Effect of Bacterial Inoculation, Bacillus Megaterium, Vermicompost, and Phosphate Pock on Growth and Yield of sunflower (Helianthus annuus L.)

Haider Makki Jahil1 and Jawad Abdal-Kadhim Kamal1

1College of Agriculture, University of Al-Qadisiyah, Iraq.
Email: jawad.alshabbany@qu.edu.iq

Abstract
This study aimed to investigate the interaction between bacterial bio-inoculation by Bacillus megaterium and vermicompost and three phosphate rock fertilizer (0, 100, and 200) kg.ha⁻¹ on the growth and yield of sunflower, Helianthus annuus L. A field experiment was conducted in the fall season of 2020 in the field of silt clay loamy soil. The results showed that inoculation with Bacillus and Vermicompost, individually or together, led to a significant increase in (plant height, leaf area, chlorophyll, and total yield) traits compared to the control treatment. The highest mean was obtained with biological and organic inoculation and phosphate rock fertilizer at the level (100) kg.ha⁻¹ valued at (134.3, 7250, 48.70, and 2808) for the studied indicators, respectively, compared to the comparison treatment.

Keywords: Bacillus, vermicompost, phosphate rock, total yield.

1. Introduction
Sunflower crop, Helianthus annuus L., is one of the most important oil crops in the world. Its seeds give a large amount of oil, up to (55)% It is one of the high-quality oils that are used in the human diet. Its seeds' coat is good fodder for farm animals since it contains (36)% protein and 20-22% carbohydrates, with up to (6)% oil and other nutrients [1]. This crop ranks third, after soybeans and colza, in the amount of oil produced. Sunflower oil is among the best-consumed vegetable oils at the global level due to its health and economic distinction. It is characterized by a high proportion of essential unsaturated fatty acids (90)% [2]. Despite the great importance of the crop, its productivity per unit area locally is still low compared to global production, as the yield rate did not exceed (310.7) kg.d⁻¹, with a production amount of (5.2) thousand tons and an area of (16.8) thousand dunums [3]. The global production rate was (1.426) tons.h⁻¹, with a production amount of (33.77) million tons and an area of (23.68) million hectares [4]. One of the reasons for the crop's low productivity is the lack of its supply of the necessary nutrients because the oil formed in its seeds as organic matter depletes the ready elements in the soil significantly, especially phosphorus, which the Plant cannot grow and develop naturally without it. Phosphorous is one of the primary and essential elements needed by plants in relatively large quantities and cannot be replaced by another element because of its significant role in many of its physiological processes. It is included in the composition of energy-rich compounds and enzymatic attachments, without which the Plant cannot carry out its vital functions and the decomposition of carbohydrates resulting from the photosynthesis process. Phosphorous helps in the division of plant cells and stimulates the growth and development of roots, maturation of plants, and seeds and fruits' formation. Its presence in sufficient quantities during the growth stages is essential for increasing productivity and improving quality [5]. Phosphate rock can be used as a source of phosphorous. It contains a fair percentage of phosphorous (10-15)% P. The phosphate rock efficiency in phosphorus preparation can be increased by grinding it and sifting it into small (0.5) mm or smaller [6]. Thus, it has importance in plant nutrition and increases the root group's growth and size, improving its resistance to diseases and encouraging organisms' growth in the root zone.

Many types of bio-inoculations can apply to the soil, including bacteria of atmospheric nitrogen fixation. These fertilizers provide (35)% of the applied nitrogen fertilizer. Its availability may be higher or lower than that, depending on its efficiency and improving crop yields and raising soil fertility. As for the other type, it contains phosphate-dissolving bacteria such as (Bacillus megaterium) that contribute to reducing the rate of phosphate chemical fertilization by up to (50)% and thus saving its costs and also reducing the rate of soil and environmental pollution with increasing production in quantity and quality. These biofertilizers
are environmentally friendly and contribute to increasing agricultural production by up to (30)%?. It replenishes the fertile state of the soil and protects plants against drought and some pathogens[7]. They expand the root system and increase seed germination. Following the correct method of serving the crop, especially when using vital vaccines, is the key to successful operations management for good crop performance. Therefore, biofertilizers are among the necessary growth requirements for their importance in preparing the Plant's nutritional needs and its positive effect in increasing the yield in quantity and quality on the one hand and improving the physical, chemical, and biological properties of the soil on the other hand.

