The Effect of Type and Method of Immobilizing Bacillus Megaterium Bacteria Inoculation in Increasing Potassium Available and Growth of Wheat Plant Triticum Aestivum L.

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Abstract

A field experiment was carried out at the second agricultural research and experiment station of the College of Agriculture, Muthanna University, for the 2019-2020 agricultural season, with the randomized complete block design RCBD, using two factors and three repeats, that's to study the effect of adding the bio inoculant from Bacillus megaterium bacteria, and when loaded the bacterium with the immobilization inoculation technology on zeolite, sodium alginate, Agarose and bentonite and it's symboled of M0, M1, M2 and M3 respectively to compare its efficiency in increasing potassium available and growth of wheat plant variety Eba’a 99. The experiment included two comparison treatments, the first being B0 without the addition of the bacterial inoculant and the second comparative treatment being B1 adding the bacterial vaccine. At the end of the experiment, potassium concentration was estimated in soil after cultivation. The results showed a clear moral effect of the use of the immobilized bacterial inoculant in Nitrogen available at a 15.65 (mg N kg\(^{-1}\) soil) and with an increase of 17.14%, Potassium available at 289.2 (mg K kg\(^{-1}\) soil) and an increase of 6.79%, and the plant height was a rate of 84.87 cm and an increase of 9.41% and bio yield at 810.25 kg dunum\(^{-1}\) and an increase of 34.98%.

Keywords: Wheat flour, Bio-inoculation, Bacillus megaterium, Immobilization.

1. Introduction

Wheat plant (Triticum aestivum L.) is a Gramineae family of strategic crops whose cultivation is the number one in Iraq and the world. The wheat crop has the important nutritional value of balancing well its grains between carbohydrates and proteins, as well as containing amounts of fat, vitamins and some of the basic amino salts that humans need in their food [1]. Therefore, the countries of the world, including Iraq, have sought to increase the productivity of this crop by adding chemical and bio-fertilizers to it, and, because bio-fertilizers have the advantage of being added to plants compared to chemical fertilizers, they have been used as inoculation added to agricultural soil or mixed with seeds in cultivation [2]. These additions are called biofilms, and they are produced from micro-organisms and serve to supply nutrients to plants by interfering with the root area and releasing plant growth organizations from natural sources in order to reduce dependence on chemical fertilizers [3]. Recent researchers have sought to introduce the Immobilization inoculant technique as a result of low numbers of microbiological added to the soil as fertilizer and less competition with the original micro-organisms present in the soil by extending the inoculant's life by using polymeric substances that can conserve bacterial cells within them for as long as possible. The possibility of exploiting the bacterial inoculant technology on the wheat crop has been and remains the primary objective of modern agriculture in the world because it increases the productive capacity of agricultural crops, since bacterial inoculants are a target factor that has been employed to improve the growth and productivity of plants. So, the goal was to study the effect of adding Bacillus megaterium to the bacteria in a ready increase.

2. Materials and Methods

The experiment was conducted in the city of Samawah, Muthanna province, during the 2019-2020 agricultural season in mixed soil, with the aim of studying the impact of adding the bio informative inoculant from Bacillus megaterium bacteria and using the loading of bacteria in the manner of immobilized inoculant.
2.1 Ground preparation and service operations

The land was plowed in an orthogonal manner and leveling, and smoothing operations were carried out on it, and then the land was divided into experimental units, the area of each experimental unit is 2x2 meters. Wheat variety Iba'a 99 was planted on 29-11-2019, and each experimental unit contains 8 lines, the distance between each line is 15 cm., phosphate fertilizer was added in the form of triple superphosphate at a rate of 100 kg H\textsuperscript{-1}, and potassium fertilizer in the form of potassium sulfate was added at a rate of 100 kg H\textsuperscript{-1}, where these two fertilizers were added in one batch before planting, then nitrogen was added in the form of urea at a rate of 150 kg H\textsuperscript{-1}. One batch upon planting and the second two months after planting. After that, the characteristics of the plant and the soil were studied after harvesting to know the development of wheat plant growth as a result of bio-pollination and other factors interfering with it.

