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Effect of different microbial inoculants on growth and physiology of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) in Vertisols of western Madhya Pradesh

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Abstract

Bio-fertilizers have prodigious potential to improve the plant nutrition by replacing synthetic fertilizers for eco-friendly agriculture. Bio-fertilizers have fabulous tendency for decreasing the requirement of synthetic fertilizers without compromising on crop yield. In order to investigate the effects of plant growth promoting rhizobacteria (PGPR) on improvement in quality parameters and was conducted at KVK (Krishi Vigyan Kendra), Ujjain. In this study, factorial experiment based on completely randomized block design with three replications was used. The microbial inoculants were mixed with compost and cocopit @15g/Kg. Results of the study showed that all treatments significantly increased all growth quality parameters. Maximum increase in germination percentage and other quality parameters were found in Phosphorus solubilizing bacteria (PSB) in case of Maize and *Azatobacter* in case of Wheat seed, respectively. Highest germination % (83.8% / 83.8%), Shoot length (25.8 / 16.13 cm), Root length (10.1 / 10 cm), Total seedling length (35.3 / 25 cm), dry wt. (0.27 / 0.06 mg), SV-I (3429 / 2475 mg), SV-II (26.3 / 6), Leaf area (16.35 / 3.7 cm²). Results suggested that applications of all microbial seed treatments did not affect adversely and proved to be beneficial for maximum seed quality parameters of the selected seeds over control one.

Keywords: Plant growth promoting rhizobacteria (PGPR) - phosphorus solubilizing bacteria (PSB)

1. Introduction

Maize is one of the most important cereals of the world as a source of energy and protein in the human diet and as raw material for large no. of individual products. It is a versatile emerging crop having wider adaptability under varied agro-climatic conditions. Globally, maize is known as miracle crop and thus termed as "King of cereals" because of its productivity potential compared to any other cereal crop. As staple foods, maize and wheat provide vital nutrients and health benefits, making up close to two-thirds of the world's food energy intake, and contributing 55 to 70 percent of the total calories in the diets of people living in developing countries, according to the U.N. Food and Agriculture Organization. CIMMYT scientists tackle food insecurity through improved nutrient-rich, high-yielding varieties and sustainable agronomic practices, ensuring that those who most depend on agriculture have enough to make a living and feed their families. The U.N. projects that the global population will increase to more than 9 billion people by 2050, which means that the successes and failures of wheat and maize farmers will continue to have a crucial impact on food security.

At global level, India ranks 4th in area and 5th in production of maize. The year 2013-14 was a remarkable year for the maize sector in several ways. As per third advanced estimates, maize production in India has grown at a CAGR of 5.5 percentage over the last ten years from 14 MnMT in 2005 to 23 MnMT in 2014, respectively. Whereas maize consumption in India has grown at a CAGR of 4 percent over the last ten years from 14 MnMT in 2005 to 19 MnMT in 2014, respectively.

Wheat is the most important crop in the world and also in the Madhya Pradesh. Currently, India is the second largest producer of Wheat in the world after China with 12% share in the total world wheat production. Wheat production in India recorded an all time highest production of 95.60 million tones, during 2014 crop season (Directorate of Wheat research annual report, 2013-14). Whereas, wheat consumption in India has grown from 69.98 MnT to 93.72 MnT in 2014, respectively. The country needs to produce 100 MnT of wheat by 2030 to feed the ever-growing population, which is a major challenge under changing climatic scenario.

Therefore, concerted efforts are needed to intensify the research on enhancing the productivity in terms of per unit area on ecologically and economically sustained basis.

The seeds are one of the most important inputs for higher grain production and the necessity of quality seed is not to be eluded. Quality seed is required for rapid and synchronous seedling emergence, a pre-requisite for successful stand, establishment and uniform plant growth and development. Vigorous seedling growth is important for the successful establishment of Maize and wheat crops. The capacity of microorganisms to stimulate germination and improve development of plants has been adapted for in vitro and in vivo conditions of some agricultural (Avyadurai et al., 2006; Tsavkelova et al., 2007). The extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer health. Consequently, there has recently been a growing level of interest in environmental friendly sustainable agricultural practices.