There are many types of fertilizers, including compost produced from earthworms, great dissolvers of various organic residues, and temperate and tropical regions. The presence of these ring organisms in the soil makes them fertile and rich in the organic matter through the production of Vermicompost (CAST), which is rich in nutrients such as nitrogen, magnesium, iron, and other nutrients and helps the growth of the Plant through its good absorption of the elements essential for its growth. Vermicompost also contains several enzymes that enhance the activities of living organisms [8]. The presence of earthworms in the soil affects its physical properties, improves its structure, water, and nutrients' movement through it, and enhances its porosity by digging channels while performing its various biological activities. These channels help spread pesticides and fertilizers to different soil layers. Also, earthworm products are excellent nutritional fertilizer that helps plant growth [9].

Based on the importance of the preceding, the field experiment was conducted to study:

1. The effect of Bacillus megaterium on sunflower growth and yield.
2. The effect of vermicompost on the growth and yield of the sunflower plant.
3. The effect of phosphate rock on the growth and yield of the sunflower plant.
4. The interaction between Bacillus megaterium, vermicompost, and phosphate rock on the sunflower plant's growth and yield.

2. Material and methods

The field experiment was conducted in the fall season of 2020. The field soil was prepared by conducting critical field operations, including orthogonal tillage, smoothing, and leveling. Three main drivers were constructed along the field, including sub-markets for each experimental unit. As the field was divided into three main sectors, each sector contains (12) experimental units, and each experimental unit contains (5) rows. The distance between a row and another is (75) cm, and between planting seedbed and the other is (25) cm. The experimental unit dimensions were (4 × 4) m² and (1) m was left between each experimental unit and another. Thus, the total number of experimental units is (36) units.

Sunflower seeds, Helianthus annuus L, and the local variety (Aishaqi 1) were planted on the 28th of July 2020. Seeds were planted in rows, and the distance between a row and another was (75) cm, and between a seedbed and another is (25) cm as the experimental unit contains five rows in a unit, with a plant density of (53333) plants. ha⁻¹. Three seeds were placed in each seedbed hole, and after (10) days of planting, the Plant was rouge out to one Plant in each seedbed. The number of plants in each unit is (64) plants. Urea fertilizer (46% N) was used as a nitrogen source at a rate (200) kg.N.ha⁻¹. It was applied three times, the first application at the six-leaf stage, the second application after 30 days of the first one, and the third application was a month after the second one. Potassium sulfate fertilizer K2SO4 (45) % K was applied as a source of potassium element at the rate (225) kg.K.ha⁻¹. Phosphate rock was applied in three levels (0-100-200) P.kg.ha⁻¹ as a phosphorus source once before planting. Bush control was carried out manually throughout the growing season and whenever needed. The experiment was irrigated with a regular flow of irrigation water according to the Plant's water needs.

2.1. Soil's chemical and physical analyzes

Soil samples were taken from the field soil before planting, and samples were taken at a depth ranging between (0-30) cm. A compound sample was taken from it after mixing it well to ensure the sample's homogeneity between them and air-dried. It was ground with a wooden hammer and then sifted with a sieve with a diameter of (2) mm holes to complete some chemical, physical, and biological analyses.

