



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(10): 2439-2444
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www.thepharmajournal.com
Received: 13-07-2023
Accepted: 19-08-2023

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Evaluating the effect of various seed treatment approaches on seed quality of wheat (*Triticum aestivum* L.)

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Abstract

The quality of seeds is a critical factor in crop establishment and has a substantial influence on overall production and productivity. Seed treatment is used to manage seed-borne pathogens, insects and pests which can directly impact seed quality, germination, and vigor. This study was conducted during 2018 to 2020 at the Department of Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University in Hisar, aimed to assess the influence of pesticide and biofertilizer treatments on the seed quality of wheat varieties (WH1105 and WH1124). The results revealed that seeds treated with *Azotobacter* exhibited improved seed quality across various parameters. The maximum speed of germination was recorded in treatment T₆-Chlorpyrifos+*Azotobacter* and the germination percentage, seedling length, seedling dry weight, vigor index-I, vigor index-II, field emergence index and seedling establishment all these parameters were recorded highest with treatment T₃-*Azotobacter*. Chlorpyrifos had a detrimental effect on seed quality when treated as individual along with different combinations. Furthermore, the performance of WH1124 was better than WH1105 in different parameters. These findings suggest that biofertilizers enhance seed quality, whereas the insecticides and fungicides have a deleterious influence on seed quality.

Keywords: *Azotobacter*, biofertilizers, seed quality, seed treatments, wheat

Introduction

Wheat is an annual crop that belongs to the Poaceae family and is believed to have originated in South West Asia (Jenkins, 1966) [12]. This crop played an indispensable role in ushering the “Green Revolution” in many developing countries including India. Globally, wheat annual production is 802.1 million tons recorded in 2022-23 (FAO, 2023) [8]. In India, total cultivated area of wheat is 31.86 million hectares in 2022-23 (DAFW, 2023) [3] which produces 109.52 million tons of grains and shares 13% of total wheat production of the world (Singh *et al.*, 2023) [20]. Seed is the most fundamental input required to sustain agriculture. Therefore, it is imperative to develop a cheap and eco-friendly seed production technology, which produces good quality seeds. Seed treatment is a prudent pest-management tactics, are becoming increasingly important as IPM-compatible measures. The process of treating seeds to provide the necessary nutrients and prevent pests and diseases involves the application of both beneficial microbes and chemical pesticides. In India, insect pests are responsible for 15.7 percent yield losses in wheat, (Dhaliwal *et al.*, 2015) [6].

Currently, the agriculture has become heavily relied on cost-intensive crop inputs such as inorganic fertilizers and pesticides (Das and Mandal, 2015) [4]. The use of chemical fertilizers in agriculture is reduced by biofertilizers. The biofertilizers never pollute the land, water, or air (Tamilkodi and Victoria, 2018) [21]. Biofertilizers are elements that comprise a multiplicity of microbes that have the capability to enhance plant nutrient uptake by colonizing the rhizosphere and making nutrients readily available to plant root hairs. Biofertilizers are rated as the one of most promising alternatives to chemical fertilizer application. *Azospirillum*, *Azotobacter*, *Azola*, blue-green algae, Phosphate solubilizing microorganisms, sinorhizobium, and mycorrhizae are among the most prominent microorganisms that function as effective biofertilizers (Me Carty *et al.*, 2017) [17]. These key soil-inhabiting microorganisms perform nitrogen fixation for plants, solubilization of potassium and phosphorus reserves in soil (Gupta *et al.*, 2012; Meena *et al.*, 2016; Hamid and Bashi, 2019) [9, 16, 10]. The *Azotobacter* is responsible for nitrogen fixing in soil, while the phosphate solubilizing bacteria is responsible for solubilizing phosphorus in soil.

The present study was commenced, taking into consideration that seed treatment serves as an environmentally friendly and cost-effective method to achieve both quality and sustainability in high-quality wheat seed production.

Materials and Methods

Experimental site

The experiments were carried out between 2018 and 2020 in the laboratories and research farm of the Department of Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, situated in the semi-tropical region in the western zone of India.

For the experiment, wheat seed cultivars WH1105 and WH1124 along with one-year-old harvested seeds and freshly harvested seed lots for each variety were used. The seeds were primed with Chlorpyrifos 20EC (1.5 ml/kg of seeds), Vitavax (2g/kg of seeds), *Azotobacter* (5 ml/kg of seeds), and their combinations of total 8 treatments including control as

shown in table 1 and important properties of treatments are mentioned in table 2. By using 10% jaggery solution, the biofertilizer stickiness on seed surface was significantly improved and then keeps the treated seeds under shade for a while. After that treated seeds were packed in plastic zipped bags.

