33.348 $dm^2 Plant^{-1}$ and 42.556, 45.546 $g plants^{-1}$ and 22.235, 21.840 $tons ha^{-1}$ for both seasons respectively, and the triple interaction treatment $A_3P_2BL_2$ was characterized by producing highest value for total sugars and specific density of 3.667, 2.453% and 1.089, 1.084 $g cm^{-2}$ for both seasons respectively, while $A_0P_0BL_0$ treatment produced lowest value of 22.235, 21.840 $ton ha^{-1}$ respectively for each of them for both seasons.

The tables (1-3) shows the effect of study factors on the vegetative growth indicators, qualitative characteristics, and marketable yield of industrial potato tubers in both the fall and spring seasons. These factors include leaves area, plant dry weight, specific density, the percentage of sugars in tubers, and marketable yield. The study factors showed a clear effect in these indicators, possibly due to the positive characteristics of the study factors in improving vegetative growth and leaves nutrient content. The influence of these factors on vegetative indicators has a positive effect on other study indicators. The biological factor has many properties that help improve the quality of growth indicators and tuber quality. This positive effect may be attributed to the positive characteristics of the study factors, represented by the fungus *Aspergillus niger* and *Bacillus megaterium* bacteria which work on mineral dissolution and facilitate availability of nutrients in the soil solution. Additionally, they release hormones, improve soil properties, enhance resistance against pathogens, and overcome stresses that the plant could be faces (Joshi *et al.*, 2021). Additionally, it has the ability to release low molecular weight compounds called siderophores, which work on chelating iron, making it available for absorption and increasing its accumulation in the leaves. Moreover, its significance effects lies in dissolution of unavailable phosphate in the soil, resistance to biotic and abiotic stresses, enhance healthy plant growth for both fungal types (Jyothi & Basaiah., 2022) and bacterial types (Kesaulya *et al.*, 2015), and the microorganisms that increase iron absorption cause a decrease in the growth of plant pathogenic microorganisms (AL-Aamel & AL-Maliky, 2023), also iron is an important element in improving qualitative traits and increasing the percentage of protein in tubers (AL-Dulaimi & AL-Amri). Gibberellins, as known, play a role in increasing element availability, helping in root system growth, and improving the growth of yield, and quality of tubers (Zainaldeen & Rasool, 2018). Also, one of the essential properties of bio-fertilizers is their support for root system growing and other plant organs including branches and leaves. This enhances the efficiency of nutrient absorption and vegetative growth, consequently reflecting on the carbon structure and the biological composition of the plant reaching to plant growth indicators, yield and its quality (Naziya *et al.*, 2019; Navarro *et al.*, 2019). In addition to releasing organic acids, bio-fertilizers work on releasing nutrients from organic matter in soil and increasing their availability in soil solution (Meyer *et al.*, 2011). This aids in improving vegetative growth indicators by increasing



nutrient absorption, positively effecting in manufactured carbohydrates and important amino acids that transfer to tuberous parts and increasing dry matter in tubers. This in turn, affects the rest of tuber quality indicators and yield (**Chowdhury, 2017**).

Another property of bio-fertilizers is increasing calcium ratio, in addition to potassium, phosphorus, and iron (**Bhatt & Maheshwari, 2020**). Phosphorus forms compounds with calcium and magnesium in alkaline soils. The phosphate-solubilizing microorganisms release phosphorus by reducing the soil pH, enhancing phosphorus availability and releasing calcium. Therefore, phosphorus increases the absorption of calcium and magnesium (**Kelling et al., 2020**). Calcium is an essential nutrient for plants which playing a role in converting sugars into starch and vice versa. It also enters the composition of the middle lamella of cell walls, regulates the respiratory process, and acts as an activator for the phosphatase enzyme. Magnesium, on the other hand considered as regulator in photosynthetic process and carbohydrate formation, activation of nucleic acid metabolism enzymes and protein constructing (**Saker, 2010**). It is important in transfer, storage and improving the structure of manufactured carbohydrates in tubers, that reflecting on qualitative characteristics and it has actively participating in sugars and proteins formation (**Taiz & Zeiger, 2010**). The results agree for both genres from biological inoculation with (**AL-Rubaye et al., 2019; Jain & Singh., 2015; Jubeir et al., 2014; AL-Mamori & Abdul-Ratha, 2020**).

