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Article

Effect of the Biofertilizer (Bacillus Megaterium) and the Addition of Yeast Spraying on the Vegetative growths in Phosphorous Availability, Growth and Yield of Onions (Allium Cepa L.)

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Abstract: A field experiment was carried out in clay mixture soil according to the randomized complete block design with three replicates in one of the fields of the College of Agricultural Engineering Sciences / University of Baghdad. The study aims to identify the effect of the bio fertilizer (Bacillus megaterium) and the addition of yeast on the availability of phosphorus, the yield, and growth of onions after adding 50% of the fertilizer recommendation for phosphorus. The study results showed the superiority of Bacillus megaterium treatment in the phosphorous availability, yield, vegetative growth and phosphatase enzyme activity (7.444 mg.kg-1, 0.540%, 0.298%, 35.521 µg) respectively. In comparison, the treatment of not adding Bacillus megaterium gave an average of (4.113 mg.kg-1, 0.363%, 0.198%, and 23.740 µg) respectively. Also, the results of the interaction between Bacillus megaterium and yeast showed the superiority of the treatment of adding yeast spraying to the vegetative part in the concentration of phosphorous in the soil. Besides, the yield, the vegetative part, the activity of phosphatase enzyme (5.925 mg.kg-1, 0.480%, 0.292%, 32.483 µg) respectively compared to the treatments of no yeast addition (5.630 mg.kg-1, 0.423%, 0.205%, and 26.778 µg).

Keywords: Biofertilizer, Yeast, Soil, Onions.

1. Introduction

The element phosphorous is called (the key of life) because of its direct role in most of the vital processes that take place inside the plant, as these processes cannot take place without it. It is likewise considered one of the essential nutrients for plant growth[1] . Phosphorous is included in the formation and division of living cells, the transfer of genetic traits, and the energy compound ATP and ADP, and it is considered one of the components of DNA and RNA, [2]. Furthermore, Phosphorous participates in the decomposition of carbohydrates and substances that are the product of photosynthesis, and it also participates with proteins in the formation of cell membranes such as the plasma membrane and the gap membrane. Plus, it has a role in increasing the yield, by increasing the plant's resistance to some diseases, as well as its role in the formation of seeds[3] In the same role, it represents one of the essential nutrients for the growth of crops as it is a basis in vital physiological processes [4] . The world is moving towards clean agricultural technologies to reduce as much as possible the pollution resulting from the use of chemical fertilizers. Thus, the natural materials used such as organic fertilizers and biofertilizers considered a suitable alternative to chemical fertilizers, [5] . Bacillus bacteria have the ability to dissolve phosphate compounds through their ability to reduce the degree of soil reaction by producing organic acids such as citric and propionic acid. These acids can release phosphate through ion exchange with calcium, iron, or aluminum ions associated with phosphate [6] . Studies have indicated that yeast extract encourages cell division and thus leads to plant growth and an increase in leaf area, as well as the percentage of chlorophyll in the plant [7] . The addition of yeast extract to the vegetative growth affected the metabolic and vital activities of the plant. This extract had an encouraging effect that led to an increase in the photosynthetic pigments and enzymes responsible for this process that work to encourage growth in the plant. Consequently, it leads to an increase in the activity of the plant members, especially the leaves because it is responsible for producing quantities of the photosynthesis products such as carotenoids. As well as, storing the products that are used later in the formation of the florets [8] . The use of yeast extract leads to an increase in the characteristics of vegetative growth because it contains amino acids and nitrogen, and also contains some mineral elements that stimulate growth and chlorophyll synthesis in the leaves. In addition to the fact that yeast is a natural source of cytokinins that have an important role in cell division and increase their size [9]. Based on the foregoing, the study objective can be

categorized as an evaluation of the role of B.megaterium inoculation in phosphorous availability in the plant rhizosphere by spraying yeast. Besides, assessment of spraying yeast to the vegetative growth in phosphorous availability, Studying and estimation of the activity of alkaline phosphatase enzyme in plant rhizosphere, growth, and yield of onions

