Annals of Plant and Soil Research 25(4): 541-548 (2023)
https://doi.org/10.47815/apsr.2023.10304

Effect of nitrogen level and herbicide management on soil microbial function under conservation agriculture in Maize-Wheat system

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Received: August, 2023; Revised accepted: November, 2023

ABSTRACT

A field experiment was started during 2008-09 to study the effect of nitrogen (N) management practices and various herbicides mixture on soil enzymatic activities under long-term conservation agriculture in maize-wheat system. Four N rates in main plots i.e. CT_{100}; conventional tillage + residue + 100% recommended dose of N (RDN) used as control, ZT_{50}; zero tillage (ZT) + residue + 50% RDN, ZT_{75}; ZT + residue + 75% RDN, ZT_{100}; ZT + residue + 100 % RDN and four herbicide treatments in sub plots i.e. W_1; sulfosulfuron + metsulfuron - methyl @ 40 g a.i. ha^{-1}, W_2; carfentrazone-ethyl + metsulfuron-methyl @ 20+4 g a.i. ha^{-1}, W_3; clodinafop + sulfentrazone+ metsulfuron-methyl @60+20+4 g a.i. ha^{-1} and U; unweeded were used with three replications and arranged in the split-plot design. Among N management with tillage practices, ZT_{100} recorded higher enzymatic activity viz. dehydrogenase, β-glucosidase, arginine deaminase and microbial biomass carbon in soil by 7.10, 11.8, 20, 10.3 and 3.75% respectively over CT_{100} and enzymatic activity followed the order; ZT_{100}>CT_{100}> ZT_{75} and ZT_{50}. Among herbicides, the enzymatic activities were found to reduce to the maximum extent in W_3 of ZT_{50}. The highest grain yield for wheat crop was observed under ZT_{100} (3.76 t ha^{-1}) and ZT_{100} treatment which was statistically at par with such treatment CT_{100} (3.71 t ha^{-1}).Therefore, a 100% recommended dose of N with zero till may be economically viable in place of conventional tillage. Also, application of a mixture of sulfosulfuron + metsulfuron-methyl can is suggested to control the weeds without affecting the soil microbial activity significantly.

Key words: Conservation agriculture, herbicides, nitrogen management, Soil enzymes, yield

INTRODUCTION

The rice-wheat cropping system is one of the most popular cropping systems of India, particularly practiced in the North- Western part of Indo-Gangetic Plains, where the soil health is strained by the loss of soil carbon. Soil health is the integral component of one health concept of biosphere. Soil health is mainly influenced by soil microbiota and due to present chemical dominated conventional clean cultivation with continuous tillage, hampering the soil microbial growth. The accelerated soil degradation activities in this region particularly by anthropogenic factors, not only deteriorates soil quality but also the quality of living of crores of human being present in this area (Kumar *et al.,* 2020). The soil biota is heavily influenced by the different agricultural activities, including the weed management approaches where the application of herbicides known to alter microbial metabolic activities and several soil enzymatic activities. But the positive or negative influence of herbicides on soil microorganisms mainly depends on the type of herbicides, type of microbe and prevailing environmental and ecological conditions. The weed management practices also indirectly regulate nutrient availability to crops by controlling competition from weeds (Jatav *et al.,* 2018). Soil enzymes plays a key role in maintaining soil ecosystem and hence the soil health (Patra *et al.,* 2021). Soil enzymes are the catalytic agent and mediators of key soil functions, which include release of mineral nutrients for growth and development of plants; biological fixation of N; breakdown of organic matter; detoxification of xenobiotic; nitrification etc. (Vishwanath *et al.,* 2020). They also play a significant role in carbon (β-glycosidase and β-galactosidase), N (urease), phosphorus (phosphatases), and sulphur (sulphatase) cycle (Vishwanath *et al.,* 2021). The dehydrogenase activity in soil indicates overall microbial respiration status, which is well correlated with soil quality. The increase in the microbial population leads to increase in the

