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Standardization of Pre-sowing Seed Treatments with Selected Biofertilizers on Seedling Parameters of Wheat (*Triticum aestivum* L.)

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ABSTRACT: This study was undertaken to investigate the effects of various microbial seed treatments on germination, seedling growth, and nutritional content in wheat plants. Results indicated that *Mycorrhiza* treatments, @10%, significantly improved seed germination, seedling length, shoot length, root length, and protein content compared to the untreated control whereas *Azotobacter* treatments exhibited only marginal enhancements in germination percentage and protein content. Conversely, *Trichoderma* treatments showed slight reductions in germination percentage and seedling growth parameters. *Rhizobium* treatments exhibited mixed effects on germination percentage and seedling growth. Notably, *Mycorrhiza* treatments showed consistent positive effects on multiple parameters, highlighting their potential as seed treatment for enhancing crop performance and nutritional quality. These findings underscore the importance of selecting appropriate microbial treatments tailored to specific crop requirements for sustainable agricultural practices.Top of Form

Keywords: Bio-Fertilizer, *Aztobacter, Azospirillum*, *Pseudomonas fluroscence*, *Rhizobium, Mycorrhiza*

INTRODUCTION

Wheat, is a vital staple food for human consumption and a crucial source of dry fodder for livestock. Nowhere is its importance more pronounced than in India, where it ranks second in production following rice. In the financial year 2023, India's wheat production during the rabi season was estimated to exceed 112 million metric tons, reflecting steady growth over the years. India, the third-largest wheat producer worldwide, exported over two billion U.S. dollars worth of wheat in 2022. Ranked second globally for wheat consumption, wheat is a major source of protein, vitamins, and carbohydrates in India, particularly in the North, where it is consumed as Roti, Chapati, and Parathas. The major wheat-producing states include Punjab, Uttar Pradesh, and Madhya Pradesh [1].

Recognizing the pivotal role of seed quality in agricultural productivity, research endeavors have increasingly focused on innovative pre-sowing treatments aimed at enhancing wheat seed vigor and subsequent crop performance [2]. Bio-fertilizers, including *Rhizobium*, *Azotobacter*, and *Trichoderma*, have emerged as promising interventions, exhibiting the potential to augment seed germination, growth, and yield attributes while reducing reliance on synthetic inputs [3, 4] . Further, bio-priming techniques have shown remarkable success in fortifying seeds against environmental stresses, prolonging viability and vigor during storage [5, 6].

This study was undertaken with the objective to determine the effect of pre-sowing seed treatments with selected biofertilizers on seed quality parameters of stored wheat seeds.

MATERIAL AND METHODS

Seeds of the wheat *cv.* variety AAIW6 were obtained from the Department of Genetics and Plant Breeding, SHUATS. The study was conducted at the Notified State Seed Testing Laboratory and Field Experimentation Center of the Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology, and Sciences, Prayagraj (U.P) during November, 2022 to March, 2023.

Wheat (variety AAIW6) seeds were obtained from the Directorate of Research, SHUATS, Prayagraj, and stored from one planting season to next planting season (April 2022 to October 2022). These seeds were primed with

Table 1. Analysis of variance for seedling parameters of Wheat

S. no.	Characters	Treatments (*d.f.*= 10)	Error (*d.f.*= 33)
1.	Germination percentage	5.422*	1.673
2.	Seedling length	5.455*	2.176
3.	Seedling fresh weight	3.399*	0.534
4.	Seedling dry weight	0.865*	0.060
5.	Root length	3.650*	0.767
6.	Shoot length	10.561*	2.371
7.	Vigour Index I	5.962*	1.589
8.	Vigour Index II	8.031*	0.559
9.	Protein	1.173*	0.520
10.	Crude fibre	0.377*	0.045
11.	Germination energy	4.999*	1.541

*significant at 5% level of significance, respectively.

biofertilizer treatments across twelve groups: T_0 (control), T_1 and T_2 (*Azotobacter* at 5% and 10%), T_3 and T_4 (*Trichoderma* at 5% and 10%), T_5 and T_6 (Mycorrhiza at 5% and 10%), T_7 and T_8 (*Rhizobium* at 5% and 10%), T_9 and T_{10} (*Pseudomonas fluorescence* at 5% and 10%), and T_{11} (*Azospirillum* at 10%).

Biofertilizer solutions were prepared at 5% and 10% concentrations and seeds were immersed for 6 hours. Post-treatment, seeds were germinated using the Between Paper method [13]. Key assessments included germination percentage, seedling length, seedling dry weight, and germination energy on the fourth day. Seed Vigour Index I and II were calculated following Abdul-Baki and Anderson [14], and seedling protein content was measured by Lowery *et al.* [15].