Table 1: Their combinations of total 8 treatments including control

Treatments	Concentrations
T ₀	Control
T ₁	Chlorpyrifos 20EC
T ₂	Vitavax
T ₃	<i>Azotobacter</i>
T ₄	Vitavax+Chlorpyrifos
T ₅	Vitavax+ <i>Azotobacter</i>
T ₆	Chlorpyrifos+ <i>Azotobacter</i>
T ₇	Chlorpyrifos+Vitavax+ <i>Azotobacter</i>

Table 2: Important properties of treatments

Active ingredient	Application rate	Properties
Chlorpyrifos 20EC	1.5 ml/kg of seeds	Insecticides for the control of termites
Vitavax	2 g/kg of seeds	Broad spectrum, dual action (systemic and contact) fungicide which controls seed and soil borne diseases.
<i>Azotobacter</i>	5 ml/kg of seeds	Improve plant health through nitrogen fixation, growth hormone production, phosphate solubilization, plant disease management

The following methodology employed for recording various observations are given below:

For speed of germination, three replications of 100 seeds each were placed in petri-plates for germination (Top of paper method). The numbers of radicle emergence were counted on daily basis. The index of the speed of germination was calculated by formula as cited by Maguire (1962) [14].

$$\text{Speed of germination} = \frac{n_1}{d_1} + \frac{n_2}{d_2} \dots + \frac{n_n}{d_n}$$

Where,

n = number of newly emerged radicle on a respective day

d = days after sowing up to the emergence of the radicle

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

From each replication of all the genotypes, thirty normal seedlings were chosen at random, and their length was measured in cm. The average length of these seedlings was calculated. For determining seedling dry weight, we used the same thirty healthy seedlings from each replication that were measured for seedling length. These seedlings were dried in a hot air oven for one day (24 hours) at a temperature of 80±1 °C. Before taking the seedling dry weight, we allowed it to equilibrate in a desiccator for at least 20 minutes. Seedling vigor indices were calculated by using the formula given by Abdul-Baki and Anderson (1973) [1] and expressed as the whole numbers.

$$\text{Field Emergence Index (FEI)} = \frac{\text{No. of seedling emerged}}{\text{1st day of sowing}} + \dots + \frac{\text{No. of seedling emerged}}{\text{Day of the last count}}$$

The seedling establishment was determined when the total number of seedlings emerged i.e. there was no more addition in the total emerged seedlings.

In the laboratory, three replications of 100 seeds were uniformly placed in between wet paper. The samples were placed in a germinator at a temperature (20°C) in a completely randomized design. For between paper methods, the final count was observed on the 8th day as per ISTA (2011) [11]. Data collected on the basis of number of germinated seeds, normal seedlings, seedling length, seedling dry weight, vigor index-I, vigor index-II, field emergence index and seedling establishment as recommended by ISTA (2011) [11]. The final count day after germination is used to collect data for the germination percentage by counting the number of germinated seeds, normal seedlings, abnormal seedlings, hard seeds, and infected seedlings.

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

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

For the observation of field parameters i.e. field emergence index, hundred seeds of all the treatments in three replications in Randomized Block Design (RBD). The number of seedling emerged out of soil were counted daily up to the seedling establishment. Field emergence index was calculated using formula as:

Statistical analysis

Statistical analysis of data collected during the study was done by using the factorial complete randomized design as

described by Panse and Sukhatme (1985) [18]. 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 use of chemical fertilizers and pesticides causes the unfavorable impact on the environment. Extensive research was carried out to minimize the use of chemical fertilizers and improve soil fertility status and enhancing the crop production by their biological activity in the rhizosphere. The effect of various seed treatment viz. *Azotobacter*, Chlorpyrifos and Vitavax are analyzed on speed of germination, germination percentage, seedling length, seedling dry weight, vigor index-I, vigor index-II, field emergence index and seedling establishment of wheat. The seed quality parameters bear up the performance of the wheat seed of WH1105 and WH1124 of both the old and fresh seed lots.

