Research Article

Effect of Biofertilizer and Farmyard Manure on Microbial Dynamics and Soil Health in Maize (*Zea Mays* L.) Rhizosphere

Amandeep Brar[1,2]*, S. K. Gosal[1] and S. S. Walia[3]

[1]Department of Microbiology, Punjab Agricultural University, Ludhiana, 141004, India
[2]Department of Microbiology, Central University of Rajasthan, Ajmer, 305801, India
[3]Department of Agronomy, Punjab Agricultural University, Ludhiana, 141004, India

Abstract

Production of sustainable crops depends upon the good soil health, which requires an optimum combination of organic and inorganic manures to enhance the microbial biomass and soil enzymatic activity. A long term experiment was conducted to evaluate the effect of different sources of nutrition on microbial dynamics and soil enzymatic activities in maize crop. The significantly higher bacterial population (285.10×10^8 cfu g^{-1} soil), fungal population (89.70×10^4 cfu g^{-1} soil), P-solubilizers population (240×10^3 cfu g^{-1} soil) and soil alkaline phosphatase activity (28.6 mg PNP g^{-1}soil hr^{-1}) were observed in plots treated with FYM + non-edible oil cakes + biofertilizers whereas, treatment having 50% N as FYM + Biofertilizers recorded significantly higher diazotrophic population (27.96×10^6 cfu g^{-1} soil), actinomycetes population (76.17×10^4 cfu g^{-1} soil), urease activity (615µg g^{-1} soil hr^{-1}) and dehydrogenase activity (475 µg TPF g^{-1}soil hr^{-1}). The application of FYM, Biofertilizer and non-edible oil cakes can be concluded as the best organic fertilizer for the maize cultivation. Thus, the results reveal the positive influence of organic farming on the biological requirements of soil for the maintanince of a sustainable environment.

Keywords: Biofertilizer, FYM, non edible oil cakes, maize, yield

***Correspondence**
Author: Aman Deep Brar
Email: braramandeep28@gmail.com

Introduction

The integral plant- microorganism system in nature undergoes short and long-term fluctuation depending upon the agro-ecological conditions as well as the development stages of the plant. Agricultural practices have a considerable impact on the size and activity of soil microbial community and biological health of the soil [1]. In India, soil is not left idle to regain its fertility due to the immense pressure of population. Therefore, the staple crops follow each other in a row and this practice leads to the decline in crop productivity due to extensive use of chemical fertilizers and deficiency of micronutrients. In order to obtain higher productivity, farming practices have undergone various changes from time to time with new technologies and heavy doses of fertilizers. These practices, even though increases the yield, but, also make the microbial and plant system more vulnerable to various stresses beside their deleterious effect on the soil environment. In Punjab, maize is the second major grain crop of the *kharif season* being grown on an area of 154 thousand hectares with a total production of 459 thousand tonnes [2]. So, maize is emerging as third most important crop after rice and wheat, being having the highest yield potential among cereals. Hence it is called as 'miracle crop' and also as 'queen of cereals' [3]. Its production requires high amount of nitrogen and phosphorus. This has led to the emergence of a movement popularly known as 'organic farming' which is based on the traditional farming philosophy. In the era of increased chemical fertilizers, biofertilizers will be an ideal choice for the sustainable farming systems and should be incorporated into the agricultural sector to extract the full potential of maize crop as a source of nutrition. The productivity of maize can be increased by using biofertilizers having nitrogen fixers, phosphate solubilizers and plant growth promoting rhizobacteria's (PGPR's). These microorganisms differ in their mode of action and when applied alone or in combination, can act as a substitute to chemical fertilizer. In this study, the combination of various organic and inorganic nutrients over inorganic nutrients have been addressed.

Material and Methods

Experimental Design and Soil Sample Collection

A long term experiment was conducted since 2002 on maize crop during the kharif period in randomized block design (in triplicate) in the research fields of Department of Agronomy, Punjab Agricultural University, Ludhiana, using

different combinations of biofertilizers, non-edible oil cakes and farmyard manure. The standard cultivation practice followed was 8 kg seed of maize per acre; row to row spacing of 60 cm; plant to plant distance of 20 and 15 cm. Five replicates of rhizospheric soil (soil associated with the roots of the plant) samples were collected from the each treatment plot. Different combinations of treatments are mentioned (**Table 1**).