Data were analyzed using WASP (ICAR-GOA) software, with the F-test determining significance at a 5% probability level, and critical differences calculated accordingly [17].

RESULTS

The comparison of various seed treatments with the control group revealed (Table 1 & 2) slight changes in germination percentages. *Azotobacter* treatment at 5% concentration showed a marginal increase of 0.18% in germination compared to the control, while the 10% concentration exhibited a slightly higher enhancement of 0.40%. Conversely, *Trichoderma* treatments at 5% and 10% concentrations resulted in slight reductions of 0.70% and 1.95% respectively. *Mycorrhiza* treatment at 5% concentration showed a decrease of 1.65%, whereas the 10% concentration displayed a significant improvement of 2.10% over the control. *Rhizobium* treatments demonstrated consistent enhancements, with a 1.275% increase at 5% concentration and a decrease of 1.35% at 10% concentration compared to the control. *Pseudomonas* treatments at both 5% and 10% concentrations resulted in slight increases of 0.175% and 0.425% respectively. *Azospirillum* treatment at 10%

Table 2. Mean Performance of different Bio-fertilizers on seedling and quality parameters of Wheat

Treatment symbols	Germination (%)	Seedling length (cm)	Shoot length (cm)	Root length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Vigour Index I	Vigour Index II	Protein content (%)	Crude fiber content (%)	Germination energy
T_0	92.23	34.30	28.45	15.98	561.50	205.75	3163	18975	10.25	1.15	81.98
T_1	92.40	34.80	28.20	16.25	573.25	214.50	3216	19820	10.68	1.18	82.15
T_2	92.63	35.43	26.68	17.00	583.00	222.00	3281	20563	10.83	1.30	82.68
T_3	91.53	33.93	28.05	16.03	631.50	234.25	3105	21440	11.05	1.28	81.25
T_4	90.28	35.10	28.48	17.03	637.75	228.20	3169	20601	10.90	1.70	81.08
T_5	90.58	34.10	29.45	17.35	642.75	233.25	3089	21127	11.23	1.55	81.75
T_6	94.33	37.70	32.00	19.08	703.50	254.00	3556	23959	12.25	2.08	84.33
T_7	93.50	36.50	31.48	18.48	680.75	240.00	3413	22440	10.93	1.60	82.50
T_8	90.88	35.10	28.93	17.38	659.75	229.00	3190	20810	10.55	1.40	79.95
T_9	92.08	34.40	27.53	16.68	628.75	218.75	3167	20141	10.25	1.18	80.78
T_{10}	91.65	35.00	28.05	17.05	580.75	219.00	3208	20071	10.38	1.03	80.00
T_{11}	91.88	33.45	26.93	16.20	581.75	205.75	3219	20737	10.95	1.08	81.00
Grand mean	91.99	34.98	28.68	17.04	622.06	225.35	3219	21	10.85	1.38	81.78
SE(d)	0.92	1.04	1.09	0.62	25.24	5.29	0.89	0.53	0.50	0.15	0.88
SE(m)	0.65	0.74	0.77	0.44	17.85	3.74	0.63	0.37	0.35	0.16	0.62
CD at 5%	1.86	2.12	2.22	1.26	5.40	1.78	1.82	1.08	1.02	0.31	1.79

Legends- T_0 - Control, T_1 and T_2 - *Azotobacter* at 5% and 10%, T_3 and T_4 - *Trichoderma* at 5% and 10%, T_5 and T_6 - Mycorrhiza at 5% and 10%, T_7 and T_8 - *Rhizobium* at 5% and 10%, T_9 and T_{10} - *Pseudomonas fluorescence* at 5% and 10%, and T_{11} - *Azospirillum* at 10%.

concentration showed a marginal improvement of 0.885%. These findings underscore the varying impacts of different treatments on seed germination percentages, highlighting the need for careful consideration of treatment concentrations in agricultural practices. Among the treatments, the 10% concentration of *Mycorrhiza* stood out significantly, exhibiting a notable improvement of 2.10% in germination compared to the control group. This result suggests that higher concentrations of *Mycorrhiza* may hold promise for enhancing seed germination in wheat.

The comparison of seedling lengths against the control reveals diverse shifts in percentage changes. *Azotobacter* treatments at 5% and 10% concentrations exhibited slight increases, with percentage changes of approximately 1.46% and 3.32%, respectively. *Trichoderma* treatments at 5% and 10% concentrations resulted in slight decreases of approximately 1.47% and increases of about 1.42% respectively. Mycorrhiza treatments at 5% and 10% concentrations showcased changes of approximately -0.29% and 7.94% respectively, with the latter showing a significant improvement. Rhizobium treatments displayed consistent enhancements, with changes of approximately 6.69% and 0.29% at 5% and 10% concentrations respectively. *Pseudomonas* treatments at both 5% and 10% concentrations resulted in changes of approximately 0.29% and 0.86% respectively. *Azospirillum* treatment at 10% concentration exhibited a decrease of approximately -2.52%. Overall, among the treatments, *Mycorrhiza* at 10% concentration emerged as the superior treatment, displaying a substantial increase of 7.94% in seedling length compared to the control group.