The mean performance specifically to the lots and the varieties of WH1105 and WH1124 are cited in table (3, 4, 5 and 6). The mean performance of speed of germination precisely to the lots and the varieties of WH1105 and WH1124 is cited in table 3. The highest speed of germination was recorded in T₆-Chlorpyrifos+*Azotobacter* (57.97) followed by T₃-*Azotobacter* (53.49), while the lowest was observed in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (46.06) as compared to T₀-control (46.36) in WH1105. The same trend was recorded in WH1124 i.e., the maximum speed of germination observed in T₆-Chlorpyrifos+*Azotobacter* (58.09) followed by T₃-*Azotobacter* (55.18), while the minimum speed of germination was recorded in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (48.01) as compared to T₀-control (49.94). From the second day, treatment T₆-Chlorpyrifos+*Azotobacter* had begun to show their impact on embryo protrusion. It might be due to the Chlorpyrifos used as solute and water as a solvent to make a solution for treatment to the seed and that solvent (water) helps to seed for early embryo protrusion along with the impact of biofertilizers for providing the required nutrient to seed.

In WH1105, the maximum enhancement of germination was observed in treatment T₃-*Azotobacter* (93.67%) which was at par with T₅-Vitavax+*Azotobacter* (91.00%) and minimum was in T₁-Chlorpyrifos (84.67%) as compared to T₀-control (89.00%). The similar trend was observed in WH1124 i.e., the maximum germination was observed in T₃-*Azotobacter* (94.83%) followed by T₅-Vitavax+*Azotobacter* (91.67%) and was minimum in T₁-Chlorpyrifos (85.67%) as compared to T₀-control (90.17%) mentioned in table 4. These findings confirm the results of Biswas *et al.* (2015) when *Azotobacter* was utilized as seed treatment and in conjunction with soil application of *A. chroococum*.

The mean performance of seedling length precisely to the lots and the varieties of WH1105 and WH1124 is shown in figure 1. In WH1105, the maximum seedling length was observed in treatment T₃-*Azotobacter* (31.39 cm), at par with T₆-Chlorpyrifos+*Azotobacter* (28.68 cm) and minimum seedling length was in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (25.24 cm) as compared to T₀-control (26.43 cm). In WH1124, the highest seedling length was observed in T₃-*Azotobacter* (31.90 cm) followed by T₆-Chlorpyrifos+*Azotobacter* (29.38 cm) and lowest seedling length was in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (25.94 cm) as compared to T₀-control (27.02 cm). These findings confirm the results of Singh and Prasad (2011) in wheat. The mean performance of seedling

dry weight specifically to the lots and the varieties of WH1105 and WH1124 is presented in figure 2. In WH1105, the maximum seedling dry weight was observed in treatment T₃-*Azotobacter* (14.18 mg), at par with T₆-Chlorpyrifos+*Azotobacter* (13.07 mg) and minimum seedling dry weight was in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (11.32 mg) as compared to T₀-control (11.82 mg). In WH1124, the highest seedling dry weight was observed in T₃-*Azotobacter* (14.44 mg) followed by T₆-Chlorpyrifos+*Azotobacter* (13.27 mg) and lowest seedling dry weight was in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (11.75 mg) as compared to T₀-control (12.24 mg).

The mean performance of vigor index-I specifically to the lots and the varieties of WH1105 and WH1124 is mentioned in table 5. The maximum vigor index-I was observed in treatment T₃-*Azotobacter* (2942), followed by T₅-Vitavax+*Azotobacter* (2559) and minimum was in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (2163) as compared to T₀-control (2355) in WH1105. The similar trend was observed in WH1124 i.e., the highest vigor index-I was observed in T₃-*Azotobacter* (3027) followed by T₅-Vitavax+*Azotobacter* (2654) and was lowest in T₁-Chlorpyrifos (2238) as compared to T₀-control (2238). The mean performance of vigor index-II precisely to the lots and the varieties of WH1105 and WH1124 is cited in table 6. The highest vigor index-II was recorded in T₃-*Azotobacter* (1330) followed by T₆-Chlorpyrifos+*Azotobacter* (1158), while the lowest was observed in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (971) as compared to T₀-control (1053) in WH1105. The same trend recorded in WH1124 i.e., the maximum vigor index-II was observed in T₃-*Azotobacter* (1371) followed by T₆-Chlorpyrifos+*Azotobacter* (1188), while the minimum vigor index-II was recorded in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (1016) as compared to T₀-control (1105). Kumar *et al.* (2018) [13] confirms the results of seedling length, seedling dry weight, vigor index I and vigor index II in maize crop.

