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The Effect of Seed Inoculation (*Pseudomonas putida+Bacillus lentus*) and Different Levels of Fertilizers on Yield and Yield Components of Wheat (*Triticum aestivum* L.) Cultivars

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***Abstract*—**In order to study of The Effect of seed inoculation with Pseudomonas putida+Bacillus lentus on yield and yield components of wheat (Triticum aestivum L.) cultivars, an experiment was carried out as factorial based on Randomized Complete Block Design (RCBD) in Agricultural Research Station of Shahrood University of Technology. Results showed that inoculation with Pseudomonas putida+Bacillus lentus promoted seed germination. Also, inoculation with Pseudomonas putida+Bacillus lentus significantly affected grain yield, Number of spikes per m^2, Number of grain per spike and 1000-seed weight and There was not statistically significant difference between Chamran and Pishtaz cultivars . Finally, the dosages of chemical fertilizers currently applied in commercial wheat field in Iran (Shahrood region) could be reduced through proper combination of Pseudomonas putida+Bacillus lentus inoculation plus fertilization.

***Keywords*—** Seed inoculation, wheat, yield, yield components

I. Introduction

MANY species and specific strains of bacteria have beeninvestigated as plant growth-promoting rhizobacteria PGPR) in different parts of the world on different plants. The impact of rhizobacteria generally on plant growth and health may be classified as neutral, deleterious or beneficial [20]. However, PGPR specifically are beneficial and the beneficial effects have been utilized in many areas including biofertilizer, disease control,microbe-rhizoremediation, biopesticide, in forestry [24] as well as probiotics [26]. Different bacteria that have been reported as PGPR belong to the following genera: *Pseudomonas*, *Bacillus*, *Azospirillum*,*Agrobacterium*, *Azotobacter*, *Arthrobacter*, *Alcaligenes*,*Serratia*, *Rhizobium*, *Enterobacter*, *Burkholderia*, *Beijerinckia*,*Klebsiella*, *Clostridium*, *Variovovax*, *Xanthomonas*, and *Phyllobacterium* (8,11,19,24, 29,35). Among these, *Pseudomonas* and *Bacillus* are the most widely reported PGPR.

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Growth promotion and disease control by *Pseudomonas* and *Bacillus* are complex interrelated processes involving direct and indirect mechanisms that include synthesis of some metabolites (auxin, cytokinin and gibberellins), induction of 1- aminocyclopropane-1-carbocylate (ACC) deaminase, production of siderophore, antibiotics, hydrogen cyanide (HCN), and volatile compounds. Others include mineral solubilization (e.g., phosphorus), competition, and induced systemic resistance (15,17,29,33,42). In these processes there are some similarities and differences between *Pseudomonas* and *Bacillus* based on the reports of different authors cited above. However, the relative competence of these two important genera is not clear. To our knowledge, no reports are available presenting a comparison between them under the same study conditions.

High-input farming practices achieving high yields have created environmental problems and degradation in natural resources. Large quantities of chemical fertilizers are used to replenish soil N and P, resulting in high costs and severe environmental contamination. Consequently, there has recently been a growing level of interest in sustainable agricultural practices to alleviate detrimental effects of intensive farming currently practiced. Increasing and extending the role of biofertilizers would reduce the need for chemical fertilizers and decrease adverse environmental effects. Microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers. The positive effects of PGPR have been correlated with increased mobilization of insoluble nutrients and consequent improvement in plant nutrient uptake [23]. These mechanism require direct contact between the bacteria and the surface or interior of root tissues, and active state of the inoculated bacteria [16]. Studies to date suggest that positive growth responses of wheat (*Triticum aestivum* L.) to inoculation with PGPR are due in part to increased root absorption capacity. Rhizosphere associated N2-fixing and P-solubilizing bacteria have increasingly been used in non-legume crop species such as sugar beet, sugar cane, rice, maize and wheat [36]. Trials with *Bacillus* species indicated yield increases in rice [37], cereals [4,10,27] and maize [28]. Asymbiotic N2 fixing bacteria were reported to replace 60% of N requirements of sugar cane amounting to 200 kg N/ha^{-1} [38]. Suggest that positive growth responses of wheat

