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Enzyme activities and crop productivity in typic *Haplustepts* soil of southern Rajasthan (India) as influenced by long-term organic and inorganic fertilization under maize-wheat cropping system

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Abstract
The effect of organic and inorganic fertilization on enzyme activities and crop productivity in an typic Haplustepts soil of southern Rajasthan (India) was studied in a long-term field experiment initiated during 1996-97 at Instructional farm, Rajasthan College of Agriculture, Udaipur (India). Application of 100% NPK + FYM 10 t ha^{-1} recorded significantly highest alkaline phosphatise, urease and β-glucosidase activity followed by 100% NPK (-NPK of FYM) treatments. However, highest dehydrogenase activity was registered under FYM @ 20 t ha^{-1} treatment. The highest grain and stover/straw yield of maize and wheat obtained by integrated application of 10 t FYM with recommended dose of NPK.

Keywords: Enzyme activities, long-term experiment, farm yard manure, soil organic carbon

Introduction
Many intensive cereal based cropping systems are under practice in India based on their respective agro-climatic regions. The maize (*Zea mays* L.) wheat (*Triticum aestivum* L.) is the most important cropping system adopted in arid and semi-arid ecological regions of Rajasthan. Soil quality does not depend just on the physical and chemical properties of the soil but also on the biological properties. Enzymes are important soil components involved in dynamics of soil nutrient transformations, and enzyme activity is considered to be a major contributor of overall soil microbial activity (Frankenberger and Dick, 1983) [8] and soil quality (Visser and Parkinson, 1992; Dick, 1994) [30]. Soil enzymes are the mediators and catalysts of important soil functions that include: decomposition of organic inputs; transformation of native soil organic matter; release of inorganic nutrients for plant growth (Dick, 1997) [6]. In addition, soil enzymes have a crucial role in C (β-glucosidase), N (urease), P (phosphatase), and S (sulphatase) cycle (Karaca *et al*., 2011) [12]. Continuous application of fertilizer and manure has a significant effect on enzyme activity (Masto *et al*., 2006). Addition of organic manures significantly increases the urease, alkaline phosphatase and dehydrogenase activity in the soil as compared to chemical fertilizers and also very little attention has been paid on maize-wheat cropping system. Hence a long term experiment was planned to study the effects of organic manures and chemical fertilizers on the soil enzymes.

Materials and Methods
The present investigation was carried out in the ongoing long-term fertilizer experiment initiated during 1996 at the Instructional farm, Rajasthan College of Agriculture, Udaipur during 2016-17 and 2017-18. The experimental site is a permanent manurial trial and its layout is on fixed site, at block B_2, situated at 24°34N' latitude, 73°42E' longitude and 582.17 m about mean sea level. The area comes under sub-humid southern plain (Zone-IVa) of Rajasthan.

The climate of the region is subtropical, characterized by mild winters and distinct summers associated with high relative humidity particularly during the months of July to September. The mean annual rainfall of the region varies from 650 to 750 mm, most of which is received in rainy season from July to September. The mean maximum and minimum temperature are 35.45 °C and 17.41 °C, respectively. The soil of the experimental field was sandy clay loam in texture, non-saline and slightly alkaline in reaction and classified taxonomically as "typic *Haplustepts*".

At the initiation of the experiment, soil of the experimental field was having pH 8.20, EC 0.48 dSm^{-1}, Organic carbon 6.80 g kg^{-1}, available Nitrogen 360 kg ha^{-1}, available phosphorus 22.4 kg ha^{-1}, available potassium 671 kg ha^{-1}, available Zn 3.76 mg kg^{-1}, available Fe 2.52 mg kg^{-1}. The 12 treatments with four replications in a randomized block design with 152 m^2 plot for each treatment were as follows: T_1-control; T_2-100% N; T_3-100% NP; T_4-100% NPK; T_5-100% NPK + Zn; T_6-100% NPK + S; T_7- 100% NPK + Zn + S; T_8-100% NPK + Azotobacter; T_9-NPK 100% NPK + FYM 10 t ha^{-1}; T_{10}- FYM 10 t ha^{-1} + 100% NPK (-NPK of FYM); T_{11}-150%; T_{12}-FYM 20 t ha^{-1}. Soil samples collected from a depth of 0-0.15 m after the harvest of maize (2016-17 and 2017-18) were used for determination of various chemical parameters.