The mean performance of field emergence index specifically to the lots and the varieties of WH1105 and WH1124 is revealed in figure 3. In WH1105, the maximum field emergence was observed in treatment T₃-*Azotobacter* (8.58), at par with T₂-Vitavax (7.91) and minimum was in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (4.58) as compared to T₀-control (6.07). The similar trend was observed in WH1124 i.e., the highest field emergence index was observed in T₃-*Azotobacter* (8.79) followed by T₂-Vitavax (8.03) and was lowest in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (4.69) as compared to T₀-control (6.48). The mean performance of seedling establishment precisely to the lots and the varieties of WH1105 and WH1124 is shown in figure 4. In WH1105, the maximum seedling establishment was observed in treatment T₃-*Azotobacter* (87.84), at par with T₂-Vitavax (84.17%) and minimum seedling establishment was in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (77.17%) as compared to T₀-control (81.50%). In WH1124, the highest seedling establishment was observed in T₃-*Azotobacter* (88.84%) followed by T₂-Vitavax (84.84%) and lowest seedling establishment was in T₇-Chlorpyrifos+Vitavax+*Azotobacter* (78.33%) as compared to T₀-control (82.50%).

The best results of germination percentage, seedling length, seedling dry weight, vigor index-I, vigor index-II, field emergence index and seedling establishment were observed due to the seed treated with T₃-*Azotobacter*. Seed treatment of

only *Azotobacter* treatment recorded significantly highest among all parameters as compared to other treatments and combinations. It might be due to the biofertilizers helped the seed in mobilizing the essential nutritional elements from non-usable to usable form via biological processes and nitrogen fixing properties of bio inoculants along with the encouragement to seed for nutrient uptake as discussed by Me Carty *et al.* (2017) [17] in wheat by using *Azotobacter* and PSB. The outcomes and results were in favour of biofertilizer treatments to enhance the performance of seed quality as reported by El-Sirafy *et al.* (2006) [7] in wheat, Zaki *et al.*

(2009) [22] in rice, Mahato *et al.* (2017) [15] in maize. Chlorpyrifos showed an adverse effect individually, as well as in combination with Vitavax and *Azotobacter* on amongst the parameters it might be due to the chlorpyrifos depressed the nitrogen metabolism, amylase, and ATPs activities, impaired respiration and caused the inhibition of normal cell division or elongation and depressed the late germination of the seedling and caused the lowest germination, seedling length, seedling dry weight, and vigor indices, Dalvi *et al.* (1972) [5] in wheat and mungbean. Using biofertilizer treatments is strongly recommended over the use of insecticides and fungicides.

Table 3: Effect of seed treatments on Speed of germination of wheat

Treatments (T)	Variety (V)					
	WH1105			WH1124		
	Old	Fresh	Mean	Old	Fresh	Mean
T ₀	43.36	49.36	46.36	48.11	51.76	49.94
T ₁	47.38	53.11	50.25	49.74	54.65	52.19
T ₂	46.11	51.24	48.68	48.82	53.80	51.31
T ₃	51.04	55.95	53.49	52.98	57.38	55.18
T ₄	45.33	50.23	47.78	47.46	52.35	49.90
T ₅	48.55	54.33	51.44	50.61	55.12	52.86
T ₆	56.92	59.01	57.97	56.66	59.53	58.09
T ₇	43.89	48.23	46.06	46.79	49.23	48.01
	V	L	T	V×L	V×T	L×T
C.D. (P=0.05)	0.570	0.570	1.139	NS	NS	NS
S.Em (±)	0.202	0.202	0.403	0.285	0.570	0.570

Table 4: Effect of seed treatments on Germination (%) of wheat

Treatments (T)	Variety (V)					
	WH1105			WH1124		
	Old	Fresh	Mean	Old	Fresh	Mean
T ₀	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	83.33 (65.88)	86.00 (68.00)	84.67 (66.94)	84.00 (66.40)	87.33 (69.13)	85.67 (67.76)
T ₂	87.00 (68.85)	92.33 (73.90)	89.67 (71.38)	88.00 (69.71)	93.67 (75.43)	90.83 (72.58)
T ₃	91.33 (72.86)	96.00 (78.49)	93.67 (75.67)	92.67 (74.29)	97.00 (80.08)	94.83 (77.19)
T ₄	87.33 (69.13)	93.00 (74.69)	90.17 (71.91)	88.33 (70.02)	94.00 (75.82)	91.17 (72.92)
T ₅	88.00 (69.72)	94.00 (76.05)	91.00 (72.88)	88.67 (70.31)	94.67 (76.70)	91.67 (73.51)
T ₆	85.67 (67.74)	91.33 (72.87)	88.50 (70.30)	86.33 (68.28)	92.67 (74.29)	89.50 (71.29)
T ₇	84.00 (66.39)	87.33 (69.16)	85.67 (67.78)	84.67 (66.93)	88.33 (70.00)	86.50 (68.47)
	V	L	T	V×L	V×T	L×T
C.D. (P=0.05)	0.473	0.473	0.946	NS	NS	1.337
S.Em (±)	0.167	0.167	0.335	0.237	0.473	0.473