Additionally, phosphate fertilization and the addition of supplementary nitrogen and potassium fertilizers could play a role in improving vegetative growth indicators, yield, and its quality. Where balancing the absorption of N, P, K nutrients using organic fertilizers with chemical fertilizers increases the availability of these nutrients (N, P, K) (**AL-Obaidi & Abdul-Ratha, 2022**). Each of these nutrients plays essential physiological role in plant biological processes, particularly in carbon constructing processes, amino acid and protein synthesis (**Yang et al., 2020**). This is reflected in plant growth, yield, and tuber quality. Phosphorus, plays a significant and effective role in growth and spread the root system in soil (**Hailu et al., 2017**). This increases the plant's ability to obtain nutrients from the soil and enhances their accumulation in the plant, that reflecting on plant growth and the quality of the yield (**Fernandes et al., 2017**). Phosphorus also plays a role in important energy related enzymes for respiratory and carbon constructing processes also it's involved in enzymes for nucleic acid synthesis (**Muthoni, 2016**). The results agree (**Soratto et al., 2015**) indicating that balanced absorption of N, P, and K nutrients improves the quality of yield by increasing dry matter and the specific density of potato tubers (**Das & Prasad, 2005**). Increasing the availability and concentrations of these nutrients plant parts lead to improving growth yield and its quality. This is due to the importance of these elements in carbon constructing, carbohydrate production, and their transfer to storage organs such as tubers and that reflecting on qualitative characteristics. While increasing sugars happen by increasing leaves area in Table 1 resulted an increase in the total sugar content due to the enhanced carbon construction and carbohydrate production (**Keller & Koblet, 1995**). The results agree with (**Martins et al., 2020**).

The positive effect could be attributed to the effective influence of the growth regulator Brassinolide on vegetative growth and increasing plants ability to resisted biotic and abiotic stresses and regulating physiological processes (**Sharma, 2021**). It has been found that using of



Brassinolide increasing vegetative growth and yield and its quality due to its role in cell elongation and cell division, also it can increasing the plant's ability to tolerate oxidative damage and salt stresses (**Li et al., 2021**). One other hand, it activates enzymes involved in chlorophyll biosynthesis, increasing the efficiency of carbon construction and carbohydrate production (**Siddiqui et al., 2018**). It has been found that external spraying with Brassinolide not only improves vegetative growth but also enhances the quality of yield. The mentioned characteristics of Brassinolide contribute positively to increased nutrient absorption from the soil solution, participating in the construction of a well-developed plant structure and root system. This results in the production of nutritional substances until surplus occurs, Improves plant growth also yield and its quality. Moreover, it increases amino acids in plants, and amino acids are basic material in protein synthesis (**Yuan et al., 2012**). The results agree with the role of Brassinolide in improving growth and qualitative characteristics (**Bideshki et al., 2019; Siddiqui et al., 2018**). And about bilateral and triple interaction effects they result from the integration individual effects with each other for the study factors.

CONCLUSIONS

The use of Bio-fertilizers, phosphatic fertilization, and foliar application of brassinolide has shown a positive impact on increasing leaf area and plant dry weight. The yield and tuber quality have also exhibited noticeable improvement, indicating the effective influence of these fertilizers on plant growth indicators and productivity. Additionally, the combination of phosphatic fertilization and brassinolide foliar application underscores the importance of these study factors in enhancing growth, yield, and production quality.

The interaction effect between these factors demonstrates integrated benefits, surpassing the individual effects and promoting plant growth, productivity, and quality. In general, the use of bio-fertilizers, phosphatic fertilization, and the plant growth regulator brassinolide suggests that they are effective tools for enhancing vegetative growth, yield, and production quality for industrial potato plants.

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