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Assessment of enzyme activity at different growth stages in groundnut-wheat cropping sequence under AICRP-LTFE soils

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Abstract
The current study was carried out during the Kharif-2023 and Rabi-2023-24 seasons as part of the All India Coordinated Research Project on "Long Term Fertilizer Experiment" at the Instructional Farm, College of Agriculture, Junagadh Agricultural University, Junagadh. This experiment, initiated in 1999, aimed to evaluate the impact of various fertilizer applications, with or without organic manure, on soil enzyme activities at different growth stages in a groundnut-wheat cropping sequence. A total of 12 treatments were evaluated: T1 - 50% NPK, T2 - 100% NPK, T3 - 150% NPK, T4 - 100% NPK + $ZnSO_4$ at 50 kg ha^{-1}, T5 - NPK as per soil test recommendations, T6 - 100% NP, T7 - 100% N, T8 - 50% NPK + FYM at 10 t ha^{-1} to groundnut and 100% NPK to wheat, T9 - FYM at 10 t ha^{-1} for groundnut and 15 t ha^{-1} for wheat, T10 - 50% NPK + Rhizobium + PSM for groundnut and 100% NPK to wheat, T11 - 100% NPK of the recommended dose for the groundnut-wheat system (P as SSP), and T12 - Control. The findings indicated that the highest dehydrogenase activity was recorded at the flowering stage of groundnut (79.22 µg TPF g^{-1} soil 24 hr^{-1}) and at the CRI stage in wheat (77.30 µg TPF g^{-1} soil 24 hr^{-1}) with the treatment of 50% NPK + FYM at 10 t ha^{-1} to groundnut and 100% NPK to wheat. Additionally, enzyme activities such as acid phosphatase, alkaline phosphatase, β-glucosidase, arylsulfatase, and urease were significantly elevated (39.46, 67.41, 71.95, 24.97 µg PNP g^{-1} hr^{-1} and 12.48 µg NH_4-N g^{-1} hr^{-1} respectively) at the pod formation stage of groundnut and during flowering in wheat (55.38, 74.75, 36.15, 12.79 µg PNP g^{-1} hr^{-1} and 36.98 µg NH_4-N g^{-1} hr^{-1} respectively) under the same treatment.

Keywords: Enzyme activity, fertilizer, organic manure, growth stages, cropping sequence

Introduction
In Indian agriculture, sustaining and enhancing soil fertility is of utmost significance to meet the increasing demand for food grains driven by the country's growing population. Restoring soil fertility involves ensuring sufficient nutrient supply to crops, thereby promoting higher yields. A robust aboveground biomass production is typically supported by an active root system, which exudes various organic substances into the rhizosphere (Bowen and Rovira, 1991) [4]. Approximately 17% of the total photosynthates produced by plants are secreted by roots, with a large portion being accessible to soil microorganisms. These root-derived organic substances serve as substrates for microbial growth, leading to an increase in microbial populations (Kent and Triplett, 2002) [13] and influencing both the diversity and functionality of the microbial community (Patra *et al*., 2006) [19].

For sustainable agricultural production that also conserves the environment, improving soil biological health is crucial. In this context, agricultural methods that promote soil quality and long-term sustainability are receiving growing interest among both scientists and farmers (Eivazi *et al*., 2003) [10]. While inorganic fertilizers are essential for maintaining crop productivity, their application can significantly alter soil chemical, physical, and biological characteristics. Over time, these changes may affect the overall quality and performance of agricultural soils.

Organic inputs like farmyard manure (FYM), crop residues, and compost have demonstrated effectiveness in enhancing soil physical and chemical attributes, boosting soil organic matter (SOM), urease activity (UA), and acid phosphatase activity, thereby improving soil health. Combining organic materials with inorganic fertilizers can further enhance soil biological

parameters such as microbial biomass and enzyme activity. Soil enzymes are integral to the transformation of essential elements required for plant growth (Burns, 1982) [6]. The activity of both extracellular and intracellular soil enzymes is influenced by environmental conditions, crop rotation patterns, amendments, tillage, and overall farm management (Eivazi *et al*., 2003) [10].

Since these enzymes are continually synthesized, accumulated, inactivated, or broken down in the soil, they play a key role in nutrient cycling and agricultural productivity. Soil biochemical processes largely rely on enzyme-driven catalytic actions, utilizing diverse substrates that also provide energy to microorganisms. Important soil enzymes—such as arylsulfatases, glycosidases, dehydrogenases, phosphatases, and ureases—originate from plant roots, animal residues, organic compounds, and soil microbes. Gaining deeper insights into these enzyme activities offers valuable opportunities for integrated biological assessment of soil health, owing to their central role in biological functions and sensitivity to variations in soil management practices.

Dehydrogenases are key enzymes that play a vital role in the biological oxidation of soil organic matter by facilitating the transfer of hydrogen from organic substances to inorganic electron acceptors. This positions dehydrogenase as a critical indicator of microbial oxidation-reduction activities and serves as a reliable measure of microbial oxygen-dependent metabolic processes in soils.

A range of enzymes contributes to the breakdown of organic phosphorus compounds (Jennings, 1995) [12]. Phosphatases, in particular, are enzymes that hydrolyze phosphate esters, playing an essential role in converting organic phosphorus into its inorganic form through dephosphorylation. Thus, soil phosphatases are central to phosphorus cycling in the soil ecosystem.

Among the extracellular enzymes found in soil, those involved in organic matter decomposition are especially significant. One such enzyme is β-glucosidase, which is instrumental in breaking down cellulose, the primary structural component of plant-derived polysaccharides.

Arylsulphatase functions by cleaving the oxygen-sulfur (O-S) bond and contributes substantially to the mineralization of ester sulfate compounds in the soil. Although ester sulfates represent the most readily degradable form of soil organic sulfur, they are not directly accessible to plants and must be hydrolyzed into inorganic sulfate (SO_4^{2-}) to be absorbed by plant roots.

