Response of two wheat cultivars to inoculation of \textit{Bacillus subtilis} and Phosphorus fertilizer

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Abstract. A pot experiment was conducted at College of Agriculture, University of Diyala during the season (2017-2018) In order to evaluate the efficiency of \textit{Bacillus subtilis} plus phosphorus chemical fertilizer on two wheat cultivars (Ala and Audi). A factorial experiment was arranged with three replications of each treatment and two factors, the first include two wheat cultivars, the second factor includes three different levels from bio and chemical fertilizers. Results showed that application of bio fertilizers \textit{B. subtilis} with a half dose of chemical fertilizer were superior significantly in Plant height and Number of branches 46.98, 65.68 cm and 3.87, 7.71 after 45 and 120 days respectively , Plant fresh weight 39.41 g, Plant dry weight 19.18 g, % Phosphor in plant 0.27% while Bio fertilizer was superior in Number of bacteria from other treatments reached 90.66×10^6 . Ala cultivar was the best in traits of Plant height and Plant dry weight , whereas Audi cultivar was the best in traits of Number of branches , % Phosphor in plant and Number of bacteria.

Key words: Biofertilizer, \textit{Bacillus subtilis}, Wheat, Phosphorus

1. Introduction

Soils have reserves of natural from plant nutrients and the largely part of the reserves are unavailable generally for plants, just only a minor amount is released through chemical processes or biological activity and this amount is too slow for compensating of crop requirements [1]. Agricultural soils usually contain huge amounts of total phosphorus (P), but there is a widespread problem in agriculture due to deficiency of phosphorus (P) because most of total P is exist as low available forms for plants [2], [3], [4], [5]. Phosphorus is one of main essential elements for biological growth, generally, the soils contain large reserves of total phosphorus, but most of these reserves remain relatively inert, and less than 10% of soil phosphorus enter the cycle of plant and animal [6]. Phosphorus availability to plants is related to the solubility of metal phosphates present in soil [7]. Phosphorus deficiency is widespread in the world, therefore it is added to soil in the phosphate fertilizers form, only a small part of phosphorus is employed by plants and the rest is converted into forms of insoluble fixed [8], also when adding phosphorus to soils the soluble phosphates react with the soil constituents and form compounds that are low soluble. The
majority of P is existed as forms with low available amounts because of reactions that occurs in the soil [4], such as precipitation in calcareous soils with low organic matter content and high pH [9]. The excessive usage of synthetic fertilizers led to changes the pH levels of the soil and groundwater pollution, thus, new alternative systems were used to reduce the negative effects of chemical fertilizers on ecological balance and human health [1]. Some researchers suggest fertilization practices that more efficient by using of microorganisms to improve P availability and productivity of crop [10], [11], [12], [2], [13]. Microorganisms in rhizosphere have additional benefits such as biocontrol of soil-borne diseases beside plant growth promotion (PGPR) [14], [15]. Microbial communities in the rhizosphere can improve uptake of some nutrients like P and it may contribute to increase nutritional efficiencies [16], [17], [18]. Using of Microbiological fertilizer is consider a new approach in order to increase the plants productivity, also many species of bacteria provide an increase in biomass and dry matter of plants, soluble protein, carotenoids, chlorophyll and antioxidant enzymes by improving availability of nitrogen, phosphorus, and potassium in soil [19]. Microbiological fertilizers have several benefits in agriculture, such as it stimulates nitrogen fixation and stimulates plant growth through increased rooting, symbiotic interaction between plant and microorganism, increase soil fertility through the production of organic material [1]. [20] reported that autotrophic and heterotrophic microorganisms including fungi, bacteria, and cyanobacteria leads to solubilize insoluble phosphate forms viz. rock phosphate, hydroxyapatite, and tricalcium phosphate. The root colonizing bacteria that called Plant Growth Promoting Rhizobacteria (PGPR) act as very useful biofertilizers and biocontrol agents [21]. There are numbers of bacterial species are well known, mostly these related to the plant rhizosphere. Phosphate solubilizing microorganisms convert these insoluble phosphates into soluble form. Thus, their use as biofertilizers in agriculture has been a focus for many years [22]. Some of P-solubilizing microorganisms have ability to increase the availability of (P) to plants by mobilizing P from the forms of the organic phosphor or insoluble Calcium phosphates that naturally present in soil [23], [24], [25], [26]. Most of PGPR genera that studied was Bacillus, Pseudomonas, Rhizobium and Enterobacter [27]. Some commercial strains of Bacillus subtilis have proved effective plant growth promotion and agents of biocontrol and facilitate solubilization of phosphor compounds [28], [29], [30]. thus, its effects occur directly from the production of acid phosphatases and organic acids, which solubilize nutrients, especially phosphates [31], [32], [29]. The bacteria B. subtilis are able to enhance the availability of limited organic nutrients to the plant such as phosphorus, nitrogen, carbon, and amino acids [33]. The aim of this study was to evaluate response of two wheat varieties to inoculation of B. subtilis and Phosphorus fertilizer.

