Original Research Article

Appraisingthe Biochemical Responses in Wheat (*Triticum aestivum* L.) Seedlings under Various Seed Treatments

Abstract

A study was conducted to analyse the effect of pesticides and biofertilizers on enzymatic activity of dehydrogenase, catalase, peroxidase and superoxide dismutase (SOD) in seedlings of wheat (*Triticum aestivum* L.) varieties (WH1105 and WH1124) and their old and fresh seed lots. This experiment was conducted in the laboratoriesat the Department of Seed Science and Technology, CCS Haryana Agricultural University in Hisar during 2020. A total of 16 treatment combinations were applied to the seeds in the experiment, including a control group, which were subsequently used for germination. The seedlings of the wheat cultivars were germinated in between paper at controlled temperature 20°C. 4 days old germinated seedlings were used for the analyses of the biochemical parameters. Thisfinding revealed that the seedlings germinated from seedstreated with T_5-*Azotobacter*+PSBexhibited highestbiochemical activity. The application of biofertilizers enhanced the dehydrogenase, catalase, peroxidase and superoxide dismutase enzyme activities, whereas the T_1-Chlorpyrifos reduces the biochemical activities of the wheat seedling. The results demonstrated that Chlorpyrifos had an adverse effect on the biochemical activities of wheat seedlings.

Keyword: Biochemical, Catalase, Dehydrogenase, Enzyme activity, Peroxidase, Superoxide dismutase, Wheat.

Introduction

Plants are equipped with a robust antioxidant defense system, consisting of both enzymatic and non-enzymatic elements. Among the non-enzymatic components are carotenoids, ascorbate, glutathione, and tocopherols (Vitamin E). During various stress conditions, it has been observed that reactive oxygen species, including hydrogen peroxide (H_2O_2), superoxide (O^{2-}), and hydroxyl radicals (OH), are produced. In conjunction with these, there are antioxidants enzymes like superoxide dismutase (SOD), catalase, and those involved in the ascorbate-glutathione cycle.SOD plays a fundamental role in this defense system by transforming two molecules of superoxide into a lesser amount of harmful substances, namely hydrogen peroxide (H_2O_2) and molecular oxygen (O_2)[1]. In plants, three distinct types of superoxide dismutase (SOD) enzymes (EC 1.15.1.1) have been identified, each characterized by its specific metal cofactor. These are Manganese SOD (MnSOD), which is located in the mitochondrial matrix. Copper/Zinc SOD (Cu/ZnSOD) primarily localised in the cytosolic. Iron SOD (FeSOD), which is localised in the chloroplastic stroma [2]. Additionally, some plants may also have Cu/Zn SOD in their chloroplasts. It's worth noting that Cu/ZnSOD is sensitive to both hydrogen peroxide (H_2O_2) and potassium cyanide (KCN), while FeSOD is responsive to H_2O_2 but not KCN. On the other hand, MnSOD displays insensitivity to both H_2O_2 and KCN, making it distinct from the other two SOD types [3]. Plant peroxidases have served as valuable biochemical indicators for different forms of biotic and abiotic stresses. This is attributed to their essential involvement in critical physiological processes, such as regulating growth through lignification, forming cross-links in pectins and structural proteins within cell walls, and participating in the breakdown of auxins[4]. Catalases and superoxide dismutases stand out as the most effective antioxidant enzymes [5]. These enzymes include superoxide dismutase (SOD, EC 1.15.1.1) which reacts with superoxide radicals and converts them to O_2 and H_2O_2. H_2O_2 is then detoxified by catalase (CAT, EC 1.11.1.6). The endosperm of mature seeds contains over 250 proteins that play a role in 13 different biochemical processes, including reactions related to ATP inter-conversion, during germination [6]. As respiration becomes more intense during germination, there is a concurrent increase in the release of inorganic phosphorus and cellular energy. This heightened respiration plays a crucial role in generating the energy needed for the normal progression of metabolic processes. Following this phase, the synthesis of new proteins, nucleic acids, and lipids occurs, while the stored reserves serve as nutrients for the growing seedling [7]. This study is focused on assessing the impact of various environmental pollutants resulting from the extensive use of substances like insecticides and fungicides, and how therole played by certain biofertilizers in the plant development process. Consequently, the current research aims to investigate how Chlorpyrifos, Vitavax, *Azotobacter* and PSB, individually and in various combinations, influenceenzymatic activity of dehydrogenase, catalase, peroxidase and superoxide dismutase of the wheat seedlings.

