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Impact of Zinc Solubilizing Bacteria on Zinc Contents of Wheat

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Abstract: To explore the possibility of reducing doses of chemical fertilizers the influence of bacterial inoculation, *viz. Rhizobium, Azospirillum* and *Pseudomonas* in different combinations with recommended dose of chemical fertilizer (NP) on wheat (*Triticum aestivum* L.) crop was tested in a field experiment during year 2011-2012. The highest shoot zinc (47.6 mg/kg) content was recorded with *Azospirillum* + ½ NP, while flag leaves showed highest zinc with *Pseudomonas* + N ½ P (34.73mg/kg) and *Azospirillum* + N ½ P (1.46mg/kg). The maximum zinc content in wheat straw was recorded with *Rhizobium* ½ NP(13.2 mg/kg) followed by *Pseudomonas* N ½ P (12.26mg/kg) and *Azospirillum* + ½ NP(12.6mg/kg). In grain, the highest zinc (32.33mg/kg) concentration was recorded with *Rhizobium* ½ N P, *Pseudomonas* N ½ P and *Azospirillum* + ½ NP. The results revealed that wheat treated with *Azospirillum, Pseudomonas and Rhizobium* significantly increased zinc contents in different parts of wheat plant at different growth stages. These microbes also facilitate efficient nutrient's uptake which ultimately produce plants of superior quality making agriculture more productive and lesser harmful to environment. It may be concluded from the study that beneficial microorganisms/biofertilizers applied in combination could be a better choice for farmers to reduce the use of chemical fertilizers for sustainable crop production.

Key words: *Pseudomonas* · *Azospirillum* Wheat *Rhizobium* Zinc · Biofertilizers · National Agricultural Research Centre (NARC)

INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to family Poaceae. Wheat is the most important staple diet of the people of Pakistan. It contributes 13.7 percent to the value added in agriculture and 3.0 percent to gross domestic product (GDP). In 2010, country produced over 23 million tons of wheat to meet its domestic needs. Irrigated areas contribute 95 % towards total national wheat production, while rain-fed areas make a payment only 5 % [1].

Area under wheat has decreased to 9180 thousand hectares in 2014-15 from last year's area of 9199 thousand hectares which shows a decrease of 0.2 percent. The production of wheat stood at 25.478 million tonnes during 2014-15, showing a decrease of 1.9 percent over the last year's production of 25.979 million tonnes. The production was decreased due to prolonged winter season and unprecedented rains during April and May and caused damages to grain at harvesting time. Wheat production decreased to 25,478 thousand tonnes in 2014-15 as compared to 25,979 thousand tonnes in 2013-14 showing a decrease of 1.9 percent [2].

The use of N-fertilizer not only spoils the ground water, soil but also have toxic effects by the discharge of harmful gases. The chemical fertilizers should be replaced with the natural and organic fertilizers which can play a key role of the conservation of the environment [3].

Therefore, the combined usage of the nutrients to get quality product without any environmental hazard is of major concern. The use of organic soil alterations and microbial culture along with the cautious use of chemical fertilizers can improve biological and physico-chemical properties of the soil, moreover improving the nutrient uptake efficiency. During the current decade, microbial

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culture proved to be an important component of integrated nutrient application in agriculture and therefore appears a viable potential for efficient use of microorganisms for maximizing crop production. Application of microbes is anvital part of environment friendly justifiable organic agriculture [4].

Soil microbes play a significant part in different ecological processes like: decomposition of organic matter (OM), homeostasis, nutrient cycling and supporting crop growth and health as bio-fertilization [5]. Certain strains of microbes are reported as plant growth-promoting rhizobacteria (PGPR), which can be applied as inoculant biofertilizer [6].

Biofertilizers used in combination with chemical fertilizers improve crop productivity and nutrient use efficacy. A diverse range of bacteria including species of *Rhizobium, Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholderia, Klebsiella, Enterobacter* and many others have been shown to promote plant growth [7]. These plant growth promoting rhizobacteria (PGPR) can be used as biofertilizer and biocontrol agents. It is not realistic to consider sustainable agriculture on a broad scale in the absence of biofertilizers. The production and application of biofertilizers for leguminous and non-leguminous crops are now common in developed world while in developing world it is now gaining pace. Pakistan is an agro-based country, largely dependent on agricultural products for its economy. Biofertilizers are used on farms at a limited scale which contributes only <2.0% of the total fertilizers used by the farmers for crop production [8]. However, there is a great prospective to increase the use of biofertilizers for sustainable agriculture systems. Presently, Pakistan is spending huge amounts (i.e., Rs. > 100 billion) on the import and manufacturing of 8.41 million nutrient tons of chemical fertilizers [9]. A 10% contribution by the biofertilizers to the total fertilizer consumption can save up to 10 billion rupees per year in Pakistan.