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respirational activities which results in enhancement of dehydrogenase activity. The phosphatase enzyme fuels the phosphorus cycle in soil by hydrolyzing the organic phosphorus forms to plant utilizable phosphates forms. Urease enzyme regulates the conversion of urea to ammoniacal form. Under the conventional intensive cropping systems, imbalanced use of fertilizers, excessive pesticides application and exhaustive tillage practices have deteriorated the microbial activities in soil. Conversely, adoptions of long-term conservation agricultural practices have improved soil microbial activity in terms of microbial biomass carbon (MBC), enzymatic activities and overall microbial metabolism. Conservation agriculture (CA), in which tillage is decreased to minimum or no tillage and crop leftovers are employed as sources of organic matter in the field, in conjunction with crop diversity in the rice-wheat cropping system, resulted in enhanced soil structure, hydrological qualities, and total plant productivity (Page *et al.,* 2020). However, the information regarding influence of N and weed management practices on soil enzymatic activities under CA practices is limited. So, in the present investigation, the significance of N and weed management practices on soil enzymatic activities and Soil microbial biomass carbon under CA in maize-wheat cropping system for sustainable soil health and weed management was evaluated.

MATERIALS AND METHODS

The experiment was initiated with Maize (*Zea mays* L.) –Wheat (*Triticum aestivum* L.) cropping system at the Agronomy Research Farm (Mid A-5 Plot), ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India during 2008. The site is situated at 28°37′–28°39′ N latitude and 77°9′–77°11′ E longitude, altitude 228.6m above mean sea level. The mean annual rainfall of the study site is 670 mm. The average annual temperature is 25.3 °C, with maximum and minimum temperatures 40.5 °C and 6.5 °C, respectively during experimental period.

Treatment details, design of experiment and crop management practices

Initially the experiment was started with cotton (*Gossypium hirsutum*) - wheat (*Triticum aestivum*) cropping system during 2008-09. However, diversified cropping system was used in order to attain higher productivity and the legume crop was added in the cropping system in 2011-12, thus the cropping system was modified to maize-wheat-green gram. During 2015-16, the cropping system was changed to soybean -wheat for crop diversification. The cropping system was changed again to maize-wheat-green gram in 2016-17 and which has been continued till now. Maize (*var* PMH-1) was grown in the *kharif* season followed by wheat (*var* HD-2967) in the *rabi* season. The recommended dose of fertilizer for maize and wheat were 150-80-60 and 120:60:40 NPK kg ha^{-1}, respectively. The experimental design was split plot with 3 replications. The four N management with tillage practice treatments were kept in the main plots *i.e.* conventional tillage + residue + 100% recommended dose of nitrogen (CT_{100}) used as control; zero tillage + residue + 50% recommended dose of nitrogen (ZT_{50}); Zero tillage + residue + 75% recommended dose of nitrogen (ZT_{75}); Zero tillage + residue + 100% recommended dose of nitrogen (ZT_{100}). The 100% nitrogen (120 kg N ha^{-1}) was applied through urea in three split doses *i.e.* 50% basal (at the time of sowing of crop), 25% at crown root initiation and 25% at booting stage. Whereas in the sub-plots there were four treatments involving weed management sulfosulfuron + metsulfuron-methyl 40 g a.i. ha^{-1} (pre-mix post-em.) at 30 days after sowing(W_1); carfentrazone-ethyl + metsulfuron-methyl (20+4 g a.i. ha^{-1}) (pre-mix post-em.) at 30 days after sowing (W_2); clodinafop + carfentrazone + metsulfuron-methyl (60+20+4 g a.i. ha^{-1}) (tank- mix post- em.) at 30 days after sowing (W_3) and unweeded/control (U). Recommended dose of nitrogen, phosphorus and potassium was applied through urea, diammonium phosphate (DAP) and muriate of potash (MOP).

Collection and preservation of soil samples

The soil samples were collected from 0-15cm depth from all the treatments at the harvesting stage of wheat crop for analysis of soil enzymatic activities. Total 48 composite soil samples (4 main plot x 4 sub plot x 3 replicates) were filled in the tagged bags and transported to the laboratory through proper mode of transport.

Later, soil was placed in open air dry condition and simultaneously sieved (2 mm mesh size), homogenized and stored at 4 °C with proper tagging.

Analysis of soil samples

The activity of dehydrogenase enzyme was determined using the method suggested by Casida *et al.* (1964). β-glucosidase activity (µg p-nitro-phenol g-1 h^{-1}) of soil were measured in terms of released amount of p-nitrophenol after 1 h of incubation with p-nitrophenyl-β-D-glucopyranoside (Eivazi and Tabatabai, 1988). Alkaline phosphomonoesterase activity was assayed using the method suggested by (Tabatabai and Bremner 1970). Activity of arginine deaminase was determined using colorimetric method (Kandeler *et al.,* 1996). Soil microbial biomass carbon (SMBC) measured by method of fumigation extraction as suggested by (Jenkinson and Powlson, 1976).