Materials and Methods

This study was carried out during the year 2020 at the laboratory of the Department of Seed Science and Technology and the seed material were obtained from breeder seed store, Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar, situated in the semi-tropical region in the north-western zone of India. For this study, wheat varieties WH1105 and WH1124 were taken, along with one-year-old harvested seeds and freshly harvested seed lots for each variety.

Seed treatment

The seeds were primed with different treatments, including 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) and their combinations of total 16 treatments including control as shown in table 1. To enhance the adhesion of biofertilizers to the seed surface, a 10 percent jaggery solution was used, followed by shade exposure for some time to the treated seeds. Afterward, the treated seeds were packaged in malleable zippered bags and used for sowing purpose.

Table 1: Various treatments and their combinations

S. No.	Treatments	S. No.	Treatments
T_0	Control	T_8	Vitavax+*Azotobacter*
T_1	Chlorpyrifos 20EC	T_9	Vitavax+*Azotobacter*+PSB
T_2	Vitavax	T_{10}	Chlorpyrifos+*Azotobacter*
T_3	*Azotobacter*	T_{11}	Choloropyrifos+PSB
T_4	Phosphate Solubilizing Bacteria (PSB)	T_{12}	Chlorpyrifos+*Azotobacter*+PSB
T_5	*Azotobacter*+PSB	T_{13}	Chlorpyrifos+Vitavax+*Azotobacter*
T_6	Vitavax+Chlorpyrifos	T_{14}	Chlorpyrifos+Vitavax+PSB
T_7	Vitavax+PSB	T_{15}	Choloropyrifos+Vitavax+*Azotobacter*+ PSB

Seedlings were grown from seeds in the between paper at controlled temperature at 20^oC. In this study, enzymatic activity of dehydrogenase (DHA), catalase, peroxidase (POX) and superoxide dismutase (SOD) were studied on the 4th day seedlings during germination after the various treatments *i.e.*, Chlorpyrifos, vitavax, *Azotobacter* and PSB with their combinations. The germinated seedlings were used for enzyme extraction.

Tissue extraction

The steps, which involve the extraction process, were carried out at a temperature range of 0-4°C. The1 g of plant tissue from 4th day germinated seedlings were finely ground in a chilled mortar and pestle while being mixed with 5 ml of 0.1 M phosphate buffer with a pH of 7.5. Three sets of biological samples (leaf tissue) were collected to create enzyme extracts. This buffer solution also contained 5% (w/v) polyvinylpyrrolidone (PVP), 10mM β-mercaptoethanol, and 1mM EDTA.The resulting homogenate was subjected to centrifugation using a centrifuge operating at 10,000 rpm for 20 minutes at 4°C. Following the centrifugation process, the liquid portion, known as the supernatant, was carefully separated and utilized as a crude enzyme preparation, following the method outlined by Sinha [8].These extracts were used for all the enzyme assays except dehydrogenase (DHA).