The sources used for applying N, P and K were urea, di-ammonium phosphate (adjusted for its N content) and muriate of potash, respectively. Gypsum and zinc sulphate ($ZnSO_4.7H_2O$) were used to supply S and Zn respectively. The other sources of nutrients were FYM (farm yard manure) and bio fertilizer (*Azotobacter* sp.). Nitrogen, Phosphorus and Potash was applied @ 100:26:25 kg ha^{-1}. The sulphur and zinc were applied @ 40 kg S ha^{-1} and 5 kg Zn ha^{-1} respectively while FYM was applied as per the treatments. The sulphur, zinc and FYM were applied once in a year to the maize crop. The bio fertilizer for seed inoculation was used at 600 g ha^{-1} during both seasons.

Fertilizer application in both wheat and maize crops was made as per the treatment. Full dose of phosphorus and potash and half dose of nitrogen were applied at sowing time by drilling in crop rows. The remaining dose of nitrogen was top dressed in two equal split doses at 25-30 DAS and 45-50 DAS depending upon the occurrence of rains to maize and top dressed just before 1st irrigation applied at CRI stage to wheat crop. Farm yard manure was thoroughly mixed in soil as per the treatment allocation one month before sowing of maize. The seeds were treated with *Azotobacter* inoculants as per the treatment. The seeds were thoroughly mixed with biofertilizer slurry in such a way that all the seeds were uniformly coated with *Azotobacter* and then allowed to dry in the shade before the sowing of crop.

Soil samples collected from a depth of 0-0.15 m after the harvest of maize (2017-2018) were used for determination of various chemical parameters. The other lot was passed through 100-mesh screen and was stored in polythene bags for determining different fractions of SOM. Four composite soil samples from 0-0.15 m were also drawn from adjacent fallow plots. The processed soil samples were analyzed for active pools of SOM *viz*., Water-soluble organic carbon (WS-OC), Water soluble carbohydrates (WS-CHO), Soil microbial biomass carbon (SMBC), Permanganate oxidizable soil carbon ($KMnO_4$-C), Particulate organic carbon (POC), Light fraction organic carbon (LFOC) and Enzymatic activity *viz*., Urease Activity, Alkaline Phosphatase Activity, Soil dehydrogenase activity and β-glucosidase activity.

Urease activity was measured following the method described by Tabatabai and Bremner (1972) [27]. Five gram of soil was incubated with 9 ml THAM buffer, 0.2 ml toluene and 1 ml of 0.2% of urea solution at 37^0C for 2 hours. Then 50 ml KCl-$AgSO_4$ solution was added and shaking was done for 30 minutes. Soil suspension was filtered. Taking 20 ml aliquot from filtrate, NH_4-N was determined by steam distillation method (Keeney and Nelson, 1982) [14]. Alkaline phosphatase activity was measured by the method described by Tabatabai and Bremner (1969) [26]. Alkaline phosphatase activity was estimated by taking 1 g of soil with 0.2 ml toluene, 4 ml of modified universal buffer (MUB, pH 11) and 1 ml of p-nitrophenyl phosphate (PNP). After incubation for 1 hour at 37 ^{0}C, the enzyme reaction was stopped by adding 4 ml of 0.5 M NaOH and 1 ml of 0.5 M $CaCl_2$. Soil suspension was filtered and absorbance was measured at 400 nm. Soil dehydrogenase activity was analyzed by Anthrone extraction method (Cassida *et al.*, 1964) [4]. 6 g of soil and a pinch of $CaCO_3$ were taken in test tube and add 1 ml of 3 per cent aqueous solution of 2,3,5-triphenyl tetrazolium chloride and 2.5 ml of distill water. After mixing, the sample incubated for 24 hours at 37 °C. 10 ml methanol was added and test tube was shaken. The suspension was filtered through a glass funnel plugged with absorbent cotton. Added methanol, until the reddish colour disappeared from the cotton plug. Diluted to 100 ml volume with methanol. The intensity of red colour of TPF was measured using a spectrophotometer at a wavelength of 485 nm. The β-glucosidase activity was assessed using 100 µl of 5 mM p-nitrophenyl-β-D-glucanopyranoside (PNG) as the substrate. Soil subsamples (100 mg of fresh soil) were incubated at 37˚C for 2 h with 400 µl of citrate phostphate buffer at pH 5.8. The p-nitrophenol (PNP) produced in the enzymatic reactions was extracted and determined at 400 nm (Wu *et al*. 2006).The results were expressed as µg PNP released $g^{-1}\cdot soil\cdot h^{-1}$.

