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Analyzing the Impact of Chemical and Biofertilizer Seed Treatments on the Quality of Wheat (*Triticum aestivum* L.) Seeds

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ABSTRACT: Seed is the most crucial input for gaining a plentiful output. The germination of the seed in the field condition is hampered by various edaphic factors along with different pests. So it becomes necessary to treat the seed before sowing with various chemical and biological agents. That's why seed treatment with insecticides, fungicides and biofertilizers has gained significant importance as a practice to ensure the initial establishment of crops. This escalating dependence has, in turn, led to a gradual decline in the production of high-quality seeds. This study was undertaken to examine the impact of chlorpyrifos, vitavax, Azotobacter and phosphate solubilizing bacteria on seed quality of wheat during 2018 to 2020 at the Department of Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. In this study, two wheat varieties WH1105 and WH1124 and their one year old harvested seeds and freshly harvested seed lots were used. The findings revealed that seeds treated with T₂-Azotobacter + PSB showed significant results across several parameters. The maximum speed of germination was recorded in treatment T₆- (Chlorpyrifos + Azotobacter + PSB) and chlorpyrifos subsequently led to the germination of abnormal seedlings. In other parameters such as, germination, seedling length, seedling dry weight, vigor indices, field emergence index and seedling establishment were observed maximum with treatment T_2 -Azotobacter+PSB. The findings suggest that biofertilizers deliver a positive enhancement to seed quality and serve as a feasible alternative to pesticides. It has been observed that biofertilizers contribute to increased speed of germination, germination percentage, vigor indices, field emergence, and seedling establishment both in the laboratory and in the field. On the other hand, it has been observed that fungicides and insecticides negatively affect seed quality.

Keywords: Azotobacter, Biofertilizers, Phosphate solubilizing bacteria, Treatments, Wheat, Quality seed.

INTRODUCTION

Wheat, being an important crop with a central role in the global food supply, is of utmost significance. Its propagation primarily relies on seeds, making seed quality a critical factor influencing crop yield. Germination and vigor index are two key attributes that serve as indicators of the physiological quality of wheat seeds (Ghorbani et al., 2008). Quick seed germination and successful seedling establishment play crucial roles in crop production. Seed treatment stands as a significant innovation to facilitate rapid and consistent seed germination and seedling emergence. Treatment of seeds ultimately enhances seed resilience and vigor and improves crop yields in unfavorable environmental conditions (Kaur et al., 2005). Pre-sowing seed treatment methods have been adopted as an alternative strategy to counter the detrimental impacts of stresses in agriculture due to their cost-effectiveness and lower risk. Seed treatment is recognized as a dual technology that serves to promote instant and uniform seedling emergence, resulting in elevated vigor and improved crop yields. Priming enables certain metabolic processes required for germination to proceed without actual germination taking place. Out of these approaches, seed treatment stands out as a technique in