Computation of data and their statistical analysis

The data was statistically analyzed using procedures laid out by Gomez & Gomez (1984) for the split plot design (SPD). The analysis of variance (ANOVA) and Tukey's Honest Significant Difference test (Tukey HSD) were conducted using Microsoft Office 2016 and R software. Significance tests were caried out at P <0.05.

RESULTS AND DISCUSSION

Dehydrogenase activity (DHA)

The weed management and N practices found to significantly influence the enzymatic activities in soil under the CA systems. The soil DHA under different nitrogen management treatments was found in the range of 3.79 to 4.72 µg TPF g^{-1} soil hr^{-1} (table 1). The treatment ZT_{100} recorded significantly higher DHA activity of 4.72µg TPF g^{-1} soil hr^{-1}amongst all treatments. DHA in soil was increased by 7.51 % in treatment ZT_{100} over CT_{100}. Among N management treatments, DHA activity in soil followed the order ZT_{100} (4.72 µg TPF g^{-1} soil hr^{-1}) > CT_{100} (4.39µg TPF g^{-1} soil hr^{-1}) > ZT_{75} (4.16µg TPF g^{-1} soil hr^{-1}) and it was found to be lowest in ZT_{50} plot (3.79µg TPF g^{-1} soil hr^{-1}). The higher DHA in ZT_{100} plots may be attributed to greater soil microbial abundance in zero tillage plots having 100% recommended dose of N because of an increase in available carbon sources through residues retention (Bhattacharya *et al*. 2019). Under different weedicide treatments, DHA was recorded in the range of 3.78 to 4.87 µg TPF g^{-1} soil hr^{-1} (table 1).Weedicide treated plots recorded significantly lower soil DHA as compared to the unweeded plot. Application of W_3 resulted in 22.3% lower DHA than unweeded plot. The diminished carbon source due to herbicide application might have caused the poor stimulus for microbial growth, which ultimately resulted in lower dehydrogenase activity in weedicide treated plots (Pertile *et al.,* 2020).

β-glucosidase activity (BG)

The activity of β-glucosidase was also affected notably by the N management and weedicide application.The soil BG under N management treatments was recorded in the range of 58.2 to 95.7µg PNP g^{-1} soil hr^{-1}. Highest BG activity was recorded in ZT_{100} treatment (95.7 µg PNP g^{-1} soil hr^{-1}) whereas the minimum enzymatic activity was found under ZT_{50} (58.2 µg PNP g^{-1} soil hr^{-1}). The lower tillage intensity under CA might have induced a general positive effect on enzymatic activity in the bulk soil (Chen *et al.,* 2019). In present study, it was also found that unweeded plot recorded significantly higher β-glucosidase activity followed by W1 (80.2µg PNP g^{-1} soil hr^{-1}), W_2 (78.1µg PNP g^{-1} soil hr^{-1}) and W_3 (68.9µg PNP g^{-1} soil hr^{-1}). The application of weedicide in W_3 was found to reduce soil BG activity to the highest extent. It can be due to the reason that W_3 treatment contained 3 herbicides belonging to different classes and mode of action. The differential residual effect of herbicides present in the mixture affected the growth of enzyme adversely (Pose *et al.,* 2017). A significant interaction was observed between N management and weedicide application in terms of β-glucosidase activity as given in the Table 1.

Table 1: Impact of nitrogen management and various herbicides on soil enzymes activity under Conservation Agriculture in Maize-Wheat system

Soil dehydrogenase activity (μg TPF g^{-1} soil hr^{-1})					
Treatments	W_1	W_2	W_3	U	Mean
CT_{100}	4.44	4.25	3.72	5.15	4.39^b
ZT_{50}	3.92	3.50	3.33	4.40	3.79^d
ZT_{75}	4.22	3.93	3.79	4.71	4.16^c
ZT_{100}	4.95	4.41	4.28	5.22	4.72^a
Mean	4.38^b	4.02^c	3.78^d	4.87^a	
LSD ($p<0.05$)	N= 0.17 H= 0.22 N*H= 0.44				
β-glucosidase activity (μg PNP g^{-1} soil h^{-1})					
CT_{100}	85.7	88.9	77.6	90.2	85.6^b
ZT_{50}	60.1	57.1	49.6	65.8	58.2^d
ZT_{75}	75.8	72.3	63.5	77.8	72.3^c
ZT_{100}	99.1	94.0	85.0	104.6	95.7^a
Mean	80.2^b	78.1^c	68.9^d	84.6^a	
LSD ($p<0.05$)	N= 3.80 H= 2.82 N*H=5.64				