2. Materials and methods

2.1. Isolation, purification, and identification:

10 soil samples were collected from the root zone (rhizosphere) of different plant hosts and different geographical locations. Then, the samples were placed in sterile polyethylene bags and brought to the laboratory to conduct the process of Bacillus megaterium isolation. The process of Bacillus megaterium isolation was carried out in the microbiology laboratory in dishes containing a solid Pikovskaya culture medium with three replicates. The dishes were incubated upside down in the incubator at a temperature of 30°C for (5) days. The growing colonies were examined to note that the colonies were surrounded by a transparent halo, which is evidence of the bacteria's ability to dissolve phosphate. The bacterial colonies were re-cultivated on the above solid culture media several times to ensure their ability to dissolve phosphate. Then, they were purified on the above culture media and then transferred to the slant medium for use in subsequent experiments. The following morphological, microscopic, and biochemical tests were carried out including colony color, colony shape, cell shape, cell arrangement, gram staining, and catalase production, oxidase production. As well, urease and starch decomposition, liquefaction of gelatin, motility, Voges-Proskauer, and the solubilization index was estimated.

2.2.Preparation of bacterial inoculum and activation of bacterial isolates

100 ml of Nutrient broth was prepared and sterilized by autoclave at a temperature of 121°C and a pressure of 15 Ib.in-2. After the medium was cooled, a portion of the isolated Bacillus megaterium, which had been previously isolated and identified, was taken by Loop. The liquid medium was inoculated with bacteria, and the inoculated medium was placed in the vibrating incubator for 48 hours. Then, the inoculum density was calculated, and a solution of gum Arabic was prepared at a concentration of 20% and sterilized with autoclave at a temperature of 121 °C for a quarter of an hour before using it in the experiment.

2.3.Cultivation and inoculation

The experiment was carried out at the Agricultural Research and Experiments Station of the College of Agricultural Engineering Sciences / University of Baghdad, where the field was divided into blocks according to the (RCBD) design, using the drip irrigation system. The experiment included two factors; the first factor was the bio-fertilizer Bacillus megaterium, and the second factor was the yeast fungus, with three replicates. Onion sets (Texas White Crano) were planted on 7/9/2021 with 8 sets per furrow, the distance between one plant and another was 20 cm, and the number of plants reached 24 plants for each experimental unit. Before planting the onion sets, the biological inoculum of Bacillus megaterium was developed and prepared in the laboratory after washing the onion set with distilled water several times, and then the bacterial inoculum was placed in a clean container. The Arabic gum was also placed at a rate of 20%, where the sets were placed in this gum, then the onion set was dipped in the biological inoculum several times for half an hour and placed on a clean cloth to dry it. After that, the set was planted in the field, whereas 5 g/l of yeast fungi were added in the form of spraying on the plant three sprays between each spray and another 15 days, 50% fertilizer recommendation was added to phosphorous.

Table 1. Chemical and physical soil analyses before planting

Measured properties		Value	Unit
Electrical conductivity EC 1:1		1.6	dS.m-1
PH 1:1		7.28	---
Available Nitrogen		20.5	Mg.kg-1 soil
Available phosphorous		2.64	
Available potassium		132.41	
Soil separates	Sand	396	g.kg-1 soil
	Clay	344	
	Silt	260	
Texture		Clay loam	

2.4.Phosphatase activity test

The activity of phosphatase enzyme in the soil before planting and after harvest was estimated, according to the (Tabatabai and Bremmer, 1965) method.

3. Results

The results of biochemical tests indicated that they are starch-degrading bacteria, and this was evidenced by the blue coloration of the medium, except for the colorless transparent halo around the area of bacterial growth. Also, the fact that the medium of gelatin liquefaction remained in its liquid state when it was placed in the refrigerator indicated the ability of bacteria to liquefy gelatin and produce the enzyme gelatinase.