1. Degree of reaction (pH): The degree of soil reaction in a 1: 1 soil: water suspension was measured using an E.C. meter according to [10,11].
2. Electrical conductivity (E.C.): Soil: water was measured in suspension 1: 1 using an E.C. meter as stated in [11].
3. Cation exchange capacitance (CEC): It was measured using sodium acetate and ammonium acetate as reported [10].
4. Soil texture: It was measured by the international pipette method, according to [12].
5. Organic matter (O.M): The organic matter was determined by wet digestion method using IN of (K2Cr2O7) as mentioned in [10].
6. Available nitrogen: Nitrogen was measured using potassium chloride KCl, and nitrogen was estimated using a keldal device as mentioned in [11].
7. Available phosphorous: The available phosphorus was measured by NaHCO$_3$ sodium bicarbonate, the color was developed with ascorbic acid and ammonium molybdate, and the Spectrophotometer was used to estimate the available phosphorus as mentioned in [11].
8. Available potassium: Soil potassium was measured using (0.5) Molar calcium chloride and was estimated using a Flame Photometer, according to [11].
9. Sodium Ione: Sodium was measured using NaCl solution by using a Flame Photometer as mentioned in [10].
10. Calcium Ca$^{2+}$ and Magnesium Mg$^{2+}$: They were measured using a buffer solution of ammonium hydroxide and ammonium chloride by adding a reagent of EBT as reported in [10].
11. Chloride Cl$: It was measured using potassium chromate guide and silver nitrate solution, as a white precipitate was formed according to [10].
12. Carbonate CO$_3^-$ and bicarbonate HCO$_3^-$: They were measured by adding sulfuric acid, methyl orange index, and phenolphthalein reagent; when the carbonate was present, the color of the solution changed to violet, after which the titration was done with sulfuric acid as mentioned in.
13. Sulfate SO$_4^{2-}$: The measurement was made using a barium chloride solution at a concentration of 1 N, hydrochloric acid, and ethanol, as reported [10].
14. +2 Cu, +2 Zn, and +2Fe

These elements were measured using an atomic absorption spectroscopy apparatus. Standard solutions were prepared for all elements by adding an extracting solution, as mentioned in [10].

| Table 1. Some physical and chemical properties of the soil before planting. |
|-------------------|-----------------|----------------|
| Trait                  | Value | Unit       |
| Reaction Degree (pH) (1:1) | 7.6   |            |
| Electrical Conductivity (EC) (1:1) | 2.90  | DesiSmens.M$^{-1}$ |
| Cation exchange capacity (CEC) | 19.7  | Cml.charge.kg$^{-1}$.soil |
| Organic matter O.M.     | 2.8   | g.kg. Soil$^{-1}$ |
| Available elements       |       | Mg. kg.Soil$^{-1}$ |
| N                        | 26.07 |            |
| P                        | 13.9  |            |
| K                        | 191.8 |            |
| Ca$^{2+}$                | 4.8   |            |
| Mg$^{2+}$                | 2.80  | Cml.charge.L$^{-1}$ |
| Na$^{+}$                 | 460   |            |
| SO$_4^{2-}$              | Nil   |            |
| HCO$_3^-$                | 1.78  |            |
| Soluble positive ions    |       | Cml.charge.L$^{-1}$ |
| Soluble negative ions    |       |            |
| CO$_3^{2-}$              | 10    |            |
| Cl$^-$                   | 7.0   |            |
| Sand                     | 180   |            |
| Silt                     | 500   |            |
| Soil separators          |       | g.kg. Soil$^{-1}$ |
| clay                     | 320   |            |
| silt clay Loamy          |       |            |
| Bulk density             | 1.40  | g.cm$^{-3}$ |

2.2. Vegetative growth Traits
1. Plant height (cm): Measured at maturity from the soil surface to the disc's base and an average of ten plants taken from the midlines for each experimental unit.
2. Leaf area (cm$^2$: Plant): The leaf area was measured upon completion of flowering, and five plants were selected from each experimental unit. The leaf area was calculated after measuring the maximum width of the plant leaves and using the following equation:

\[
\text{Leaf area} = \text{Sum of Squares of leaf Width} \times 0.65
\]
\[
\text{L.A.} = 0.65 \sum \text{Li}\text{2}
\]
Results and discussion

The effect of biological inoculation (bacterial), vermicompost, and phosphate rock on plant height (cm.Plant\(^{-1}\)). Table (2) presents that the bacterial inoculation \(B_1\) application led to a significant increase in the trait of plant height (103.2 cm.Plant\(^{-1}\)) compared to the comparison treatment \(B_0\). The reason is attributed to the positive role of the applied bio-inoculation. It works to increase plant growth and increase the solubility of non-dissolved phosphorous compounds in the soil, convert them from non-available forms into available forms, produce organic acids, and their role in fixing nitrogen liberating potassium. It has a role in producing growth regulators such as indole acetic acid, gibberellins, auxin, and cytokinins.