Table 1 Different combinations of treatments

	Treatments
T1	NPK (50% of recommended level) + FYM (50% N)
T2	FYM(@ 10t/ha) +Non edible oil cakes
T3	T2 + Intercropping (soyabean)
T4	T2 without any agropesticides
T5	50% N as FYM (@ 10t/ha) +Biofertilizer *(Azotobacter* + PSB)
T6	T2 + Biofertilizer (*Azotobacter* +PSB)
T7	Control (Recommended dose of NPK)

Enumeration of Microbial population

Soil samples were procured from the rhizospheric soil of maize grown with treatments under the field conditions. Five samples were randomly collected from different areas of the same treatment field and were mixed to get one representative sample. Enumeration of different microbial population (viz. Bacteria, Diazotrophs, Fungi, Actinomycetes and P-solubilizers) were done in their specific media, sterilized in an autoclave at 15 psi pressure and 121˚C temperature for 20 minutes using serial dilution spread plating technique (at different intervals of time).

Soil Enzyme Activity Assay

Soil enzymatic activities were analyzed from soil samples collected at different time intervals, i.e. 0 Days, 45 DAS (days after sowing) and at harvest. Dehydrogenase activity was assayed by the method of Mersi and Schinner [4] which uses iodonitrotetrazolium chloride (INT) as substrate. This substrate gets reduced, the colored complex, so formed was highly stable and was measured spectrophotometrically at 460 nm. Alkaline phosphatase enzyme activity was determined by the method of Bessey *et al.,* [5]. It employs β nitrophenyl phosphate that allows an instantaneous color development at high pH. Urease activity was estimated by method of McGarity *et al.,* [6] which measures the amount of urea remaining in the soil solution.

Statistical analysis

The results were expressed as mean ± SD of three replicates. Data were subjected to analysis of variance (ANOVA) and the difference between various treatments with respect to control were analyzed by Duncun's test using a SPSSv16.0 software at 5% significance level. The simple correlation between microbial population and organic carbon were also worked out.

Results and Discussion

Effect of different treatments on microbial population and soil enzymatic activity

The microbial population was found significantly higher in organically treated plots as compared to inorganic fertilizers. At all intervals of time, bacterial population showed significantly greater values in all treatments over control and treatment T6 recorded maximum population (285.1×10^8cfu g^{-1} soil) (**Table 2**). Treatments T1, T5 and T7 exhibited significant variation according to Duncun's test at $P \leq 0.05$. These results were in accordance with Martin *et al.,* [7] who reported that the application of *Azotobacter* spp. supports the growth of the bacterial population. Statistically higher diazotrophic population (279.7×10^5cfu g^{-1} soil) was observed in the treatment T5 followed by T6 (274.9×10^5cfu g^{-1} soil) (Table 2) over control (206.3×10^5cfu g^{-1} soil). All the treatments exhibited significant variation at 45 DAS according to Duncun's test at $P \leq 0.05$. There was a positive correlation between the application of biofertilizers, farmyard manure and diazotrophic population as it was maximum in treatments having biofertilizers. According to Mikanova *et al.,* [8] nitrogen fertilization in organic form (FYM) increased the counts of *Azotobacter* spp. The application of FYM led to significantly increase the fungal and bacterial population up to 45 DAS as compared to inorganic sources of nutrition. The mean fungal population reached the greatest value 89.7×10^4cfu g^{-1}soil for treatment T6 over control (27.1×10^4cfu g^{-1} soil) at 45 DAS (Table 2).