Results and Discussion

Effect of organic and inorganic fertilization on enzyme activities

Dehydrogenase Activity

Highest dehydrogenase activity was observed in FYM alone followed by combined application of FYM with inorganic fertilizer. Application of FYM 20 t ha^{-1} gave 53.28 and 19.19 per cent higher dehydrogenase activity as compare to control (30.59 µg TPF 24 hr^{-1} g^{-1}) and recommended dose of fertilizer (39.34 µg TPF 24 hr^{-1} g^{-1}) (Table 1). Application of organic manures alone or in combination with NPK fertilizers significantly increased dehydrogenase activities as compared to NPK fertilizers applied alone. This might be due to the reason that sources of potential beneficial microbes in organic manures may possibly provide microbial diversity and activity of microorganisms accompanied by better dehydrogenase activity. Moreover, the applied organic sources were able to get mineralized rapidly in early days of incubation hence, there was more mineralization than immobilization which consequently provided sufficient nutrition for the proliferation of microbes and their activities in terms of soil dehydrogenase (Lakshmi *et al*., 2014; Tripura *et al*., 2018; Swati *et al*., 2018; Sheoran *et al*., 2018) [17, 28, 25, 22] also found the similar results. Dehydrogenase activity in 100% NPK (T_4) being 39.34 µg TPF 24 hr^{-1} g^{-1} was found to be significantly higher than treatment receiving 100% NP (37.20 µg TPF 24 hr^{-1} g^{-1}) and 100% N (34.38 µg TPF 24 hr^{-1} g^{-1}). This indicated that imbalanced application of fertilizers exerted adverse effect on dehydrogenase activity.

Alkaline phosphatase activity

Application of balanced dose of chemical fertilizers (100% NPK) either alone or combination with FYM resulted in significantly increased phosphatase activity as compared to the control. Application of optimal and super optimal dose of

NPK *viz;* 100 and 150% NPK increased the phosphatase activity by 40.82 and 42.22 per cent, respectively over the control (Table 1). Addition of organic manures resulted in significant higher build up in alkaline phosphatase activity over NPK fertilizers alone. It might be due to added quantity of organic matter, which in turn increased the organic carbon and nitrogen. The organic acids produced during decomposition of organic matter tend to reduce soil pH which enhanced the enzyme activity (Reddy and Reddy, 2009) [20]. The lowest enzyme activity was observed under control treatments due to less substrate availability. In the present study, alkaline phosphatase activity was higher with application of conjunctive use of organic and inorganic fertilizers over application of organic manures or chemical fertilizers alone. This increase in activity may be due to the release of more organically bound P due to faster decomposition of organic matter in presence of mineral N and P which stimulate the synthesis of the enzyme. Increase in alkaline phosphatase activity with application of organic manures over chemical fertilizers application was reported by several workers Kumar *et al.* (2005) [16], Hojati and Nourbakhsh (2006) [9] and Mohammadi *et al.* (2011) [19] while superiority of combined application of organic manures and NPK fertilizers over organic manures alone has also been observed in several studies (Aher *et al.*, 2015; Jat and Singh., 2017; Sheoran *et al.*, 2018) [1, 11, 22].

