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EFFECT OF BIO PRIMING OF WHEAT WITH *TRICHODERMA HARZIANUM* AND INTEGRATED USE OF ORGANIC AND INORGANIC SOURCES OF NITROGEN ON PHYSICAL, CHEMICAL AND BIOLOGICAL PARAMETERS OF EXPERIMENTAL SOIL

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ABSTRACT

A field experiment was conducted for two consecutive *rabi* seasons with the objective to find out the effect of bio priming and best combination of organic and inorganic fertilizers on soil physical, chemical and biological parameters after harvesting of wheat (*Triticum aestivum* L.). The experiment was laid out in randomized complete block design (factorial) consisting of factor I bio priming with two levels and factor II nitrogen management with seven levels. Thus, a total of 14 treatment combinations were tested in the study and were replicated thrice. There was positive effect of treating wheat seeds with *Trichoderma harzianum* and nitrogen management on biological parameters of soil collected as well as chemical parameters especially available nitrogen from the harvested field of experimental wheat during both the seasons.
***Keywords*:** organic source of nitrogen, bio priming, available nitrogen, biological parameters

Introduction

Wheat plays a crucial role in global food security, providing 20% of the world's carbohydrates and protein (Langridge *et al*., 2022). As of 2020, global wheat production reached 761 Mt, making it the most widely grown food crop (Guarin *et al*., 2022). The average global yield has increased from just over 1 tonne per hectare in the early 1960s to around 3.5 tonnes in the past decade, thanks to innovations in wheat breeding and agronomy (Langridge *et al*., 2022). However, wheat production faces significant challenges. Heat stress affects up to 57% of the wheat area in surveyed developing countries, while competition with weeds and diseases each affect up to 55% of the wheat area (Kosina *et al*., 2007). Climate change, rising costs of fertilizers and pesticides, and declining water availability for irrigation in many regions threaten the gains made in wheat production (Langridge *et al*., 2022). Despite these challenges, there is potential for increasing global wheat production. The estimated mean global genetic yield gap is 51%, suggesting that global wheat production could benefit greatly from exploiting this untapped potential through optimal cultivar designs and improved crop management (Senapati *et al*., 2022). Wheat is a staple cereal crop cultivated worldwide for its nutritional value and versatility. As a member of the grass family, it is known for its ability to adapt to various climatic conditions, making it one of the most widely grown crops globally. Wheat grains are rich in carbohydrates, proteins and essential nutrients. The top wheat-producing countries include China, India, Russia, the United States, and France. Climate change continues to impact wheat production, with extreme weather events and shifting precipitation patterns affecting crop yields in various regions.

India is the second largest producer of wheat globally, with production ranging from 68-75 million tons in recent years (Joshi *et al*., 2007). However, the country faces significant challenges in maintaining food security due to its growing population and increasing demand for wheat. The estimated demand for wheat in 2020 was approximately 87.5 million tons, which is about 13 million tons more than the record production of 75 million tons harvested in 1999-2000 (Joshi *et al*., 2007). Interestingly, recent studies have shown contradicting trends in wheat production across different Indian states. While some regions have experienced stagnation in wheat yields, others continue to show improvement. According to (Madhukar *et al*., 2019), wheat yields in 13 Indian states are no longer improving, affecting approximately 76% of the wheat harvested area (about 18.5 million hectares). This stagnation is particularly concerning given the need for increased production to meet growing demand. In conclusion, India's wheat production status is complex and faces numerous challenges. The country must address issues such as heat stress, water scarcity, disease threats, and yield gaps to increase production (Chatrath *et al*., 2007; Joshi *et al*., 2007). Additionally, the impact of climate change, as evidenced by the 2022 Indian heatwave, which reduced national wheat production by an estimated 4.5% (Sidhu, 2023), highlights the need for adaptive strategies and improved agricultural practices to ensure food security in the face of changing environmental conditions.