Table 2. Results of diagnostic culture, morphological and biochemical tests for isolates of B. megaterium

Isolate name and type / Test type	Eggplant B2	Alfalfa B5	Pepper B6
Colonial color	White	White	White
Colonial shape	Circular	Circular	Circular
Cell shape	Rod	Rod	Rod
Cell arrangement	Short strings	Short strings	Short strings
Gram stain	+	+	+
Catalyst production	+	+	+
Oxidase production	+	+	+
Decomposition of urease	-	-	-
Decomposition of starch	+	+	+
Liquefying gelatin	+	+	+
Motility	+	+	+
Voges-Proskauer	-	-	-

3.1. Results of determination of solubilization index (SI)

The results showed the superiority of isolate (2), as it gave the highest solubilization index of 3.10 compared to the two isolates belonging to B. megaterium. In comparison, isolate (6) had the lowest solubilization index of 1.95.

Table 3. Values of the solubilization index of B. megaterium isolates

Isolation number Solubility	Plant host	SI
B2	Eggplant	3.10
B5	Alfalfa	2.89
B6	Pepper	1.95

3.2.Effect of the biofertilizer (Bacillus megaterium) and the addition of yeast by spraying on the vegetative growth on the concentration of available phosphorus in the soil (mg.kg^{-1} soil)

The results showed the superiority of the bio-fertilizer treatment in the phosphorous concentration in the soil with an average of (7.442) mg.kg^{-1} soil over the comparison treatment without Bacillus megaterium that was recorded (4.113) mg.kg-1 soil. The reason for this may be due to the ability of bacteria to dissolve insoluble phosphate compounds, as they have mechanisms in making phosphorous available for uptake by the plant to produce organic acids such as (succinic, lactic, and acetic acids) that increase the dissolution of insoluble phosphorous compounds. These results are consistent with [14] findings. However, the results of the interaction between biofertilizer and yeast showed that the treatment of adding yeast sprayed on the vegetative growth was superior to an average of (5.925) mg.kg-1soil compared to the treatment of not adding yeast (5.630) mg.kg-1 soil. The reason for this is that yeast contains many substances that encourage better plant growth, which is reflected in an increase in the efficiency of phosphorous uptake [15]

Table 4. The effect of biofertilizer (Bacillus megaterium) and the addition of yeast by spraying on the vegetative growth on the concentration of available phosphorus in the soil (mg.kg^{-1} soil)

Biofertilizer	Yeast		Average
	Without addition	Addition by spraying	
Without addition	4.103	4.123	4.113
Bacillus megaterium	7.157	7.727	7.442
Average	5.630	5.925	
LSD 0.05	1.205	1.221	1.522

3.3Effect of biofertilizer (Bacillus megaterium) and the addition of yeast by spraying to the vegetative growth on the concentration of phosphorous in the vegetative part %

The results showed the superiority of the biofertilizer treatment in the phosphorous concentration of the vegetative part with an average of (0.298%) over the comparison treatment without Bacillus megaterium which achieved (0.198%). The reason for this increase is attributed to the positive role of B. megaterium, which stimulates plant growth. Among, it has the ability to increase the solubility of phosphate compounds in the soil by secreting organic acids such as oxalic, citric, gluconic, humic, acetic, and formark, and transforming phosphorous forms from unprepared forms to available forms of plants. However, the results of the interaction between the bio-fertilizer and yeast showed that the treatment of adding yeast sprayed on the vegetative part exceeded by an average of (0.292 %) compared to the treatment of not adding yeast achieved (0.205 %). The reason for this increase is attributed to the role of yeast in increasing the percentage of nitrogen and phosphorous in the vegetative growth [17]

Table 5. Effect of the biofertilizer (Bacillus megaterium) and the addition of yeast by spraying on the vegetative growth on the concentration of phosphorous in the vegetative part %

Biofertilizer	Yeast		Average
	Without addition	Addition by spraying	
Without addition	0.140	0.257	0.198
Bacillus megaterium	0.270	0.327	0.298
Average	0.205	0.292	
LSD 0.05	0.012	0.023	0.058