Examining the shoot lengths in relation to the control group reveals varied percentage changes across different treatments. *Azotobacter* treatments at 5% and 10% concentrations exhibited decreases of approximately -1.75% and -6.04% respectively. *Trichoderma* treatments at 5% and 10% concentrations showed marginal changes, with percentage increases of about 0.14% and 0.96% respectively. *Mycorrhiza* treatments at 5% and 10% concentrations demonstrated significant increases, with 3.56% and 11.78% respectively, highlighting substantial improvements, especially at the higher concentration. *Rhizobium* treatments displayed enhancements, with changes of approximately 10.42% and 0.94% at 5% and 10% concentrations, respectively. *Pseudomonas* treatments at both 5% and 10% concentrations resulted in changes of shoot lengths by approximately -3.35% and -1.37% respectively. *Azospirillum* treatment at 10% concentration exhibited a decrease of approximately -5.31%. In conclusion, among the treatments, Mycorrhiza at 10% concentration emerged as the best treatment, demonstrating a significant increase of approximately 11.78% in shoot length compared to the control group.

Similarly root lengths revealed distinct percentage changes over control across various treatments. Azotobacter treatments at 5% and 10% concentrations displayed increases of approximately 1.58% and 6.24% respectively. *Trichoderma* treatments at 5% and 10% concentrations showed marginal changes, with percentage increases of about 0.31% and 6.42% respectively. *Mycorrhiza* treatments at 5% and 10% concentrations demonstrated significant increases, with 8.64% and 19.28% changes respectively, indicating substantial improvements, particularly at the higher concentration. *Rhizobium* treatments exhibited enhancements, with changes of approximately 15.97% and 8.76% at 5% and 10% concentrations, respectively. *Pseudomonas* treatments at both 5% and 10% concentrations resulted in changes of root lengths by approximately 4.33% and 6.53% respectively. *Azospirillum* treatment at 10% concentration exhibited a change of approximately 1.58%. In conclusion, among the treatments, *Mycorrhiza* at 10% concentration emerged as the best treatment, demonstrating a significant increase of 19.28% in root length compared to control.

Comparing the seedling fresh weights against the control group also revealed distinct percentage changes across various treatments. *Azotobacter* treatments at 5% and 10% concentrations displayed increases of approximately 2.09% and 3.89% respectively. *Trichoderma* treatments at 5% and 10% concentrations showed further enhancements, with percentage changes of about 12.52% and 13.59%, respectively. *Mycorrhiza* treatments at both 5% and 10% concentrations demonstrated significant increases, with changes of approximately 14.51% and 25.25%, respectively, indicating substantial improvements, particularly at the higher concentration. *Rhizobium* treatments also exhibited enhancements, with changes of approximately 21.25% and 17.55% at 5% and 10% concentrations respectively. *Pseudomonas* treatments at both 5% and 10% concentrations resulted in changes of seedling fresh weights by approximately

11.87% and 3.55% respectively. *Azospirillum* treatment at 10% concentration exhibited a change of approximately 3.57%. In conclusion, among the treatments, *Mycorrhiza* at 10% concentration emerged as the superior treatment, demonstrating a significant increase of approximately 25.25% in seedling fresh weight compared to the control group.

Effect of seed treatment with microbial inoculum showed a similar effect on seedling dry weights also. *Azotobacter* treatments at 5% and 10% concentrations displayed increases of approximately 4.22% and 7.93% over control, respectively. *Trichoderma* treatments at 5% and 10% concentrations showed further enhancements, with percentage changes of about 13.82% and 10.97% respectively. *Mycorrhiza* treatments at both 5% and 10% concentrations demonstrated significant increases, with changes of approximately 13.53% and 23.48% respectively, indicating substantial improvements, particularly at the higher concentration. *Rhizobium* treatments also exhibited enhancements, with changes of approximately 16.52% and 11.29% at 5% and 10% concentrations respectively. *Pseudomonas* treatments at both 5% and 10% concentrations resulted in changes of seedling dry weights by approximately 6.32% and 6.68%, respectively. *Azospirillum* treatment at 10% concentration exhibited a change equal to that of the control, 0%. In conclusion, among the treatments, *Mycorrhiza* at 10% concentration emerged as the superior treatment, demonstrating a significant increase of approximately 23.48% in seedling dry weight compared to the control group.