Among nitrogen-associated extracellular enzymes, urease plays a key role in nitrogen cycling by breaking down urea. Generally, amidohydrolases serve as good indicators of nitrogen mineralization due to their ability to convert small nitrogen-containing organic molecules into inorganic nitrogen compounds like ammonia (Sinsabaugh and Follstad Shah, 2012) [21]. Urease specifically catalyzes the conversion of urea into ammonia (NH_3) and carbon dioxide (CO_2).

Given the significance of enzyme functions, it becomes essential to consider soil enzyme activity as a valuable indicator of soil quality. These enzymatic activities are closely associated with key soil quality factors such as organic matter content, physical structure, and microbial dynamics. Notably, changes in enzyme activities can manifest within a relatively short time frame (1-2 years), offering early insight into the direction of soil health in response to different management practices—well before changes in parameters like soil organic carbon are observable.

In light of this, the present study was undertaken to evaluate soil enzyme activities at various growth stages within the groundnut-wheat cropping system under the Long Term Fertilizer Experiment (LTFE) of the All India Coordinated Research Project (AICRP) at Junagadh Agricultural University (JAU), Junagadh.

Materials and Methods

Experimental details

The experiments was carried out at the Instructional Farm, College of Agriculture, Junagadh Agricultural University, Junagadh during *kharif* season-2023 and *rabi* -2023-24. Crop and variety of groundnut was GG-20 and of wheat was GW-496. The experimental design was randomized block design (RBD) involving 12 treatments and 4 replication. Net plot size for groundnut was 6m (10 rows) x 16m, and for wheat 6.75m (30 rows) x 16m with spacing between rows for groundnut was 60cm and for wheat were 22.5cm.

The treatment details are given below

T1 involved 50% of the recommended NPK dose for the groundnut-wheat cropping sequence; T2 involved 100% of the recommended NPK dose; T3 involved 150% of the recommended NPK dose; T4 comprised 100% of the recommended NPK dose along with zinc sulfate ($ZnSO_4$) applied at a rate of 50 kg ha^{-1} every three years specifically to groundnut (years: 1999, 2002, 2005, 2008, 2011, 2014, 2017, 2020, 2023); T5 consisted of NPK application based on soil test recommendations; T6 included only 100% of recommended NP fertilizers (excluding potassium); T7 included only nitrogen fertilizer at 100% of the recommended dose; T8 included 50% of the recommended NPK dose combined with farmyard manure (FYM) at 10 t ha^{-1} for groundnut and 100% of the recommended NPK dose for wheat; T9 involved the sole use of FYM at 10 t ha^{-1} for groundnut and 15 t ha^{-1} for wheat; T10 included 50% of the recommended NPK dose combined with Rhizobium inoculation and phosphate-solubilizing microorganisms (PSM) applied to groundnut, and 100% recommended NPK dose to wheat; T11 involved 100% of the recommended NPK dose; and T12 Control.

Collection of soil sample at different growth stages of groundnut-wheat

In the experiments, groundnut crops was grown in *kharif* -2023 and wheat crops was grown in *rabi* season during 2023-24, respectively under LTFE (AICRP). The soil samples were collected during the different growth stages of groundnut i.e., flowering (30-40DAS), pod formation (60-80DAS) and maturity (120-130DAS) and wheat i.e., CRI (15-25 DAS), flowering (65-70DAS), and maturity (100-120DAS) for analysis. The collected fresh soil samples of the *kharif* season-2023 and *rabi* -2023-24 were used for the investigation.

Preparation of soil sample

Fresh soil samples collected during the Kharif season of 2023 and Rabi season of 2023-24, corresponding to various growth stages of groundnut and wheat crops, were first air-dried in the shade. These samples were then ground using a wooden mortar and pestle and passed through a 2 mm sieve to obtain uniform particles. For enzyme activity analysis, the processed samples were stored at 4°C in a refrigerator to preserve their biological integrity. The enzyme assays included the measurement of dehydrogenase activity by estimating the formation rate of triphenyl formazan (TPF) from triphenyl tetrazolium chloride (TTC), following the protocol outlined by Klein *et al*. (1971) [14] and Tabatabai (1982) [24]. Acid and alkaline phosphatase activities were quantified by measuring the amount of p-

nitrophenol released, and results were expressed in terms of µg of p-nitrophenol released per gram of soil per hour, as per the method of Tabatabai and Bremner (1969) [23]. β-glucosidase activity was determined using spectrophotometric analysis according to the method proposed by Eivazi and Tabatabai (1988) [9]. Arylsulphatase activity was assessed following the procedure described by Tabatabai and Bremner (1970) [22]. Urease activity was estimated by measuring the quantity of ammonium released and expressed as mg of NH_4^+ released per gram of soil per hour, based on the method developed by Bremner and Douglas (1971). [5] The collected data were subjected to statistical analysis using the analysis of variance (ANOVA) technique appropriate for a randomized block design (RBD), as recommended by Panse and Sukhatme (1978) [17].

Results and Discussion

Dehydrogenase activity

The data presented in Table 1 on soil dehydrogenase activity at various growth stages of groundnut and wheat reveal that treatment T8—comprising the combined application of 50% recommended NPK and 10 t ha⁻¹ FYM to groundnut, along with 100% NPK to wheat—significantly enhanced dehydrogenase enzyme activity in the soil. Specifically, the activity levels recorded were 79.22, 75.41, and 72.06 µg TPF g⁻¹ soil 24 hr⁻¹ at the flowering, pod formation, and maturity stages of groundnut, and 77.30, 65.64, and 60.83 µg TPF g⁻¹ soil 24 hr⁻¹ at the CRI, flowering, and maturity stages of wheat, respectively. These values were statistically comparable to those observed in treatment T9, which involved only the application of FYM (10 t ha⁻¹ for groundnut and 15 t ha⁻¹ for wheat).