Table 5: Effect of seed treatments on Vigor index-I of wheat

Treatments (T)	Variety (V)					
	WH1105			WH1124		
	Old	Fresh	Mean	Old	Fresh	Mean
T ₀	2202	2507	2355	2296	2581	2438
T ₁	2087	2263	2175	2165	2310	2238
T ₂	2286	2604	2445	2417	2700	2559
T ₃	2759	3126	2942	2848	3207	3027
T ₄	2220	2497	2359	2325	2566	2445
T ₅	2387	2730	2559	2485	2822	2654
T ₆	2367	2716	2542	2454	2810	2632
T ₇	2079	2247	2163	2159	2329	2244
	V	L	T	V×L	V×T	L×T
C.D. (P=0.05)	42.152	42.152	84.303	NS	NS	NS
S.Em (±)	14.917	14.917	29.834	21.095	42.191	42.191

Table 6: Effect of seed treatments on Vigor index-II of wheat

Treatments (T)	Variety(V)					
	WH1105			WH1124		
	Old	Fresh	Mean	Old	Fresh	Mean
T ₀	981	1124	1053	1049	1160	1105
T ₁	918	1038	978	982	1064	1023
T ₂	987	1122	1055	1042	1153	1098
T ₃	1259	1401	1330	1312	1429	1371
T ₄	1084	1210	1147	1111	1248	1180
T ₅	1048	1182	1115	1080	1217	1149
T ₆	1090	1226	1158	1121	1256	1188
T ₇	916	1026	971	981	1052	1016
	V	L	T	V×L	V×T	L×T
C.D. (P=0.05)	19.068	19.068	38.137	NS	NS	NS
S.Em (±)	6.748	6.748	13.496	9.543	19.086	19.086