Bio-fertilizers are well recognized as an important component of integrated plant nutrient management for sustainable agriculture and hold a great promise to improve quality parameters (Narula et al., 2005; Wu et al., 2005). Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from complex and non-usable form to simple and usable form through biological processes (Cakmakc et al., 2007). Most plant growth promoting rhizobacteria are advantageous and their positive impacts have been greatly put to practical use in numerous ways, using as bio-fertilizers, checking diseases in plants as well as having probiotic properties (Nicholson et al., 2002). Moreover, PGPR protect the plants against soil borne phytopathogens by production of antimicrobial metabolities including siderophores e.g., production of azotobactin by Azatobacter vinelandii (Husen, 2003). Emerging importance of bio-fertilizers will decrease the requirement of synthetic fertilizers and in result it will be helpful in the restoration of environment (White law, 2000). Hence, the present study was undertaken to observe the effect of seed treatments PGPR on seedling growth and other quality parameters of the selected crops.

2. Materials and Methods 2.1 Materials

Maize seeds JM-216 and Wheat seeds HI-1479 were collected from Krishi Vigyan Kendra Ujjain. Bio-fertilizers used includes; Phosphorus solubilizing bacteria (PSB), Trichoderma *Viride* and Azatobacter were collected from Indore Inputs and Research P. Ltd. Protrays were filled with compost and cocopit and the whole experiment was done in open field environmental conditions.

2.2 Treatments

The treatments consisted of a control (no fertilizer applied) and three other seed treatments used in this experiment are furnished below-

- Phosphorus solubilizing bacteria (T₁) @ 15g/Kg of compost and 5g/Kg of seeds
- Trichoderma viride (T₂) @ 15g/Kg of compost and 5g/Kg of seeds
- Azatobacter (T₃) @ 15g/Kg of compost and 5g/Kg of seeds
- Untreated control (T₄)

The experiment was laid out in a completely randomized block design with three replications for each treatment.

2.3 Experimental detail

For evaluating the effects of microbial (Bio-fertilizer) treatments on quality parameters of Maize and Wheat, a field experiment was conducted by using pro-trays method filled with compost and coco-pit for growing plants at Krishi Vigyan Kendra (KVK) Ujjain, (M.P.). The experiment was arranged in a randomized block design consisting of four microbial treatments and each microbial seed treatment contains three replications. Microbial seed treatments included (1) Control: Untreated. (2) Phosphorus solubilizing bacteria (PSB). (3) Trichoderma viride. (4) Azatobacter. These microbial seed treatments were thoroughly mixed with compost in a bucket @ 15g/Kg of compost or 5g/Kg of seeds. About 300 seeds were planted for each treatment in three replicated pro-trays. These pro-trays were placed in green house net and these pro-trays were regularly giving water spray. Seeds were observed for germination in all the set ups every day for a period of about 10 days until no further germination occurred.

2.4 Statistical analysis

The data obtained in this study were subjected to analysis by one way ANOVA using CRD design for all quality parameters. The data on percentage of germination were transferred into the actual values by online analysis using OPSTAT.

2.5 Assessment of growth parameters

- **2.5.1** Germination Percentage (%)
- 2.5.2 Shoot Length (cm)
- 2.5.3 Root Length (cm)
- **2.5.4** Dry weight (mg)
- 2.5.5 Seedling vigour index

SV-I = (Total germination. %) X (Total seedling length) SV-II = Total Ger. % X Total seedling dry wt.

2.5.6 Chlorophyll content (Arnons Method 1949).

2.5.7 Leaf area (Cm^2)

Calculated Leaf area was calculated as per Hoyt and Brandfield (1962) ^[12] by using the following equation as shown below;

 $LA = LL \times LB \times 0.75$

3. Result and Discussion

3.1 Germination Percentage (%)

According to the results of variance analysis that PGPR isolates were enhanced seed germination of Maize and wheat seedlings over control. However, the rate of enhancement varied with bacterial strains. Data from (Table 1 and Table II) observed that highest seed germination percentage in Maize and wheat seeds was observed in seeds treated with Phosphorus solubilizing bacteria (PSB) T1 (83.8%) in case of Maize and Azatobacter T_3 (83.8%) in case of Wheat. Whereas, lowest value of seed germination percentage was recorded in seeds of untreated control T₄ (81.3%) / 83.1%), respectively. Overall, results showed that bio-fertilizers (Phosphorus solubilizing bacteria, Azatobacter and Trichoderma viride) increased the percentage of seed germination which is comparable with the results of Gholami, et al., (2009) who reported that increment of maize seed germination over control due to plant growth promoting rhizobacteria inoculants. The increment of seed germination with inoculants could be due to the isolates ability to synthesis seed germination hormone. This is in conformity with the results of Amruthesh, *et al.*, (2003) and Hameeda, *et al.*, (2008) ^[11] who observed such increased germination due to bio-fertilizers applications.