Table 2 Effect of different treatment combinations on soil microbial population and enzymatic activity at different time intervals in field

Treatments	Time interval	Soil microbial population (cfu g^{-1})					Soil enzymatic activities (/g of soil/hr)		
		Bacteria ($\times 10^8$)	Diazotrophs ($\times 10^5$)	Fungi ($\times 10^4$)	Actinomycetes ($\times 10^4$)	P-solubilizers ($\times 10^3$)	Dehydrogenase(µg TPF)	Alkaline phosphatase (mg PNP)	Urease (µg)
T1	0 Days	150.8±1b	143.6±1b	5.4±0.4e	36.7±0de	50.0±0d	112.5±1.4d	19.2±0.2c	295±5f
	45DAS	247.1±0.31d	247.8 ±0.8c	41.6±2d	26.6±2d	90±5d	250.0±5c	22.2±2.2cd	**245**±1e
	Harvest	144.5±0.5c	182.4±2b	25.7±1d	20.9±0.9e	50.0±5c	275.0±1c	25.2±1c	105±2e
T2	0 Days	133.0±1d	107.8±1f	14.3±1b	47.4±0.4c	35.0±5f	75.0±5e	20.4±0.4bc	450±5d
	45DAS	225.2±0f	165.4 ±2g	62.3±2b	25.9±1d	110.0±10c	109.0±1g	24.6±0.6bc	**260**±1d
	Harvest	132.4±2a	117.7±0.7e	34.5±1.5c	18.0±1c	90.0±5d	200.0±5f	26.8±1d	115±1d
T3	0 Days	135.7±5d	131.5±0c	9.6±1d	40.7±5d	40.0±2e	200.0±1a	15.8±0.8d	555±5b
	45DAS	242.7±1e	195.9 ±1f	44.7±3c	29.6±2c	80.0±1e	275.0±2b	24.0±1bcd	**315**±2b
	Harvest	108.5±2d	130.9±5d	23.5±3d	13.6±0.6e	50.0±3e	300.0±1b	29.6±1b	200±0b
T4	0 Days	143.9±0c	151.1±0.13a	6.6±0e	50.8±2c	60.0±0c	175.0±5b	19.8±0.1bc	400±1e
	45DAS	261.2±0.17b	210.2±1.36d	34.8±1e	32.3±0.3b	80.0±2e	212.5±1d	21.6±1d	**289**±1c
	Harvest	111.0±4d	136.9±0c	22.7±0.03d	21.0±0d	58.0±1.5c	237.5±0.5d	25.3±0.6c	115±4d
T5	0 Days	134.5±2.31d	113.3±0.67e	5.9±0e	76.2±4.9a	100.0±1a	150.0±2c	21.9±3ab	615±1a
	45DAS	253.5±1c	279.7 ±0.01a	53.8±2c	34.2±1.01ab	210.0±5b	337.5±1a	23.4±1bcd	**380**±1a
	Harvest	124.5±0b	120.0±0e	39.2±3b	61.7±0b	111.0±0a	475.0±5a	28.2±0.2b	210±2a
T6	0 Days	175.4±1a	126.9±5d	19.1±1a	57.4±0b	80.0±1b	31.25±1f	21.2±0.2bc	515±0c
	45DAS	285.1±0.1a	274.9± 1b	89.7±0.7a	34.9±0.9a	240.0±6a	152.0±0f	25.6±2b	**205**±0g
	Harvest	129.7±1a	207.2±0.23a	50.2±0a	24.6±2a	140.0±2b	175.0±1g	28.6±0.6b	140±5c
T7	0 Days	125.4±2e	128.9±1cd	11.8±2.18c	33.6±0e	30.0±1g	25.0±2g	23.4±0.4a	510±1c
	45DAS	176.1±1g	206.3 ±3e	27.1±0f	13.1±0.1e	40.0±0f	175.0±4e	30.4±0a	**210**±4f
	Harvest	99.5±0e	81.4±0f	24.7±1d	7.0±0f	20.0±2f	212.0±0e	33.8±2a	100±5e