2.2 Soil samples analysis

The random samples of the field's soil were taken before agriculture at the depth 0-30 cm. for the chemical and physical analysis purpose of for the soil and shows its results in the table 1.

| Measurements       | Value | Unit of measurement |
|--------------------|-------|---------------------|
| PH 1:1             | 7.8   | ....                |
| EC 1:1             | 6.1   | ds m\textsuperscript{-1} |
| organic matter     | 2.9   | gm kgm\textsuperscript{-1} |
| N available        | 15.8  | mg kgm\textsuperscript{-1} soil |
| P available        | 23.15 | mg kgm\textsuperscript{-1} soil |
| K available        | 289.3 | mg kgm\textsuperscript{-1} soil |
| counts total bacteria Bacillus megaterium | 5.7x10\textsuperscript{5} | CFU g\textsuperscript{-1} dry soil |
| soil separates     | 16    | gm kgm\textsuperscript{-1} soil |
| sand               | 68    | gm kgm\textsuperscript{-1} soil |
| silt               | 16    | gm kgm\textsuperscript{-1} soil |
| clay               | ....  | loamy silt |
| soil texture       | ....  | loamy silt |

2.3 Biological inoculate

It was obtained 200 ml of bacterial isolate Bacillus megaterium From the Graduate Studies Laboratory at the College of Agriculture - University of Al-muthanna, then loaded 100 ml of zeolite and abentonite inoculant and restricted 100 ml of the inoculant with agarose and sodium alginate and add the seeds to it after that.

2.4 Method and type of immobilization

- followed method by [4], in restricting cells by weight 3 grams of sodium alginate substance and put it in 100 ml distilled water with the addition of 15 gm of material starch and mixing the solution then add 50 ml of his biological inoculant.
- it used the described method by [5], To restrict cells to the media of Nutrient Agar, where preparation 28 gm from the media in 1000 ml of distilled water and dissolved until the solution is homogeneous, then placed in the purifier (Autoclave) for 20 min. under temperature 150 Celsius and after the solution has cooled, it is placed in it 50 ml of the biological inoculant.
- The biological inoculant is loaded with zeolite by weight 200 gm of zeolite in 1000 ml of distilled water with the addition of 50 ml of the biological inoculant.
• Loading of the bio-inoculant on bentonite by weight 150 ml of bentonite in 1000 ml of distilled water with the addition of 50 ml of the biological inoculant.

2.5 Study's treatment

The first factor is the addition of the bacterial inoculant and symbolizes it B

- B0 Without adding an inoculant
- B1 Add Bacterial inoculant Bacillus megaterium

The second factor is the type and method of immobilization of the inoculant and is symbolized by its M

- M0 Loading of bacteria on zeolites
- M1 immobilization of bacteria with sodium alginate
- M2 Bacteria immobilization with Agarose
- M3 Loading bacteria with bentonite

2.6 Soil and plant analysis after planting

Plant height (cm): The height of the plant was measured from the soil surface to the end of the spike without the stalk for ten plants selected from the two middle lines of each experimental unit before harvest.

2.7 Bio yield (kg acres⁻¹)

Have been calculated (B.Y.) at harvest per square meter from the two middle lines of each experimental unit.

2.8 Plant analysis

The plant samples were dried in an electric oven at a temperature 65 Then it was ground using an electric grinder and the samples were digested after taking (0.2 gm per sample ) using sulfuric and perchloric acids in a ratio of 3:1, leave the beaker on Hot Plate until the solution becomes clear and then transferred to a volumetric flask 50 ml and supplement the volume with distilled water as stated in [6],in order to estimate:

Nitrogen N: The amount of nitrogen in plants and grains using the (micro kijeldahl) device according to the method Bremner, as mentioned in [7].

Potassium K: Estimated in plant sample extracts and grain samples using the (Flame photometer) device, as stated in [6].

2.9 Statistical analysis

The study was a statistical analysis of the data according to the randomized complete block design RCBD According to [8], using a program GENSTAT RELEASE 12.1 The averages were compared using the least significant difference test (L.S.D.) at a morale level 0.05.

3. Results and Discussion

3.1 Effect of the type and method of immobilization inoculant Bacillus megaterium In availability of nutrient elements

3.1.1 Nitrogen in the soil (mg N kg⁻¹ soil)

It is clear from the table 2 in addition to the inoculant the bacterial led to the existence of significant differences in the values of nitrogen available, as it has given treatment B1 Adding the bacterial inoculant B. megaterium percentage increase of 17.14% Compared to the comparison treatment B0 that reached 13.36 (mg N kg⁻¹ soil). This is due to the fact that bacteria Bacillus It has a role in fixing atmospheric nitrogen, as well as secreting many growth regulators, organic acids and chelates that increase the concentration of elements in the soil, including nitrogen, which positively affects plant growth, and this was confirmed by [9]. And also, the results of the table showed the T. ether type and method of immobilization in the availability of nitrogen in the soil exceeds the immobilization method of sodium alginate M1 On the rest of the immobilization methods where it's registered 16.46 (mg N kg⁻¹ soil) with an increase of 24.60 % Compared to the zeolite loading treatment recorded 13.21 (mg N kg⁻¹ soil). This was confirmed [10], In a study for them when they proved that the immobilization of the bacteria nitrogen - fixing by sodium alginate have increased the availability of nitrogen in the soil.
Table results also showed the effect of interaction between the inoculant and the bacterial type and method immobilization its superior treatment B1M1 where recorded 18.13 (mg N kg$^{-1}$ soil) With an increase of 44.69 % Ratio for comparison treatment B0M0 which recorded 12.53 (mg N kg$^{-1}$ soil). And attributed this increasing of the role of the Sodium alginate to increase the effectiveness and activity of the bacteria Bacillus as well as the secretion of some growth regulators (Gibberellin GA3 and Auxin), which stimulate the root hairs of the plant to absorb the elements of nutrients [11].