Determination of the Dehydrogenase Activity (O D $g^{-1} ml^{-1}$)

The Dehydrogenase activity (DHA) test was conducted following the method outlined by Kittock and Law [9]. Each seedling lot, represented by one gram of seedlings and replicated thrice, was ground properly. The resulting 200 mg of extract was soaked in 5 ml of a 0.5% tetrazolium solution at 38°C for 3 hours. Afterward, it was centrifuged at 10,000 rpm for 3 minutes, and the supernatant was carefully decanted. The formazan, a red-colored compound, was extracted from the mixture using 10 ml of acetone over a 16-hour period. Following this, another round of centrifugation was performed, and the absorbance of the resulting solution was measured at 480 nm using an Eppendorf Biospectrometer. These observations were expressed as changes in optical density per gram per milliliter (OD g^{-1} ml^{-1}).

Determination of the Catalase activity (CAT)

In this process of Catalase activity (CAT) (EC 1.11.1.6), a mixture was created by combining 0.55 ml of 0.1 M potassium phosphate buffer at pH 7.0, 0.4 ml of 0.2 M H_2O_2, and 50 µl of enzyme extract. This mixture was thoroughly mixed and incubated for one minute. Subsequently, 3.0 ml of a 5% potassium dichromate: acetic acid (1:3) solution was added. A control sample was also prepared, which included 0.6 ml of assay buffer and 0.4 ml of 0.2 M H_2O_2, but without the enzyme extract, alongside the test samples. The tubes containing these mixtures were then placed in a boiling water bath for 10 minutes, allowed to cool, and their absorbance was measured at 570 nm using the dichromate: acetate solution as a reference. Calculations were carried out by subtracting the absorbance of the samples from that of the control, and the quantity of H_2O_2 was determined using a standard curve. One catalase (CAT) unit is defined as the amount of enzyme required to decompose one µmol of H_2O_2 per minute or per milligram of protein.

Determination of the Peroxidase activity (POX)

In Peroxidase activity (POX) (EC 1.11.1.7) a glass cuvette was filled with 2.15 ml of 0.1 M Tris-HCl buffer at pH 7.0 was carefully measured. To this, 0.6 ml of a 1% guaiacol solution and 0.1 ml of the enzyme extract were added. The solution was thoroughly mixed and adjusted to achieve 100% light transmission at 470 nm. Then, 0.15 ml of 100 mM H_2O_2 was introducedand mixed, and then the initial reading was taken at 470 nm. Subsequently, the increase in absorbance was recorded at 15-second intervals for duration of 3 minutes. The enzyme activity was calculated using the linear segment of the optical density (OD) change, utilizing a molar extinction coefficient of 26.6 mM^{-1} cm^{-1} for guaiacol oxidation. One unit of peroxidase (POX) activity is defined as the amount of enzyme needed to oxidize one nmol of guaiacol per minute, per milliliter, or per milligram of protein.

Determination of the SOD activity

To measure superoxide dismutase (SOD, EC 1.15.1.1) (SOD) activity, the method employed involved evaluating its capacity to impede the photochemical reactions involving nitro blue tetrazolium (NBT), following the procedure outlined by Beauchamp andFridovich [10]. In this procedure, a reaction mixture of 3.0 ml was prepared, consisting of 2.5 ml of 60 mM Tris-HCl at pH 7.8, along with 0.1 ml each of 420 mM L-methionine, 1.80 mM NBT, 90 µM riboflavin, 3.0 mM EDTA, and 0.1 ml of the enzyme extract. Riboflavin was introduced at the end of the preparation. The tubes were thoroughly shaken and positioned 30 cm below a light source comprising three 20 W fluorescent lamps. The reaction was initiated by switching on the light and halted after a 40-minute incubation period by turning off the light source. To shield the tubes from further light exposure, they were covered with a black cloth. A control was maintained, consisting of a non-irradiated reaction mixture that did not exhibit any color change. The maximum color development was observed in the reaction mixture without the enzyme extract. The absorbance was measured at 560 nm, and one enzyme unit was defined as the amount of enzyme required to inhibit the photo-reduction of one µmol of NBT. To express the enzyme activity in terms of units per gram of fresh weight, it was calculated using the standard formula by Giannopolitis and Ries [11], which is commonly used for assessing kinetic and regulatory properties.