MATERIALS AND METHODS

The experiments were carried out in the Soil Biology and Biochemistry Group (SBB), Land Resources Research Institute (LRRI), National Agricultural Research Centre (NARC) Islamabad. Wheat variety Afaq 2000 was sown on 15th November during winter season of 2011. With average minimum temperature of 1°C and average maximum temperature of 36°C, a total rainfall of 430 mm, relative humidity of 80% and day length ranging from 10-13 h, plant growth in the region is restricted to the period between November and May.The experiment was laid out in randomized complete block design with three replications. Three plant growth promoting rhizobacteria (PGPR) inoculants containing *Rhizobium, Pseudomonas* and *Azospirillum* separately were prepared in NARC carrier. The treatments as follow:

T1= ½ NP@ 50:80 Kg h^{-1}

T2= N ½P@100:40 Kg h^{-1}

T4=*Rhizobium*+½NP @50:80 Kg h^{-1}

T5=*Pseudomonas*½ NP@23:10Kg h^{-1}

T6= *Azospirilum* + ½NP@ 50:80 Kg h^{-1}

Both fertilizers were applied at the time of sowing. Urea and single super phosphate (SSP) were applied as source of N and P, respectively. Plants above ground parts and below ground parts samples were collected at 105,125 and 160 days after sowing (DAS) for the analysis of zinc.To finely ground plant parts and seed sample (0.25g), were taken in conical flask 10ml mixed acid (perchloric and nitric acid in 2:1) was added. These flasks were heated on a hot plate at a temperature of 100-300 °C until the solution samples (about 2-3ml) were colorless. Flasks were removed from hot plates and cooled. The digest was transferred into a 50ml volumetric flask and volume (50ml) was made with distilled water (Steckel and Flannery, 1965). Zinc was determined in simple extract in Atomic Absorption Spectrophotometer.

Statistical Analysis: The data obtained in the study were subjected to Analysis of variance using STATISTIX, computer software arranged as a randomized complete block (RCBD) and means were compared by LSD test at 5% ($p < 0.05$) level of significance.

RESULTS

In this study zinc concentration was significantly affected by bacterial inoculation treatments over chemical fertilizers alone.

Effects of Different Treatments on Concentration of Zinc in Wheat Shoot: Wheat shoot zinc due to the effect of different inoculants at 105 DAS are presented in Fig.1.

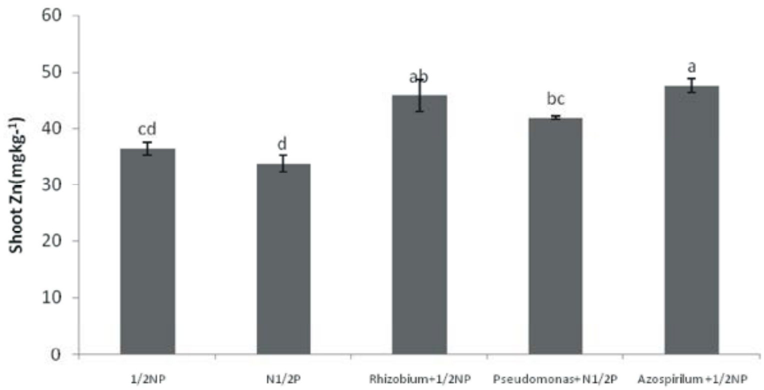

Fig. 1: Zinc content (mg kg^{-1}) of wheat shoot as affected by different inoculants at 105 DAS. Bars sharing the same letter (s) are statistically non-significant (= 0.05).

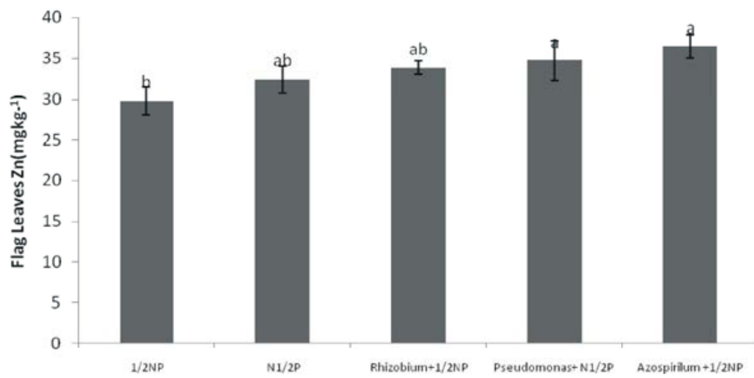

Fig. 2: Zinc content (mg/Kg) of wheat flag leaves as affected by different inoculants at 125 DAS. Bars sharing the same letter (s) are statistically non-significant (= 0.05).