Analyzing the percentage changes in Vigour Index I and Vigour Index II against the control group revealed distinct trends across various treatments. *Azotobacter* treatments at 5% and 10% concentrations exhibited increases in both indices, with percentage changes ranging from approximately 1.86% to 3.64%. *Trichoderma* treatments at 5% and 10% concentrations resulted in varied changes, ranging from approximately -1.74% to 0.18%. *Mycorrhiza* treatments at both 5% and 10% concentrations demonstrated substantial increases in both indices, with percentage changes ranging from approximately 2.42% to 12.35%, indicating significant improvements, particularly at the higher concentration. Rhizobium treatments also showed enhancements, with percentage changes ranging from approximately 7.85% to -1.27% at 5% and 10% concentrations respectively. *Pseudomonas* treatments at both 5% and 10% concentrations resulted in changes ranging from approximately 0.12% to 1.78%. *Azospirillum* treatment at 10% concentration exhibited changes ranging from approximately -2.73% to -0.36%. In conclusion, among the treatments, *Mycorrhiza* at 10% concentration emerged as the superior treatment, demonstrating substantial percentage increases in both Vigour Index I and Vigour Index II compared to the control group, indicating enhanced plant vigor.

Analyzing the percentage changes in protein content against the control group reveals diverse impacts of the treatments. *Azotobacter* treatments at 5% and 10% concentrations exhibited increases of approximately 4.88% and 5.37% respectively compared to the control. *Trichoderma* treatments at 5% and 10% concentrations resulted in changes of approximately 7.32% and 6.83% respectively. *Mycorrhiza* treatments at both 5% and 10% concentrations demonstrated significant increases, with changes of approximately 9.76% and 20.73% respectively, indicating substantial improvements, particularly at the higher concentration. *Rhizobium* treatments exhibited changes of approximately 6.34% and 2.68% at 5% and 10% concentrations respectively. *Pseudomonas* treatments showed minimal changes compared to the control. *Azospirillum* treatment at 10% concentration exhibited an increase of approximately 6.34%. In conclusion, among the treatments, *Mycorrhiza* at 10% concentration emerged as the superior treatment, demonstrating a significant increase of approximately 20.73% in protein content compared to the control group.

Examining the percentage changes in germination energy against the control group reveals varied effects of the treatments. *Azotobacter* treatments at 5% and 10% concentrations exhibited increases of approximately 0.22% and 0.71%, respectively, compared to the control. *Trichoderma* treatments at 5% and 10% concentrations resulted in decreases of approximately -0.85% and -1.03%, respectively. *Mycorrhiza* treatments at both 5% and 10% concentrations demonstrated increases of approximately -0.28% and 3.11%, respectively, indicating substantial improvements, particularly at the higher concentration. *Rhizobium* treatments showed changes of approximately 0.73% and -2.52% at 5% and 10% concentrations, respectively. *Pseudomonas* treatments at both 5% and 10% concentrations resulted in changes of approximately -1.47% and -2.29%, respectively. *Azospirillum* treatment at 10% concentration exhibited a change of approximately -0.98%. Among all the treatments, *Mycorrhiza* at 10% concentration emerged as the superior treatment, demonstrating a significant increase in germination energy compared to the control group.

DISCUSSION

The results of the study shed light on the diverse effects of various microbial seed treatments on seed germination, seedling growth, and nutritional content. Notably, *Mycorrhiza* treatments, particularly at higher concentrations, consistently demonstrated superior outcomes across multiple parameters, including seed germination percentage, seedling growth, and protein content. These findings align with previous research by Arati [7] and Balemi [8], highlighting the beneficial role of mycorrhizal fungi in enhancing plant growth and nutrient uptake [6]. Additionally, treatments such as *Trichoderma* and *Rhizobium* showed significant improvements in vigor index and germination energy [9, 10, 11, 12].

However, not all treatments yielded significant improvements across all parameters. *Azospirillum* treatment, for instance, did not show substantial changes in seedling growth parameters or nutritional content compared to the control [16]. This corresponds with previous findings by Singh *et al.* [12], suggesting variability in the effectiveness of microbial inoculants based on factors such as soil conditions and plant species. In conclusion, the study showed the potential of *Mycorrhiza* treatments, notably at a 10% concentration in augmenting seed germination rates, seedling growth metrics, and protein content and underscores the importance of selecting appropriate treatments tailored to specific crop requirements and environmental conditions to optimize agricultural productivity and sustainability. Further study into mechanistic elucidations and refinement of the application methodologies is needed for using these for the advancement of sustainable agriculture.

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