CT_{100} - Conventional tillage + residue + 100% recommended dose of nitrogen; ZT_{50} - Zero tillage + residue + 50% recommended dose of nitrogen; ZT_{75} - Zero tillage + residue + 75% recommended dose of nitrogen; ZT_{100} - Zero tillage + residue + 100% recommended dose of nitrogen. W1- Sulfosulfuron + metsulfuron-methyl 40 g a.i. ha^{-1} (pre-mix post-em.) at 30 days after sowing (DAS); W2 -Carfentrazone-ethyl + metsulfuron-methyl (20+4 g a.i. ha^{-1}) (pre-mix post-em.) at 30 DAS; W3- Clodinafop + carfentrazone + metsulfuron-methyl (60+20+4 g a.i. ha^{-1}) (tank- mix post- em.) at 30 DAS; U- Unweeded/control; N- Nitrogen management; H- Herbicide management

Arginine deaminase activity (ADA)

The results revealed that arginine deaminase activity was significantly influenced by N and weed management (table 2). Zero tillage with 100% recommended dose of nitrogen plot had highest ADA activity (16.2 μg $NH4^+$ g^{-1} soil hr^{-1}) followed by the CT_{100} (13.5 μg $NH4^+$ g^{-1} soil hr^{-1}) > ZT_{75} (12.0 μg $NH4^+$ g^{-1} soil hr^{-1}) and ZT_{50} (9.3 μg $NH4^+$ g^{-1} soil hr^{-1}) (Table 2). However the application of weedicide significantly reduced soil ADA activity and the decrement was maximum under W_3 treatment (27.5%) as compared to unweeded plot.The interaction effect between CT_{100}, ZT_{50}, ZT_{75} and ZT_{100} treatments with unweeded plot was found to be significant as given in the table 2.The continuous crop residue retention with optimum N fertilization can lead to build up of total as well as different pools of soil organic carbon along with soil microbial biomass carbon. The addition of residue or carbon substrate increased ADA activity in ZT_{100} because soil microbes used residues as energy source for their growth. The ADA in soil has been found to be strongly correlated with carbon content, microbial respiration (Choudhary *et al.,* 2018).

Table 2: Impact of nitrogen management and various herbicides on Soil enzymes activity under Conservation Agriculture in Maize-Wheat system

Arginine deaminase activity (μg NH^{4+} g^{-1} soil hr^{-1})					
Treatments	W_1	W_2	W_3	U	Mean
CT_{100}	13.7	13.1	12.4	14.7	13.5^b
ZT_{50}	9.8	8.8	7.6	11.0	9.3^d
ZT_{75}	13.2	12.3	8.3	14.1	12.0^c
ZT_{100}	17.5	15.1	13.9	18.4	16.2^a
Mean	13.6^b	12.3^c	10.5^d	14.5^a	
LSD ($p<0.05$)	N=0.88 H= 0.68 N*H= 1.37				
Alkaline phosphomonoesaterase activity (μg PNP g^{-1} soil h^{-1})					
CT_{100}	256	245	239	269	252^b
ZT_{50}	228	207	197	261	223^c
ZT_{75}	253	234	215	264	242^b
ZT_{100}	296	266	236	315	278^a
Mean	258^b	238^c	222^d	277^a	
LSD ($p<0.05$)	N=17.6 H= 12.6 N*H= 25.2				

Alkaline phosphormonoesterase activity (ALP)

Alkaline phosphormonoesterase in soil hydrolyse the soil's organic phosphorus compounds into inorganic form and maintain the soil P cycling. Similar to the trend followed by DHA, ADA and β-glucosidase, significantly higher ALP activity was noticed with ZT_{100} treatment and the enhancement was found to be 10.3% higher than that of CT_{100} treatment (Table 2). The enhanced microbial population under CA might have immobilized carbon in microbial pool more than that of conventional farming (Li et al. 2018). The CT_{100} and ZT_{75} treatments were found to be statistically at par. The weedicide treatment for controlling weed significantly reduced ALP activity of soil and the decrement was found to follow the order W3 (20.1%)>W2 (14.1%)>W_1 (6.81%) (Table 2).The residual effect of Clodinafop, metsulfuron-methyl and carfentrazone-ethyl (W_3) may have affected the microbial population in the plot adversely so the reduction of ALP activity was found to be highest (Pose *et al.,* 2017).The N management and weedicide application showed a significant interaction in terms of ALP activity.