which external substances, such as polymers, fungicides, biofertilizers, and insecticides, are directly applied to the seed to augment its quality and production potential (Chaudhary et al., 2022). Importantly, this treatment does not substantially alter the seed size or weight, nor does it obscure its original shape. Insecticides such as imidacloprid, fipronil, and chlorpyrifos have proven their effectiveness in controlling termites and serve as viable integrated pest management (IPM) choices for termite control in India. Biofertilizers are regarded as one of the most promising alternatives to the application of chemical fertilizers (Khan et al., 2023). Bio-fertilizers consist of a range of components, including nitrogen-fixing microorganisms (Nfixer), potassium and phosphorus solubilizing agents, growth-promoting rhizobacteria (PGPRs), cyanobacteria, and other beneficial microscopic organisms (Barman et al., 2017). In the case of wheat cultivation, non-symbiotic species such as Azotobacter and Streptomyces may serve as valuable sources of plant growth promoters (Kumar and Brar 2021). The microorganisms contained in biofertilizers are of great importance as they generate essential nutrients like nitrogen, potassium, phosphorus, and others that are essential for plant well-being. Additionally, many biofertilizers produce hormones such as auxins, cytokinins, biotins, and vitamins, which are critical for stimulating plant growth (Murugaragavan et al., 2020). Furthermore, biofertilizers contribute to plant protection by releasing antibiotics that prove effective against plant pathogens. The application various of biofertilizers results in enhanced nutrient availability and water absorption, promotes plant growth, and increases plant resilience to both abiotic and biotic stressors. Soil fertility can be enhanced through the comprising application of biofertilizers, microorganisms such as fungi, bacteria, and protozoa, which have the capability to fix nitrogen, solubilize phosphorus, and capture iron. Biofertilizers consist of a mix of both living and dormant microorganisms, serving as a source of essential nutrients for promoting plant growth (Ranipa et al., 2023). This approach is not only driven by the goal of reducing the expenses associated with chemical fertilizers but also aims to mitigate the detrimental impacts of chemical fertilizers on soil and the overall plant environment (Seenivasagan et al., 2021). Biofertilizers, being cost-effective are also a good preference for organic farming because they replace the chemical load in the plants and soil(Sahoo et al., 2012; Khanna et al., 2019). Seed treatments play a crucial role in addressing the issue of seed-borne pathogens responsible for diseases. These seed treatments can negatively impact radicle emergence, seed germination, seedling emergence, and crop growth. Many chemicals are utilized for controlling these pathogens, but they can have adverse effects on the environment and soil. The utilization of biofertilizers and organic manure in agriculture is gaining widespread popularity in contemporary farming practices. Ultimately, it seeks to secure higher crop productivity. Applying an appropriate and efficient insecticide, fungicide, or other treatment to the seeds is one of the ways for managing pests, fungal diseases, and improving germination, vigor and field emergence. There has been a surge in the dependency on chemical within conventional fertilizers and pesticides agriculture. Hence, the present investigation was undertaken to study the effect of chlorpyrifos and vitavax seed treatments both alone or in combination with biofertilizers i.e. Azotobacter and PSB on seed quality of wheat.

MATERIAL AND METHODS

Experimentation. The experiments were conducted during 2018 to 2020 at the laboratories and research farm of the Department of Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University. This institution is located in Hisar, within the semi-tropical region situated in the north-western zone of India.

Seed Material. In the experiment, wheat seed cultivars WH1105 and WH1124 were taken, and one year old harvested seeds and freshly harvested seed lots were used for each variety. These seeds underwent priming using a total of nine treatments, which included chlorpyrifos 20EC (1.5 ml/kg of seeds), Vitavax (2g/kg of seeds), *Azotobacter* (5ml/kg of seeds), Phosphate solubilizing bacteria (PSB) (5ml/kg of seeds) as well as

various combinations, including a control as displayed in Table 1. The application of a 10% jaggery solution significantly enhanced the adhesion of biofertilizer to the seed surface. Subsequently, the treated seeds were placed in the shade for a period. Following this, the treated seeds were packed in plastic zip-top bags and used for sowing.

 Table 1: List of Treatments and their combinations used during the study.

Sr. No.	Treatments							
T ₀	Control							
T ₁	Phosphate solubilizing bacteria (PSB)							
T ₂	Azotobacter+PSB							
T ₃	Vitavax+PSB							
T_4	Vitavax+Azotobacter+PSB							
T ₅	Chlorpyrifos+PSB							
T ₆	Chlorpyrifos+Azotobacter+PSB							
T ₇	Chlorpyrifos+Vitavax+PSB							
T ₈	Chlorpyrifos+Vitavax+Azotobacter+PSB							

The following section outlines the methodology used to record various observations: To assess the speed of germination, the method involved placing three sets of 100 seeds each in petri-plates (utilizing the "Top of the paper method") and kept in the germinator under the same experimental conditions for 8 days. The relative humidity while performing of experiments was maintained at $90\pm2\%$. Radicle emergence was observed daily, and the speed of germination index was computed using the formula as specified by Maguire (1962)

Speed of germination = $\frac{X_1}{t_1} + \frac{X_2 - X_1}{t_2} + \dots + \frac{X_n - X_n - 1}{t_n}$

Where,

 x_1 , x_2 and x_n = number of seeds germinated on the first, second and nth day, respectively

 t_1 , t_2 and t_n = number of days from sowing to first, second and nth count, respectively.