In contrast, the enzyme activity was markedly lower under the control treatment (T12) across all stages and showed only modest improvement with increasing doses of inorganic fertilizers. The enhanced enzyme activity in T8 and T9 is likely due to the increased soil organic carbon content, which supports microbial proliferation. These microorganisms, arising from the decomposition of organic matter, serve as the primary source of soil enzymes and utilize the carbon-rich organic substrates as energy, thereby contributing to higher enzyme activity.

These observations align with those of Patel *et al*. (2018) [18], who also reported that the integrated application of chemical fertilizers with FYM significantly enhanced soil enzymatic activity, particularly dehydrogenase. Regarding crop growth stages, maximum dehydrogenase activity was recorded during the flowering stage in groundnut and at the CRI stage in wheat (as shown in Figures 1 and 2). Similarly, Bhadoria *et al*. (2011) [1] concluded that regardless of treatment, dehydrogenase activity peaked around 25 days after sowing and subsequently declined gradually until 50 DAS, followed by a steeper decrease up to 75 DAS, and then a steady decline until 100 DAS.

Table 1: Dehydrogenase activity (µg TPF g^{-1} 24 hr^{-1}) at different growth stages of groundnut and wheat

Treatment		Groundnut			Wheat		
		Flowering (30-40 DAS)	Pod formation (60-80 DAS)	Maturity (120-130 DAS)	CRI (15-25 DAS)	Flowering (65 -70 DAS)	Maturity (100-120 DAS)
T_1	50% NPK	67.04	48.50	16.31	46.71	41.99	26.46
T_2	100% NPK	69.01	56.92	44.70	50.39	54.32	48.65
T_3	150% NPK	69.40	57.50	46.69	65.65	58.60	50.15
T_4	100% NPK+$ZnSO_4$ 50 kg ha^{-1} once in three year to groundnut *(i.e.*'99,02,05,08, 11,14,17,20,23 *etc.*) &100% NPK to wheat	70.05	54.33	30.09	54.80	52.36	37.98
T_5	NPK as per soil test	68.85	53.67	23.14	54.47	47.47	30.51
T_6	100% NP	66.44	38.00	15.27	44.83	39.04	19.40
T_7	100% N	57.13	32.94	12.27	41.44	36.39	18.41
T_8	50% NPK+10 t ha^{-1} FYM to groundnut & 100% NPK to wheat	79.22	75.41	72.06	77.30	65.64	60.83
T_9	Only FYM 10 t ha^{-1} to groundnut and 15 t $ha^{-1}$to wheat	75.51	70.00	69.66	71.22	63.97	55.98
T_{10}	50% NPK + *Rhizobium*+ PSM to Groundnut & 100% NPK to wheat	67.66	52.67	19.84	51.94	43.94	27.06
T_{11}	100% NPK (P as SSP)	67.52	54.83	33.68	56.41	53.65	46.92
T_{12}	Control	17.57	16.50	14.95	28.97	28.58	22.73
S.Em. ±		2.39	1.90	1.36	2.35	1.71	1.92
C.D. 5%		6.89	5.46	3.92	6.76	4.91	5.52
C.V.%		6.42	6.45	7.10	7.58	6.05	8.95

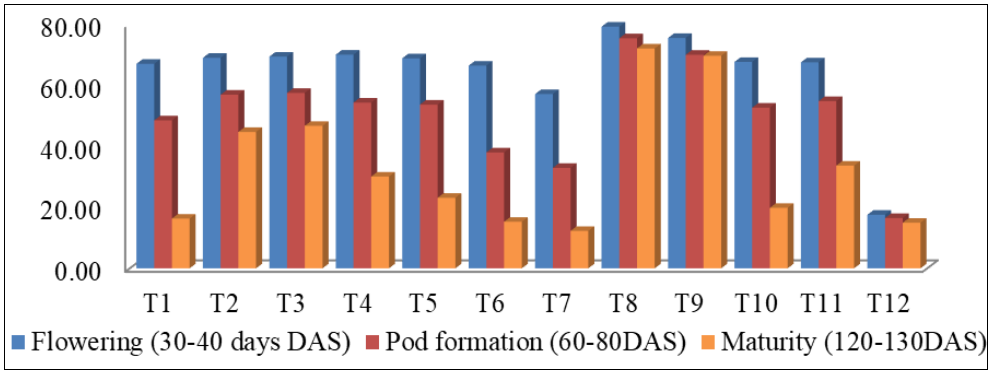

Fig 1: Dehydrogenase activity (µg TPF g^{-1} 24 hr^{-1}) at different growth stages of groundnut

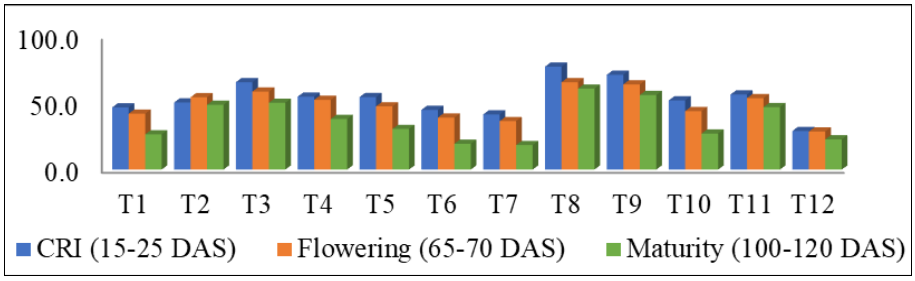

Fig 2: Dehydrogenase activity (µg TPF g^{-1} 24 hr^{-1}) at different growth stages of wheat

Acid phosphatase activity

According to the data presented in Table 2 on acid phosphatase activity in soil at various growth stages of groundnut and wheat, treatment T8—comprising 50% of the recommended NPK dose along with 10 t ha^{-1} of FYM for groundnut and 100% NPK for wheat—significantly enhanced enzyme activity. The observed values were 21.53, 39.46, and 17.52 µg TPF g^{-1} soil 24 hr^{-1} at the flowering, pod formation, and maturity stages of groundnut, respectively, and 30.46, 55.38, and 26.09 µg TPF g^{-1} soil 24 hr^{-1} at the CRI, flowering, and maturity stages of wheat. These results were statistically comparable to those obtained under treatment T9, which involved the sole application of FYM (10 t ha^{-1} for groundnut and 15 t ha^{-1} for wheat), across all growth stages.