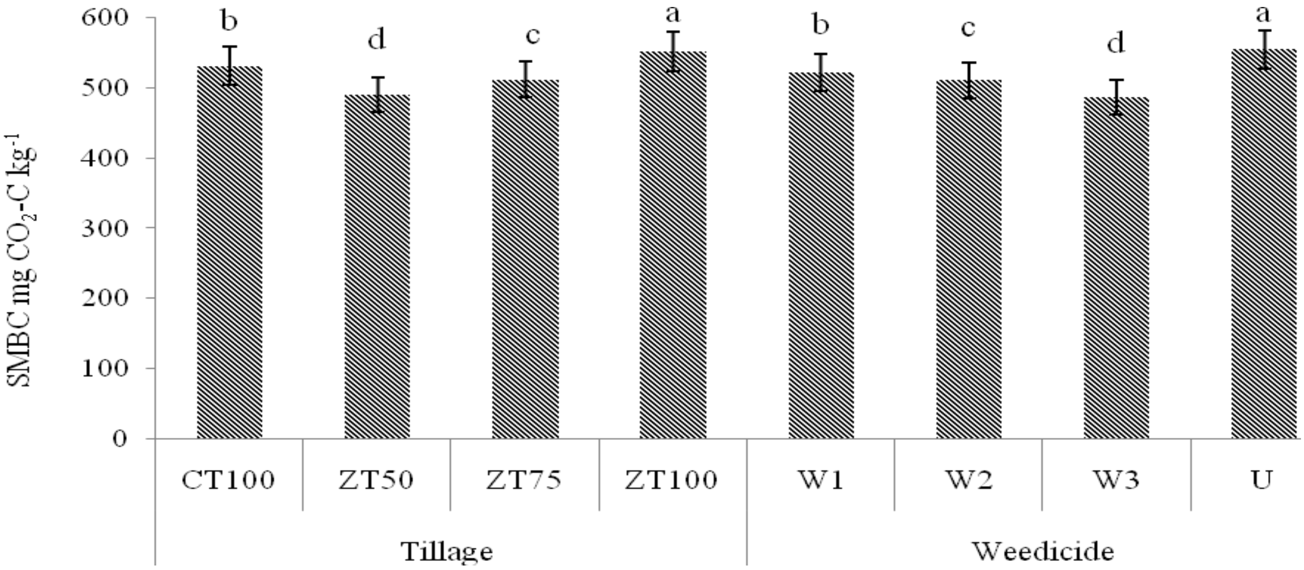

Figure 1: Impact of nitrogen management and various herbicides on soil microbial biomass carbon (mg CO_2-C kg^{-1} soil) under Conservation Agriculture in Maize-Wheat system. Bars indicate standard errors of mean. Means followed by same letter are not significantly different according to Tukey's honest significant difference test (at $P < 0.05$); SMBC- soil microbial biomass carbon

***Soil microbial biomass carbon* (SMBC)**

N management, weed management and tillage practices under CA significantly influence the SMBC.The SMBC under N management treatments were found in the range of 490 to 552 mg CO_2-C kg^{-1}(Figure 1). The SMBC of soil among different treatment was found to followthe order: ZT_{100}>CT_{100}>ZT_{75} and ZT_{50}. The values for SMBC in ZT_{100} (552 mg CO_2-C kg^{-1}) treatment was found to be significantly higher than CT_{100} (532mg CO_2-C kg^{-1}) (Figure 1). The absence of soil disturbance under zero tillage might have improved the aggregate distribution and stability under ZT_{100} treatment. This improvement in structure in terms of macro aggregates might have provided favorable micro-habitat for soil micro-flora (Talebbeigi 2020). Balota *et al*. (2011) also reported improved SMBC under soil cultivated with different summer legumes in coffee crop. The SMBC under weedicide treatment was recorded in the range of 497 to 555 mg CO_2-C kg^{-1}(Figure 1). Weedicide treated plot recorded the lower SMBC as compared to unweeded plot and the order being followed was W_3> W_2 and W_1.The maximum decrement was found in W_3 treatment (10.5%) followed by the W_2 (7.75%) and W1(5.95%) over unweeded plot. Higher SMBC under unweeded plot might be attributed to the incorporation of easily degradable carbonaceous material like manure, root biomass, rhizodeposits. These compounds provide the readily available C source for native microorganisms (Vishwanath *et al.,* 2020).