Values with different letter(s) are significantly different at $p \leq 0.05$

Fungal population exhibited significant variation among treatments T2, T5 and T6 at harvest according to Duncun's test at P≤ 0.05 and no significant variation was exhibited in treatments T1 and T3 at 45 DAS and T1, T3, T4 and T7 at harvest. The results were in accordance to Chaturvedi *et al.,* [9] who observed a decline in the fungal population at the time of crop harvest due to the proceeding of crop to maturity stage. It had also been observed that bacterial proliferation after the addition of labile organic substrates had antagonistic effects on fungal growth [3] which could explain the absence of response of the fungal population to organic fertilizers at the time of harvest. In the present study, the actinomycetes population decreased significantly towards harvesting. The population of actinomycetes decreased significantly from 0 DAS to the time of harvest. Maximum decrease in actinomycetes population was observed for the treatment T6 as compared to other treatments (Table 2). The results were in accordance with Mandic *et al.,* [10] who observed that less secretion of root exudates and the high doses of nitrogen repressed the number of microorganisms, especially in the early stages of vegetation and at low soil moisture period (mid-growing season). The toxic effect of high doses of nitrogen was significantly lower in the rhizosphere as well as during the periods of increased humidity that supported the growth of actinomycetes population. The significantly higher population of P-solubilizers (240×10^4cfu g^{-1} soil) was found in the treatment T6 and it was statistically at par with T5 at 45 DAS. The application of biofertilizers had significantly increased the population of phosphate solubilizing bacteria over control.

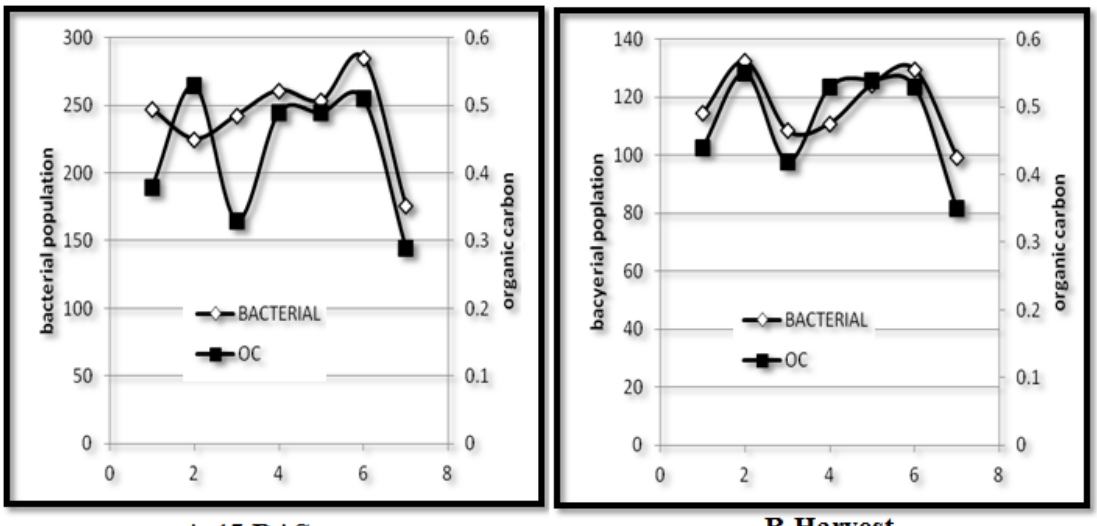
Soil enzyme activities

Soil enzyme activities were strongly influenced by long-term organic and inorganic fertilization as evidenced by highly significant F-values for the treatment effects on enzyme activities (Table 2). Moreover, the impact of organic manuring on soil enzyme activity was more conspicuous when compared with control. The dehydrogenase and alkaline phosphatase activity increased up to flowering and then declined thereafter, whereas, the urease activity increased as the crop progressed towards maturity. The dehydrogenase enzyme showed significant variation among