In contrast, acid phosphatase activity was drastically reduced under the untreated control (T12) and exhibited only slight improvement with increasing doses of inorganic fertilizers. The enhanced enzyme activity observed in the organic-amended treatments is attributed to higher organic carbon levels in the soil, which promote microbial activity. These microbes, deriving from the decomposition of organic inputs, serve as key contributors to enzyme production, as they utilize the organic matter as a carbon and energy source, thereby supporting increased microbial populations and enzymatic functions.

These findings are consistent with those of Patel *et al*. (2018) [18], who demonstrated that long-term integrated nutrient management in Vertisols under a soybean-wheat cropping system significantly improved soil biochemical properties. They specifically noted that the combined application of chemical fertilizers and farmyard manure was more effective than other treatments in enhancing soil enzyme activities, including acid phosphatase.

Table 2: Acid phosphatase (µg PNP $g^{-1}hr^{-1}$) activity at different growth stages of groundnut and wheat

Treatment		Groundnut			Wheat		
		Flowering (30-40 DAS)	Pod formation (60-80 DAS)	Maturity (120-130 DAS)	CRI (15-25 DAS)	Flowering (65-70 DAS)	Maturity (100-120 DAS)
T_1	50% NPK	18.09	25.34	15.51	18.21	25.53	12.84
T_2	100% NPK	18.18	34.02	15.30	24.60	32.20	17.94
T_3	150% NPK	19.92	36.52	16.70	29.08	52.18	24.29
T_4	100% NPK+$ZnSO_4$ 50 kg ha^{-1} once in three year to groundnut *(i.e.*'99,02,05,08, 11,14,17,20,23 *etc.*) &100% NPK to wheat	19.26	28.18	15.51	22.96	27.93	14.49
T_5	NPK as per soil test	18.38	24.30	15.56	19.24	26.85	13.60
T_6	100% NP	18.09	25.11	15.38	19.08	25.69	13.28
T_7	100% N	16.52	23.85	14.73	16.72	17.95	7.44
T_8	50% NPK+10 t ha^{-1} FYM to groundnut & 100% NPK to wheat	21.53	39.46	17.52	30.96	55.38	26.09
T_9	Only FYM 10 t ha^{-1} to groundnut and 15 t $ha^{-1}$to wheat	20.15	36.65	16.73	30.95	54.38	25.39
T_{10}	50% NPK + *Rhizobium*+ PSM to Groundnut & 100% NPK to wheat	16.58	23.95	15.48	17.72	22.91	8.38
T_{11}	100% NPK (P as SSP)	19.25	36.33	15.57	23.25	31.92	15.07
T_{12}	Control	13.07	14.43	14.72	6.04	7.58	6.10
S.Em. ±		0.74	1.04	0.67	0.77	1.22	0.64
C.D. 5%		2.13	2.99	1.92	2.21	3.50	1.85
C.V.%		7.01	6.19	7.36	6.17	6.65	7.23

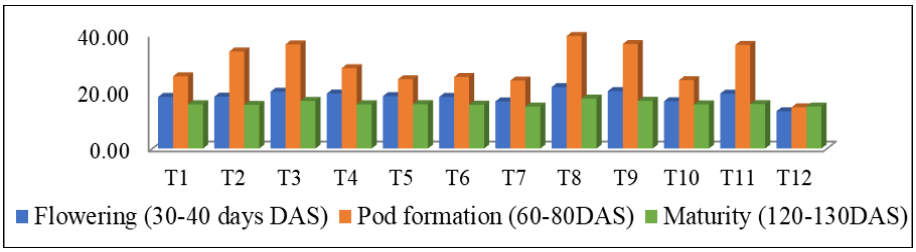

Fig 3: Acid phosphatase (µg PNP g^{-1} hr^{-1}) activity at different growth stages of groundnut

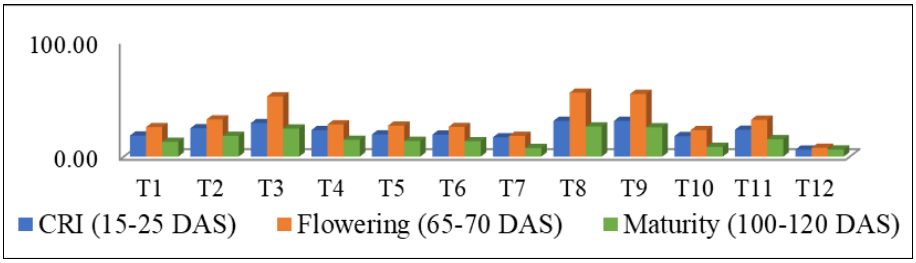

Fig 4: Acid phosphatase (µg PNP g^{-1} hr^{-1}) activity at different growth stages of wheat

With respect to growth stages, the higher acid phsphatase activity observed at pod formation in groundnut and at flowering in wheat (Fig. 1 & 2). Bhavani *et al.* (2017) [2] reported that in all the treatments, the acid phosphatase activity exhibited the highest activity at 60 DAT (days after transplanting of rice) and there after the activity decreased gradually to 90 DAT.