In the laboratory, three sets of 100 seeds were uniformly placed in between paper method. These samples were then positioned in a germinator at a controlled temperature of 20°C, following a completely randomized design. For the between paper method, the final assessment was conducted on the 8th day, following the guidelines outlined in ISTA (2011). Data was gathered, taking into account the number of germinated seeds, normal seedlings, seedling length, seedling dry weight, vigor index-I, vigor index-II, field emergence index, and seedling establishment, as per the recommendations of ISTA (2011). The data for germination percentage, including the count of germinated seeds, normal seedlings, abnormal seedlings, hard seeds, and infected seedlings, was collected on the final day after germination.

$$Germination (\%) = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds kept for germination}} X \ 100$$

In each replication for all genotypes, a total of thirty randomly selected normal seedlings were picked. Their length was measured in centimeters, and the average length of these seedlings was then calculated. To determine seedling dry weight, the same thirty healthy seedlings from each replication, previously measured for length, were employed. These seedlings were subjected to drying in a hot air oven for a period of one day (24 hours) at a constant temperature of 80±1°C. Before taking the seedling dry weight, they were allowed to reach equilibrium in a desiccator for a minimum of 20 minutes. The seedling vigor indices were calculated according to the formula provided by Abdul-Baki and Anderson (1973) and presented as whole numbers.

Seedling vigor index-I = Germination percentage \times Average seedling length (cm)

Seedling vigor index-II = Germination percentage \times Average seedling dry weight (mg)

To observe field parameters, including the field emergence index, a total of one hundred seeds from all the treatments were employed across three replications in a Randomized Block Design (RBD). The count of seedlings that emerged from the soil was recorded daily until seedling establishment. The field emergence index was determined using the following formula Field Emergence Index (FEI) =

No. of seedlings emerged	+ + No. of seedlings emerged	
1 st day of sowing	Day of the last count	

Seedling establishment was ascertained when the cumulative count of emerged seedlings reached a point where no further additions were observed.

Statistical Analysis. The laboratory data were analyzed in Completely Randomized Design (CRD) along with Randomized Block Design (RBD) for field parameters and all the values described as mean of the replicates with the evaluation of CD at 5% level of significance by using software OPSTAT.

RESULTS AND DISCUSSION

The study examines how different seed treatments, such as Azotobacter, PSB, chlorpyrifos, and vitavax, impact the various aspects of wheat seeds *i.e.* speed of germination, germination percentage, seedling length, seedling dry weight, vigor index-I, vigor index-II, field emergence index, and seedling establishment. The seed quality parameters influence the performance of both old and fresh seed lots of WH1105 and WH1124 wheat varieties. The performance of various seed quality parameters is mentioned in Tables 2 and 3 along with Fig. 1-4. Table 2 provides the results for speed of germination pertaining to the different lots of WH1105 and WH1124. In WH1105, the highest speed of germination (59.57) was recorded in T_6 -(Chlorpyrifos + Azotobacter + PSB), followed by (58.72) T₅-(Chlorpyrifos + PSB). On the other hand, the slowest speed of germination observed (45.27) in T₈-(Chlorpyrifos + Vitavax + Azotobacter + PSB), as compared to T₀-control (46.36).A similar pattern was observed in WH1124, where the highest speed of germination (59.95) was obtained in T_{6} -(Chlorpyrifos + Azotobacter + PSB), at par speed of germination (58.85) in T_5 -(Chlorpyrifos + PSB). The slowest speed of germination occurred in T₈-(Chlorpyrifos + Vitavax + Azotobacter + PSB) (47.00) compared to the T_0 control (49.94).In speed of germination, T₆-Chlorpyrifos + Azotobacter + PSB facilitate the early

emergence of the radicle, potentially due to the use of a solution as a carrier for seed treatment, with water serving as a solvent. This solvent (water) aids in the early protrusion of the seed embryo, with the support of biofertilizers that supply the necessary nutrients to the seeds. Later on, this chlorpyrifos caused abnormal seedlings, which were not counted in germination percentage or in other seed quality parameters. That is why other seed quality parameters result better in T₂-Azotobacter + PSB treatment. Karthika and Vanangamudi (2013) results also confirmed that seed priming with biofertilizer enhanced the speed of germination, germination, root length, shoot length, and vigor index as compared to no primed seeds in the case of maize crop. Sithik and Mahapatro (2017) found that chlorpyrifos germinated the malformed seedlings in wheat. Bakshi et al. (2021) revealed that chlorpyrifos had a toxic effect on Brassica juncea L. which reduced the germination potential, proper radicle development and vigor index.