each other according to Duncun's test at P≤ 0.05. Maximum dehydrogenase activity (337.5 µg TPF/g of soil/hr at 45 DAS and 475.0 µg TPF/g of soil/hr at harvest) was found in treatment T5 (50% N as FYM (@ 10t/ha) + Biofertilizer *(Azotobacter* + PSB). A significant correlation was observed between microbial biomass and soil dehydrogenase activity, i.e. as microbial population increased, enzyme activity was improved. According to Basu *et al.,* [11] dehydrogenase activity is a measure of overall microbial activity and being an intracellular enzyme, it is related to oxidative phosphorylation processes. This result was in agreement with Lee *et al.,* [12] who reported that soil treated with FYM and organic manure showed higher levels of dehydrogenase activity as compared to soil treated with mineral fertilizers. In case of alkaline phosphatase, control (T7) was statistically different from all other treatments at all time intervals according to to Duncun's test at P≤ 0.05. Higher alkaline phosphatase activity, i.e. between 0-45 DAS (with a difference between activity of 45 DAS and 0 days -8.2mg PNP/g of soil/hr) and 45 DAS- harvest (with a difference of activity between harvest and 45 DAS -5.6mg PNP/g of soil/hr) was recorded in T2 + soyabean Intercropping (T3). Hojati and Nourbakhsh [13] observed a significant increase in enzyme activities, including phosphatases, due to the addition of chemical fertilizer and organic manure over only chemical fertilizer or control. Therefore, addition of FYM as a source of organic manure influenced the alkaline phosphatase activity. According to Duncun's test, treatments T6 & T7 at 0 days and treatments T2 & T4 and T1 & T7 were statistically at par with each other. Bhattacharyya *et al.,* [14] and Krishnamurthy *et al.,* [15] reported that the addition of organic manures increased the urease activity over mineral N and control to the significant extent which were in accordance with the present study. Low level of urease activity in fertilizer treated soil indicated that mineral N without sufficient amount of available organic substrate may not have an impact on urease activity. Significantly higher urease activity was observed in the treatment T5 (615µg/ g of soil/hr) at 0 Days followed by urease activity (555 µg/ g of soil/hr) in treatment T3 (Table 2).

Correlation analysis between different Microbial population and Organic carbon

Bacterial population had a significant positive correlation with organic carbon, i.e. r = 0.633 at 45 DAS p≤ 0.10 and r = 0.850 p≤ 0.10 at harvest (**Figure 1** (A, B)). The organic nutrient management had greatly influenced the bacterial population in the rhizospheric soil and was found to have a positive correlation with crop maturity and decreased thereafter, irrespective of treatment combinations. Fungal population had a positive correlation with organic carbon (r = 0.655) at 45 DAS and at harvest (r = 0.574) p ≤ 0.10 (Figure 1C-1D). Present results were supported by the findings of Qureshi *et al.*,[16] and Saini *et al.*,[17] that at 45 DAS, the increase in fungal population was might be due to more degradation of organic matter in soil which leads to the increase in the organic carbon sequentially enhancing the microbial populations. Actinomycetes population had a positive correlation with organic carbon (r = 0.687) at 45 DAS, p ≤ 0.10 and at harvest (r = 0.573), p ≤ 0.10 (Figure 1E-1F). P-solubilizers population had a positive correlation with organic carbon (r = 0.658) at 45 DAS, p ≤ 0.20 and at harvest (r = 0.796), p ≤ 0.05 (Figure 1G-1H). According to Chand *et al.*, [18] the PSB had a significant positive correlation coefficient with P and N-uptake and higher doses of biofertilizers thereby accelerating their (PSB) population. Diazotrophic population had a non- significant correlation with organic carbon.