(Triricum aestivum L.) to inoculation with PGPR are due in part to increased root absorption capacity. Bacterial genera studied in this regard include *Azospirillum* [3,9], *Azotobacter* [30], Bacillus [12], inoculation of wheat [31] and barley [10] with Bacillus sp. Increased biomass and grain yield. Similarly, inoculation of wheat with Bacillus sp. Increased the mass of soil adhering to the roots [14], enhanced the stability of soil aggregates [5] and stimulated plant growth [32]. The actual mechanism of the rhizobacteria in plant interaction and its positive effect on plant growth is still unclear [6,35]. There are some evidence that plant growth and yield increase may be stimulated by plant growth promoting bacteria due to their ability of N2 fixing, phosphate solubilizing and production of plant growth hormones [34]. Yield responses of cereal to inoculation may also depend on plant genotype [18,25], bacterial strains and soil type [2] as well as environmental conditions ([6]. *Bacillus* species used as biofertilizers may have direct effects on plant growth through the synthesis of plant growth hormones [1], N2-fixation [41] and synthesis of the enzymes modulating the level of plant growth promoting rhizobacteria [22]. Some of the above bacteria may also solubilize inorganic phosphate, making soil phosphorus otherwise remaining fixed available to the plants [22,40] due to excretion of organic acids [21,39] and through carbon and nitrogen sources. Phosphate solubilizing *Bacillus* spp. stimulates plant growth through P nutrition [40], increasing the uptake of N, P, K and Fe [7]. Phosphorus biofertilizers could help increase the availability of accumulated phosphates for plant growth by solubilization, enhancing plant growth by the increasing the efficiency of biological nitrogen fixation and the availability of Fe, Zn through production of plant growth promoting substances [21]. Combined inoculations with N2-fixing and P-solubilizing bacteria were more effective than single microorganisms controlling soil-borne pathogens [13] and providing a more balanced nutrition for plants [4]. Dual inoculations increased yields in sorghum and barley [4] compared to single inoculations with N2-fixing or P-solubilizing bacteria.

II. Material and methods

Studies were performed at the experimental field site of the Shahrood University of Technology in Shahrood , Iran. The soil was a clay loam with organic matter content of 0.75 % and pH= 7.88; Electrical conductivity (3.9 ds/m), Nitrogen percent 0.04 %, available P, 6.4 ppm. The site has a dry and cold climate, with average annual rainfall 150-160 mm and mean annual temperature 14.4 °C. We used a factorial experiment based on randomized complete block design with 4 replications. First factor included two cultivars of wheat (Chamran and Pishtaz) and second factor included six levels included: 1- Inoculated of seeds, unfertilized soil; 2-100% dose fertilized (100 kg ha^{-1} urea -100 kg ha^{-1} amonium phosphate); 3- Inoculated plus 100% dose fertilized; 4-50% dose fertilized (50 kg ha^{-1} urea -50 kg ha^{-1} amonium phosphate); 5- Inoculated plus 50% dose fertilized and 6- non priming, unfertilized soil (control). Each block consisted of 12 plots, plots were separated by a distance of 1 m. Seeds were hand sown on 2* 8 m plots. Seed sowing density was 120 kg ha^{-1}.. Wheat seed were placed in bacteria suspension for 30 min before sowing and then transferred to soil. Weeds were removed manually. Plants were watered as needed in all growth stages. At emergence of seedlings stage, the number of seedlings emerging per m^2 was evaluated using a 1/2 m^2 iron ring. Plants in plots were harvested 220 days after sowing. Yield parameters evaluated were: grain yield (kg ha^{-1}), 1000-seed weight, number of spikes per m^2, and number of grains per spike. These parameters were determined after creating clearances of 1 m at the edges of each plot, and 2 sowing lines at each side. Data were subjected to analysis of variance (ANOVA). When ANOVA showed treatment effects ($P<0.05$), the least significant difference test (LSD) was applied to make comparisons among the means ($P<0.05$). For analysis of data, Mstat-C program was used.