Alkaline phosphatase

The data on soil alkaline phosphatase activity (Table 3) at various growth stages of groundnut and wheat indicate that the integrated application of organic manure and inorganic fertilizers (NPK + FYM) proved to be the most effective among all treatments. Specifically, treatment T8—comprising 50% NPK combined with FYM at 10 t ha^{-1} for groundnut and 100% NPK for wheat—significantly enhanced enzyme activity. The observed values were 76.78, 87.41, and 60.33 µg PNP g^{-1} hr^{-1} at flowering, pod formation, and maturity stages of groundnut, and 65.25, 74.75, and 64.50 µg PNP g^{-1} hr^{-1} at CRI, flowering, and maturity stages of wheat. These results were statistically on par with those from treatment T9, which involved only FYM application (10 t ha^{-1} for groundnut and 15 t ha^{-1} for wheat).

The increase in enzyme activity during the flowering stage may be attributed to the elevated microbial biomass and intensified phosphomonoesterase activity in the soil. This is likely due to increased enzyme synthesis by soil microbes and root activity during peak plant growth stages, as supported by the findings of Tang *et al.* (2014) [26]. These observations are consistent with Meshram *et al.* (2016) [16], who reported a significant rise in alkaline phosphatase activity (160.65 µg PNP g^{-1} soil hr^{-1}) in a soybean-safflower cropping system with continuous application of 100% NPK along with organic manure. Similar results were noted by Tekam *et al.* (2024) [27].

In terms of crop growth stages, the highest alkaline phosphatase activity was recorded during the pod formation stage in groundnut and the flowering stage in wheat (Figures 3 & 4). These findings are supported by Islam and Borthakur (2016) [11], who observed a peak in phosphatase activity during the vigorous growth phase, followed by a decline post-maturity. Bhavani *et al.* (2017) [2] also reported that alkaline phosphatase activity in rice peaked at 60 days after transplanting and then gradually declined by 90 DAT.

Table 3: Alkaline phosphatase (µg PNP $g^{-1}hr^{-1}$) activity at different growth stages of groundnut and wheat

Treatment		Groundnut			Wheat		
		Flowering (30-40 DAS)	Pod formation (60-80 DAS)	Maturity (120-130 DAS)	CRI (15-25 DAS)	Flowering (65-70DAS)	Maturity (100-120 DAS)
T_1	50% NPK	37.05	34.34	28.42	41.50	46.25	26.00
T_2	100% NPK	56.67	40.58	28.44	43.50	50.00	26.50
T	150% NPK	64.42	74.50	49.28	58.50	65.25	49.75
T_4	100% NPK+$ZnSO_4$ 50 kg ha^{-1} once in three year to groundnut (*i.e.*'99,02,05,08,11, 14,17,20,23 *etc.*) &100% NPK to wheat	48.78	57.20	47.17	53.00	54.50	46.75
T_5	NPK as per soil test	46.78	52.58	38.28	51.75	54.00	32.75
T_6	100% NP	42.11	42.58	31.84	47.00	51.25	32.25
T_7	100% N	21.17	23.14	25.25	35.75	41.00	18.00
T_8	50% NPK+10 t ha^{-1} FYM to groundnut & 100% NPK to wheat	76.78	87.41	60.33	65.25	74.75	64.50
T_9	Only FYM 10 t ha^{-1} to groundnut and 15 t $ha^{-1}$to wheat	72.83	83.84	56.54	63.50	68.00	59.68
T_{10}	50% NPK + *Rhizobium*+ PSM to Groundnut & 100% NPK to wheat	26.80	33.56	26.53	38.50	41.75	19.00
T_{11}	100% NPK (P as SSP)	53.42	63.86	47.22	54.00	56.00	48.25
T_{12}	Control	18.01	21.33	21.61	34.00	33.25	17.00
S.Em. ±		1.67	1.81	1.39	1.79	2.46	1.77
C.D. 5%		4.82	5.21	4.00	5.15	7.07	5.10
C.V.%		6.16	6.12	6.26	6.34	8.03	8.36

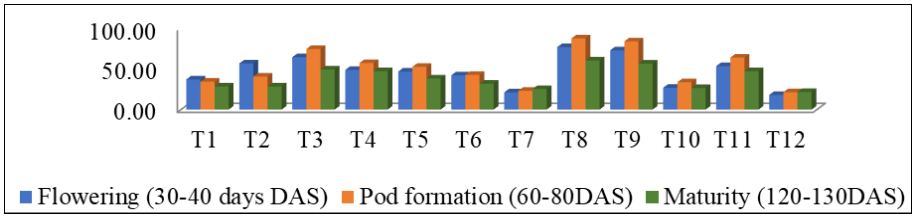

Fig 5: Alkaline phosphatase (µg PNP g^{-1} hr^{-1}) activity at different growth stages of groundnut

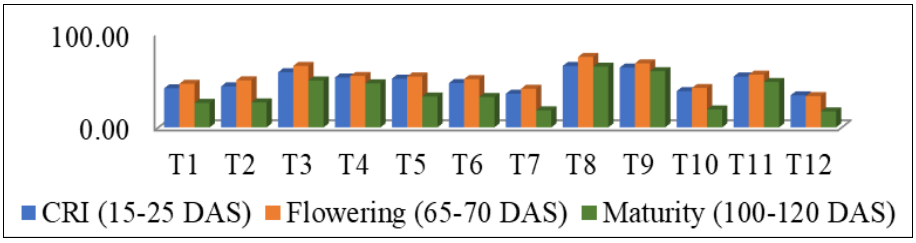

Fig 6: Alkaline phosphatase (µg PNP g^{-1} hr^{-1}) activity at different growth stages of wheat