The bio-fertilizer facilitates the nutrients in the soil and increases its availability for absorption by the Plant, which positively affects plant height, and these results are consistent with [15]. The results showed that the vermicompost \(V_1\) worm-fertilizer application led to a significant increase in plant height (109.4 cm.Plant\(^{-1}\)) compared with the comparison treatment \(V_0\). It is attributed to the applied vermicompost (earthworm fertilizer) and its role in increasing the numbers and activity of microorganisms in the soil and its enzymatic activity that works on the decomposition of organic compounds. It leads to improving the soil's physical and chemical properties and its role in increasing the release of nutrients that increase cell division rates. These results were in agreement with what was obtained from [16-18].

The application of different levels of phosphate rock resulted in significant differences in the characteristic of plant height compared with the comparison treatment (120.2 and 110.8 cm.Plant\(^{-1}\)) for the application levels (P\(_1\), P\(_2\)), respectively, compared with the comparison treatment \(P_0\). The increase in sunflower's plant height trait is attributed to phosphorus's positive role in forming plant cells, their division, and their elongation. It contributes to the formation of RNA, which is essential in building protein-based on building plant cells. This result is consistent with the findings of [19,20]. These results also agree with the finding of [21], who confirmed that phosphate rock's application in a complementary manner with organic fertilizer caused a significant increase in the maize plant's height.

The interaction between the Bacillus inoculation and phosphate rock (B + P) at the level (P\(_1\)) achieved the highest average (125.3 cm.Plant\(^{-1}\)). It is attributed to the role of phosphorus-dissolving organisms that can increase plant growth and height by producing phytohormones such as indole acetic acid. It is what indicated to the mechanism [22], that their overlap has an important role that confirms the correlation between them in height and most of the growth traits. Bacillus bacteria also can secrete enzymes and hormones that stimulate growth, such as Gibberellins, Auxin, and Cytokinins. The nature of these hormones works to increase plant cells. It leads to an increase in plant cell division and its activity in fixing the atmospheric nitrogen, which prepares some of the Plant's nitrogen needs during the various growth stages [23-26]. As for the bilateral interaction between the biological pollen and Vermicompost (B + V), there were significant differences (112.0 cm.Plant\(^{-1}\)) compared with the comparison treatment (99.6 cm.Plant\(^{-1}\)). The increase may be attributed to biological and organic fertilizers, which increase the stimulation of lateral root formation and increases the roots' surface area. They secret important plant hormones such as auxins and gibberellins, which increase cell division and the formation of root capillaries [27,28]. In addition to its role in increasing nitrogen fixation, phosphorous mineralization, potassium release, and increased absorption of water and essential nutrients, and this is what each of [29,30] obtained.

The results of the statistical analysis of the two-way interaction between vermicompost and phosphate rock (V + P) indicated that significant differences were at the level (P\(_1\)) (127.8 cm.Plant\(^{-1}\)) compared to the treatment of no application of vermicompost and phosphate rock (81.2 cm.Plant\(^{-1}\)). The increase in plant height may be due to nutrient processing since vermicompost is an organic matter rich in essential nutrients such as nitrogen, phosphorous, and potassium. It is shown by analyzing vermicompost samples.
that are fast dissolving and easy to absorb by the plant and stimulating vital processes throughout branch production and the beginning of stem elongation, leading to an increase in plant height.

Phosphorous plays an essential role in increasing growth and cell division\(^{11}\). The results of the table confirmed that the triple interference B\(_1\)V\(_1\)P\(_1\) (Bacillus + vermicompost + phosphate rock) led to a significant increase in the plant height trait for sunflower at the level (100) kg.ha\(^{-1}\) (134.3) cm.plant\(^{-1}\) compared to the comparison treatment B\(_0\)V\(_0\)P\(_0\) (75.3) cm.plant\(^{-1}\).