The data indicated that shoot zinc is significantly different in all the treatments (p 0.00). The highest shoot zinc concentration was recorded with *Azospirillum*+½ NP (47.60mg kg^{-1}). Shoot zinc content with chemical fertilizer in ½ NP was 36.47mg kg^{-1} whereas inoculants i.e. *Rhizobium* + ½ NP has 45.87 mg kg^{-1} and *Azospirillum*+½ NP has 47.60mg kg^{-1}, while N ½ P has 33.80mg kg^{-1}whereas *Pseudomonas*+ N ½ P has 41.87mg kg^{-1}.

Effects of Different Treatments on Concentration of Zinc in Wheat Flag Leaves: Wheat flag leaves zinc content due to the effect of different inoculants at 125 DAS is presented in Fig.2. The data directed that flag leaves zinc is significantly different in all the treatments (p 0.06). The highest flag leaves zinc was recorded with *Rhizobium* + ½ NP (33.80mg/ Kg), *Pseudomonas*+ N ½ P (34.73mg/ Kg) and *Azospirillum*+½ NP (36.47mg/ Kg). Flag leaves zinc concentration with chemical fertilizer T1was 29.73mg kg^{-}1, while T2 has 32.40mg kg^{-1}.

Effects of Different Treatments on Concentration of Zinc in Wheat Straw: Wheat straw zinc due to the effect of different inoculants at 160 DAS are presented in Fig. 3. The data directed that straw zinc content is significantly different in all the treatments (p 0.00). The highest straw zinc was recorded with *Rhizobium* + ½ NP (13.20mg kg^{-1}) and *Azospirillum*+½ NP (12.60mg kg^{-1}), *Pseudomonas*+ N ½ P ranked second with straw zinc content12.27mg kg^{-1}. Straw zinc concentration with chemical fertilizer T1was 8.80mg kg^{-1}, while T2 has 9.47mg kg^{-1}.

Effects of Different Treatments on Concentration of Zinc in Wheat Grain: Wheat grain zinc due to the effect of different inoculants at 160 DAS is presented in Fig. 4. The data publicized that grain zinc is significantly different in all the treatments (p 0.00). The highest grain zinc was recorded with *Azospirillum* + ½ NP (37.40 mg kg^{-1}). Grain zinc content with T1 ½ NP has 22.53mg kg^{-1} and T2 has 21.27mg kg^{-1},whereas inoculants *Rhizobium* + ½ NP has 32.33mg kg^{-1} and *Pseudomonas*+ N ½ P has 32.93mg kg^{-1}.

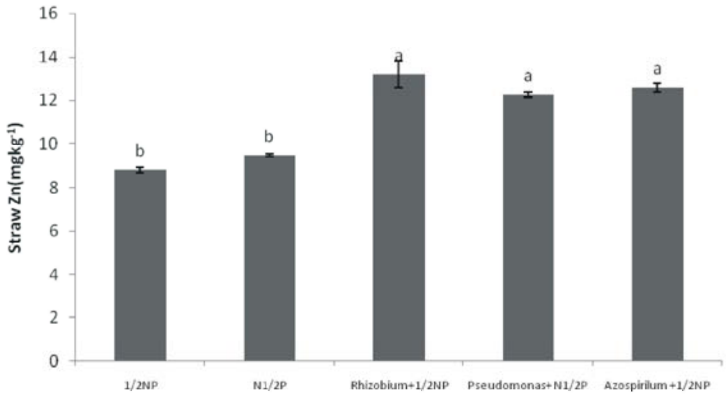

Fig. 3: Zinc content (mg kg^{-1}) of wheat straw as affected by different inoculants at 160 DAS. Bars sharing the same letter (s) are statistically non-significant (= 0.05).

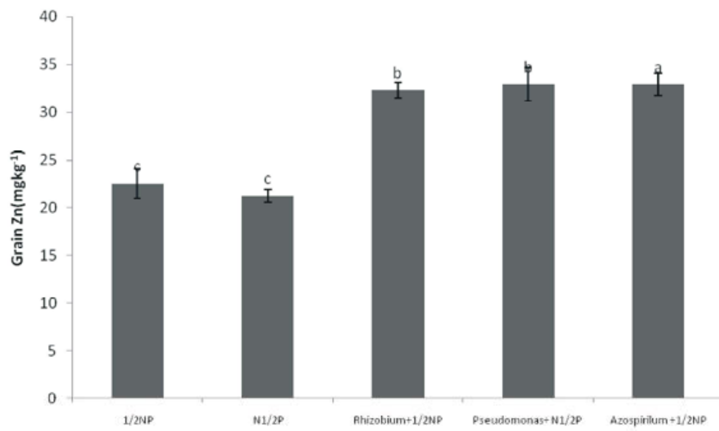

Fig. 4: Zinc content (mg kg^{-1}) of wheat grain as affected by different inoculants at 160 DAS. Bars sharing the same letter (s) are statistically non-significant (= 0.05).