β-glucosidase activity

The data presented on β-glucosidase activity in soil (Table 4) at different growth stages of groundnut and wheat were indicates that significantly increased β-glucosidase enzyme activity in soil at flowering, pod formation and at maturity (62.80, 71.95 and 47.17 µg PNP $g^{-1}hr^{-1}$) in groundnut and at CRI, flowering and maturity (25.44, 36.15 and 25.29 µg PNP $g^{-1}hr^{-1}$) in wheat with treatment T_8 (50% NPK + FYM 10 t ha^{-1} to groundnut and 100% NPK to wheat), which were remain at par with treatment T_9 (Only FYM 10 t ha^{-1} to groundnut and 15 t ha^{-1} to wheat) at all three stages. Changes in β - glucosidase enzyme activity can be were due to various variables, including changes in overall climate and local climate conditions, differences in nutrient availability, and changes in the amount of leaf litter intake (Dash *et al.* 2014) [8]. This result is supported by Biswas *et al.* (2023) [3] who reported that the highest β-glucosidase activity under NPK + FYM treatment, and the lowest under control. With respect to growth stages, higher β-glucosidase activity were observed at pod formation in groundnut and at flowering in wheat (Fig. 7 & 8).

Table 4: β-glucosidase activity (µg PNP $g^{-1}hr^{-1}$) at different growth stages of groundnut and wheat

Treatment		Groundnut			Wheat		
		Flowering (30-40 DAS)	Pod formation (60-80 DAS)	Maturity (120-130 DAS)	CRI (15-25 DAS)	Flowering (65-70 DAS)	Maturity (100-120 DAS)
T_1	50% NPK	51.06	57.54	40.34	20.19	25.10	20.15
T_2	100% NPK	51.07	54.46	41.70	21.69	27.84	21.63
T_3	150% NPK	51.08	56.24	41.79	23.49	27.88	21.84
T_4	100% NPK+$ZnSO_4$ 50 kg ha^{-1} once in three year to groundnut (*i.e.*'99,02,05,08,11,14, 17, 20,23 *etc.*) &100% NPK to wheat	48.39	63.47	42.11	21.50	26.49	20.82
T_5	NPK as per soil test	47.27	60.53	39.91	20.34	25.24	20.29
T_6	100% NP	47.84	61.23	41.05	20.81	26.57	20.75
T_7	100% N	48.37	57.46	39.75	19.31	25.00	18.29
T_8	50% NPK+10 t ha^{-1} FYM to groundnut & 100% NPK to wheat	62.80	71.95	47.17	25.44	36.15	25.29
T_9	Only FYM 10 t ha^{-1} to groundnut and 15 t ha^{-1} to wheat	61.50	66.62	44.16	24.96	35.87	24.81
T_{10}	50% NPK + *Rhizobium*+ PSM to Groundnut & 100% NPK to wheat	44.43	59.73	39.88	20.13	25.09	19.74
T_{11}	100% NPK (P as SSP)	51.75	63.71	39.87	21.57	27.26	21.25
T_{12}	Control	44.87	46.79	38.89	16.72	19.56	14.07
S.Em. ±		3.82	2.25	1.51	0.81	0.99	0.75
C.D. 5%		10.99	6.49	4.36	2.32	2.86	2.17
C.V.%		13.00	6.51	6.33	6.54	6.29	6.30

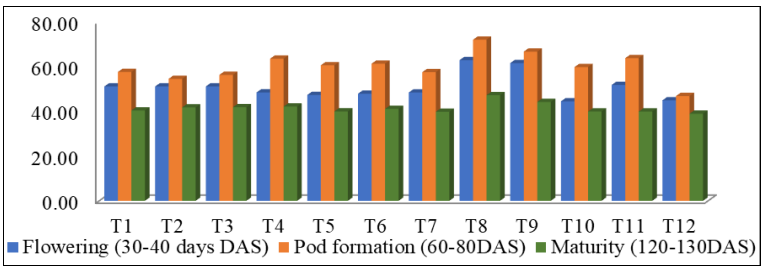

Fig 7: β-glucosidase activity (µg PNP $g^{-1}hr^{-1}$) at different growth stages of groundnut

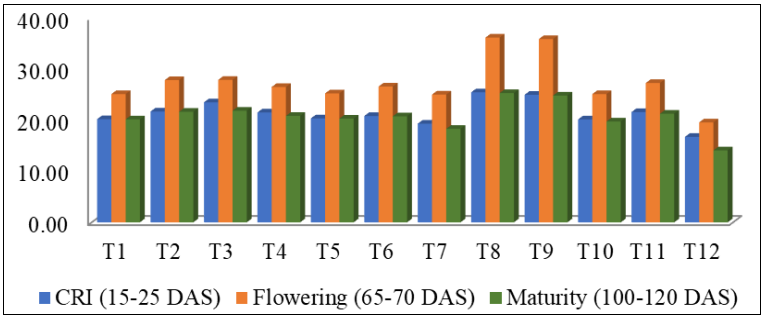

Fig 8: β-glucosidase activity (µg PNP $g^{-1}hr^{-1}$) at different growth stages of wheat

This result is supported by Dash *et al.* (2018) [7] who considering the three growth stages *viz.*, vegetative, flowering and pod formation, β-glucosidase was found more at pod formation stage and more under GM-CLCC N (Green maurig + 75% N + CLCC based N management) (65.02 µg g^{-1} hr^{-1}) and less under ZT (Zero tillage) (48 µg g^{-1} hr^{-1}). Similar finding also done by Sheoran *et al.* (2024) [20] at different growth stages of wheat.

Arylsulphatase activity

Table 5 presents the data on arylsulphatase activity in soil across different growth stages of groundnut and wheat. The results indicate a significant increase in enzyme activity with treatment T8—consisting of 50% NPK combined with FYM at 10 t ha^{-1} for groundnut and 100% NPK for wheat. In groundnut, arylsulphatase activity reached 21.23, 24.97, and 13.70 µg PNP g^{-1} hr^{-1} at the flowering, pod formation, and maturity stages, respectively. For wheat, the corresponding activity levels were 7.63, 12.79, and 9.50 µg PNP g^{-1} hr^{-1} at the CRI, flowering, and maturity stages. These values were statistically comparable to those recorded under treatment T9, which included only FYM (10 t ha^{-1} for groundnut and 15 t ha^{-1} for wheat), and both treatments outperformed the other fertilizer regimes.