**Table 2.** Effect of the type and method of bacterial immobilization inoculant *B. megaterium* In increasing the nitrogen availability in the soil (mg N kg$^{-1}$ soil).

| 2nd Factor | M0  | M1  | M2  | M3  | The Average |
|------------|-----|-----|-----|-----|-------------|
| B0         | 12.53 | 14.80 | 13.57 | 12.57 | 13.36      |
| B1         | 13.90 | 18.13 | 14.57 | 16.03 | 15.65      |
| The Average| 13.21 | 16.46 | 14.07 | 14.30 | 14.50      |

L.S.D. B M BM  2.983  2.983  5.966

### 3.1.2 Potassium in the soil (mg K kg$^{-1}$ soil)

Table No. 3 There were significant differences in the values of available potassium when adding the bacterial inoculant to the soil, as the results gave superiority to the treatment of adding the inoculant. B1 by an increase in the amount 6.79 % Compared to a treatment without a inoculant B0 that reached 270.8 (mg K kg$^{-1}$ soil). This superiority may be attributed to the role of Bacillus in increasing potassium in the soil through its secretion of organic acids that lower the pH in the soil, which in turn frees B and T from the compounds it binds with, and this was confirmed by [12], in a study by him showing the role of bacillus in the mung plant.

The results of the table also showed the superiority of the immobilization method with sodium alginate M1 on the rest of the immobilization methods in the available of potassium in the soil by an increase of its amount 9.44 % Compared to the load on zeolite, which was 264.7 (mg K Kg$^{-1}$ soil), due to be sugars multiple of sodium alginate and the role of Bacillus in T. Quinn organic acids [13]. Which you turn liberally potassium ions from the metal a carrier for her through the replacement of ion hydrogen ion replaces potassium [14].

The table also showed the effect of the interaction between the bacterial inoculant and the type and method of immobilization it over treatment B1M2 immobilization inoculant bacterial with agarose by an increase of 19.01 % compared to the treatment B0M0 Without an inoculant that was the amount of 257.7 (mg K kg$^{-1}$ soil), and this is due to the efficiency of agar in preserving bacterial cells around the roots of plants for a longer period, which provides efficiency for their work throughout the growing season because bacteria are susceptible to environmental conditions such as heat, humidity and salinity [15].

**Table 3.** Effect of the type and method of immobilization of the bacterial inoculant *B. megaterium* In the availability of potassium in the soil (mg K kg$^{-1}$ soil).

| 2nd Factor | M0  | M1  | M2  | M3  | The Average |
|------------|-----|-----|-----|-----|-------------|
| B0         | 257.7 | 297.7 | 262.7 | 265.3 | 270.8      |
| B1         | 271.7 | 281.7 | 306.7 | 296.7 | 289.2      |
| The Average| 264.7 | 289.7 | 284.7 | 281  | 280        |

L.S.D. B M BM 41.47 41.47 82.93

### 3.2 Effect of the type and method of immobilization inoculant bacterial *Bacillus megaterium* On the characteristics of the plant

#### 3.2.1 Plant height (cm)

Shows Table 4 effect of adding the bacterial inoculant *B. megaterium* There were significant differences in the rate of plant height, where the treatment of adding bacterial inoculant was superior B1 by an increase in the amount 9.41 % Compared to the comparison treatment B0 Without inoculate, which was (77.57) Cm, it attributed the rise to the effectiveness of the

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**Table 4.** Effect of the type and method of immobilization of the bacterial inoculant *B. megaterium* On the characteristics of the plant.

| 2nd Factor | M0  | M1  | M2  | M3  | The Average |
|------------|-----|-----|-----|-----|-------------|
| B0         | 257.7 | 297.7 | 262.7 | 265.3 | 270.8      |
| B1         | 271.7 | 281.7 | 306.7 | 296.7 | 289.2      |
| The Average| 264.7 | 289.7 | 284.7 | 281  | 280        |

L.S.D. B M BM 41.47 41.47 82.93
bacteria in the secretion of growth regulators indole acetic acid, influential in the elongation of the cells and the division and these mechanisms are increasingly material and protein, which is reflected positively in the rate of plant height, and this was confirmed by both [16,17].