2. Materials and Methods

A pot trial was conducted at College of Agriculture, University of Diyala during the season (2017-2018) to evaluate response of two wheat varieties to inoculation by phosphate solubilizing bacteria (Bacillus subtilis) as a commercial product and Phosphorus fertilizer. A factorial experiment design was carried out with three replications of each treatment and two factors, the first include two wheat varieties viz. local cultivar (Ala) and European cultivar (Audi), the second factor include three levels from bio and chemical fertilizers (triple super phosphate P₂O₅ 50 kg /hectare, triple - super phosphate P₂O₅ 25 kg /hectare & bio fertilizer (Bacillus subtilis) and bio fertilizer (Bacillus subtilis)), 15 seeds were planted and thinned to ten plants in each pot (25 cm diameter and 25 cm depth) containing soil with Ec (13.7 ds/m) which collected from College of Agriculture, Urea (46% N) at the rate 360 kg ha⁻¹ were applied in three batch, the first is during farming, the second after 45 days and the third after 90 days. Bio fertilizer (Bacillus subtilis) has been added at rate 2 g/50 ml water / pot after seeds planting, plant growth traits were recorded after 75 and 120 days such as Plant height, Number of branches, Plant fresh weight, Plant dry weight, % Phosphor in plant which was estimated by spectrophotometer and Number of bacteria.
2.1. Method of counting bacteria (*Bacillus subtilis*)

Ten grams of soil mixture were weighed and added to 90 ml of distilled water and shake the suspension well, then worked a series of dilutions till $10^{-6}$ and transfer 1 ml from each dilution into 3 sterile petri dishes contain picovskaya media then incubate all the plates at $28\pm 2\,\text{C}^0$ for 4 days. Phosphate-solubilizing bacteria will grow on this medium and form a clear zone around the colony, formed due to phosphate solubilization in the vicinity of the colony. $10^{-6}$ dilution was adopted in counting the bacteria, the number of colony forming units (c.f.u.) in 1 g of soil was calculated by the following formula.

\[
\text{Number of cfu per g soil} = \frac{\text{Number of colonies}}{\text{dilution factor}}
\]

Number of cfu for bacteria per g soil before planting was $40 \times 10^6$.

2.2. Statistical analysis

The experiment was conducted in a factorial experiment design and the data was analyzed by one way Analysis of Variance (ANOVA) [34].

3. Results and Discussion

As shown in Table (1), Ala cultivar was superior and achieved the highest rate of plant height 46.98, 65.68 cm with significant differences from Audi cultivar 23.42, 30.21 cm after 45 and 120 days respectively, Chemical fertilizer and Chemical & Bio fertilizer got the highest rate of plant height 36.68, 37.10 cm respectively with significant differences from Bio fertilizer 31.83 cm after 45 days, whereas Chemical & Bio fertilizer was superior in rate of plant height reached 52.60 cm from other treatments after 120 days, while the interfere between Wheat cultivars and fertilizers was significant, Ala cultivar with Chemical & Bio fertilizer recorded the highest plant height 50.80, 73.66 cm after 45 and 120 days respectively. The results indicated that Audi cultivar was superior in rate of Number of branches 3.87, 7.71 from Ala cultivar 1.94, 3.45 after 45 and 120 days respectively, while Chemical & Bio fertilizer was recorded highest rate of Number of branches reached 3.55 after 45 days, whereas no significant differences after 120 days, the interfere between wheat cultivars and fertilizers was also significant, Audi cultivar with Chemical & Bio fertilizer were recorded significant increase in Number of branches 4.83, 8.13 after 45 and 120 days respectively.