Materials and Methods

A field study was conducted at the Agricultural Research farm of Banaras Hindu University, Varanasi, using a Randomized Block Design (Factorial). The study included two factors: factor I involved bio priming with two levels, P0 (no bio priming) and P1 (bio priming with *Trichoderma harzianum*), while factor II focused on nitrogen management with seven levels: N0 (Control), N1 (90 kg N ha^{-1} through urea), N2 (63 kg N ha^{-1} through urea + 27 kg N ha^{-1} through FYM), N3 (120 kg N ha^{-1} through urea), N4 (84 kg N ha^{-1} through urea + 36 kg N ha^{-1} through FYM), N5 (150 kg N ha^{-1} through urea), and N6 (105 kg N ha^{-1} through urea + 45 kg N ha^{-1} through FYM). Nitrogen from urea was applied in four equal split doses (25% each at basal, Z_{21}, Z_{31}, and Z_{51}; Z= Zadoks scale; Z_{21}= main shoot and one tiller; Z_{31}= 1st node detectable; Z_{51}= tip of inflorescence emerged). In total, 14 treatment combinations were evaluated and each was replicated three times. The soil in the experimental field was analyzed before sowing of the test crop and recorded values summarized in the below mentioned table:

Table 1: Soil parameters analyzed before sowing of wheat

Soil parameters	Values		Method employed	Reference
	2020-21	2021-22		
pH	7.41	7.47	Glass electrode digital pH meter	Jackson (1973)
Electrical conductivity (dSm^{-1})	0.258	0.283	Using ELICO conductivity bridge	Jackson (1973)
Organic carbon (g kg^{-1})	3.70	3.90	Walkley and Black rapid titration method	Walkley and Black (1934)
Available nitrogen (kg ha^{-1})	203.05	210.74	Alkaline permanganate method	Subbiah and Asija (1956)
Available phosphorus (kg ha^{-1})	18.74	19.48	0.5 M $NaHCO_3$ (pH 8.5) spectrophotometric method	Olsen *et al*. (1954)
Available potassium (kg ha^{-1})	178.46	185.61	Flame photometric method using neutral normal ammonium acetate extract	Jackson (1973)

The observations on soil parameters *viz.* physical parameters (pH and EC), chemical parameters (organic carbon (g kg^{-1}), available nitrogen (kg ha^{-1}), phosphorous (kg ha^{-1}) and potassium (kg ha^{-1})) and biological parameters (dehydrogenase activity (µg TPF g^{-1} soil day^{-1}), phosphatase activity (µg pNP g^{-1} soil hr^{-1}) and AMF spore count (100 g^{-1} soil)) were recorded from the soil processed after collecting it from the harvested field of the experimental crop through standard procedure. Soil pH was determined by suspension of soil and water (1:2.5) using glass-calomel electrode (Jackson, 1973). EC was determined by salt bridge measurement from the suspension of soil and water (1:2.5) used for pH determination (Jackson, 1973). Organic carbon was determined by Walkley and Black's (1934) rapid titration method. Available nitrogen was determined by modified alkaline potassium permanganate method (Subbiah and Asija, 1956) and was expressed in kg ha^{-1}. Available phosphorus was determined by using 0.5 M sodium bicarbonate extractable P method (Olsen *et al.,* 1954). The intensity of colour developed by stannous chloride was measured at 660 nm on spectrophotometer and was expressed as P_2O_5 kg ha^{-1}. Available potassium (K) was extracted with ammonium acetate extractable K method (Jackson, 1973) and potassium was determined by flame photometer and expressed as K_2O kg ha^{-1}. Dehydrogenase activity was determined by using extracting agent 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) method as outlined by Klein *et al.* (1971). The dehydrogenase activity was expressed as µg Triphenyl formazan (TPF) formed g^{-1} soil day^{-1}.