A-45 DAS **B-Harvest**

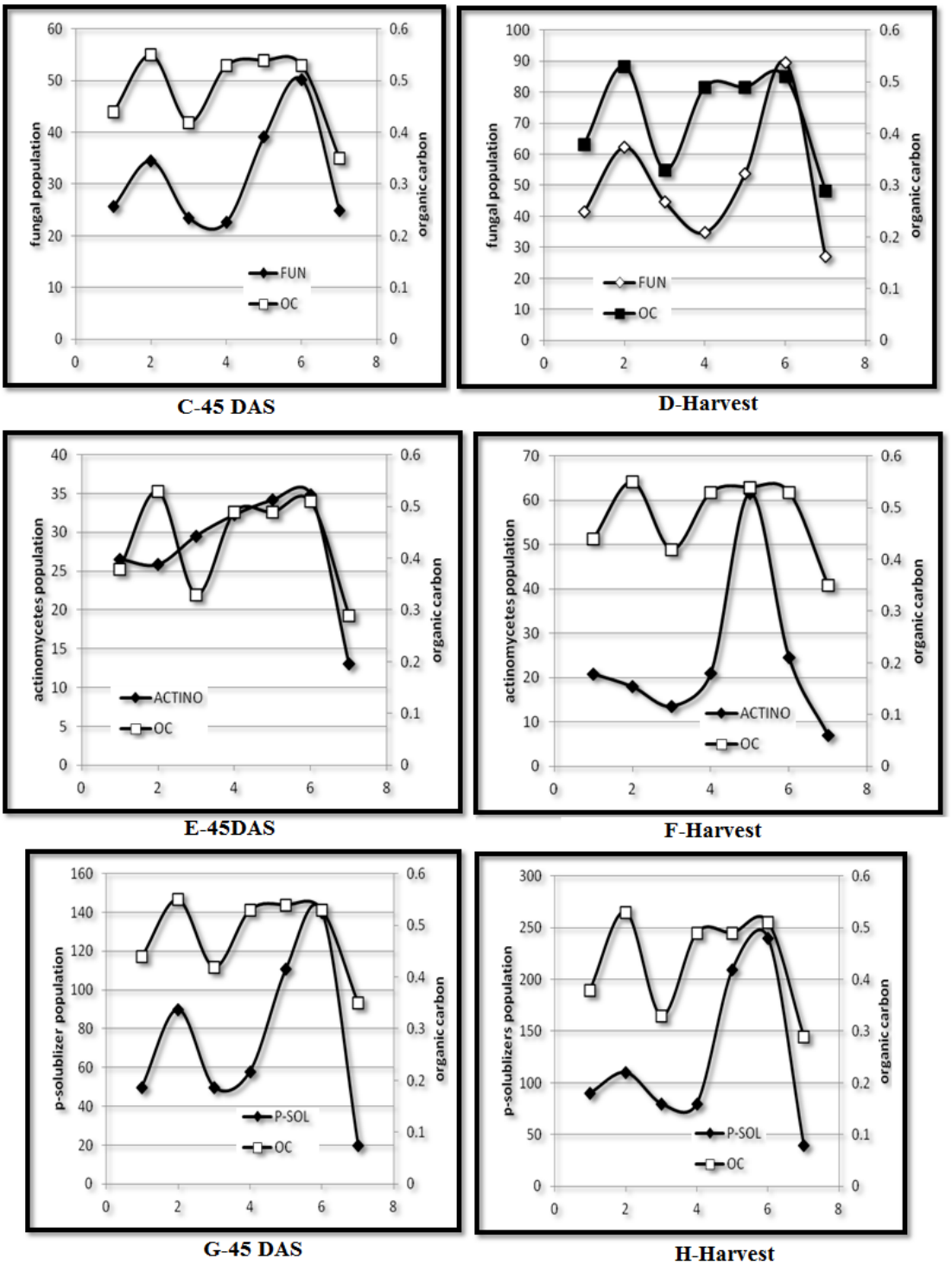


Figure 1 Correlation between different microbial population with organic carbon in soil in different treatment (A-H). A - Bacterial population at 45DAS, B- Bacterial population at Harvest, C- Fungal population at 45DAS, D- Fungal population at Harvest , E- Actinomycetes population at 45DAS, F- Actinomycetes population at, G- P-solubilizers population at 45DAS, H- P-solubilizers population at Harvest

Conclusion

The periodic changes prevailing in the soil during the growing season has advocated the diversification of microbial communities in the soil. The long-term application of biofertilizer (containing N-fixers and P-solubilizers) and farmyard manure in maize crop have improved the microbial population and soil enzymatic activities over the chemical fertilizers. Farmyard manure supplies the additional organic matter to the soil, which supported the survival of rhizospheric microbial population and hence lead to increased soil enzymatic activities.

References

[1] Das, B. B. and Dkhar, M. S. 2011. Rhizosphere Microbial Populations and Physico Chemical Properties as Affected by Organic and Inorganic Farming Practices. Am-Eur J. Agric. Environ. Sci., 10(2): 140-50.

[2] Anonymous. 2006. Package and Practices for Kharif crops of Punjab. Pp: 26. Punjab Agricultural University, Ludhiana.