SOD units =R-r divided by r

Where,

R = Rate of assay reaction in absence of SOD.

r = Rate of assay reaction in presence of SOD.

However, calculate the percent inhibition by the following formula of Asada *et al*. [12]:

Per cent inhibition = R-r divided by R × 100

Statistical analysis

The final data attained was subjected to the analysis using online statistical analysis tool OPSTAT by Sheoran [13].

Results and Discussion

This study focuses on evaluating the effects of different seed treatments, both when applied individually and in various combinations like *Azotobacter*, PSB, Chlorpyrifos, and Vitavax, on the biochemical activities of wheat seedlings.The dehydrogenase activity of seedlings, germinated from treated seeds is presented in table2 of wheat in both the lots old and fresh of WH1105 and WH1124. Dehydrogenase (DHA) activity was enhanced with treatment of biofertilizers, the activity indicated as (optical density) OD value (O.D. g^{-1} ml^{-1}). The overall highest DHA activity was recorded in treatment T_5-*Azotobacter*+PSB (0.250), followed by T_4-PSB (0.238) and lowest was observed in T_1-Chlorpyrifos (0.183) as compared to T_1-control (0.200).The mean performance includesthe both lots of the variety WH1105, the maximum enhancement of DHA activity was observed in treatment T_5-*Azotobacter*+PSB (0.248), followed by T_4-PSB (0.237) and lowest was observed in T_5-Chlorpyrifos (0.181) as compared to T_1-control (0.199). The same performance was recorded in both the lots of the variety WH1124, the maximum DHA activity was observed in treatment T_5-*Azotobacter*+PSB (0.251), followed by T_4-PSB (0.239), while the lowest observedT_1-Chlorpyrifos (0.185) in comparison of T_1-control (0.202).

Table 3 displays the catalase activities of seedlings that have germinated from treated seeds reported in µmoles g^{-1} fresh weight of wheat in old and fresh lots of WH1105 and

WH1124. The overall maximum catalase activity was observed in treatment T_5-*Azotobacter*+PSB (5637), followed by T_4-PSB (5400) and minimum was recorded in T_1-Chlorpyrifos (4305) as compared to T_1-control (4430). The mean observations involves the

Table 2:- Effect of various treatments on Dehydrogenase activity (DHA) (O.D. g^{-1} ml^{-1}) in wheat seedlings

S. No.	**VARIETY(V)**						**Overall Mean**
	WH1105			**WH1124**			
	Old	**Fresh**	**Mean**	**Old**	**Fresh**	**Mean**	
T_0	0.195	0.202	0.199	0.198	0.206	0.202	0.200
T_1	0.177	0.185	0.181	0.181	0.188	0.185	0.183
T_2	0.203	0.210	0.207	0.207	0.214	0.211	0.209
T_3	0.231	0.236	0.234	0.233	0.237	0.235	0.234
T_4	0.234	0.239	0.237	0.236	0.241	0.239	0.238
T_5	0.243	0.253	0.248	0.246	0.256	0.251	0.250
T_6	0.205	0.213	0.209	0.209	0.218	0.214	0.211
T_7	0.211	0.217	0.214	0.214	0.220	0.217	0.216
T_8	0.208	0.214	0.211	0.209	0.218	0.214	0.212
T_9	0.224	0.228	0.226	0.227	0.231	0.229	0.228
T_{10}	0.197	0.204	0.201	0.203	0.206	0.205	0.203
T_{11}	0.209	0.215	0.212	0.212	0.219	0.216	0.214
T_{12}	0.214	0.221	0.218	0.218	0.226	0.222	0.220
T_{13}	0.181	0.187	0.184	0.183	0.193	0.188	0.186
T_{14}	0.201	0.208	0.205	0.205	0.211	0.208	0.206
T_{15}	0.184	0.190	0.187	0.186	0.194	0.190	0.189
Mean	0.207	0.214		0.210	0.217		
	V	**L**	**T**	**V×L**	**V×T**	**LxT**	**VxLxT**
C.D. (P=0.05)	0	0	0.001	NS	NS	0.001	NS
SE.m (±)	0	0	0	0	0.001	0.001	0.001