III. Results and discussion

Analysis of variance are shown in table (1). Effects of inoculation of wheat with *Pseudomonas putida* + *Bacillus lentus* were evaluated at germination and ripening stages of wheat. Inoculation affected germination or emergence of seedlings. The number of plants per m^2 was larger for the inoculation treatment than for fertilization without inoculation in each of wheat cultivars. (Figure 1 and figure 2). There was not statistically significant difference between Chamran and Pishtaz cultivars. Inoculated plus 100% dose fertilized and inoculated plus 50% dose fertilized treatments (table 1 and table2) produced maximum plants per m^2 for Chamran and Pishtaz cultivars respectively. There was not statistically difference between inoculated plus 100% dose fertilized and inoculated plus 50% dose fertilized treatments. Regarding the yield parameters, kg ha^{-1} value was significantly higher than control by 1710 for inoculation plus 50% dose fertilized and by 1729 for inoculation plus 100% dose fertilization for Pishtaz cultivar (table 2). In Chamran cultivar, yield parameters, kg ha^{-1} value was significantly higher than control by 983 for inoculation plus 50% dose fertilized and by 1105 for inoculation plus 100% dose fertilization (table3). Grain yield for 1- Inoculated of seeds, unfertilized soil; 2-100% dose fertilized (100 kg ha^{-1} urea -100 kg ha^{-1} amonium phosphate); 3- Inoculated plus 100% dose fertilized; 4-50% dose fertilized (50 kg ha^{-1} urea -50 kg ha^{-1} amonium phosphate); 5- Inoculated plus 50% dose fertilized and 6-non priming, unfertilized soil (control) tratments was 3115, 3427, 3629, 3205, 3610 and 1900 kg/ha for Pishtaz cultivar (table2) and 2225, 2590, 2995, 2420, 2873 and 1890 kg/ha for Chamran cultivar (table3) respectively. There was not statistically difference between Pishtaz and Chamran cultivars (Table 1). Number of spike per m^2 was significantly higher than control by 55.6 for inoculation plus 50% dose fertilized and by 163.8 for inoculation plus 100% dose fertilization for Pishtaz cultivar (table 2). In Chamran cultivar, Number of spike per m^2 was significantly higher than control by 79 for inoculation

50% dose fertilized and by 104 for inoculation plus 100% dose fertilization (Table 3). Number of spikes per m^2 for 1-Inoculated of seeds, unfertilized soil; 2-100% dose fertilized (100 kg ha^{-1} urea -100 kg ha^{-1} amonium phosphate); 3-Inoculated plus 100% dose fertilized; 4-50% dose fertilized (50 kg ha^{-1} urea -50 kg ha^{-1} amonium phosphate); 5-Inoculated plus 50% dose fertilized and 6-non priming, unfertilized soil (control) tratments was 435.1, 480.4, 553.8, 445.6, 490.3 and 390 for Pishtaz cultivar (table 2) and 317, 362, 395, 370, 360 and 291 for Chamran cultivar (table 3) respectively. There was not statistically differences between Chamran and Pishtaz cultivars (Table1). Number of grain per spike was significantly higher than control by 4.8 for inoculation plus 50% dose fertilized and by 10.2 for inoculation plus 100% dose fertilization for Pishtaz cultivar (table 2). In Chamran cultivar, Number of grain per spike was significantly higher than control by 2 for inoculation 50% dose fertilized and by 7 for inoculation plus 100% dose fertilization (Table 3). Number of grain per spike for 1-Inoculated of seeds, unfertilized soil; 2-100% dose fertilized (100 kg ha^{-1} urea -100 kg ha^{-1} amonium phosphate); 3-Inoculated plus 100% dose fertilized; 4-50% dose fertilized (50 kg ha^{-1} urea -50 kg ha^{-1} amonium phosphate); 5-Inoculated plus 50% dose fertilized and 6-non priming, unfertilized soil (control) tratments was 18.7, 27, 26.3, 20.9, 22.3 and 16.1 for Pishtaz cultivar (table2) and 13, 15, 19, 14, 15 and 12 for Chamran cultivar (table 3) respectively. 1000-Seed weight was significantly higher for control by 4.4 than inoculation plus 50% dose fertilized and by 6.5 than inoculation plus 100% dose fertilization for Pishtaz cultivar (table 2). In Chamran cultivar, 1000- seed weight was significantly higher for control by 5 than inoculation plus 50% dose fertilized and by 4 than inoculation plus 100% dose fertilization for Pishtaz cultivar (Table 3). 1000- seed weight for 1- Inoculated of seeds, unfertilized soil; 2-100% dose fertilized (100 kg ha^{-1} urea -100 kg ha^{-1} amonium phosphate); 3- Inoculated plus 100% dose fertilized; 4-50% dose fertilized (50 kg ha^{-1} urea -50 kg ha^{-1} amonium phosphate); 5-Inoculated plus 50% dose fertilized and 6-non priming, unfertilized soil (control) tratments was 39.1, 34.7, 37.8, 39.9, 38.8 and 44.3 for Pishtaz cultivar (table2) and 24, 29, 30, 29, 28 and 34 for Chamran cultivar (table 3) respectively. There was not statistically differences between Chamran and Pishtaz cultivars (Table1). Interaction between treatments and cultivars were not statistically significant differences for all of traits (Table 1). This is the first field study in Shahrood University of Technology in Iran of *Pseudomonas putida+Bacillus lentus* inoculation effects. Emergence promotion effects in wheat inoculated with fluorescent *pseudomonas* is reported by Luz (2001). Important conclusions from this study are: 1) inoculation with *Pseudomonas putida+Bacillus lentus* promoted emergence of seedlings and yield of wheat. 2) The dosages of chemical fertilizers currently applied in commercial wheat field in Iran (Shahrood region) could be reduced through proper combination of *Pseudomonas putida+Bacillus lentus* inoculation plus fertilization.

