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Effect of sowing methods and nutrient management on soil enzymatic activity of wheat (*Triticum aestivum* L.) under organic farming

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Abstract

A field experiment was conducted during the *rabi* seasons of 2022-23 and 2023-24. The experiment was laid out in a strip plot design with three replications and horizontal strip four sowing methods treatments viz. S₁: Line sowing, S₂: Criss cross sowing, S₃: Line sowing with 150% seed rate and S₄: Criss cross sowing with 150% seed rate and vertical strip six organic nutrient management viz. N₁: 100% RDN, N₂: 75% RDN + Azotobacter + PSB, N₃: 100% RDN + Azotobacter + PSB, N₄: 75% RDN + Azotobacter + 4 foliar spray of 10% Cow urine at 20 days interval and N₆: 125% RDN. Different sowing methods recorded non-significant effects on Soil enzymatic activity at flowering stage of wheat during both the years and on mean basis. Among different nutrient management practices, the highest alkaline phosphatase activity, DHA and MBC was found under 125% RDN (N₆) which was at par with 100% RDN + Azotobacter + PSB + 4 foliar spray of 10% cow urine at 20 days interval (N₅) and 100% RDN + Azotobacter + PSB (N₃) and lowest was observed under 75% RDN + Azotobacter + PSB (N₂) during both the years and on mean basis.

Keywords: Wheat, alkaline phosphatase activity, dehydrogenase activity, soil health

Introduction

Soil enzymes are sensitive integrative indicators of microbial activity and nutrient cycling; they respond rapidly to changes in soil management. In organic farming, where synthetic fertilizers are not used, nutrient supply and physical soil conditions (influenced by sowing/tillage) are key drivers of microbial processes. Sowing methods that reduce soil disturbance (direct drill/zero-till) can preserve soil structure and microbial habitats, while varied organic inputs (FYM, vermicompost, liquid manures) supply organic substrates of different quality. Understanding how combinations of sowing method and organic nutrient management affect key enzymes (dehydrogenase — overall microbial activity; phosphatases — P cycling;) will help optimize organic wheat systems for both productivity and soil health.

Methods and Materials

Technical programme of work

(Including location and place of work, facilities available etc.)

Location: Instructional-cum-Research Farm, IGKV, Raipur (C.G)

Season: *Rabi* 2022-23 and 2023-24

Crop & variety: Wheat (Amber)

Design: Strip plot design

No. of replications: Three

Row spacing: 20 cm

Gross plot size: 4 m x 4 m = 16 m²

Seed rate: As per treatment

Recommended nutrient dose: 100:60:40 NPK kg ha⁻¹

Details of treatment

Symbol	Treatments
A. Horizontal strip- Sowing Methods	
S ₁	Line sowing
S ₂	Criss cross sowing
S ₃	Line sowing with 150% seed rate
S ₄	Criss cross sowing with 150% seed rate
B. Vertical strip - Organic Nutrient Management	
N ₁	100% RDN
N ₂	75% RDN + Azotobactor* + PSB*
N ₃	100% RDN + Azotobactor + PSB
N ₄	75% RDN + Azotobactor + 4 foliar spray of 10% cow urine at 20 days interval
N ₅	100% RDN + Azotobactor + PSB + 4 foliar spray of 10% cow urine at 20 days interval
N ₆	125% RDN

Note- All nutrient applied through organic sources. (75% nitrogen through FYM and 25% nitrogen vermicompost)

* Seed treatment of PSB @ 5 g kg⁻¹ seed

* Seed treatment of Azotobactor @ 10 g kg⁻¹ seed

* RDN (Recommended Dose of Nitrogen)

Microbial studies

1. Dehydrogenase activity (mg TPF/kgsoil/hr)

The Dehydrogenase activity in soil was determined using the method described by Nayak (2016) [7]. A one-gram air-dried soil sample was placed in an airtight screw-capped test tube (15 mL capacity). A 0.2 mL solution of 3% TTC (Triphenyl Tetrazolium Chloride) was used to saturate the soil. After that, 0.5 mL of 1% glucose solution was poured to the tube, followed by gentle tapping to remove any trapped oxygen. The tubes were incubated at 28±0.5 °C for 24 hours. Following incubation, 10 mL of methanol was added, forcefully shaken and allowed to stand for six hours. The clear pink supernatant was removed and measured using a spectrophotometer at 485 nm (blue filter). The amount of TPF (Triphenyl Formazan) formed was calculated from the standard curve drawn in the range of 10 mg to 90 mg TPF/mL. The result was expressed as mg Triphenyl formazan formed per g of soil. Dehydrogenase activity in soil was calculated as:

$$\frac{\text{Concentration} \times \text{Dilution}}{\text{Incubation time} \times \text{Soil weight}}$$

Where

Concentration = Absorbance value x Y value of standard curve (78.58)