The germination performance is revealed in Table 2. When considering the mean of both lots in WH1105,the highest improvement in germination was observed with treatment T_2 -Azotobacter + PSB(95.00%), which was at par with T₁-PSB (94.17%) and minimum germination was observed in T₈-Chlorpyrifos + Vitavax + Azotobacter + PSB (86.33%) as compared to T_0 -control (89.00%). The similar trend was observed in WH1124 *i.e.*, the maximum germination was observed in T₂-Azotobacter + PSB(96.17%), followed by T_1 -PSB (95.33%) and lowest germination observed in $T_{\rm 8^{-}}$ Chlorpyrifos + Vitavax + Azotobacter + PSB (87.00%) as compared to T₀-control (90.17%). Bakonyi et al. (2013) reported that biofertilizer significantly increased the number of germinated seeds in comparison to the untreated control. Mubeen et al. (2006) treated the seeds with fungicides and pelleted them with biofertilizer prior to sowing in wheat, and his findings indicated that fungicides do indeed exert detrimental effects on the inoculated microorganisms. This confirms that the combined treatment of pesticide and biofertilizers reduced germination of wheat.

The mean performance of old and fresh seed lots of WH1105 and WH1124 in terms of seedling length is presented in Fig. 1. When examining the mean values for both lots within WH1105, the highest seedling length was observed in treatment T_2 -Azotobacter+PSB(32.31 cm), at par with T₁-PSB (31.81 cm) and the minimum seedling length was in T₈-Chlorpyrifos+Vitavax+Azotobacter+PSB(24.11 cm) as compared to T₀-control (26.43 cm). In WH1124, the highest seedling length was observed in T₂-Azotobacter + PSB (32.93 cm), followed by T_1 -PSB(32.40 cm) and the lowest seedling length was in T₈-Chlorpyrifos + Vitavax +Azotobacter + PSB(24.84 cm) as compared to T₀-control (27.02 cm). Fig. 2 shows the mean performance of both older and fresher seed lots for WH1105 and WH1124 in terms of seedling dry weight. In WH1105, the maximum seedling dry weight was observed in treatment T_2 -Azotobacter + PSB(14.65 mg), at par with T_1 -PSB (14.43 mg) and the minimum seedling dry weight was in T₈-Chlorpyrifos + Vitavax + Azotobacter + PSB(11.07 mg) as compared to T₀-701

Singh et al.,

Biological Forum – An International Journal 15(10): 699-705(2023)

control (11.82 mg). In WH1124, the highest seedling dry weight was observed in T₂-Azotobacter+PSB(15.22 mg), followed by T₁-PSB(14.68 mg) and the lowest seedling dry weight was recorded in T₈-Chlorpyrifos + Vitavax + Azotobacter + PSB(11.50 mg) as compared to T₀-control (12.24 mg). Boubekri *et al.* (2021) also found substantial increase in the seedling length and seedling dry weight wheat treated with phosphate solubilizing bacteria. Ansari *et al.* (2015) unveiled a study that in controlled environments, Azotobacter biofertilizers showed favorable outcomes in terms of seed germination and average shoot length in chickpea. However, when field studies were conducted, phosphate solubilizing biofertilizers generally delivered superior overall crop performance.

The performance of vigor index-I precisely to the lots of WH1105 and WH1124 is revealed in Table 3. When considering the mean of both the lots of WH1105, the maximum vigor index-I was observed in treatment T₂-Azotobacter+PSB (3072), followed by T₁-PSB (2998) and the minimum was in T₈-Chlorpyrifos + Vitavax + Azotobacter + PSB (2083) as compared to T_0 -control (2355) in WH1105. A similar trend was observed in WH1124, the highest vigor index-I was observed in T₂-Azotobacter+PSB(3169) followed by T₁-PSB (3090) and the lowest in T₈-Chlorpyrifos + Vitavax + Azotobacter + PSB(2162) as compared to T_0 control(2438). The performance of vigor index-II specifically to the lots of WH1105 and WH1124 is cited in Table 3. From the mean of both lots of WH1105, the highest vigor index-II was recorded in T₂-Azotobacter + PSB(1393), followed by T_1 -PSB(1359) while the lowest was observed in T₈-Chlorpyrifos+ Vitavax + Azotobacter + PSB(957) as compared to T₀control(1053) in WH1105. The same trend was recorded in WH1124 i.e., the maximum vigor index-II was observed in T2-Azotobacter+PSB(1465), followed by T_1 -PSB(1400), while the minimum vigor index-II was recorded in T₈-Chlorpyrifos + Vitavax + Azotobacter + PSB(1001) as compared to T_0 -control (1105). The analysis of harvested seeds from the field

revealed that seed pelleting with liquid Rhizobium and 80% nitrogen of recommended dose of fertilizer (RDF) resulted in the production of high vigor seeds compared to the control and other treatments (Singh and Thakur 2022). Thube *et al.* (2023) highlighted that chlorpyrifos had a negative impact on various seedling parameters, including germination, seedling length and vigor indices I and II in soybean.