3.4.Effect of the biofertilizer (Bacillus megaterium) and the addition of yeast by spraying on the vegetative growth on the concentration of phosphorous in yield %

The results showed the superiority of the biofertilizer treatment in the phosphorous concentration in the yield with an average of (0.540 %) over the comparison treatment without Bacillus megaterium recorded (0.363 %). Though, the results of the interaction between the biofertilizer and yeast showed that the treatment of adding yeast sprayed on the vegetative part exceeded by an average of (0.480 %) compared to the treatment of not adding yeast (0.423 %). This is due to the role of bread yeast in supplying the plant with essential mineral elements, some micro-elements, and vitamin B,

Table 6. Effect of biofertilizer (Bacillus megaterium) and the addition of yeast by spraying on the vegetative growth on the concentration of phosphorous in yield %

Biofertilizer	Yeast		Average
	Without addition	Addition by spraying	
Without addition	0.323	0.403	0.363
Bacillus megaterium	0.523	0.557	0.540
Average	0.423	0.480	
LSD 0.05	0.420	0.098	0.128

3.5.Effect of biofertilizer (Bacillus megaterium) and the addition of yeast by spraying on the vegetative growth in the phosphatase activity in the soil (µg.g^{-1} soil hour^{-1})

The results showed the superiority of the biofertilizer treatment in the activity of the phosphatase enzyme, with an average of (35.521) µg.g^{-1} soil hour^{-1}, over the comparison treatment without Bacillus megaterium (23.740) µg.$^{g-1}$ soil hour^{-1}.. Even though, the results of the interaction between biofertilizer and yeast showed the superiority of the yeast treatment spraying on the vegetative part

with an average of (32.483) µg.gm^{-1} soil hour^{-1} compared to the treatment without yeast addition (26,778) µg.gm^{-1} soil hour^{-1}.

Table 7. The effect of the biofertilizer (Bacillus megaterium) and the addition of yeast by spraying on the vegetative growth on the phosphatase activity in soil µg.gm^{-1} soil hour^{-1}

Biofertilizer	Yeast		Average
	Without addition	Addition by spraying	
Without addition	20.527	26.953	23.740
Bacillus megaterium	33.030	38.013	35.521
Average	26.778	32.483	
LSD 0.05	2.881	2.982	3.411

4.Discussion

The reason for the superiority of the bacterial bio fertilization treatment in the alkaline phosphatase activity is due to the ability of B. megaterium to secrete enzymes, especially the phosphatase enzyme, which increases the availability of phosphorous[12]. The reason for this discrepancy in the solubilization index may be due to its difference in genetic traits and secretion of enzymes, especially phosphatase enzymes and organic acids, this was mentioned [12,13]. The reason for the increase in phosphorus in the yield is attributed to the role of B. megaterium bacteria in the ability to dissolve phosphate compounds by producing organic acids and creating an appropriate PH reaction degree to increase the phosphorus availability. Accordingly, increase its concentration in the soil and plants, which was reflected in the absorbed amount from the plant, led to an increase in plant growth,[18]. Moreover, bacteria can move through the spread of growth outside the stabbing area, and the bacteria are oxidase-positive, evidenced by the change in color to dark violet, and negative for the Voges-Proskauer test for its inability to convert the color to red. These results are similar to what was found by several researchers in this domain[10]. However these results present a good agreement with [16] findings. Finally, the role of yeast in increasing the percentage of nitrogen and phosphorous in the vegetative growth is consistent with [17].

5. Conclusion

The microscopic culture tests showed that three phosphate-soluble isolates belonging to B. megaterium were obtained. The colonies were characterized by a circular shape surrounded by a round transparent halo with regular edges. The cell's shape was short rods, either singly or grouped, and they were gram-positive. Moreover the examination showed a negative result of the urease test, and the inability of the bacteria to change the medium color from yellow to pink, which is a catalase-positive bacteria by producing gas bubbles due to its ability to break hydrogen peroxide.

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