3.2 Shoot Length (cm)

Results of the experimental study showed that shoot length of Maize and wheat was significantly influenced by microbial treatments over untreated seeds. Data presented in the tables I & II showed that Phosphorus solubilizing bacteria (PSB) T1 recorded maximum shoot length (25.8 cm) in case of maize followed by Azatobacter which was at par with the seeds treated with Trichoderma Viride shown in table III. Whereas minimum results were observed in seeds of untreated (Control) T4 recorded (18.4 cm). In case of Wheat seeds treated with Azatobacter T3 recorded maximum results (16.13 cm) followed by seeds treated with Phosphorus solubilizing bacteria (PSB) T1 and Trichoderma Viride T3. Minimum results were found in seeds of untreated control T4 (14.9 cm). Above findings are also related with the findings of Shanmugaiah, et al., (2009) [19] who observed that shoot length, fresh weight were significantly increased by growth promoting rhizobacteria (PGPRs) as compared with the control. This might be due to the positive effect of organic fertilizer and bio-fertilizers on better root development which resulted in more nutrient uptake.

3.3 Root Length (cm)

Data regarding the effects of bio-fertilizers on quality parameters of Maize and Wheat are given in the table I & II. Seed treatments with bio-fertilizers significantly increased the root length in Maize and Wheat seeds. In general, maximum root length (10.1 cm) was obtained to seeds treated with Phosphorus solubilizing bacteria (PSB), while least value (9.5 cm) was recorded in untreated seeds T4 in case of Maize. Whereas, in case of Wheat seeds maximum root length (8.7 cm) was recorded in seeds treated with Azatobacter T3, while least value was recorded in seeds of un- treated (7.8 cm). These findings are also related with the findings of Omar, *et al.*, (2014) who observed that root length of seedling was significant increase in response to treatments with PGPR isolates. Findings were also supported by Kumar and Vessey (2004).

3.4 Total seedling Length (cm)

Data regarding the effects of bio-fertilizers on quality parameters of Maize and Wheat are given in the table I & II. Seed treatments with bio-fertilizers significantly increased the total seedling length in Maize and Wheat seeds. In general, maximum total seedling length (35.3cm) was obtained to seeds treated with Phosphorus solubilizing bacteria (PSB), while least value (28.6 cm) was recorded in untreated seeds T4 in case of Maize. Whereas, in case of Wheat seeds maximum total seedling length (25 cm) was recorded in seeds treated with Azatobacter T₃, while least value was recorded in seeds of un- treated (22.7cm). The results of the present study was supported by Sharma, *et al.*, (2007) and Rawat and Prasad (2011) reported that seed quality was enhanced through microbial seed inoculation.

3.5 Seedling Dry Weight (mg)

As shown in the table I & II seeds of Maize treated with Phosphorus solubilizing bacteria (PSB) T1 recorded the highest seedling dry weight (0.27 mg), while least value (0.231 mg) was recorded in untreated seeds. Whereas in case of Wheat seeds treated with Azatobacter T_3 maximum seedling dry weight was recorded (0.06 mg) followed by Phosphorus solubilizing bacteria (PSB) (0.05 mg) and Trichoderma Viride (0.04 mg), respectively. Whereas, least value was obtained in untreated seeds T_4 (0.02 mg). Omar, A. *et al.*, (2011) also supported the above findings who observed that lowest shoot dry weight was recorded in untreated, while the highest shoot dry matter was recorded in seeds treated with S. marcences followed by *P. putida*, *B. cereus*, *B. subtilis* and *P. Fluorescens*.

3.6 Vigour Index

The results of variance analysis of the measured vigour index of Maize and Wheat are presented in table I and II. It was revealed that significant increase in seedling vigour index SV-I and SV-II was observed in all microbial seed treatments. Maximum value of SV-I and SV-II was recorded in Phosphorus solubilizing bacteria (PSB) T1 (3429 & 26.3) followed by Azatobacter T₃ (3253 & 23.8 mg) and Trichoderma Viride (3241 & 22.5mg), respectively. Whereas, least value was obtained in untreated seeds T₄ (2838 mg & 20.5 mg). Whereas, least value were obtained in seeds of untreated one T4 (2838 & 20.5 mg), respectively in Maize. Whereas, in case of Wheat seeds treated with Azatobacter T_3 maximum value of vigour index SV-I and SV-II was recorded (2475 & 6 mg) followed by Phosphorus solubilizing bacteria (PSB) (2286 & 5mg) and Trichoderma Viride (2240 & 4.8mg), respectively. Whereas, least value was obtained in untreated seeds T₄ (2222 mg & 4.6 mg). Similar results were found by Jiriaie, M. et al., (2013) ^[13] stated that non-treated seeds with bio-fertilizer could cause decrease of seed vigour index by 25% and 17%, respectively for Azosprillium and Mycorrhiza. It seems that treated seeds with bio-fertilizer, moved nutrition material better to embryo. Same findings were also reported by Karthikeyan, et al., (2007) [14] who found that seeds treated with Azatobacter and Azosprillium increased activities of antioxidant enzymes such as superoxide dismutase, Peroxidase and catalase of seeds and seedlings therefore vigour index were improved.