Table 3: Effect of various treatments on Catalase activity (µmoles g^{-1}fresh weight) in wheat seedlings

S. No.	VARIETY(V)						Overall Mean
	WH1105			WH1124			
	Old	**Fresh**	**Mean**	**Old**	**Fresh**	**Mean**	
T_0	4,392	4,448	4,420	4,405	4,473	4,439	4,430
T_1	4,194	4,320	4,257	4,277	4,428	4,353	4,305
T_2	4,442	4,630	4,536	4,474	4,726	4,600	4,568
T_3	4,927	5,320	5,123	5,060	5,491	5,276	5,200
T_4	5,039	5,562	5,300	5,226	5,775	5,501	5,400
T_5	5,282	5,767	5,524	5,553	5,947	5,750	5,637
T_6	4,467	4,706	4,586	4,505	4,822	4,664	4,625
T_7	4,642	4,985	4,814	4,840	5,043	4,942	4,878
T_8	4,495	4,808	4,652	4,677	4,935	4,806	4,729
T_9	4,838	5,174	5,006	4,951	5,329	5,140	5,073
T_{10}	4,401	4,460	4,430	4,424	4,491	4,458	4,444
T_{11}	4,532	4,911	4,722	4,715	4,989	4,852	4,787
T_{12}	4,723	5,036	4,880	4,893	5,135	5,014	4,947
T_{13}	4,245	4,357	4,301	4,311	4,432	4,372	4,336
T_{14}	4,403	4,518	4,461	4,438	4,591	4,515	4,488
T_{15}	4,311	4,406	4,359	4,380	4,437	4,409	4,384
Mean	4,583	4,838		4,696	4,940		
	V	**L**	**T**	**V×L**	**V×T**	**LxT**	**VxLxT**
C.D. (P=0.05)	3.53	3.53	9.98	4.99	14.11	14.11	19.95
SE.m (±)	1.26	1.26	3.57	1.78	5.04	5.04	7.13

both the lots of WH1105, the maximum catalase activity in treatment T_5-*Azotobacter*+PSB (5524), followed by T_4-PSB (5300) and lowest was observed in T_5-Chlorpyrifos (4257) as compared to T_1-control (4420). The same findings were observed in both the lots of the variety WH1124, the maximum catalase activity was recorded in treatment T_5-*Azotobacter*+PSB (5750), followed by T_4-PSB (5501), while the minimum observed T_1-Chlorpyrifos (4353) in comparison of T_1-control (4439).These findings confirms by Singh *et al.* [14] Catalase (CAT) activity showed an increase in maize leaves when they were inoculated with *Azotobacter chroococcum* and the combination of *Azotobacter chroococcum*with *Bacillus polymyxa*.Seyed Sharifi *et al.* [15] revealed that the application of biofertilizers and nano-oxides enhanced the proline content, soluble sugars, catalase, peroxidase, and polyphenol oxidase enzyme activities of wheat.

The table 4 comprises the peroxidase activities (POX)of seedlings that have germinated from the various treatment combinations, presented as μmoles per gram of fresh weight, in both old and fresh seed lots of WH1105 and WH1124 wheat varieties.The overall maximum peroxidase activity was observed in T_5-*Azotobacter*+PSB (31.71) followed by T_4-PSB (29.21) whereas the minimum peroxidase activity was observed in T_2-Chlorpyrifos(11.26)as compared to T_1-control (13.53).The mean results includes thelots of WH1105, the maximum peroxidase activity in treatment T_5-*Azotobacter*+PSB (29.84), followed by T_4-PSB (27.51) and lowest was observed in T_5-Chlorpyrifos (9.58) as compared to T_1-control (11.85).The similar result was observed in both the lots of WH1124, the highest peroxidase activity in treatment T_5-*Azotobacter*+PSB (33.57), followed by T_4-PSB (30.92) and lowest was observed in T_5-Chlorpyrifos (12.94) as compared to T_1-control (15.21).Several reports confirms the results in terms of biochemicalenzyme activities, the use of a double full dose of organic manure, in combination with the *Azotobacter* and *Pseudomonas* inoculum, resulted in an increase in both peroxidase and catalase activities. These findings in sorghum supported by Amal *et al.* [16].These results also confirmed in wheat by Babaei *et al.* [17].