**Table 2. The Effect of Bacillus bacteria, vermicompost, and rock phosphate on plant height (cm).**

| Rock phosphate (P) | Vermicompost (V) | Bacterial Bio-fertilizer Mean of binary overlap P * V | Bacterial Bio-fertilizer Mean of (P) B0 B1 | Bacterial Bio-fertilizer Mean of (V) B0 B1 |
|-------------------|------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| P\(_0\) V0        | 75.3             | 87.1                                          | 81.2                                          | 84.2                                          | 96.8                                          |
| P\(_0\) V1        | 93.2             | 106.5                                         | 99.8                                          | 115.1                                         | 125.3                                         |
| P\(_1\) V0        | 108.9            | 116.3                                         | 112.6                                         | 110.3                                         | 111.2                                         |
| P\(_2\) V0        | 114.7            | 127.4                                         | 121.0                                         | 106.0                                         | 95.2                                          |
| P\(_1\) V1        | 121.4            | 134.3                                         | 127.8                                         | 106.0                                         | 95.2                                          |
| P\(_2\) V1        | 114.7            | 127.4                                         | 121.0                                         | 106.0                                         | 95.2                                          |
| LSD .05           | 6.2              | 4.4                                           |                                               |                                               |                                               |

The effect of biofertilizer (bacterial), vermicompost, and phosphate rock on total chlorophyll content (mg / kg\(^{-1}\).plant. Fresh weight)

Table (3) presents that the bacterial inoculation B\(_1\) application led to a significant increase in the trait of chlorophyll (39.97 mg/kg\(^{-1}\).plant. Fresh weight) compared to the comparison treatment B\(_0\). It is attributed to the role of microorganisms and their ability to secrete gibberellins, cytokines, and oxins, which increase the availability of nutrients in leaves, leading to an increase in chlorophyll within the plant tissues [31]. These organisms increase the availability of most of the nutrients involved in the formation of chlorophyll, such as nitrogen. Nitrogen plays a significant role in building the chlorophyll molecule through its entry into the synthesis of proteins and organic acids involved in building chloroplasts. It is also included in Porphyrin, which participates in the synthesis of the chlorophyll molecule. 51% of the nitrogen present in the leaves is part of the chlorophyll molecule. The results showed that the application of vermicompost V\(_1\) led to a significant increase in the trait of chlorophyll (39.69 mg/kg\(^{-1}\).plant. Fresh weight) compared with the comparison treatment V\(_0\). It is attributed to the fact that the use of vermicompost led to an increase in the specific traits of the lettuce plant and an increase in the percentage of chlorophyll in the leaves. In addition to increasing the availability of the necessary elements for plants in the soil, especially nitrogen, on the other hand, it reduced the concentrations of some toxic and heavy elements in the soil, as found [32]. It is evident from a table in the statistical analysis results and at a significant level (0.05) that applying different phosphate rock levels resulted in significant differences in chlorophyll trait compared with the comparison treatment (41.77 and 40.32 mg/kg\(^{-1}\).plant. Fresh weight) compared to the levels of application (P\(_1\) and P\(_2\)), respectively, compared to the comparison treatment (P\(_0\)), which had the least mean of (33.76) mg/kg\(^{-1}\).plant. Fresh weight. It is attributed to the role of released and direct phosphorous in most vital processes. These processes
cannot take place in plant tissues without it. It participates in the decomposition of carbohydrates and materials resulting from the photosynthesis process that releases the Plant's energy in its vital processes [1, 31]. The interaction between the bacterial inoculum and phosphate rock (B + P) at (P1) level achieved the highest average of (43.85) mg/kg⁻¹.plant. Fresh weight. It is attributed to the fact that the added microorganisms that can dissolve the applied phosphates (phosphate rock) present in the soil work to prepare and dissolve many other nutrients. It is based on the biological nitrogen fixation and contributes to building pigments, including chlorophyll [32]. As for the two-way interaction between the biological fertilizer and vermicompost (B + V), it produced significant differences (40.54 mg/kg⁻¹.plant. Fresh weight) compared with the comparison treatment (35.68 mg/kg⁻¹.plant. Fresh weight). The increase may be attributed to the applied biological and organic fertilizers' role, which affects the chlorophyll pigment increase. The applied worm fertilizer and some types of bacteria to the field planted with lettuce for two years have led to an increase in the number of leaves, an increase in the percentage of chlorophyll and the percentage of fats in the leaves and leaf area, an increase in the weight of the head and the total and a reduction in the number of days of maturity [33]. The results of the statistical analysis of the two-way interaction between vermicompost and phosphate rock (V + P) indicated that significant differences were achieved at the level (P1) (45.97 mg/kg⁻¹.plant. Fresh weight) compared to the treatment of not applied vermicompost and phosphate rock, which gave less average (31.48) mg/kg⁻¹.plant. Fresh weight. These results are consistent with much research confirming that applying worm fertilizer to the soil improves its fertility state and increases the availability of the Plant's nutrients, which thus improves the leaf area and increases the chlorophyll pigment, plant height, and other qualities of the Plant. The application of vermicompost to the lettuce fields was the best in improving growth, production, and quality traits than chemical fertilizers [34]. The use of vermicompost fertilizer led to an increase in the Plant's total production, an increase in vitamin C, and a reduction of harmful nitrates to the permissible limit. Also, applied organic materials with phosphate rock and the soil helped decomposed phosphate rock and phosphorus availability. It affects many of the quality traits of the sunflower plant. The organic matter affects phosphorus availability due to the release of CO₂ during its decomposition and dissolution in water, forming carbonic acid, which reduces the soil's pH and dissolves phosphorous compounds in the soil release phosphorous from phosphate rock [35,36]. The results of the table confirmed that the triple interference B₁V₁P₁ (Bacillus + vermicompost + phosphate rock) led to a significant increase at the level of (100) kg.ha⁻¹ in chlorophyll (48.70 mg/kg⁻¹.plant. Fresh weight) compared to the comparison treatment B₀V₀P₀ that resulted in the least average (30.03) mg/kg⁻¹.plant. Fresh weight.