3.7 Leaf Area (cm²)

Variance of data indicated that application of bio-fertilizer treatments had non-significant effects on leaf area in both Maize and Wheat seeds table I & II. Data mentioned in the table showed that maximum leaf area was obtained in maize seeds treated with Phosphorus solubilizing bacteria T1 (16.35 cm²) followed by seeds treated with Azatobacter T3 (15.9 cm²) which was at par with the results of seeds treated with Trichoderma Viride T2 (14.8 cm²). Whereas, least value of leaf area was found in seeds of untreated control T4 (13.93 cm²). Whereas, in case of Wheat seeds the highest value of leaf area was recorded in seeds treated with Azatobacter T3 (3.7cm²) followed by Phosphorus solubilizing bacteria (PSB) T1 (3.3 cm^2) which was at par with the results of seeds treated with Trichoderma viride T2 (3cm²) with no significant difference between them. Whereas, least value of leaf area was noticed in seeds of untreated control T4 (2.3 cm²), respectively. Similar findings were also recorded by Peng, et al., (2013) [16] which stated that maximum leaf area and weight of plants were recorded at N-fixing Azatobacter enhanced bio-fertilizer. Panhwar, et al., (2011) [15] which stated that PSB inoculation significantly increased leaf area in the aerobic rice genotype tested. Leaf area index and dry

biomass yield increased with the increase in solubilisation and P uptake due to the influence of PSB.

3.8 Chlorophyll Estimation (mg/g)

As shown in the Fig. 1 & 2 application of bio-fertilizers had significant effects on chlorophyll contents of both the selected plants. The highest chlorophyll contents of Maize Chl. a, Chl. b and total chlorophyll was recorded in seeds treated with Phosphorus solubilizing bacteria T_1 (0.4948mg/g, 0.4521mg.g and 0.9466mg/g) followed by seeds treated with Azatobacter T_3 recorded (0.4886mg/g, 0.4268mg/g and 0.9103mg/g), respectively which was at par with the seeds treated with Trichoderma Viride T_2 (0.4837mg/g, 0.4219mg/g and 0.9103mg/g). Least value of all chlorophyll contents were obtained in seeds of untreated. Similarly in case Wheat maximum chlorophyll content *viz.*, Chl. a, Chl. b and total

chlorophyll were observed in seeds treated with Phosphorus solubilizing bacteria (PSB) T1 (0.4765 mg/g, 0.4338 mg/g and 0.9103 mg/g) followed by seeds treated with Azatobacter T3 (0.4724 mg/g, 0.3679 mg/g and 0.8401 mg/g), respectively which was at par with the results of seeds of Trichoderma viride T3 (0.4724 mg/g, 0.3679 mg/g and 0.8401 mg/g), respectively. Whereas minimum chlorophyll contents were recorded in seeds of untreated control T₄. Saharan and Nehra (2011) ^[17] reported that inoculation of crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of chlorophyll content, plant vigour, seedling germination, plant height and shoot weight. Above findings were also supported by Shanthi, et al., (2012) which stated that N- fixing Azatobacter is able to supply high amount of nitrogen for tissue growing and therefore increases chlorophyll content.

 Table 1: Effect of Different Microbials on Germination %, Shoot and Root Length, Total Seedling Length, Seedling dry Weight, Vigour Index and Leaf area of Maize (Zea mays L.)

Treatment (T)	Germination (%)	Shoot Length(cm)	Root Length(cm)	Total seedling Length(cm)	Seedling dry Weight (mg)	SV-I	SV-II	Leaf area (cm ²)
T1	83.8	25.8	10.1	35.3	0.27	3,429	26.3	16.35
T ₂	83.4	23.3	9.5	33	0.23	3241	22.5	14.8
T ₃	83.5	23.4	10	33.4	0.23	3,253	23.8	15.9
T 4	83.1	18.4	9.5	28.6	0.21	2838	20.5	13.93
C.D.	0.381	1.367	N/A	3.309	0.028	318.091	2.92	N/A
S.Em.±	0.108	0.388	0.621	0.938	0.008	90.169	0.83	1.89

Where; $T_1 = Phosphorus$ Solubilizing bacteria (PSB), $T_2 = Trichoderma$ viride, $T_3 = Azatobacter$, $T_4 = Control$ (Untreated)



- Where, $T_1 = Phosphorus$ Solubilizing bacteria (PSB).
- $T_2 = Trichoderma viride.$

 $T_3 = Azatobacter.$

 $T_4 = Control$ (Untreated).