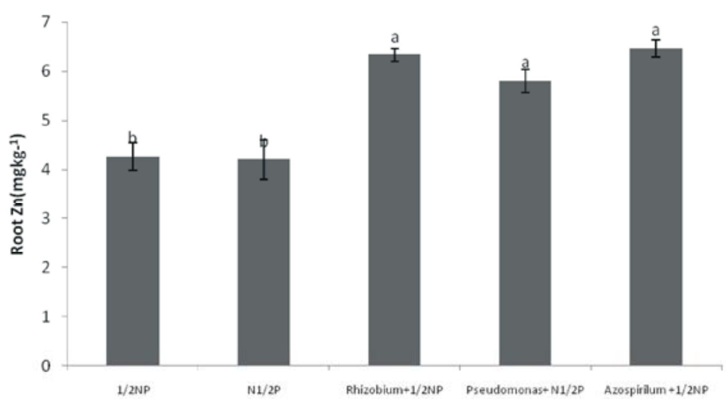

Fig. 5: Zinc content (mg kg^{-1}) of wheat root as affected by different inoculants at 160 DAS. Bars sharing the same letter (s) are statistically non-significant (= 0.05).

Effects of Different Treatments on Concentration of Zinc in Wheat Root: The effect of different inoculants on wheat root zinc at 160 DAS are presented in Fig. 5. The data indicated that root zinc is significantly different in all the treatments (p 0.00).The highest root zinc content was recorded with *Rhizobium* + ½ NP (6.33mg kg^{-1}), *Pseudomonas* + N ½ P (5.80mg kg^{-1}) and *Azospirillum*+ ½ NP (6.47mg kg^{-1}). Root zinc concentration with chemical fertilizer T1has 4.27mg kg^{-1},whereasT2 has 4.20mg kg^{-1}.

DISCUSSION

Results and statistical analysis of present study revealed that application of bio inoculants with fertilizer NP increased zinc concentration in different parts of wheat significantly at alpha level 0.05. Similar results were reported by Agricultural Statistics of Pakistan [1] who confirmed our findings that the bacteria that are accommodating to plant growth support including *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Rhizobium*. These rhizobacteria mineralized organic element in soil through enzymatic procedures and turn into plant nutritive value as well as plant growth factor. Inoculation of wheat and maize with *Azotobacter* and *Azospirillum* increased plant growth, nutrient uptake and yield were also reported by Dobbelaere *et al.* [11]. According to the findings of Vyas and Gulati [12] *Pseudomonas* strains are effective PGPR as they exhibit a wide range of properties *viz.* production of phytohormones like indoleacetic acid (IAA), gibberellic acid and cytokinins; phosphate solubilization and other nutrients. Our findings are in agreement with those obtained by Khan and Prakash [13], who concluded that *Rhizobium* culture significantly increased nitrogen, zinc and molybdenum uptake inurd bean.

Biari *et al.* [14] reported that Plants nutrient uptake of N, P, K, Fe, Zn, Mn and Cu were also significantly influenced by application of *Azospirillum.* Azospirillum Inoculation to improve yields has been extensively discovered in cereal crops [15, 16] and [17] proposed that a large array of bacteria including species of *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Enterobacter*, *Arthrobacter*, *Burkholderia* and *Bacillus* have reported to enhance plant growth and development. Plant inoculation with *Azospirillum brasilense* promoted better uptake of nutrients in corn, sorghum and wheat [18]. *Azospirillum* enhances growth and development of plants, such as phytohormone production and nitrate reduction studied that *Azorhizobium caulinodans* has been shown to colonize wheat roots through crack access of the lateral roots [19, 20]. *Azospirillum* and *Pseudomonas* symbiosis with barley root and increased the root parameters which influence and increase water and nutrition absorption causing better plant growth [21].

CONCLUSION

The obtained results from the study revealed that wheat treated with *Azospirillum, Pseudomonas* and *Rhizobium* considerably increased zinc content in different parts of wheat plant at different stage of growth. Therefore, it can be concluded that microbial inoculation facilitate efficient nutrient's uptake which ultimately produce plants of superior quality.The increased use of various biological processes in soil, will decisively contribute to make agriculture more productive less harm to environment. Therefore, the study concluded that the beneficial microorganisms / biofertilizers applied in combination were a better choice for farmers to reduce the use of chemical fertilizers for sustainable crop production.

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