| Rock phosphate (P) | Vermicompost (V) | Bacterial Bio-fertilizer Mean of binary overlap p * V | p * V | V | p * V | p * V | p * V |
|-------------------|----------------|---------------------------------|-------|---|-------|-------|-------|
|                   | B₀ | B₁ |                   |      |   |       |       |       |
| P₀                | V₀ | 30.03 | 32.93 | 31.48 |     |       |       |       |
|                   | V₁ | 34.70 | 37.37 | 36.03 |     |       |       |       |
| P₁                | V₀ | 36.13 | 39.00 | 37.57 |     |       |       |       |
|                   | V₁ | 43.23 | 48.70 | 45.97 |     |       |       |       |
| P₂                | V₀ | 40.87 | 46.23 | 43.55 |     |       |       |       |
|                   | V₁ | 38.60 | 35.57 | 37.08 |     |       |       |       |
| LSD .05           |    | 2.14 |       | 1.51  |     |       |       |       |

Table 3. Effect of Bacillus bacteria, vermicompost, and phosphate rock on leaves' chlorophyll content.

| Rock phosphate (P) | Bacterial Bio-fertilizer Mean of (P) | B₀ | B₁ | Mean of (P) |
|--------------------|-------------------------------------|---|---|-------------|
|                   |                                     |   |   |             |
| P₀                | 32.37 | 35.15 | 33.76 |
| P₁                | 39.68 | 43.85 | 41.77 |
| P₂                | 39.73 | 40.90 | 40.32 |
| LSD .05           | 1.51  |       | 1.07  |

| Vermicompost (V) | Bacterial Bio-fertilizer Mean of (V) | B₀ | B₁ | Mean of (V) |
|------------------|-------------------------------------|---|---|-------------|
| V₀               | 35.68 | 39.39 | 37.53 |
| V₁               | 38.84 | 40.54 | 39.69 |
| LSD .05          | 1.23  |       | 0.87  |

Mean of (B) 37.26 | 39.97 |
LSD .05 0.87
The effect of biological fertilizer (bacterial), vermicompost, and phosphate rock on the leaf area (cm².plant⁻¹)