[3] Meidute, S., Demoling, F. and Baath, E. 2008. Antagonistic and synergistic effects of fungal and bacterial growth in soil after adding different carbon and nitrogen sources. J. Soil Biol. Biochem., 40: 2334-43.

[4] Mersi, W. V. and Schinner, F. 1991. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitro-tetrazolium chloride. J. Biol. Fertil. Soils, 11: 216-220.

[5] Bessey, O. A., Lowry, O. H. and Bruck, M. J. 1946. A method for the rapid determination of alkaline phosphatase with fine cubic millimeters of serum. J. Biol. Biochem., 164: 321-29.

[6] McGarity, J. W. and Myers, M. G. 1967. A survey of urease activity in soils of northern New South Wales. J. Pl. Soil, 27: 217-38.

[7] Martin, X. M., Sumathi, C. M. and Kannan, V. R. 2011. Influence of agrochemicals and Azotobacter sp. application on soil fertility in relation to maize growth under nursery conditions. Eur. Asia J. Bio. Sci., 5: 19-28.

[8] Mikanova, O., Friedloa, M. and Simon, T. 2009. The influence of fertilization and crop rotation on soil microbial characteristics in the long-term field experiment. J. Pl. Soil Environ., 55: 11-16.

[9] Chaturvedi, S., Chandel, A. S., Dhyani, V. C. and Singh, A. P. 2010. Productivity, profitability and quality of soybean (Glycine max) and residual soil fertility as influenced by integrated nutrient management. Indian J. Agro., 55: 133-37.

[10] Mandic, L., Dukic, D., and Stevovic, V. 2005. The effect of different kinds of fertilizers on the number of Azotobacters in the smonitza type of soil under maize and the yield of maize. J. Acta. Agric. Serb., 20: 11-22.

[11] Basu, T. K. 2011. Effect of Cobalt, Rhizobium and Phosphobacterium Inoculations on Growth, Yield, Quality and Nutrient Uptake of Summer Groundnut (Arachis hypogaea). Am. J. Exp. Agric., 1:21-26.

[12] Lee, J. J., Park, R. D., Kim, Y. W., Shim, J. H., Chae, D. H., Rim, Y. S. and Kyoon, B. 2004. Effect of food waste compost on microbial population, soil enzyme activity and lettuce growth. J. Biores. Technol., 93: 21-28.

[13] Hojati, S. and Nourbakhsh, F. 2006. Enzyme Activities and microbial biomass carbon in a soil amended with organic and inorganic fertilizers. J. Agron., 5: 563-69.

[14] Bhattacharyya, P., Chakrabarti, K. and Chakraborty, 2005. Microbial biomass and enzyme activites in submerged rice soil amended with muncipal solid waste compost and decomposed cow manure. J. Chemosphere, 60: 310-18.

[15] Krishnamurthy, R., Raveendra, H. R. and Manjunatha, R. T. M. 2011. Effect of waterlogging and weed as organic manure on enzyme activities under Typic Paleustalf soil. Int. J. Sci. Nat., 2(2): 275-78.

[16] Qureshi, A. A., Narayanasamy, G., Chhonkar, P. K. and Balasundaram, V. R. 2005. Direct and residual effect of phosphate rocks in presence of phosphate solubilizers and FYM on the available P, organic carbon and viable counts of phosphate solubilizers in soil after soybean, mustard and wheat crops. J. Indian Soc. Soil Sci., 53: 97-100.

[17] Saini, V. K., Bhandari, S. C., Sharma, S. K. and Tarafdar, J. C. 2005. Assessment of microbial biomass under integrated nutrient management in soybean-winter maize cropping sequence. J. Indian Soc. Soil Sci., 53: 346-51.

[18] Chand, S., Somani, L. L. and Bhandari, S. C. 2010. Effect of fertilizer, farmyard manure (FYM) and biofertilizer on the population of Azotobacter and phosphate solubilising bacteria (PSB) in the Soil. J. Indian Soc. Soil Sci., 58: 460-63.

Publication History
Received 08th June 2017
Revised 22nd June 2017
Accepted 04th July 2017
Online 30th July 2017