Phosphatase activity was measured as outlined by Tabatabai and Bremner (1969). The phosphatase activity expressed as µg p-Nitrophenol (PNP) released g^{-1} soil h^{-1}. The wet sieving and decanting technique is used for sieving the coarse particles of the soil and retaining AMF spores and organic particles on sieves of different sizes. 50 g of soil was mixed with 250 ml of water in the 500 ml conical flask. The soil mixture was agitated vigorously to free the AMF spores from soil and allowed to settle for 15-45 minutes and the supernatant was decanted through standard sieves. Spores were purified by re-suspending the sieving in the 40% sucrose solution and centrifugation was carried out at 2000 rpm for 5 minutes. The supernatant was removed and promptly transferred onto a filter paper. The filter paper was viewed under dissecting microscope.

Results and Discussion

Soil pH

Soil pH (Table 2) failed to show any significant effect due to bio priming levels during both the years. Among the treatments, P_0 (control) registered higher numerical values (7.36 and 7.43) for soil pH during both the years. In case of nitrogen management levels, soil pH also could not reach the level of significance during both the experimental year. Maximum soil pH was recorded under N_0 (control) treatment. While, minimum pH value was found under application of N_6 (105 kg N ha^{-1} through urea + 45 kg N ha^{-1} through FYM) during both the years.

Electrical conductivity

Bio priming levels failed to show any significant effect on soil electrical conductivity during both the years of study. Among the treatments, P_0 (control) attained maximum soil electrical conductivity (0.275 and 0.290 dSm^{-1}) during both the years. Whereas, least soil electrical conductivity was noted under P_1. In case of nitrogen management levels, soil electrical conductivity also failed to reach the level of significance during both the experimental years. Maximum soil electrical conductivity (0.279 and 0.295 dSm^{-1}) was recorded under N_0 (control) treatment.

Organic carbon

Soil organic carbon is presented in Table 2, unable to show any significant effect due to bio priming levels during both the years. Nitrogen management levels also could not reach the level of significance during both the experimental years. Maximum numerical of organic carbon content was recorded under N_5 (3.90 and 4.20 g kg^{-1}) treatment. While, least value of organic carbon was found under N_0 (control) treatment.

Available nitrogen

Bio priming levels had non-significant effect on available nitrogen in wheat field. Among the levels, maximum availability of nitrogen (203.45 and 208.11 kg ha^{-1}) was obtained with P_0 treatment during both the years. In regard to nitrogen management levels, significantly higher available nitrogen (223.28 and 230.81 kg ha^{-1}) was registered with application of N_0 (control) which was statistically at par with N_1 (90 kg N ha^{-1} through urea), N_2 (63 kg N ha^{-1} through urea + 27 kg N ha^{-1} through FYM) and N_3 (120 kg N ha^{-1} through urea), respectively during both the years. While least available nitrogen in soil after harvest of test crop was found under N_6 (105 kg N ha^{-1} through urea + 45 kg N ha^{-1} through FYM) treatment.

Available phosphorus

Bio priming levels failed to show any significant effect on available phosphorous during both the years of study. Among the treatments, P_0 (control) attained maximum numerical value of available phosphorous (18.00 and 18.70 kg ha^{-1}) during both the years. Whereas, least phosphorous availability was noted under P_1. In case of nitrogen management levels, available phosphorous in soil also failed to reach the level of significance during both the experimental year. Maximum phosphorous availability was recorded under N_0 (control) treatment i.e., 19.87 and 20.83 kg ha^{-1}.

Available potassium

Potassium availability in soil after harvesting of wheat failed to show any significant effect due to bio priming levels during both the years. Although, P_0 (control) registered maximum available potassium (175.64 and 184.62 kg ha^{-1}) during both the years. In case of nitrogen management levels, available potassium also unable to reach the level of significance during both the experimental years. However, maximum potassium availability (184.55 and 195.64 kg ha^{-1}) in soil after harvesting of test crop was recorded under N_0 (control) treatment.