Urease activity

Application of 100% NPK + FYM gave 197.54 and 64.43 per cent higher urease activity as compare to control and recommended dose of fertilizer (Table 1). Highest urease activity obtained with application of conjunctive use of organic and inorganic fertilizers over application of organic manures or chemical fertilizers alone. High urease activity might be due to increased population of microorganisms with increased availability of substrate through production of diverse extra cellular enzymes. Application of optimal and super optimal dose of NPK *viz;* 100 and 150% NPK increased the urease activity by 80.94 and 85.04 per cent, respectively over the control. Urease activity in 100 per cent NPK (T_4) being 8.83 µg NH_3 g^{-1} hr^{-1} was found to be significantly higher than treatment receiving 100% NP (7.43 µg NH_3 g^{-1} hr^{-1}) and 100% N (5.27 µg NH_3 g^{-1} hr^{-1}). Lowest urease activity was reported in control treatment and where only 100% N and 100% NP were applied. These results are in line with findings of Mishra *et al.* (2008) [18] who reported that imbalanced fertilization (only N) deteriorated soil health and gave adverse effect on enzyme activity. Urease activity was found to be increased with application of organic manures alone or in combination with chemical fertilizers over application of chemical fertilizers alone as reported in several studies (Lakshmi *et al.*, 2014; Kashyap and Khokhar., 2017; Jat and Singh., 2017) [17, 13, 11].

β-glucosidase activity

The highest β-glucosidase activity was recorded under 100% NPK + FYM 10 t ha^{-1} treatment (286.0 µg PNP g^{-1} hr^{-1}) which was significantly superior to rest of the treatments (Table 1). Application of 100% NPK + FYM 10 t ha^{-1} treatment gave 64.36 and 34.58 per cent higher β-glucosidase activity as compare to control (174.0 µg PNP g^{-1} hr^{-1}) and recommended dose of fertilizer (212.5 µg PNP g^{-1} hr^{-1}). This might be due to the reason that sources of potential beneficial microbes in organic manures may possibly provide microbial diversity and activity of microorganisms accompanied by better β-glucosidase activity. The β-glucosidase activity in 100% N, 100% NP and 100% NPK treatments was higher by 8.90, 12.93 and 22.12 per cent, respectively over the control. Application of 100% NPK (-NPK of FYM) and FYM 20 t ha^{-1} showed significant increase in the β-glucosidase activity by 26.82 and 15.05 per cent, respectively over 100% NPK alone. β-glucosidase activity in 100 per cent NPK being 212.5 µg PNP g^{-1} hr^{-1} was found to be significantly higher than treatment receiving 100% NP (196.5 µg PNP g^{-1} hr^{-1}) and 100% N (189.5 µg PNP g^{-1} hr^{-1}). The treatment receiving 100% N alone was statistically at par with 100% NP treated plots.

Table 1: Effect of organic and inorganic fertilization on enzymatic activity of soil after harvest of maize under wheat-maize cropping sequence