Fig 1: Effect of Different Microbials on Chlorophyll Contents of Maize (Zea mays L.)

 Table 2: Effect of Different Microbials on Germination %, Shoot and Root Length, Total Seedling Length, Seedling dry Weight, Vigour Index and Leaf area of Wheat (*Triticum aestivum* L.)

Treatment	Germination	Shoot Length	Root Length	Total seedling	Seedling dry	SV-I	SV-II	Leaf area
(T)	(%)	(cm)	(cm)	Length (cm)	Weight (mg)			(cm ²)
T_1	83.2	14.96	8.7	24.7	0.05	2,286	5	3.3
T_2	82	14.96	8	23	0.04	2,240	4.8	3
T3	83.8	16.13	10	25	0.06	2,475	6	3.7
T_4	81.3	14.9	7.8	22.7	0.02	2,222	4.6	2.3
C.D.	0.286	0.906	0.803	1.547	0.006	149.898	0.038	N/A
S.Em.±	0.081	0.257	0.227	0.438	0.002	42.491	0.011	0.31



Where; $T_1 = Phosphorus$ Solubilizing bacteria (PSB). $T_2 = Trichoderma$ viride. $T_3 = Azatobacter$. T_4 =Control (Untreated)

Fig 2: Effect of Different Microbials on Chlorophyll Contents of Wheat (Triticum aestivum L.)



Photographs of Maize (Zea mays L.) variety (JM-216)



Photographs of Wheat (Triticum aestivum L.) variety (HI-1479)

4. Discussion and Conclusion

Soil and rhizosphere of crop plants supports a conglomerate of microorganisms with a high degree of diversity (Doran and Zeiss, 2000). The interactions between microorganisms or with the plant result into symbiotic, associative, neutralist or antagonistic effects (Benizri *et al.*, 2001). These effects can be either pathogenic, saprophytic and/or plant growth promoter.

Various microbial treatments like solubilizing bacteria (PSB), Trichoderma viride, Azatobacter have been founded to be effective in plant growth characteristics and enhance biomass production. Use of chemical fertilizers and manures cannot be eliminated without avoiding a consequent drastic decrease in food production. Application of microbial seed treatments offers an additional approach for prior sowing and is suggested as a proper treatment for enhancement of seed indices and improvement of seedling growth and induction system resistance. Utilization of biological fertilizer increased leaf area, seedling length and other parameters of Maize and Wheat. Utilization of chemical phosphorus fertilizer with biophosphorus fertilizer increased the biological efficiency of bacteria in the bio-fertilizer, thus root and shoot growth increased by increasing nutrients absorption by plant. Hence, there is an urgent need for integrated management of nutrients that are incorporated into the soil as agricultural inputs to reduce the adverse environmental impacts of chemical fertilizers. In this research we directed our efforts to know the effect of microbial treatments of PSB, Trichoderma spp. and Azatobacter on seed characteristics and other quality parameters of Wheat (Triticum aestivum L.) plant. Shoresh, et al., (2010) found that PSB & Trichoderma bio-fertilizer, affect plants directly; they would increase plants growth potential and their nutrient uptake, enhance efficiency of fertilizer, increase rate and percentage of seed germination, and stimulation plants defenses quirts biotic and abiotic damages.

In view of the potential application of these bio-fertilizers to enhance the availability of nutrients leading to plant growthpromoting effects. The benefits of bio-fertilizers in improving plant growth can be realized through several mechanisms which include mycoparasitism, antibiosis, degradation of toxins, inactivation of pathogenic enzymatic pathways, resistance to pathogens, enhanced nutrient uptake, solubilization, sequestration of inorganic nutrients and root hair development (Harman, 2006; Lorito *et al.*, 2010). Thus characterization of beneficial bio-fertilizers is a promising area of research to achieve maximum benefits for improvement of quality parameters.

Finally, in respect to our observations in this study, we propose a beneficial role of studied PGPB application for seed enhancement properties which can improve not only seed quality including seedling vigour, but also considered as suitable approach in sustainable agriculture. Under the changing agricultural scenario, the only technology that seems promising to enhance seed quality parameters without dwisturbing the equilibrium of environment and ecosystem is the use of more and more biological controlling agents or bio-fertilizers.

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