Table (4) presents that the application of bacterial fertilizer B1 resulted in a significant increase in leaf area index (4875) cm².plant⁻¹ compared to the comparison treatment B0. This increase may be attributed to the improvement of metabolic processes and the encouragement of the absorption of nutrients and water and the excretion of these organisms of some growth regulators in the Rhizosphere. These secretions have a role in elongating the roots and stimulating the growth of root hairs, increasing the density of the roots, and this is reflected positively on the growth of the Plant, and these results are consistent with what was found in the mechanism [37,38]. The results showed that the application of vermicompost V1 resulted in a significant increase in the leaf area (7431) cm².plant⁻¹ compared to the comparison treatment V0. It is attributed to worm compost's role in improving the soil's fertility status by preparing sunflower plants' nutrients. It increased the Plant's leaf area, increased the chlorophyll pigment, and increased the plant height and other qualitative traits. These results are consistent with the findings of [39], who confirmed that vermicompost use increased the lettuce plant's specific traits, an increase in the percentage of chlorophyll in the leaves, and an increase in their surface area. The application of different levels of phosphate rock resulted in significant differences in the leaf area compared with the comparison treatment with an average (5467) and 5013 cm².plant⁻¹ for the levels of application of (P1, P2), respectively, compared with the comparison treatment (P0), which had the least average of (3006) cm².plant⁻¹. The reason is that the kinetic parameters of phosphorus release are associated with growth indicators in calcareous soils, and these results are consistent with [40].

The two-way interaction with the bacterial inoculation and vermicompost (V + B) resulted in significant differences. As the average leaf area (5005) cm².plant⁻¹ compared to the comparison treatment (3773) cm².plant⁻¹. It is attributed to bacterial and organic interference's cooperative role through the secretion of growth regulators such as gibberellins and auxins that increase vegetative growth and production. The two-way interaction with the bacterial pollen and phosphate rock (P + B) made significant differences (P1). The average leaf area index is (6025) cm².plant⁻¹ compared to the comparison treatment (2616) cm².plant⁻¹. The reason is attributed to the ability of bacteria to dissolve phosphate rock and convert it into ions that the plant roots can absorb and thus will increase the surface area of the leaf[41], and these results are consistent with [42]. It agrees with the findings of [43], who confirmed that bio-inoculation with the application of phosphate rock with phosphorous dissolving organisms increased the amount of phosphorus absorbed by a plant. The highest overlap was achieved for vermicompost and phosphate rock fertilizer (V + P) at (P1) level. The highest obtained average was (6730) cm².plant⁻¹. It is attributed to the increase in the percentage of ready phosphorous from phosphate rock. It may be due to the microorganisms' consumption of organic matter as an energy source and their increased activity in dissolving phosphate rock and liberating phosphorus. It is consistent with what was found by [44], who mentioned that applying phosphate rock with organic fertilizer caused a significant increase in the relative leaf content of phosphorus for both maize and beans due to increased numbers of microorganisms to dissolve phosphate rock and their secretions of organic acids. It is consistent with what was mentioned by [45], who found that the phosphorous concentration of soybean shoots increased by using phosphate rock with phosphate solvents and various organic wastes. The triple interference B1V1P1 (Bacillus + vermicompost + phosphate rock) led to a significant increase at the level (100) kg.ha⁻¹ in the leaf area index. The highest average was (7250) cm².plant⁻¹ for the paper area index, compared to the comparison treatment B0V0P0, which gave the lowest average (2133) cm².plant⁻¹.

Table 4. Effect of Bacillus bacteria, vermicompost, and phosphate rock on leaf area (cm²).

| Rock phosphate (P) | Vermicompost (V) | Bacterial Bio-fertilizer | Mean of binary overlap p * V |
|-------------------|-----------------|-------------------------|-----------------------------|
| B0                | B1              | B0                      | B1                          |
| P0                | V0              | 2133                    | 2574                        | 2353                        |
| V1                | 3100            | 4217                    | 3659                        |
| P1                | V0              | 3606                    | 4801                        | 4203                        |
| V1                | 6210            | 7250                    | 6730                        |
| V0                | 5581            | 6860                    | 6220                        |
| V1                | 4063            | 3548                    | 3806                        |
| LSD .05           |                 |                         | 294                         |

The highest overlap was (416) cm².plant⁻¹ for the paper area index, compared to the comparison treatment B0V0P0, which gave the lowest average (2133) cm².plant⁻¹.
The effect of biological fertilizer (bacterial), vermicompost, and phosphate rock on seeds yield (kg.ha⁻¹)

Table (5) presents that the bacterial inoculation B1 application resulted in a significant increase in the total grain yield. The highest average grain yield was (2293 kg.ha⁻¹) compared to the comparison treatment B₀. It is attributed to the role of Bacillus megaterium, which works to release phosphorus from its non-available sources, thus increasing the number of dissolved orthophosphates and increasing the Plant's ability to absorb it, as well as the ability of this bacteria to secrete the enzyme phosphatase [46].