2. Microbial biomass carbons

The plate count method was used to analyze the microbial population through serial dilution. 1 g of soil sample is suspended in 9 mL of sterile water in a dilution tube and agitated for 15 minutes. It was serially diluted by transferring 1 mL from the stock. Fresh tips were utilized to prepare sterile serial dilution (10⁻¹ to 10⁻⁶). Different mediums were produced for the isolation of microorganisms. Nutrient agar media for bacteria and Rose Bengal agar media for fungi were utilized. Media were sterilized at 121 °C for 15 minutes. Using a sterile micro-pipette tip, 1 mL of the necessary dilution of the freshly mixed suspension was put into the sterile Petri dish. Stock solution to 10⁻⁶ dilute were spreader in specific media for particular microorganism, about 15 mL of partially cooled appropriate medium was poured into each plate and carefully thoroughly mixed. Incubate the plates at the appropriate temperature for microorganisms (28 °C ± 2 °C for fungus and 25 °C ± 2 °C for

bacteria) once the media has hardened. After a predetermined period of growth (24 hours for bacteria and 6-7 days for fungi), colonies were counted and the population was estimated using the method provided by Nayak (2016) [7]. Bacteria and fungus from chickpea and rice farmed soils can grow on Petri plates under various treatments.

$$\text{CFU} \times 10^n - 1 \text{ per g of soil} = \frac{\text{Number of Colony} \times \text{Dilution factor}}{\text{Aliquot taken} \times \text{dry weight of 1 gm soil}}$$

3. Phosphate activity

Acid phosphatase enzyme was extracted from a known amount of soil by the procedure of Nayak (2016) [7]. Five gram of soil was taken in 50 mL conical flask. In each conical flask 1 mL toluene, 20 mL of modified universal buffer MUB (pH 6.5) and 5 mL of Na- beta- glycerophosphate were added. The flasks were incubated in water bath at 37°C for 90 minutes. The flasks were replaced from water bath and kept in boiling water bath for 15 minutes to kill the enzyme activity. The content was filtered. Five mL of filtrate was taken colour development.”

Procedure

Each soil sample was taken into two sets of 1 g (oven dry equivalent) soil in 50 mL conical flasks. Out of the two sets, one was used as control. 0.2 mL toluene was added to each flask. Then 1 mL p-nitrophenol phosphate solution was added to one flask only. For few seconds both the flasks were swirled to mix the contents. Flasks were stoppered and were placed in an incubator at 37 °C for one hour. After incubation, stopper was removed and 1 mL of 0.5 M CaCl₂ was added followed by 4 mL of 0.5 M NaOH and the flasks were swirled for few seconds. Then 1 mL of p-nitrophenol phosphate was added to the remaining set of control samples. All the suspensions were filtered quickly through Whatman No. 2 filter paper. Then the yellow color intensity was measured with a blue filter or at 440 nm.

Calculation

Phosphomono-esterases activity is expressed as µg p-nitrophenol released g⁻¹ soil h⁻¹.

$$\mu\text{g p - nitrophenol} = \frac{\text{Absorbance value of sample} \times \text{factor}}{\text{hrs. of incubation} \times \text{wt. of soil}}$$

Where,

Factor-Drawn from std. curve of p-nitrophenol phosphate

Results and Discussion

1. Dehydrogenase activity (µg TPF g⁻¹ of dry soil hr⁻¹)

Dehydrogenase activity (DHA) is a key indicator of microbial respiration and overall microbial activity in soil. The results showed that dehydrogenase activity significantly varied with nutrient management treatments. The data on dehydrogenase activity (DHA) at flowering stage are presented in the Table 1. Soil dehydrogenase activity assess microbial soil quality and depends on the intensity of biological conversion of organic compounds.

It was evident from the data that non-significant difference in DHA activity due to sowing methods was observed at flowering stage during both the years and on mean basis. However, highest and lowest DHA activity were observed under the criss-cross with 150% seed rate (S₄) and line sowing (S₁) method during both the years and on mean basis.

Among different nutrient management practices, the highest

DHA activity was found under 125% RDN (N_6) which was at par with 100% RDN + Azotobacter + PSB + 4 foliar spray of 10% cow urine at 20 days interval (N_5) and 100% RDN + Azotobacter + PSB (N_3) and lowest was observed under 75% RDN + Azotobacter + PSB (N_2) during both the years and on mean basis. Saha *et al.* (2008) ^[10] studied the long-term application of FYM and mineral fertilizers on soil enzyme activities, and their results showed that manure application increased dehydrogenase, acid and alkaline phosphatases, cellulase and protease activities.

The DHA activity of wheat remained unchanged due to the interaction between sowing methods and nutrient management practices during both the years and on mean basis.

2. Phosphatase activity ($\mu\text{g p-NP h}^{-1} \text{g}^{-1} \text{soil}$)

Phosphatase enzymes play a crucial role in phosphorus mineralization, making soil phosphorus more available to plants. The results demonstrated a significant variation in phosphatase activity on nutrient management strategies. The data on alkaline phosphatase activity at flowering stage are presented in the Table 1.