This enhanced enzyme activity may be attributed to the influence of crop plants on the soil microbial community structure and function. Plants can alter the abundance, diversity, and enzymatic potential of microbes in the rhizosphere, thereby affecting arylsulphatase production. These findings align with the work of Kumari *et al.* (2024) [15], who reported peak arylsulphatase activity (12.56 µg PNP g^{-1} soil hr^{-1}) under combined application of 15 Mg FYM, 150 kg N, and 30 kg P_2O_5 ha^{-1}.

In terms of growth stages, the highest arylsulphatase activity was observed at the pod formation stage in groundnut and at the flowering stage in wheat (Figures 9 & 10). Tamilselvi *et al.* (2015) [25] also noted that, irrespective of the treatment applied, arylsulphatase activity was relatively low during early plant stages but peaked at flowering, followed by a decline in later stages.

Table 5: Arylsulphatase activity (µg PNP $g^{-1}hr^{-1}$) at different growth stages of groundnut and wheat

Treatment		Groundnut			Wheat		
		Flowering (30-40 DAS)	Pod formation (60-80 DAS)	Maturity (120-130 DAS)	CRI (15-25 DAS)	Flowering (65-70 DAS)	Maturity (100-120 DAS)
T_1	50% NPK	15.07	15.98	7.50	5.98	8.38	7.15
T_2	100% NPK	18.12	21.78	10.00	6.46	9.87	8.10
T_3	150% NPK	18.25	19.85	8.08	6.56	10.27	8.12
T_4	100% NPK+$ZnSO_4$ 50 kg ha^{-1} once in three year to groundnut (*i.e.*'99,02,05,08,11,14, 17, 20,23 *etc.*) &100% NPK to wheat	16.43	21.90	11.52	6.19	9.54	8.23
T_5	NPK as per soil test	15.43	18.07	8.02	6.08	9.37	8.04
T_6	100% NP	13.92	15.97	7.45	5.41	7.71	6.48
T_7	100% N	12.07	16.47	7.15	5.38	7.69	5.75
T_8	50% NPK+10 t ha^{-1} FYM to groundnut & 100% NPK to wheat	21.23	24.97	13.70	7.63	12.79	9.50
T_9	Only FYM 10 t ha^{-1} to groundnut and 15 t ha^{-1} to wheat	20.21	24.12	13.04	7.06	11.85	9.06
T_{10}	50% NPK + *Rhizobium*+ PSM to Groundnut & 100% NPK to wheat	15.13	16.37	7.62	6.02	8.56	7.90
T_{11}	100% NPK (P as SSP)	16.78	21.77	9.37	6.23	9.85	8.15
T_{12}	Control	9.90	9.49	7.57	5.38	5.91	5.13
S.Em. ±		0.90	0.75	0.33	0.22	0.37	0.35
C.D. 5%		2.60	2.16	0.95	0.63	1.05	1.02
C.V.%		9.73	6.87	6.15	6.07	6.81	8.04

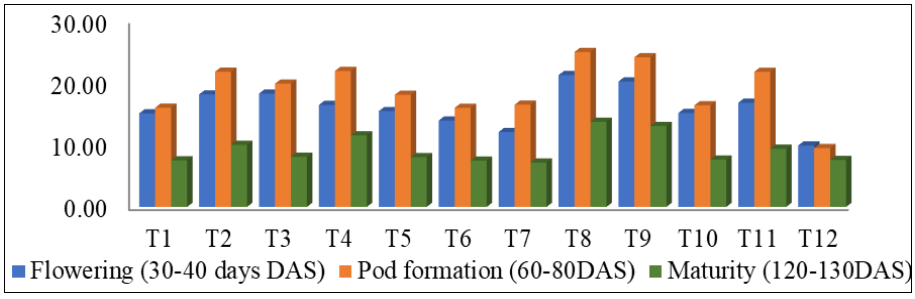

Fig 9: Arylsulphatase activity (µg PNP $g^{-1}hr^{-1}$) at different growth stages of groundnut

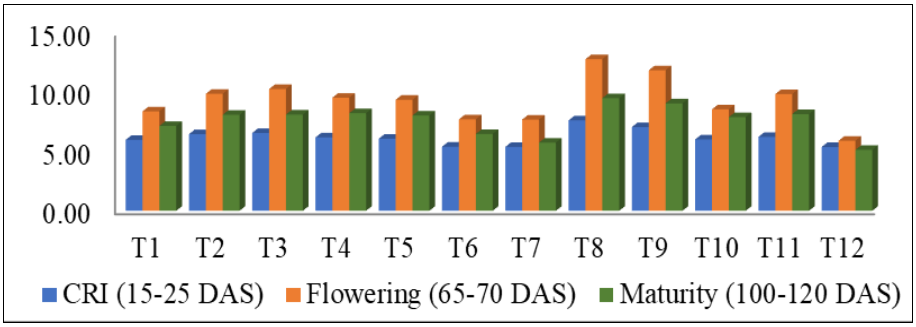

Fig 10: Arylsulphatase (µg PNP $g^{-1}hr^{-1}$) activity at different growth stages of wheat