Treatments	Dehydrogenase (mg TPF 24 hr^{-1} g^{-1})			Alkaline phosphatase (µg PNP g^{-1} hr^{-1})			Urease (µg NH_3 g^{-1} hr^{-1})			β-glucosidase (µg PNP g^{-1} hr^{-1})		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T1 = Control	30.62	30.55	30.59	135.87	135.40	135.64	4.96	4.80	4.88	175.00	173.00	174.00
T2 = 100% N	34.38	34.37	34.38	155.44	153.47	154.46	5.24	5.30	5.27	189.00	190.00	189.50
T3 = 100% NP	37.18	37.22	37.20	169.57	170.60	170.09	7.25	7.60	7.43	195.00	198.00	196.50
T4 = 100% NPK	39.29	39.38	39.34	189.78	192.24	191.01	8.75	8.90	8.83	210.00	215.00	212.50
T5 = 100% NPK + Zn	39.50	39.60	39.55	195.20	198.35	196.77	9.10	9.60	9.35	213.00	217.00	215.00
T6 = 100% NPK+ S	39.51	39.62	39.57	200.14	203.17	201.66	8.60	8.95	8.78	209.00	213.00	211.00
T7 = 100% NPK+ Zn + S	39.57	39.65	39.61	205.24	206.25	205.75	9.25	9.80	9.53	214.00	218.00	216.00
T8 = 100% NPK + Azotobacter	39.70	39.78	39.74	192.43	195.40	193.92	8.65	8.74	8.70	210.00	214.00	212.00
T9 = 100% NPK + FYM 10 t ha-1	45.80	45.96	45.88	217.47	220.19	218.83	14.24	14.80	14.52	280.00	292.00	286.00
T10 = FYM 10 t ha-1 + 100% NPK (-NPK of FYM)	45.67	45.77	45.72	215.10	218.40	216.75	13.90	14.20	14.05	266.00	273.00	269.50
T11 = 150% NPK	44.10	44.22	44.16	192.35	193.48	192.92	8.90	9.15	9.03	215.00	219.00	217.00
T12 = FYM 20 t ha-1	46.80	46.98	46.89	209.20	214.44	211.82	11.10	11.45	11.28	240.00	249.00	244.50
S.Em.±	0.97	0.97	0.69	4.51	4.56	3.21	0.25	0.24	0.17	5.46	5.55	3.89
C.D. (P = 0.05)	2.80	2.80	1.94	13.00	13.12	9.06	0.71	0.70	0.49	15.71	15.99	11.00

Effect of organic and inorganic fertilization on productivity of maize

The grain and stover yield of maize significantly varies under the treatments. The highest grain and stover yield obtained by combined application of FYM with recommended dose of NPK. This treatment increase grain yield by 209.45 and 24.45

per cent as compared to control (1322 kg ha^{-1}) and recommended dose of fertilizer (3287 kg ha^{-1}) and 145.77 and 14.71 per cent higher stover yield as compared to control (2189 kg ha^{-1}) and recommended dose of fertilizer (4702 kg ha^{-1}) (Table 2). The highest biological yield was also obtained by combined application of FYM with recommended dose of NPK. The low yield levels in zero fertilized plots are understandable and could be explained due to poor inherent capacity of the soils under study to meet the requirement of crops in respect of the essential nutrients. Application of P along with N considerably increased yield of both the crops compared to the application of N alone. Comparatively lower yields in sub-optimal level of NPK (50%) might be due to less exhaustive effects on native reserves of the soil NPK as compared to optimal treatment and thus, sustained yield at low levels. The results of the present study are in line with those reported by Durani *et al.* (2017) [7], Shah and Wani (2017) [21] and Singh *et al*. (2017) [11]. Application of 100% NPK with *Azotobacter* seed treatment increased the yield of maize over control. This was in confirmation with findings of Jaipaul *et al.* (2008) [10], Das *et al.* (2010) [5] and Kumar and Dhar (2010) [15].

Table 2: Effect of organic and inorganic fertilization on grain, stover and biological (kg ha-1) yield of maize under wheat-maize cropping sequence

Treatments	Grain yield			Stover yield			Biological yield		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T_1 = Control	1324	1320	1322	2197	2180	2189	3521	3500	3511
T2 = 100% N	2110	2107	2109	3475	3460	3468	5585	5567	5576
T3 = 100% NP	2844	2860	2852	4142	4170	4156	6986	7030	7008
T4 = 100% NPK	3278	3295	3287	4683	4720	4702	7961	8015	7988
T5 = 100% NPK + Zn	3402	3425	3413	4837	4867	4852	8239	8292	8265
T6 = 100% NPK+ S	3340	3356	3348	4802	4840	4821	8142	8196	8169
T7 = 100% NPK+ Zn + S	3566	3585	3576	4975	5010	4993	8541	8595	8568
T8 = 100% NPK + *Azotobacter*	3398	3420	3409	4870	4905	4888	8268	8325	8297
T9 = 100% NPK + FYM 10 t ha^{-1}	4083	4098	4091	5365	5394	5380	9448	9492	9470
T10 = FYM 10 t ha^{-1} + 100% NPK (-NPK of FYM)	3440	3458	3449	4628	4658	4643	8068	8116	8092
T11 = 150% NPK	3646	3663	3655	5278	5315	5297	8924	8978	8951
T12 = FYM 20 t ha^{-1}	2400	2405	2403	3080	3092	3086	5480	5497	5489
S.Em.±	70	71	50	99	99	70	169	170	120
C.D. (P = 0.05)	202	201	140	285	286	198	486	488	338

