



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) dm⁻² 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⁻³ 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 كأسدة حيوية والتسميد الفوسفاتي مع الرش بالبراسيونلايد في نمو النبات وجودة وحاصل درنات البطاطا

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الخلاصة

نُفذت التجربة في جنوب غرب بغداد منطقة الرضوانية الشرقية للموسم الخريفي 2021 والموسم الربيعي 2022 بهدف دراسة تأثير المخصبات الحيوية والتسميد الفوسفاتي والرش بالبراسيونلايد في نمو وانتاجية ونوعية البطاطا الصناعية صنف ارسنال (Arsenal)، ونُفذت تجربة عاملية بثلاث عوامل (4×3×3) بترتيب الألواح المنشقة (split plot) ضمن تصميم القطاعات العشوائية الكاملة (RCBD)، وتم توزيع العامل الأحيائي ضمن الألواح الرئيسية والتدخل بين مستويات الفسفور ومنظم النمو البراسيونلايد ضمن الألواح الثانوية وبثلاث مكررات، تضمنت المخصبات الأحيائية فطر *Bacillus megaterium* لوحده وبكتيريا *Aspergillus niger* لوحدها والتوليفة بينهما اضافة الى معاملة المقارنة والفسفور بثلاث مستويات هي 0، 0.1، 0.2 ملغم P_2O_5 h^{-1} والرش بالبراسيونلايد بثلاث مستويات 0.1، 0.2، 0.3 ملغم P_2O_5 h^{-1} . اظهرت النتائج تفوق التوليفة الثلاثية بين كلا المخصبين معاً مع الفسفور عند المستوى 0.2 ملغم P_2O_5 h^{-1} والرش بالبراسيونلايد بالتركيز 0.1 ملغم h^{-1} ملغم h^{-1} معيونياً في المساحة الورقية (141.110 و 142.856) دسم 2 نبات $^{-1}$ والوزن الجاف للنبات (58.778 و 75.000) غم h^{-1} والحاصل القابل للتسويق (35.606 و 32.136) طن h^{-1} مقارنة لكلا الموسمين، ومعاملة التوليفة بين كلا المخصبين معاً مع الفسفور عند المستوى 0.2 ملغم P_2O_5 h^{-1} والرش بالبراسيونلايد بالتركيز 0.2 ملغم h^{-1} في النسبة المئوية للسكرات الكلية (3.667 و 3.453) % والكثافة النوعية (1.084 و 1.089) غم h^{-2} مقارنة بمعاملة المقارنة التي أعطت أقل القيم لكلا الموسمين.

الكلمات المفتاحية: بطاطا صناعية، مخصبات احيائية، سدام P_2O_5 ، *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 propionic 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 = $1.0988 + \{(\text{dry matter percentage} - 24.182) / 211.04\}$

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 dm⁻² 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 g plant⁻¹ and the specific density in the fall season and amounted to 1.086 g cm⁻² compared with A₀ treatment which produced lowest value for each of them, The P₂ 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 dm² 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 P₀ treatment that produced lowest value for each of them. The BL₁ 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 dm² Plant⁻¹, 51.731, 65.046 g plant⁻¹ and 29.254, 30.327 ton ha⁻¹ for both seasons respectively, while the BL₀ treatment produced lowest value for each of them and for both seasons, the BL₂ 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 BL₀ 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(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 | 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 A₃P₂ 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 |



| | | | | | | | | | | | |
|-----------------------------|-----------------------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|---------------|--------------|--------|
| | BL₁ | 122.563 | 52.519 | 2.889 | 1.0850 | 29.559 | 102.671 | 67.296 | 1.604 | 1.0750 | 32.800 |
| | BL₂ | 114.313 | 51.370 | 3.078 | 1.0850 | 29.724 | 100.172 | 66.296 | 1.766 | 1.0780 | 31.797 |
| A₂ | BL₀ | 100.964 | 47.704 | 2.722 | 1.0850 | 27.701 | 80.784 | 62.407 | 1.198 | 1.0740 | 29.671 |
| | BL₁ | 115.332 | 51.037 | 2.911 | 1.0850 | 29.773 | 99.138 | 65.963 | 1.360 | 1.0770 | 30.830 |
| A₃ | BL₂ | 108.154 | 47.185 | 3.078 | 1.0870 | 27.830 | 100.845 | 62.963 | 1.517 | 1.0780 | 29.911 |
| | BL₀ | 112.034 | 50.407 | 2.967 | 1.0860 | 29.554 | 102.062 | 64.407 | 1.667 | 1.0780 | 31.791 |
| P₀ | BL₁ | 124.802 | 54.370 | 3.200 | 1.0850 | 31.076 | 117.363 | 69.815 | 1.889 | 1.0780 | 33.048 |
| | BL₂ | 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 | |
| P₁ | BL₀ | 71.334 | 45.028 | 2.042 | 1.0820 | 25.218 | 54.621 | 55.000 | 1.149 | 1.0740 | 27.391 |
| | BL₁ | 97.596 | 50.083 | 2.358 | 1.0820 | 27.537 | 74.483 | 57.806 | 1.364 | 1.0750 | 28.570 |
| P₂ | BL₂ | 92.734 | 47.389 | 2.550 | 1.0850 | 27.542 | 74.907 | 55.667 | 1.493 | 1.0770 | 28.110 |
| | BL₀ | 100.660 | 49.722 | 2.600 | 1.0850 | 28.755 | 82.371 | 59.639 | 1.276 | 1.0750 | 29.568 |
| P₁ | BL₁ | 112.177 | 50.889 | 2.792 | 1.0860 | 29.796 | 96.490 | 65.222 | 1.473 | 1.0780 | 30.580 |
| | BL₂ | 108.466 | 49.278 | 2.983 | 1.0860 | 29.068 | 94.362 | 63.000 | 1.673 | 1.0780 | 29.500 |
| P₂ | BL₀ | 117.294 | 51.778 | 2.875 | 1.0860 | 29.566 | 103.372 | 68.306 | 1.483 | 1.0780 | 30.165 |
| | BL₁ | 126.827 | 54.222 | 3.083 | 1.0860 | 30.428 | 117.769 | 72.111 | 1.673 | 1.0780 | 31.830 |
| P₂ | BL₂ | 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^{-3}) 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 |
| A ₁ | 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 |
| | 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 |
| A ₂ | 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 ₃ | 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 |
| A ₃ | 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 |
| | 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 |
| A ₃ | 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 genuses 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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