The data shown in table 5 presents the superoxide dismutase (SOD) activities in germinated seedlings using various treatments, measured in μmoles per gram of fresh weight. It specifically focuses on superoxide dismutase activities in both old and fresh seed lots of WH1105 and WH1124 wheat varieties. The overall mean performance superoxide dismutase activities (SOD) was recorded highest in T5-*Azotobacter+*PSB (112.23) treatment, followed by T_4- Phosphate Solubilizing Bacteria (109.59) and minimum in T_2-Chlorpyrifos (89.26) in comparison ofT_1-control (93.35). The mean performance of superoxide dismutase activities of both the lots of WH1105, the maximum SOD activity was observed in treatment T_5-*Azotobacter*+PSB (110.21) which was at par with T_4-PSB (107.19) and minimum in T_2-Chlorpyrifos (88.90) as compared to T_1-control (91.98). The same performance was recorded in both the lots of the WH1124, the maximum superoxide dismutase activity was observed in treatment T_5-*Azotobacter*+PSB (114.25), followed by T_4-PSB (111.99), while the lowest observed T_1-Chlorpyrifos (89.63) in comparison of T_1-control (94.71). The fresh seed lot of WH1124 recorded the highest enzymatic activities among other lots.In maize, the presence of microbes such as., *Serendipita indica, Rhizophagusintraradices,* and *Azotobacter chroococcum* led to increase in the activities of antioxidant enzymes, including peroxidase (POX), catalase (CAT), polyphenol oxidase (PPO), and superoxide dismutase (SOD), when compared to control plants, these results were supported by Tyagi *et al.* [18].*Paenibacillus* sp.+*Bacillus subtilis* (PGPR) (biofertilizers) showed a maximum increasein superoxidase dismutase (SOD), peroxidase dismutase (POD), catalase (CAT) in wheat. These results confirmed by Iqbal *et al.* [19]. Ithas been reported by Srivalli [20] that the activity of superoxide dismutase and peroxidase was recorded higher as compared to the control depending on plant species, tissue and stage of development.The present study revealed that

Table 4:- Effect of various treatments on Peroxidase activity (µmoles g^{-1}fresh weight) in wheat seedlings

S. No.	**VARIETY(V)**						**Overall Mean**
	WH1105			**WH1124**			
	Old	**Fresh**	**Mean**	**Old**	**Fresh**	**Mean**	
T_0	9.66	14.04	11.85	12.14	18.27	15.21	13.53
T_1	7.84	11.32	9.58	10.50	15.37	12.94	11.26
T_2	12.14	19.19	15.67	15.24	22.14	18.69	17.18
T_3	20.20	30.47	25.33	24.27	33.36	28.82	27.07
T_4	22.46	32.55	27.51	26.34	35.49	30.92	29.21
T_5	24.47	35.22	29.84	28.77	38.37	33.57	31.71
T_6	12.95	21.23	17.09	16.84	23.56	20.20	18.65
T_7	15.41	25.31	20.36	19.32	27.87	23.60	21.98
T_8	13.43	22.76	18.10	18.22	25.20	21.71	19.90
T_9	17.42	28.56	22.99	22.25	31.47	26.86	24.92
T_{10}	9.69	15.42	12.55	13.18	19.35	16.26	14.41
T_{11}	14.18	23.88	19.03	18.87	26.26	22.57	20.80
T_{12}	16.18	27.31	21.75	20.23	29.15	24.69	23.22
T_{13}	8.37	12.31	10.34	11.09	16.70	13.90	12.12
T_{14}	10.17	18.13	14.15	13.44	21.23	17.34	15.74
T_{15}	9.06	13.07	11.07	11.93	17.20	14.57	12.82
Mean	13.98	21.92		17.66	25.06		
	V	**L**	**T**	**V×L**	**V×T**	**LxT**	**VxLxT**
C.D. (P=0.05)	0.041	0.041	0.117	0.058	0.165	0.165	0.233
SE.m (±)	0.015	0.015	0.042	0.021	0.059	0.059	0.083