The results showed that V1 worm fertilizer's application resulted in a significant increase in the total grain yield. The highest average grain yield was (2289) kg.ha⁻¹ compared with the control treatment V₀. It is attributed to the role of vermicompost in increasing the readiness of nutrients and then increasing the absorption of NPK, which will positively affect the growth of the Plant and then increase the activity and growth of the root system and increase the yield and the early growth and ripening and increase the weight of the seeds.

The application of different levels of phosphate rock resulted in significant differences in the total grain yield compared with the comparison treatment, achieving averages of (2407 and 2313) kg.ha⁻¹ for the levels of treatments of (P₁ and P₂), respectively, compared to the comparison treatment (P₀), which had the least average of (1926) kg.ha⁻¹. It is attributed to the increased availability of phosphorus, which is very important for storing and transporting energy. It is essential in photosynthesis and carbohydrates' metabolic activities and essential for growth and production [47]. The results are consistent with [48].

The two-way interaction with the bacterial biological inoculation and worm fertilizer (V + B) resulted in significant differences at the level (P₁) in increasing the total grain yield, and the highest average was (2524) kg.ha⁻¹. The micro-soil regeneration works to weather the types of rocks when present on surfaces or in minute cracks slowly with time [49]. The results are consistent with [50].

The double overlap of vermicompost and phosphate rock (V + P) resulted in the highest mean increase in the total grain yield at the level (P₁), (2656) kg.ha⁻¹. It is due to the role of vermicompost and phosphate fertilizer in increasing the efficiency of the photosynthesis process and transporting the metabolites from the sites of their manufacture in the leaves to the storage sites in the grains, as well as increasing energy production, the formation of ATP, building sugars, starches, and proteins, building sebum and forming nucleic acids that are stored in seeds. It leads to an increase in her weight, and this was obtained by both [51,52].

The triple interference B₁V₁P₁ (Bacillus + vermicompost + phosphate rock) resulted in a significant increase in the total grain yield at the level (100) kg.ha⁻¹, as it reached the highest average (2808) kg.ha⁻¹, compared to the comparison treatment, which had the lowest average of (1692) kg.ha⁻¹.
Table 5. Effect of Bacillus bacteria, vermicompost, and phosphate rock on seed yield (kg ha\(^{-1}\)).

| Rock phosphate (P) | Vermicompost (V) | Bacterial Bio-fertilizer | Mean of binary overlap B * P
|-------------------|------------------|--------------------------|---------------------------|
|                   | B0               | B1                       |                           |
| P0                | V0               | 1692                     | 1852                      | 1772                      |
|                   | V1               | 1993                     | 2167                      | 2080                      |
| P1                | V0               | 2076                     | 2240                      | 2158                      |
|                   | V1               | 2504                     | 2808                      | 2656                      |
| P2                | V0               | 2083                     | 2446                      | 2318                      |
|                   | V1               | 2504                     | 2808                      | 2656                      |

LSD .05 = 130

Binary overlap B * P

| Rock phosphate (P) | Bacterial Bio-fertilizer | Mean of (P) | B0 | B1 |
|-------------------|--------------------------|-------------|----|----|
|                   | B0                       | 1843        | 2009| 1926|
| P0                | B1                       | 2290        | 2524| 2407|
| P1                | B0                       | 2283        | 2344| 2313|

LSD .05 = 92

Binary overlap B * V

| Vermicompost (V) | Bacterial Bio-fertilizer | Mean of (V) | B0 | B1 |
|------------------|--------------------------|-------------|----|----|
| V0               | B0                       | 2038        | 2246| 2142|
| V1               | B0                       | 2238        | 2339| 2289|

LSD .05 = 75

Mean of (B) = 2138

LSD .05 = 53

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