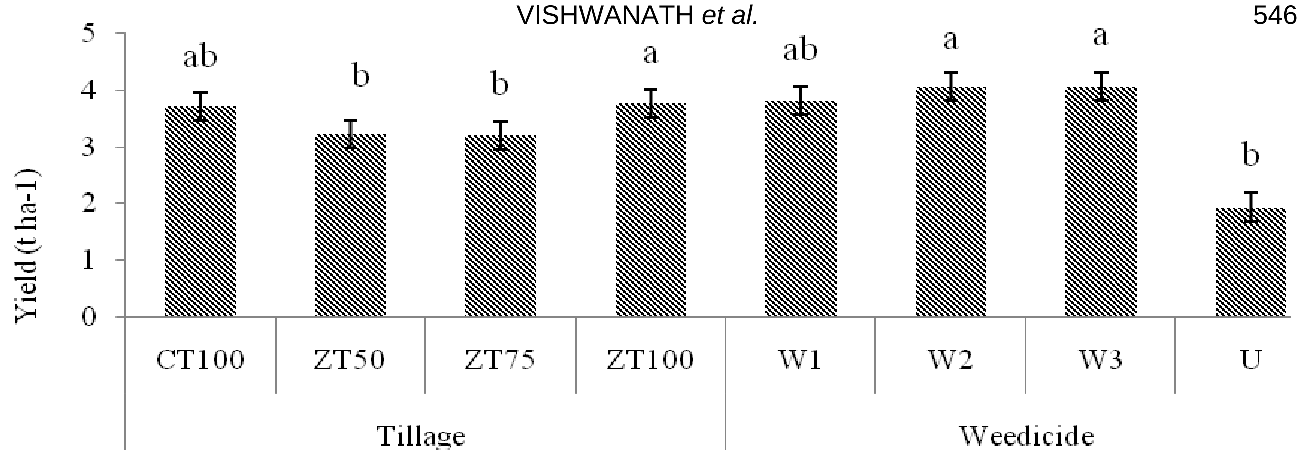

Figure 2: Impact of nitrogen management and various herbicides on yield (t ha^{-1}) of Wheat under Conservation Agriculture. Bars indicate standard errors of mean. Means followed by same letter are not significantly different according to Tukey's honest significant difference test (at $P < 0.05$)

Yield of crop

Grain yield of wheat was significantly influenced by N management and different herbicides in both tillage practices under conservation agriculture. The maximum yield of wheat was found under ZT_{100} (3.76 t ha^{-1}), which was at par with CT_{100} (3.71 t ha^{-1}). The other treatments ZT_{75} (3.22 t ha^{-1}) and ZT_{50} (3.22 t ha^{-1}) were found to be statistically similar to each other (Figure 2). In the case of weedicide treated plots, the lowest yield was recorded in the unweeded plot (1.93t ha^{-1}) whereas the highest yield was obtained in W_2 (4.07 t ha^{-1}) and W_3 (4.07 t ha^{-1}) treatments (Figure 2). The lowest yield in the unweeded plot was due to competition between the crop and the weeds for sunlight and food whereas the plots treated with herbicides were able to reduce the competition and hence had a higher yield.