Dehydrogenase activity

Dehydrogenase activity in soil after harvesting of wheat showed significant effect due to bio priming levels during both the years. Among the levels, P_1 registered maximum value for dehydrogenase activity (33.49 and 35.87 µg TPF g^{-1} soil day^{-1}) during both the years and was found statistically superior over control. In case of nitrogen management levels, dehydrogenase activity reached the level of significance during both the experimental years. Higher dehydrogenase activity in soil after harvesting of test crop was recorded under N_6 (105 kg N ha^{-1} through urea + 45 kg N ha^{-1} through FYM) treatment i.e., 43.12 and 46.47 µg TPF g^{-1} soil day^{-1} for the first and second year respectively and was found statistically at par with N_5.

Phosphatase activity

Phosphatase activity in soil after harvesting of wheat exhibited significant effect due to bio priming levels during both the experimental years. Among the levels, P_1 recorded higher value for phosphatase activity (84.43 and 88.15 µg pNP g^{-1} soil hr^{-1}) during both the years and was found statistically superior over level P_0. For second factor i.e., nitrogen management levels, phosphatase activity attained the level of significance during both the experimental years. Higher phosphatase activity in soil after harvesting of test crop was obtained with level N_6 (88.46 and 91.28 µg pNP g^{-1} soil hr^{-1}) and was found statistically superior over rest of the levels except N_5.

AMF (Arbuscular Mycorrhizal Fungi) spore count

Bio priming levels exhibited significant effect on AMF spore count in soil after harvesting of wheat during both the experimental years. Higher values for AMF spore count (44.58 and 49.62 per 100 g of soil) were observed with level P_1 during both the years of study and was found statistically superior over level control. For nitrogen management levels, AMF spore count was found significant during both the experimental years. Maximum AMF spore count in soil after harvesting of wheat was obtained with level N_6 (44.95 and 50.01 per 100 g of soil) and was found statistically superior over rest of the levels except N_5. AMF spores belonging to two different genera, viz., *Glomus* and *Acaulospora* were commonly recorded from the soils but genus *Glomus* was most dominant.

Table 2: Effect of bio priming and nitrogen management on pH, electrical conductivity (dSm^{-1}) and organic carbon content (g kg^{-1}) of soil after harvesting of wheat

Treatments	pH		EC (dSm^{-1})		Organic carbon (g kg^{-1})	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
Bio priming (P)						
P_0	7.36	7.43	0.275	0.290	3.72	3.84
P_1	7.32	7.35	0.270	0.284	3.82	3.94
SEm±	0.11	0.13	0.007	0.009	0.03	0.03
CD (P = 0.05)	NS	NS	NS	NS	NS	NS
Nitrogen management (N)						
N_0	7.38	7.47	0.279	0.295	3.60	3.70
N_1	7.37	7.43	0.275	0.289	3.80	3.90
N_2	7.35	7.41	0.274	0.288	3.70	3.90
N_3	7.34	7.39	0.273	0.286	3.80	4.10
N_4	7.33	7.36	0.271	0.285	3.80	3.80
N_5	7.31	7.35	0.270	0.284	3.90	4.20
N_6	7.29	7.33	0.268	0.281	3.80	3.90
SEm±	0.09	0.1	0.004	0.005	0.02	0.02
CD (P = 0.05)	NS	NS	NS	NS	NS	NS

Treatment details: P_0: Without Bio priming (Control); **P_1:** With Bio priming **N_0:** 0 Kg N ha^{-1} (Control); **N_1:** 90 kg N ha^{-1} through urea; **N_2:** 63 kg N ha^{-1} through urea + 27 kg N ha^{-1} through FYM; **N_3:** 120 kg N ha^{-1} through urea; **N_4:** 84 kg N ha^{-1} through urea + 36 kg N ha^{-1} through FYM; **N_5:** 150 kg N ha^{-1} through urea; **N_6:** 105 kg N ha^{-1} through urea + 45 kg N ha^{-1} through FYM

Table 3: Effect of bio priming and nitrogen management on Available nitrogen, phosphorous and potassium (kg ha^{-1}) of soil after harvesting of wheat