Urease activity

The data shown in table 6 regarding urease activity at different growth stages of groundnut and wheat. Data indicated that significantly higher urease activity in soil at flowering, pod formation and at maturity (11.72, 12.48 and 10.59 µg NH_4-N $g^{-1}hr^{-1}$) in groundnut and at CRI, flowering and maturity (35.27, 36.98 and 29.93 µg NH_4-N $g^{-1}hr^{-1}$) in wheat with treatment T_8 (50% NPK + FYM 10 t ha^{-1} to groundnut and 100% NPK to wheat), which were remain at par with treatments T_3 (150%NPK), T_7 (100% N), T_9 (Only FYM 10 t ha^{-1} to groundnut and 15 t ha^{-1} to wheat) and T_{11}(100%NPK, P as SSP) at all three stages as comparison to other treatments. The increased microbial population under organics and integrated nutrient management was responsible for increase in urease activity of the soil, this can be attributed to the organic manure which acts as a source of carbon and energy for heterotrophs and provides sufficient nutrition for the increase population of microbes and their activities in terms of soil enzymes. The result is followed by Verma *et al.* (2022) [28] who showed in their finding with the integrated nutrient management involving the use of organic manure together with inorganic fertilization exerted a significant positive effect on urease activity. The higher urease activity were found at pod formation in groundnut and at flowering in wheat (Fig. 11 & 12). Bhavani *et al.* (2017) [2] reported in all the treatments, enzymes exhibited higher activity at flowering stage (60 DAT) and thereafter the activity was decreased towards 90 DAT.

Table 6: Urease activity (µg NH_4- N $g^{-1}hr^{-1}$) at different growth stages of groundnut and wheat

Treatment		Groundnut			Wheat		
		Flowering (30-40 DAS)	Pod formation (60-80 DAS)	Maturity (120-130 DAS)	CRI (15-25 DAS)	Flowering (65-70 DAS)	Maturity (100-120 DAS)
T_1	50% NPK	8.92	9.08	8.90	18.94	26.65	14.99
T_2	100% NPK	10.21	10.85	9.24	27.38	32.83	23.36
T_3	150% NPK	10.98	11.89	9.85	31.55	33.91	28.45
T_4	100% NPK+$ZnSO_4$ 50 kg ha^{-1} once in three year to groundnut *(i.e.*'99,02,05,08,11,14, 17,20,23 *etc.*) &100% NPK to wheat	9.24	10.15	8.88	24.93	29.71	19.99
T_5	NPK as per soil test	9.81	11.26	9.41	21.51	31.98	23.32
T_6	100% NP	9.16	10.00	8.75	26.80	27.30	19.92
T_7	100% N	11.22	12.07	9.96	33.01	35.13	30.40
T_8	50% NPK+10 t ha^{-1} FYM to groundnut & 100% NPK to wheat	11.72	12.48	10.59	35.27	36.98	29.93
T_9	Only FYM 10 t ha^{-1} to groundnut and 15 t ha^{-1} to wheat	10.70	11.77	9.87	31.66	33.73	26.78
T_{10}	50% NPK + *Rhizobium*+ PSM to Groundnut & 100% NPK to wheat	9.92	10.40	9.39	23.90	31.95	22.79
T_{11}	100% NPK (P as SSP)	10.82	12.03	9.92	31.66	34.62	27.01
T_{12}	Control	8.33	8.64	7.58	16.16	18.19	15.56
S.Em. ±		0.42	0.48	0.39	1.31	1.20	1.08
C.D. 5%		1.22	1.37	1.11	3.77	3.45	3.12
C.V.%		7.28	7.56	7.13	8.42	6.68	7.97

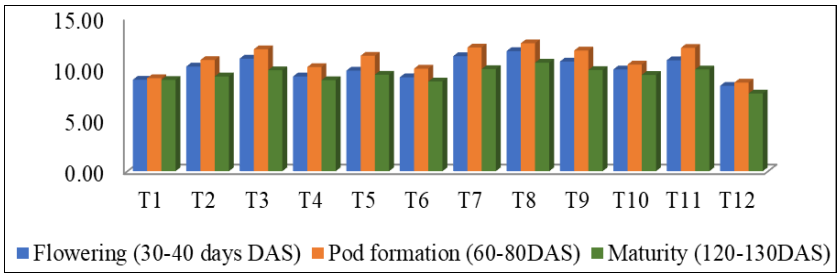

Fig 11: Urease activity (µg NH_4-N $g^{-1}hr^{-1}$) at different growth stages of groundnut

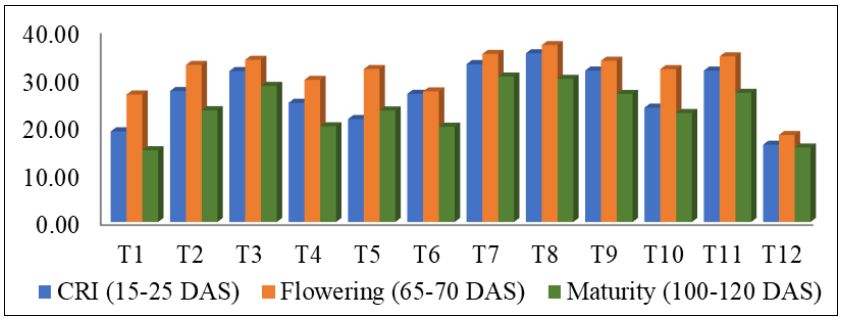

Fig 12: Urease activity (µg NH_4-N $g^{-1}hr^{-1}$) at different growth stages of wheat

Conclusion

The findings of the present study, concluded that the treatment with 50% NPK + 10 t FYM ha^{-1} to groundnut and 100% NPK to wheat significantly increased enzyme activity *viz*, dehydrogenase, acidphosphatse, alkaline phosphates, β-glucosidase, arylsulphatse and urease. With respect to enzyme activity at different growth stages of groundnut (flowering, pod formation and maturity) and wheat (CRI, flowering and maturity), the higher dehydrogenase activity observed at flowering stage in groundnut and at CRI stage in wheat. Higher enzyme activity *i.e.* acidphosphatse, alkaline phosphates, β-glucosidase, arylsulphatse and urease were obtained at pod

formation in groundnut and at flowering in wheat with treatment 50% NPK + 10 t FYM ha^{-1} to groundnut and 100% NPK to wheat.

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