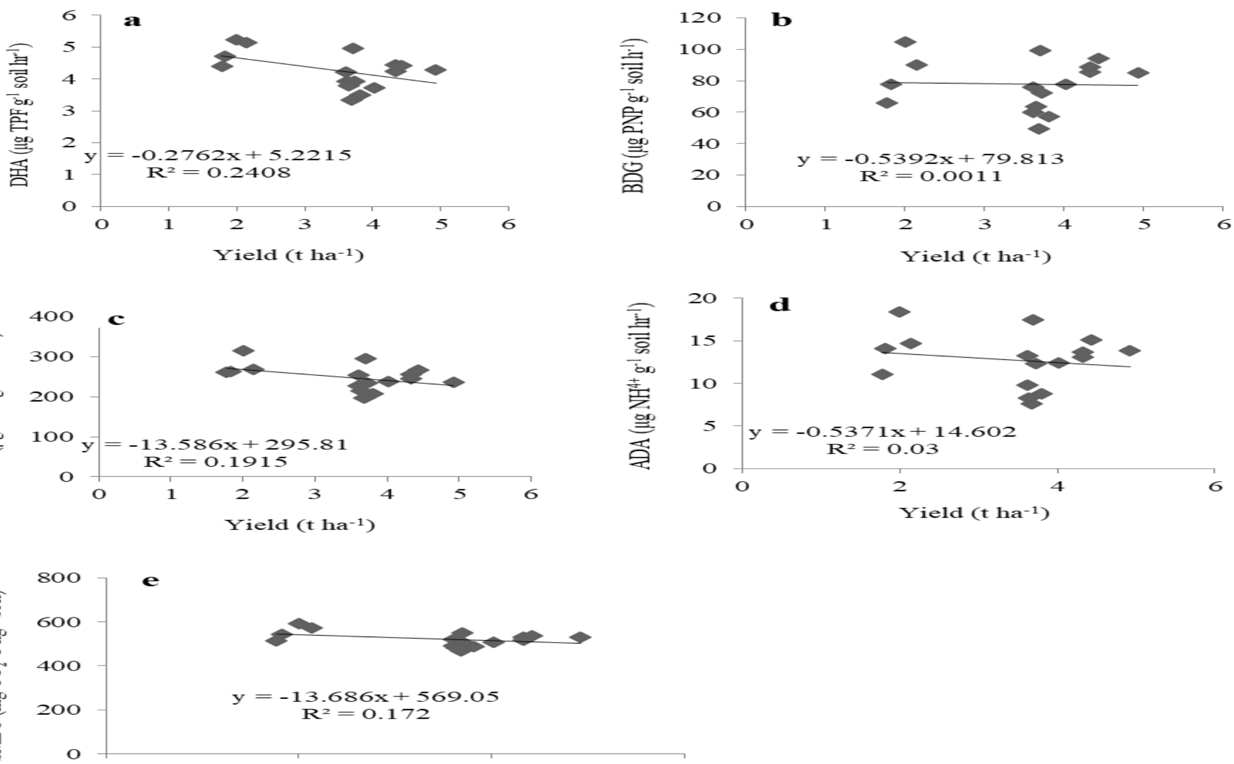

Figure 3: Correlation between soil (a) dehydrogenase (DHA) (b) β-glucosidase (BDG) (c) Alkaline phosphomonoesaterase (ALP) (d) Arginine deaminase activity (ADA) and (e) Soil microbial biomass carbon (SMBC) with wheat yield under Conservation Agriculture

Correlation between enzymes activities and soil microbial biomass carbon with wheat crop yield

In present study, correlation coefficients between different soil enzymes and yield are reported in Figure 3. Result revealed that the soil dehydrogenease (R^2 = 0.240), beta glucosidase (R^2 = 0.001), alkaline phosphatase (R^2 = 0.191) and arginine deaminase activities (R^2 = 0.03) and SMBC (R^2 = 0.172) were significantly and positively correlated with wheat yield. Among correlation study between enzymes activities and yield, the yield was highly correlated with DHA (R^2 = 0.240), ALP (R^2 = 0.191) SMBC (R^2 = 0.172) than arginine deaminase (R^2 = 0.03) and beta glucosidase (R^2 = 0.001). It is noted that a positive effect on soil enzymes activities under zero tillage along with 100% recommended dose of N and organic matter input may also contribute to C availability in this soil conferring greater yield and microbial activity.

CONCLUSIONS

Based on the present investigation, it can be concluded that the application of recommended dose of nitrogen with zero tillage practice improved the microbial activity in the soil whereas the maximum reduction in enzymatic activity was found in the W_3 subplot of ZT_{50} main plot. The yield of wheat crop in ZT_{100} was found to be at par with CT_{100}. Therefore, 100% recommended dose of nitrogen with zero till may be economically viable in place of conventional tillage. Also, the application of a mixture of sulfosulfuron + metsulfuron-methylcan be suggested for weed control without significantly affecting soil microbial activity.

ACKNOWLEDGMENT

Authors are highly grateful to ICAR-IARI, New Delhi, for continuous support during the course of this work and timely provision required facilities to conduct this research work.

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