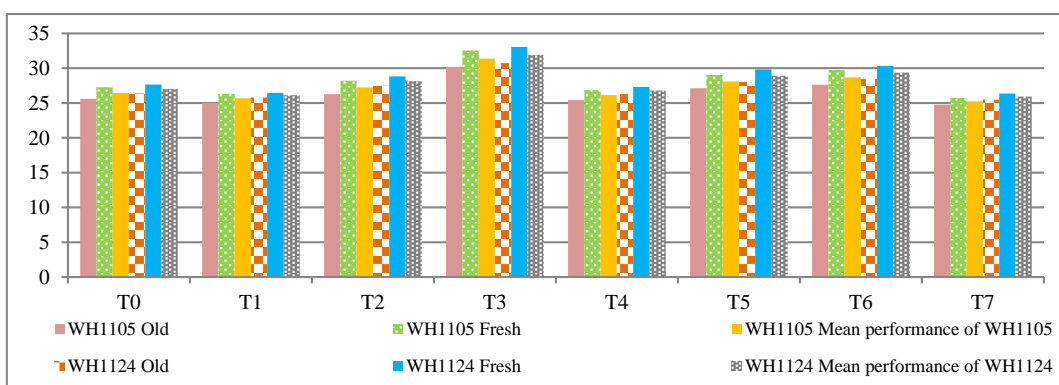


Fig 1: Effect of seed treatments on Seedling length (cm.) of wheat

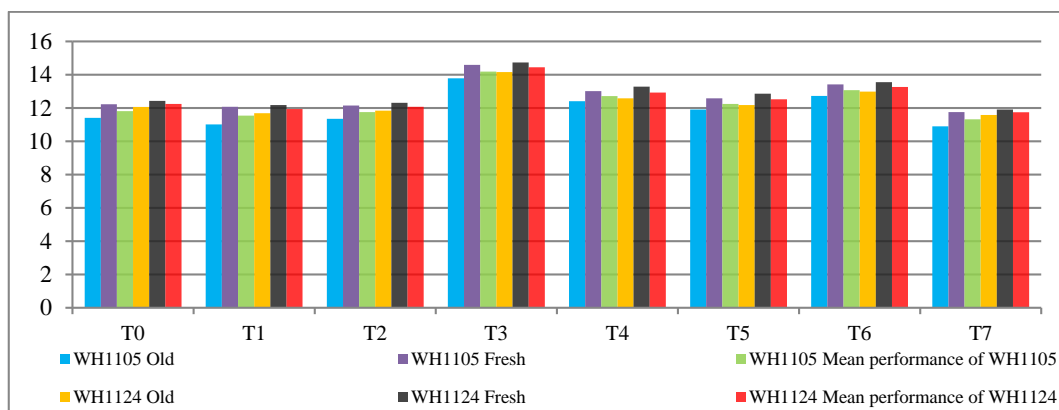


Fig 2: Effect of seed treatments on seedling dry weight of wheat

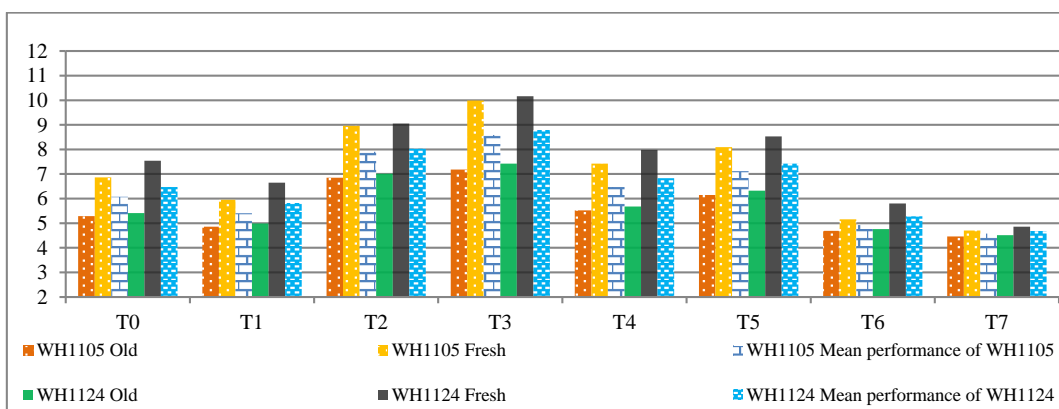


Fig 3: Effect of seed treatments on field emergence index of wheat

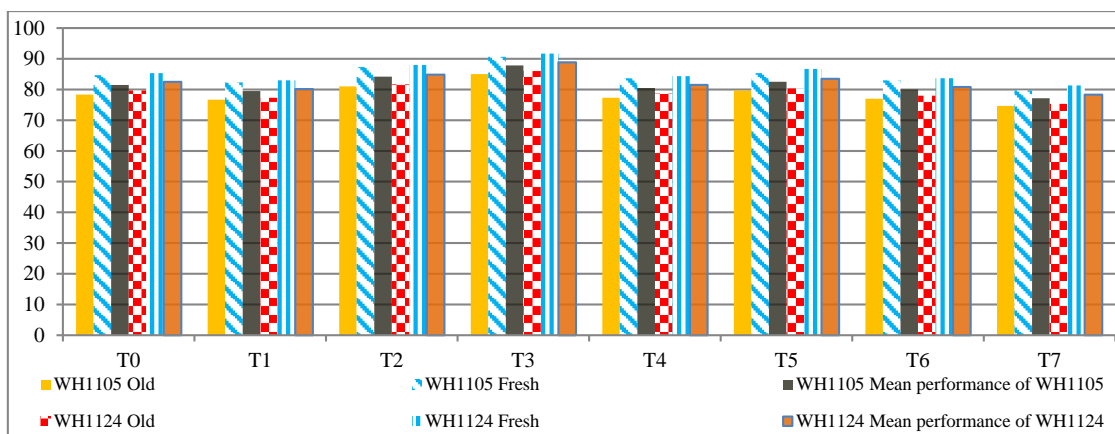


Fig 4: Effect of seed treatments on seedling establishment (%) of wheat

Conclusion

Based on the current research findings, it can be affirmed that the application of *Azotobacter* in seed treatment significantly improved various aspects of wheat seed quality. On the other hand, the use of Chlorpyrifos had a detrimental effect on the quality of wheat seeds.

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