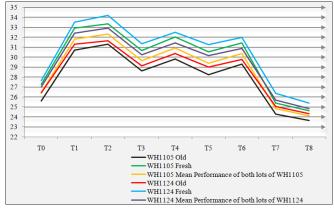
In Fig. 3, the field emergence index with a highlight on the mean performance of old and fresh lots of WH1105 and WH1124 is revealed. In WH1105, the maximum field emergence was observed in treatment T2-Azotobacter+PSB (9.52), at par with T_1 -PSB(9.03) and the minimum was in T₈-Chlorpyrifos + Vitavax+ Azotobacter + PSB(4.67) as compared to T_0 -control (6.07). A similar trend was observed in WH1124, i.e., the highest field emergence index was observed in T₂-Azotobacter + PSB (9.65), followed by T_1 -PSB(9.22) and was lowest in T₈-Chlorpyrifos + Vitavax + Azotobacter + PSB(4.80) as compared to T_0 -control (6.48). Fig. 4 illustrates the seedling establishment with a focus on the mean performance of old and fresh lots of WH1105 and WH1124.In WH1105, the maximum seedling establishment was observed in treatment T₂-Azotobacter + PSB (89.50), at par with T_1 -PSB(88.50) and minimum seedling establishment was recorded in T_8 -Chlorpyrifos+Vitavax+Azotobacter+PSB(77.83) as compared to T_0 -control (81.50%). In WH1124, the highest seedling establishment was observed in T₂-Azotobacter+PSB(90.34%), followed by T_1 -PSB (89.33%) and the lowest seedling establishment was recorded in T₈-Chlorpyrifos + Vitavax + Azotobacter + PSB(79.00%) as compared to T₀-control (82.50%).The application of nano biofertilizer increased spike length, spike number, seed number, seed weight, and the number of days until physiological maturity in wheat crop. Overall, the use of nano biofertilizer enhanced crop growth, improved yield, and positively influenced vield components by extending the growing period (Mardalipour et al., 2014).

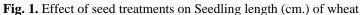
Table 2: Effect of seed treatments on Speed of gern	mination and Germination (%) of wheat.

	Speed of germination							Germination (%)						
Treatments (T)	Variety(V)							Variety(V)						
	WH1105			WH1124			WH1105			WH1124				
	Old	Fresh	Mean	Old	Fresh	Mean	Old	Fresh	Mean	Old	Fresh	Mean		
T ₀	43.36	49.36	46.36	48.11	51.76	49.94	86.00 (68.01)	92.00 (73.62)	89.00 (70.82)	87.00 (68.84)	93.33 (75.02)	90.17 (71.93)		
T ₁	52.96	56.42	54.69	53.33	57.69	55.51	92.00 (73.56)	96.33 (79.01)	94.17 (76.29)	93.33 (75.01)	97.33 (80.61)	95.33 (77.81)		
T ₂	53.31	57.67	55.49	54.84	58.11	56.47	93.00 (74.65)	97.00 (80.08)	95.00 (77.37)	94.33 (76.21)	98.00 (81.84)	96.17 (79.02)		
T ₃	54.87	58.75	56.81	55.04	58.98	57.01	89.67 (71.24)	95.00 (77.09)	92.33 (74.16)	90.67 (72.21)	95.67 (78.03)	93.17 (75.12)		
T ₄	50.20	55.12	52.66	51.78	56.90	54.34	91.00 (72.53)	95.67 (78.07)	93.33 (75.30)	92.00 (73.56)	96.33 (79.01)	94.17 (76.29)		
T ₅	57.78	59.65	58.72	57.53	60.18	58.85	88.67 (70.32)	94.33 (76.34)	91.50 (73.33)	89.00 (70.61)	95.33 (77.61)	92.17 (74.11)		
T ₆	58.44	60.71	59.57	58.87	61.04	59.95	90.33 (71.86)	95.33 (77.55)	92.83 (74.71)	91.33 (72.86)	96.00 (78.59)	93.67 (75.72)		
T ₇	44.01	48.76	46.38	47.23	51.21	49.22	85.00 (67.19)	89.00 (70.61)	87.00 (68.90)	85.67 (67.74)	89.67 (71.24)	87.67 (69.49)		
T ₈	43.22	47.31	45.27	45.66	48.34	47.00	84.67 (66.93)	88.00 (69.74)	86.33 (68.33)	85.00 (67.19)	89.00 (70.65)	87.00 (68.92)		
	v	L	Т	V×L	V×T	L×T	v	L	Т	V×L	V×T	LxT		
C.D. (P=0.05)	0.574	0.574	1.218	NS	NS	NS	0.454	0.454	0.962	NS	NS	NS		
SE.m (±)	0.204	0.204	0.432	0.288	0.611	0.611	0.161	0.161	0.341	0.228	0.483	0.483		