Treatments	Available nitrogen (kg ha^{-1})		Available phosphorus (kg ha^{-1})		Available potassium (kg ha^{-1})	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
Bio priming (P)						
P_0	203.45	208.11	18.00	18.70	175.64	184.62
P_1	188.87	193.06	15.94	16.53	166.65	174.97
SEm±	6.43	6.96	2.23	3.54	13.65	15.65
CD (P = 0.05)	NS	NS	NS	NS	NS	NS

Nitrogen management (N)						
N_0	223.28	230.81	19.87	20.83	184.55	195.64
N_1	212.31	218.41	17.97	18.98	177.51	188.68
N_2	208.64	212.20	17.21	17.39	174.23	182.44
N_3	202.96	209.51	16.72	17.62	170.51	179.12
N_4	190.87	192.28	16.43	16.5	166.47	174.28
N_5	171.79	175.16	15.62	16.77	164.29	170.49
N_6	163.25	165.78	14.98	15.28	160.46	167.97
SEm±	7.8	7.84	1.96	2.12	8.24	9.64
CD (P = 0.05)	21.46	22.53	NS	NS	NS	NS

Treatment details: P_0: Without Bio priming (Control); **P_1:** With Bio priming **N_0:** 0 Kg N ha^{-1} (Control); **N_1:** 90 kg N ha^{-1} through urea; **N_2:** 63 kg N ha^{-1} through urea + 27 kg N ha^{-1} through FYM; **N_3:** 120 kg N ha^{-1} through urea; **N_4:** 84 kg N ha^{-1} through urea + 36 kg N ha^{-1} through FYM; **N_5:** 150 kg N ha^{-1} through urea; **N_6:** 105 kg N ha^{-1} through urea + 45 kg N ha^{-1} through FYM

Table 4: Effect of bio priming and nitrogen management on dehydrogenase activity, phosphatase activity and AMF (Arbuscular Mycorrhizal Fungi) spore count of soil after harvesting of wheat

Treatments	Dehydrogenase activity (µg TPF g^{-1} soil day^{-1})		Phosphatase activity (µg pNP g^{-1} soil hr^{-1})		AMF spore count (100 g^{-1} soil)	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
Bio priming (P)						
P_0	28.62	29.78	60.49	64.07	42.82	46.26
P_1	33.49	35.87	84.43	88.15	44.58	49.62
SEm±	0.47	0.49	1.07	1.13	0.23	0.36
CD (P = 0.05)	1.37	1.42	3.11	3.28	0.67	0.98
Nitrogen management (N)						
N_0	24.03	25.04	63.05	67.57	42.61	45.12
N_1	25.49	27.04	64.37	67.92	42.71	46.31
N_2	26.18	27.81	66.19	69.52	43.27	47.52
N_3	27.80	29.40	68.01	71.46	43.43	47.76
N_4	33.77	35.35	74.67	78.55	44.24	48.89
N_5	37.01	38.67	82.48	86.46	44.72	49.95
N_6	43.12	46.47	88.46	91.28	44.95	50.01
SEm±	0.88	0.92	2.01	2.11	0.24	0.37
CD (P = 0.05)	2.56	2.66	5.99	6.14	0.68	1.11

Treatment details: P_0: Without Bio priming (Control); **P_1:** With Bio priming **N_0:** 0 Kg N ha^{-1} (Control); **N_1:** 90 kg N ha^{-1} through urea; **N_2:** 63 kg N ha^{-1} through urea + 27 kg N ha^{-1} through FYM; **N_3:** 120 kg N ha^{-1} through urea; **N_4:** 84 kg N ha^{-1} through urea + 36 kg N ha^{-1} through FYM; **N_5:** 150 kg N ha^{-1} through urea; **N_6:** 105 kg N ha^{-1} through urea + 45 kg N ha^{-1} through FYM

Conclusions

From the above overall study, it is recommended that to obtain better enzymatic activity and higher spore count in soil, wheat should be grown by following bio priming treatment of seeds (Priming material: *Trichoderma harzianum*) and nitrogen management as application of 105 kg N ha^{-1} through urea + 45 kg N ha^{-1} through FYM under agro-climatic conditions of Eastern Uttar Pradesh.

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