It was evident from the data that non-significant difference in alkaline phosphatase activity due to sowing methods was observed at flowering stage during both the years and on mean basis. However, highest and lowest alkaline phosphatase activity were observed under the criss-cross with 150% seed rate (S_4) and line sowing (S_1) method during both the years and on mean basis.

Among nutrient management, the significantly highest alkaline phosphatase activity was found under 125% RDN (N_6) which was statistically at par with 100% RDN + Azotobacter + PSB + 4 foliar spray of 10% cow urine at 20 days interval (N_5) and 100% RDN + Azotobacter + PSB (N_3) and lowest was observed under 75% RDN + Azotobacter + PSB (N_2) during both the years and on mean basis. Phosphatase activity has been shown to be correlated with organic C, and organic C could have an important role in protecting and maintaining acid phosphatase activity. (Deng, 1997) ^[3].

The alkaline phosphatase activity of wheat remained unchanged due to the interaction between sowing methods and nutrient management practices during both the years and on mean basis.

3. Microbial biomass carbon ($\mu\text{g g}^{-1} \text{soil}$)

The data on microbial biomass carbon at flowering stage are presented in the Table 1. Significant difference in microbial biomass carbon (MBC) was observed under different sowing method and nutrient management practices.

The data on microbial biomass carbon in soil at flowering stage of wheat showed non-significant difference on sowing methods. However, treatment criss-cross with 150% seed rate (S_4) recorded highest microbial biomass carbon in soil and the lowest microbial biomass carbon in soil was recorded under line sowing (S_1) method during both the years and on mean basis.

Among the different nutrient management practices, higher microbial biomass carbon was observed under treatment 125% RDN (N_6) which was statistically at par with 100% RDN + Azotobacter + PSB + 4 foliar spray of 10% cow urine at 20 days interval (N_5) and 100% RDN + Azotobacter + PSB (N_3) and lowest was observed under 75% RDN + Azotobacter + PSB (N_2) during both the years and on mean basis.

The microbial biomass carbon of wheat remained unchanged due to the interaction between sowing methods and nutrient management practices during both the years and on mean basis.

Discussion on soil enzymatic analysis

The observed increase in DHA under denser sowing conditions (criss-cross with 150% seed rate (S_4)) suggests enhanced microbial activity due to greater root exudation and organic carbon inputs, which corroborates findings from Singh *et al.* (2020) ^[12]. Increased microbial proliferation under higher plant density has been previously linked to improved soil aeration and moisture retention, which supports microbial metabolism (Fereidooni *et al.*, 2013) ^[4].

The increased microbial respiration under N_6 (125% RDN) could be attributed to higher nitrogen availability, which enhances microbial proliferation and enzymatic turnover. In contrast, lower DHA under N_2 (75% RDN + Azotobacter + PSB) suggests microbial nutrient stress due to inadequate nitrogen supply. Several studies highlighted the role of the microbial biomass in decomposition of substances such as carbohydrates and lipids originating from plant and microbial activity in the improvement of soil quality (Tisdall, 1994) ^[13].

The significant increase in microbial biomass under high-density sowing (S_4) is likely due to higher root biomass and rhizodeposition, which stimulates microbial proliferation, as reported by Sharma *et al.* (2021). Additionally, increased soil organic matter inputs under higher seed rates enhance microbial activity and carbon sequestration (Klose *et al.*, 1999) ^[6].

Higher nutrient supplementation in N_6 (125% RDN) resulted in increased microbial proliferation and biomass accumulation, supporting findings from Yadav *et al.* (2020) ^[15], who reported that optimal nitrogen application enhances microbial growth and nutrient cycling. The lower MBC in N_2 (75% RDN + Azotobacter + PSB) suggests nutrient limitations, which restrict microbial proliferation and carbon sequestration. The observed increase under denser sowing conditions (S_4) can be linked to higher root exudation and rhizospheric microbial activity, supporting previous findings by Pagliari and Laboski, (2013) ^[8], who demonstrated that increased root biomass enhances phosphatase enzyme production and phosphorus availability.

Higher phosphorus availability under N_6 promoted greater microbial phosphatase production, in agreement with studies by Singh *et al.* (2020) ^[12], who found that higher nutrient application increases microbial enzyme activity and nutrient turnover in soil. Conversely, lower phosphatase activity under N_2 (75% RDN + Azotobacter + PSB) suggests lower microbial phosphorus mobilization, which can limit phosphorus bioavailability for plant uptake (Gurmu, G. (2019) ^[5]. Similar to β -glucosidase activity, soil phosphomonoesterase increases with the addition of organic matter, likely as a result of increased organic P, which must be first mineralized before it can be used by microbes and plants (Pagliari and Laboski, 2014) ^[9].

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