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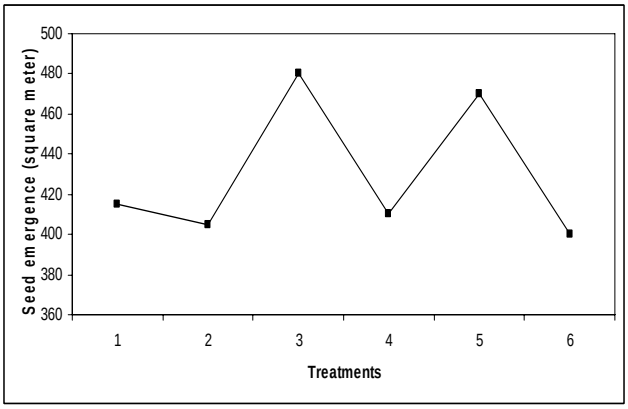

Fig. 1: Emergence of Pishtaz cultivar (plants per m^2)

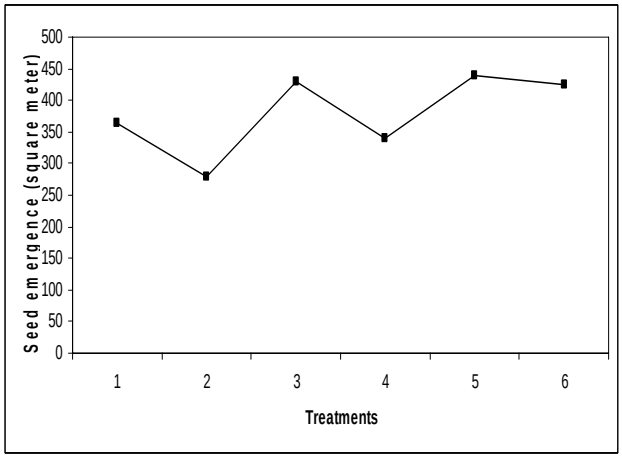

Fig. 2: Emergence of Chamran cultivar (plants per m^2)

TABLE I ANALYSIS OF VARIANCE

Source	D.F.	Yield	Number of spikes per m^2	Number of grain per spike	Weight of thousand grains
Cultivar (A)	1	NS	NS	NS	NS
Treatments (B)	5	*	*	*	*
A*B	5	NS	NS	NS	NS

TABLE II WHEAT GRAIN YIELD AND YIELD COMPONENTS

Pishtaz cultivar

Treatments	Grain yield	Number of spikes per m^2	Number of grain per spike	1000 seed Weight
1-Inoculated seeds, unfertilized soil	3115b	435.1b	18.7c	39.1b
2- 100% dose fertilized (100 kg ha^{-1}	3427a	480.4a	27a	34.7d
3- Inoculated plus 100% dose fertilized	3629a	553.8a	26.3a	37.8c
4- 50% dose fertilized (50 kg ha^{-1} urea-50 kg ha^{-1}ammonium phosphate)	3205b	445.6b	20.9bc	39.9b
5- Inoculated plus 50% dose fertilized	3610a	490.3a	22.3b	38.8b
6- Uninoculated seeds, unfertilized soil (control)	1900c	390 c	16.1d	44.3a

a, b, c significant differences by LSD test (P<0.05)

TABLE III WHEAT GRAIN YIELD AND YIELD COMPONENTS

Chamran cultivar

Treatments	Grain yield	Number of spikes per m^2	Number of grain per spike	1000 seed Weight
1-Inoculated seeds, unfertilized soil	2225cd	317c	13c	24c
2- 100% dose fertilized (100 kg ha^{-1}	2590b	362b	15b	29b
3- Inoculated plus 100% dose fertilized	2995a	395a	19a	30b
4- 50% dose fertilized (50 kg ha^{-1} urea-50 kg ha^{-1}ammonium phosphate)	2420c	370b	14b	29b
5- Inoculated plus 50% dose fertilized	2873a	360b	15b	28b
6- Uninoculated seeds, unfertilized soil (control)	1890d	291d	12c	34a

a, b, c significant differences by LSD test (P<0.05)