Effect of organic and inorganic fertilization on productivity of wheat

The highest grain and straw yield of wheat obtained by integrated application of FYM 10 t ha^{-1} with recommended dose of NPK. The highest grain yield 5146 kg ha^{-1} was obtained under 100% NPK + FYM 10 t ha^{-1} treatment which followed by 4719, 4631 and 4661 kg ha^{-1} under 150% NPK, FYM 10 t ha^{-1} + 100% NPK (-NPK of FYM, T_{10}) and 100% NPK + Zn + S respectively (Table 3). This treatment *i.e.* 100% NPK + FYM 10 t ha^{-1} gave 215.70 and 17.38 per cent higher grain yield as compared to control (1630 kg ha^{-1}) and recommended dose of fertilizer (4384 kg ha^{-1}) and 210.12 and 12.44 per cent higher straw yield as compare to control (2360 kg ha^{-1}) and recommended dose of fertilizer (6509 kg ha^{-1}). The biological yield was also in same trends. Highest yield in 100% NPK + FYM 10 t ha^{-1} may be due to addition of FYM which besides supplying all the essential nutrients might have also improved the physic-chemical properties of the soil. Singh and Agarwal (2005) [24] also reported the similar results. The application of P along with N (T_3) significantly increased the grain and stover yield as compared to unfertilized plot (T_1). Application of K along with N and P (T_4) resulted in higher grain and stover yield of wheat over 100% NP only. Similar effect of K nutrition on wheat was reported earlier by Basak *et al.* (2012) [2].

Table 3: Effect of organic and inorganic fertilization on grain, stover and biological (kg ha^{-1}) yield of wheat under wheat-maize cropping sequence

Treatments	Grain yield			Stover yield			Biological yield		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T_1 = Control	1640	1620	1630	2389	2330	2360	4029	3950	3990
T2 = 100% N	3052	3045	3049	4456	4440	4448	7508	7485	7497
T3 = 100% NP	3648	3682	3665	5478	5510	5494	9126	9192	9159
T4 = 100% NPK	4373	4395	4384	6488	6530	6509	10861	10925	10893
T5 = 100% NPK + Zn	4541	4555	4548	6680	6720	6700	11221	11275	11248
T6 = 100% NPK+ S	4462	4480	4471	6650	6690	6670	11112	11170	11141
T7 = 100% NPK+ Zn + S	4657	4665	4661	6892	6950	6921	11549	11615	11582
T8 = 100% NPK + *Azotobacter*	4459	4476	4468	6966	7015	6991	11425	11491	11458
T9 = 100% NPK + FYM 10 t ha^{-1}	5137	5154	5146	7298	7340	7319	12435	12494	12465
T10 = FYM 10 t ha^{-1} + 100% NPK (-NPK of FYM)	4622	4640	4631	7096	7152	7124	11718	11792	11755
T11 = 150% NPK	4708	4730	4719	6484	6515	6500	11192	11245	11219
T12 = FYM 20 t ha^{-1}	3166	3170	3168	4658	4665	4662	7824	7835	7830
S.Em.±	93	93	66	139	139	98	231	232	164
C.D. (P = 0.05)	266	267	185	400	402	278	666	669	463

Conclusion

Based on the results, it can be inferred that integrated organic and inorganic fertilization significantly improved alkaline phosphatise, urease and β-glucosidase activity as compared to organic and inorganic fertilization alone. However, highest dehydrogenase activity was registered under FYM @ 20 t ha^{-1} treatment. The Grain and stover yield of maize and wheat significantly varies under the treatments. The highest grain and straw yield of maize and wheat obtained by integrated application of 10 t FYM ha^{-1} with recommended dose of NPK.

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