#Values in the parenthesis are arc-sine transformed of the original

Singh et al.,





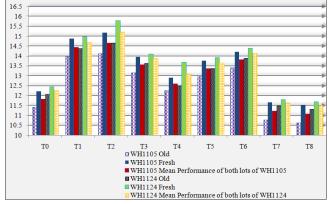
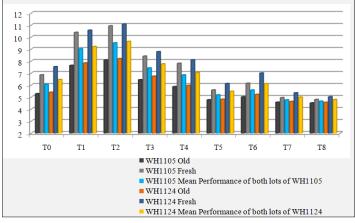
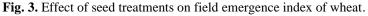


Fig. 2. Effect of seed treatments on seedling dry weight (mg) of wheat

Table 3: Effect of seed treatments on vigor index-I and vigor index-II of wheat.

	Vigor index-I						Vigor index-II						
Treatments (T)	Variety(V)							Variety(V)					
	WH1105			WH1124			WH1105			WH1124			
	Old	Fresh	Mean	Old	Fresh	Mean	Old	Fresh	Mean	Old	Fresh	Mean	
T ₀	2202	2507	2355	2296	2581	2438	981	1124	1053	1049	1160	1105	
T ₁	2825	3170	2998	2920	3260	3090	1286	1432	1359	1342	1457	1400	
T ₂	2910	3233	3072	2985	3353	3169	1313	1473	1393	1383	1547	1465	
T ₃	2565	2913	2739	2639	2996	2818	1180	1325	1253	1236	1348	1292	
T ₄	2713	3065	2889	2792	3132	2962	1116	1234	1175	1148	1318	1233	
T ₅	2205	2880	2542	2582	2981	2781	1149	1298	1224	1189	1327	1258	
T ₆	2645	2994	2820	2720	3068	2894	1212	1355	1284	1269	1380	1325	
T ₇	2062	2258	2160	2145	2363	2254	916	1037	977	984	1057	1021	
T ₈	2000	2165	2083	2065	2259	2162	899	1014	957	962	1040	1001	
	V	L	Т	V×L	V×T	LxT	V	L	Т	V×L	V×T	LxT	
C.D. (P=0.05)	49.764	49.764	105.566	NS	NS	NS	22.378	22.378	47.472	NS	NS	NS	
SE.m (±)	17.649	17.649	37.439	24.959	52.946	52.946	7.936	7.936	16.836	11.224	23.809	23.809	





Singh et al.,

Biological Forum – An International Journal 15(10): 699-705(2023)

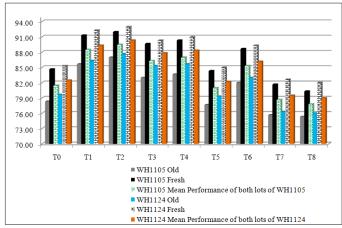


Fig. 4. Effect of seed treatments on seedling establishment (%) of wheat.

CONCLUSIONS

Based on the current investigation, it can be ascertained that seed treatment with T_2 -*Azotobacter*+PSB had maximum improvement in all seed quality parameters and it was the best treatment combination for enhancing the quality of the wheat seeds. During the combined application of chlorpyrifos, vitavax, and biofertilizers, it leads to a decline in the quality of wheat seeds.

FUTURE SCOPE

Chemical treatment has residual effects on the soil as well as the environment. It ultimately enters the food chain, which has adverse effects on the health of humans, while biofertilizers have a synergistic effect on seed quality without causing any harm to human health or the environment. Seeds should be sown with treatments exclusively involving biofertilizers and without the inclusion of insecticides and pesticides. If the seed treatment combines all these elements simultaneously, it will result in abnormal germination in the crops, causing a reduction in both quality and yield.

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Singh et al.,

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