The results also showed the superiority of the treatment M0 The method of loading bacteria on zeolites over the rest of the immobilization methods at a high rate of (88.63) cm and an increase of its amount 15.96% compared to the treatment M1 Sodium alginate binding method that reached (76.43) cm, and attributed this rise of role metal zeolites in improving soil physical properties and because this material is fragile, and help to keep the water in the root zone and help in soil aeration and increase the exchange capacity of cationic and adsorption ions feeder [18], which contributed to increasing the length of the internodes, as well as its role in the activity of meristematic tissues and cell division of the plant [19]. The results of the table also showed the effect of the interaction between the bacterial inoculant and the type and method of immobilization it over treatment B1M0 The method of loading the zeolite, where the rate of plant height reached (95.50) cm and an increase of its amount (34.75)% compared to the treatment B0M1 Sodium alginate immobilization method that reached (70.87) cm, and this is attributed to the role of zeolite mineral in stimulating cell division and elongation and the installation of cell membranes that lead to increased vegetative growth and plant height [20], This was confirmed by [21].

Table 4. Effect of the type and method of immobilization of the bacterial inoculant B. megaterium In average plant height (cm).

| 2nd Factor | M0  | M1  | M2  | M3  | The Average |
|------------|-----|-----|-----|-----|-------------|
| B0         | 81.77 | 70.87 | 79.83 | 77.80 | 77.57       |
| B1         | 95.50 | 82.00 | 78.30 | 83.76 | 84.87       |
| The Average | 88.63 | 76.43 | 79.06 | 80.73 | 81.22       |
| L.S.D.     | 3.31 | 3.31 | 3.31 | 3.31 | 6.63        |

3.2.2 Bio yield (kg dunum⁻¹)

The results of the table 5 showed the effect of adding a biological inoculant B. megaterium There were significant differences in the values of the biological yield, as the treatment of adding the inoculant was superior B1 and rate 810.25 (kg⁻¹ dunums) with an increase of 34.98% compared to the treatment B0 Without an inoculant, which was at a rate 600.25 (Kg acres⁻¹). Has attributed the cause of superiority to the role of the positives for a micro-organism in the soil played by providing major nutrients, especially N, P, K [22]. Which amounts to a tissue plant, leading to increased activity and growth of root and increased unwieldy and thus increasing the vegetative, which is reflected on most of the growth traits including bio - winning, and this is consistent with the findings of the [23]. The results of the table also showed the superiority of the treatment M0 Loading zeolite over other methods of loading with an increase in its amount 74.01% compared to the treatment M3 Loading on bentonite which reached 510 (kg Dunam⁻¹). And because zeolite, as mentioned previously, increases the improvement of the physical properties of the soil and increases the soil’s water retention, as well as the role of this mineral in the availability of the important nitrogen element for plants, which affected the characteristics of the yield and its components, including the biological yield, and this is consistent with what her found [24].

The table also showed the effect of the interaction between the bacterial vaccine and the type and method of immobilization it, and there were significant differences in the interaction treatment B0M1 inoculant B. megaterium Loaded on zeolite and at a rate 1008 (kg dunam⁻¹) With an increase of 77.77% compared to the treatment B0M2 agarose immobilized reached 567 (kg dunam⁻¹). This is attributed to the role of zeolite in its effect on the physical, chemical and biological properties of the soil and in general on the soil-plant system, which positively effects the productivity of agricultural crops, and this is what he found [25].
Table 5. Effect of the type and method of immobilization of the bacterial inoculant B. megaterium In biological yield (kg⁻¹ dunam).

| 1st Factor | M0 | M1 | M2 | M3 | The Average |
|------------|----|----|----|----|-------------|
| B0         | 767| 657| 567| 410| 600.25      |
| B1         | 1008| 663| 960| 610| 810.25      |
| The Average| 887.5| 660| 763.5| 510| 705.25      |
| L.S.D.     | B  | M  | BM |    |             |
|            | 153.1| 153.1| 306.3|    |             |

Conclusions

- It was found from the study that the bacterial inoculant carrying and binding bacteria B. megaterium and of the added with seeds may outweigh the lack of addition to increasing the availability of the nutrient's nitrogen N and potassium K As well as increasing growth and yield characteristics.
- The method of sodium alginate immobilization has outperformed the loading method to increase the availability of nutrients N and K.
- The method of loading on zeolite has outperformed in growth characteristics and yield of wheat plant.

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