| Plant height (cm) after 45 days | A (Wheat cultivars) | Rate |
|--------------------------------|---------------------|------|
|                                | Ala                | Audi |      |
| Chemical fertilizer            | 47.36              | 26.00| 36.68|
| Chemical & Bio fertilizer      | 50.80              | 23.40| 37.10|
| Bio fertilizer                 | 42.80              | 20.86| 31.83|
| Rate                           | 46.98              | 23.42|      |

Table 1: Effect of *Bacillus subtilis* and Phosphorus fertilizer on Plant height and Number of branches to two wheat cultivars after 45 and 120 days.
Data presented in Table (2) showed that there were no significant differences between Ala and Audi cultivars in Plant fresh weight while Chemical & Bio fertilizer was recorded a significant increase in this trait 39.41 g, the interfere between Wheat cultivars and fertilizers was recorded highest rate of Plant fresh weight with Audi cultivar and Chemical & Bio fertilizer 39.90 g. Ala cultivar was superior on Audi cultivar in Plant dry weight 21.92 g, also Chemical & Bio fertilizer and Bio fertilizer were superior on Chemical fertilizer 19.18 and 16.86 g respectively, whereas the interfere showed a higher rate in this trait between Ala cultivar and Chemical & Bio fertilizer reached 24.49 g. Audi cultivar was superior on Ala cultivar in percentage of phosphor 0.31%, Chemical & Bio fertilizer was superior on other treatments 0.27%, whereas the interfere showed a higher rate in this trait between Audi cultivar and Chemical & Bio fertilizer reached 0.32%. The number of bacteria was increased in Audi cultivar reached 95.77 ×10^6 as compared with Ala cultivar 63.22×10^6, while Bio fertilizer was superior in Number of bacteria from other treatments reached 90.66×10^6 followed by Chemical & Bio fertilizer 78.50×10^6 and Chemical fertilizer 69.33×10^6, whereas the interfere between wheat cultivars and fertilizers were recorded significant increase in Number of bacteria with Audi cultivar and Bio fertilizer that reached 109.66 ×10^6.

Table 2: Effect of *Bacillus subtilis* and Phosphorus fertilizer with two wheat cultivars on Plant fresh weight, Plant dry weight, % Phosphor in plant and Number of bacteria after 120 days

| B                                | A (Wheat cultivars) | Rate |
|----------------------------------|--------------------|------|
| Chemical fertilizer              | 25.94              | 30.98| 28.46 |
| Chemical & Bio fertilizer        | 38.91              | 39.90| 39.41 |
| Bio fertilizer                   | 39.60              | 32.63| 36.11 |
| Rate                            | 34.82              | 34.50|      |
| CD 0.05                          | A=2.62, B=3.20, A x B=4.53 |      |
According to the result obtained in this research, Ala cultivar was the best in traits of Plant height and Plant dry weight, whereas Audi cultivar was the best in traits of Number of branches, % Phosphor in plant and Number of bacteria, also the inoculation of *B. subtilis* with Chemical fertilizer led to increase all studied traits as compared with Chemical fertilizer or biofertilizer alone. The findings agree with [29], [30], [35] that reported inoculation with *B. subtilis* will prove their efficient related to mobilizing Phosphorus, therefore total phosphor will increase in shoots and roots. [36] reported that *B. subtilis* increases phosphor uptake by plants regardless of the phosphor availability level and improving plants growth which inoculated with other *B. subtilis* strains due to their production of auxins. *B. subtilis* was effective in improving growth of plants, this increase in growth may be result of increased plant nutrition, inoculation with *B. subtilis* significantly increased dry matter yield in roots, increased total phosphor in shoots and roots, and also total phosphor uptake [7]. [37]and [38] reported that using of chemical fertilizer with biological fertilizer can produce the highest yield compared to either chemical or biological treatments alone. [39] reported that plant growth promoting rhizobacteria (PGPR) might improve plant height by enhancing the availability of nutrients and synthesizing phytohormones. [40], [41], [42], [43] who reported that inoculation of crop plants by microbial led to increased plant height.

4. Conclusion

The findings of this study were supported possibility of decreasing chemical fertilizer using up to 50% with applying bio fertilizer, otherwise there were no additional improvements in most mentioned traits by using an entire dose of chemical fertilizer or using bio fertilizer alone, adding of bio fertilizers such as *B. subtilis* with lower levels of chemical fertilizers such as phosphor was very effective, this approach can reduce the excessive application of fertilizers, thus reducing fertilizer costs and providing economic benefits to the farmers and granting promising results, this approach can be applied to other crops.
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