Table 5:- Effect of various treatments on Superoxide dismutase activity (µmoles g^{-1}fresh weight) in wheat seedlings

S. No.	VARIETY(V)						Overall Mean
	WH1105			WH1124			
	Old	Fresh	Mean	Old	Fresh	Mean	
T_0	90.01	93.95	91.98	91.24	98.19	94.71	93.35
T_1	87.73	90.06	88.90	88.09	91.16	89.63	89.26
T_2	91.70	97.15	94.42	94.84	99.87	97.35	95.89
T_3	102.33	108.38	105.36	107.48	112.55	110.02	107.69
T_4	103.55	110.83	107.19	108.52	115.46	111.99	109.59
T_5	106.81	113.61	110.21	110.95	117.55	114.25	112.23
T_6	93.63	99.15	96.39	95.22	101.16	98.19	97.29
T_7	97.07	103.46	100.27	101.22	105.22	103.22	101.75
T_8	95.07	101.17	98.12	97.25	101.91	99.58	98.85
T_9	99.77	105.52	102.65	105.23	109.54	107.39	105.02
T_{10}	90.92	94.45	92.69	92.85	95.20	94.03	93.36
T_{11}	95.83	102.25	99.04	99.23	102.48	100.86	99.95
T_{12}	98.17	104.34	101.25	103.38	107.31	105.34	103.30
T_{13}	88.67	91.35	90.01	89.27	92.10	90.69	90.35
T_{14}	91.35	95.82	93.59	93.37	98.19	95.78	94.68
T_{15}	89.23	92.17	90.70	90.09	93.07	91.58	91.14
Mean	95.12	100.23		98.01	102.56		
	V	**L**	**T**	**V×L**	**V×T**	**LxT**	**VxLxT**
C.D. (P=0.05)	0.054	0.054	0.152	0.076	0.215	0.215	0.304
SE.m (±)	0.019	0.019	0.054	0.027	0.077	0.077	0.109

the combined application of biofertilizers enhanced the biochemical activities of wheat seedlings.The maximum enzymatic activity of dehydrogenase, catalase, peroxidase and superoxide dismutase was observed with the treatment T_5-*Azotobacter*+PSB. The increasing amount of chemical pesticides and fertilizers has had a negative impact on the ecosystem. To minimize this, we should be more and more aware of the need to reduce their dependence on chemical fertilizers. Instead of chemicals the efforts should be focused on utilizing the biofertilizers and increase biological activity of microorganisms in the rhizosphere to increase soil fertility and promote crop growth.

Conclusion

The combined application of the biofertilizers could be recommended for high quality and profitable wheat production. Seedlings germinated from seeds treated with T_5-*Azotobacter*+PSB exhibited highest biochemical activity. The application of biofertilizers enhanced the dehydrogenase, catalase, peroxidase and superoxide dismutase enzyme activities, whereas the T_1-Chlorpyrifos reduces the biochemical activities of the wheat seedling. The rise in enzyme activity leads to enhanced stress tolerance in wheat seedlings. This coordinated defense mechanism assists the plants in sustaining their growth and recuperating from stress related challenges.

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