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Effect of Integrated Nutrient Management on Soil Enzymes, Microbial Biomass Carbon and Microbial Population under Okra Cultivation

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VK and JS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VK and NB managed the analyses of the study. Authors VK and TD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A field experiment was conducted at the Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat during March to July 2016 to study the "Effect of integrated nutrient management on soil enzymes, microbial biomass carbon and microbial population under okra cultivation". The results of the study indicated that there was the improvement in soil biological properties and soil enzymes in all plots over the initial value. However, the highest biological properties like Microbial Biomass Carbon (MBC) (244.86 µg g^{-1}), bacterial population (8.24 log cfu g^{-1} soil), fungal population (3.89 log cfu g^{-1} soil), soil enzymes like fluorescein di-acetate (FDA) (7.28 µg fluorescein g^{-1} soil h^{-1}), phosphomonoesterase (PME) (50.15 µg p-nitrophenol g^{-1} h^{-1}), Deydrogenase (DH) (136.90 µg TPF g^{-1} soil 24 h^{-1}), Arylsulphatase (14.16 µg p-nitrophenol g^{-1} h^{-1}) and Arylesterase activity (113.92 µg p-nitrophenol g^{-1} h^{-1}) was found in the treatment T_3 [at 50% recommended dose of N, P, K + Vermicompost at the rate of 2 t ha^{-1} (mixed with microbial

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consortium)]. Increased in microbial population and soil enzymatic activity is the indicator of good soil condition for crop growth. Therefore the addition of organic manure and biofertilizers along with the reduced amount of inorganic fertilizers should be advocated for maintaining high soil quality for longer the period.

Keywords: Phosphomonoesterase; dehydrogenase; arylsulphatase; arylesterase; MBC; bacterial population; fungal population.

1. INTRODUCTION

Good soil condition for crop growth not only related to its ability to produce healthy and abundant crops but also includes the soil's capacity to function as a mature and sustainable agroecosystem [1]. Soil enzymatic activities have been proposed as appropriate indicators because of their intimate relationship to soil biology and rapid response to changes in nutrient management. Microbial biomass provides an insight into the composition and activity of microorganisms and signifies the main source of soil enzymes. Fluorescein di-acetate (FDA) is hydrolyzed by a number of different enzymes, such as proteases, lipases, and esterases to produce fluorescent compound fluorescein and provide comprehensive microbial activity [2]. Phosphomonoesterase (PMEase) is a group of enzymes which catalyze the hydrolysis of esters of phosphoric acid to release phosphate and is of paramount importance as a soil quality indicator [1]. Dehydrogenase (DH) exists as an integral part of intact cells, involved in oxidative phosphorylation, and reflects in the total oxidative potential of the soil microbial community [3]. Arylsulphatase help in catalyzing the hydrolysis of aromatic sulphate esters and releasing sulphate for plants uptake.

Okra is the most important vegetable crop which is widely grown in India. India is the largest producer of okra in the world. Okra is the heavy feeder of nutrient and the nutrient demand of the crop largely provided by heavy incorporation of chemical fertilizers. Excessive use of chemical fertilizers to obtained high yield resulted in several hazards to the soil, deficiency of micronutrients and nutrient imbalance, ultimately resulting in the reduction of crop yield. Therefore, it is important to understand how soil biological properties respond to integrated management of nutrients in okra. Estimations of soil enzymes, soil microbial biomass and, microbial population are the primary phase in characterizing soil metabolic potential, fertility and quality, as well as pondering guidance to the resilience of the soil since they can anticipate changes in soil quality before they are detected by other soil analyses [4].

2. MATERIALS AND METHODS

A field experiment was conducted at the Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat during March to July 2016. The experimental area is located at 26°47′N latitude and 91°12′E longitude at an elevation of 86.8 m above mean sea level and under Upper Brahmaputra Valley Agro Climatic Zone of Assam. The experiment was laid out with Randomized Block Design (RBD) and replicated three times. There were seven treatments consisting of T_1 [recommended dose of fertilizers (50:50:50 kg NPK ha^{-1} + Farm yard manure at the rate of 10 t ha^{-1})], T_2 [75% recommended dose of NPK + Vermicompost at the rate of 1 t ha^{-1} (mixed with microbial consortium)], T_3 [50% recommended dose of NPK + Vermicompost at the rate of 2 t ha^{-1} (mixed with microbial consortium)], T_4 [75% recommended dose of NPK + Microbial consortium as seed coat + Vermicompost at the rate of 1 t ha^{-1}], T_5 [50% recommended dose of NPK + Microbial consortium as seed coat + Vermicompost at the rate of 2 t ha^{-1}], T_6 [Farm yard manure at the rate of 10 t ha^{-1} (mixed with microbial consortium)] and T_7 [Microbial consortium as seed coat + Farm yard manure at the rate of 10 t ha^{-1}]. The microbial consortium was the mixture of four different groups of biofertilizers (Phosphate solubilizing bacteria, *Azotobacter*, *Azospirillum,* and *Rhizobium*) in 1:1:1:1 ratio. The crop was raised with a spacing of 45 cm × 30 cm and plot size of 2.7 m × 1.5 m. Standard cultural practices recommended for Okra was followed uniformly for all the experimental plots. Organic manures, inorganic fertilizers, and biofertilizers were applied at different doses as per the treatment requirement. Farmyard manure (FYM) was applied at the rate of 10 t ha^{-1} and vermicompost was applied at the rate of 1 t ha^{-1} and 2 t ha^{-1} after final land preparation. The microbial consortium was applied through inoculation in two ways *i.e*. with seed and with organic manures (FYM and

vermicompost). Consortium applied at the rate of 500 g per 10 kg of seed as a seed treatment and along with organic manures at the rate of 3.5 kg ha^{-1}. Inorganic fertilizers, *i.e.* Urea, Single Super Phosphate (SSP), and Muriate of Potash (MOP) were applied three days before sowing as a basal application. Half of Urea, a full dose of SSP and MOP was applied as basal. The second half of Urea was applied at 30 days after sowing. Prior to the estimation of soil enzymatic activities, microbial biomass carbon and microbial population the moist field samples were collected after final harvest and preserved in the refrigerator at 4 °C. Dehydrogenase activity was determined by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) as described by Casida et al. [5]. The method of Tabatabi and Bremner [6] is followed to estimate the phosphomonoesterase activity. FDA hydrolysis activities were carried out following the method described by Green et al*.,* [7]. The assay for arylsulphatase activity was carried out by using *p*-nitrophenyl sulphate (*p*-NPS) as substrate [8]. The assay for arylesterase activity was carried out by following standard protocol given by Zornoza et al. [9]. Microbial biomass carbon was determined by chloroform fumigation- extraction technique following the method of Joergensen and Mueller [10]. The classical serial dilution technique was used for isolation of total bacterial (PSB, Azotobacter and Rhizobium and fungal (Azospirillum) population from the soil sample by spread plate technique on appropriate media. Data on all parameters were subjected to analysis of variance (ANOVA) as suggested by Fisher and Yates [11]. When ANOVA showed significant differences, mean separation was carried out using Critical Difference (CD) test at 5% level of significance to draw the valid conclusion.

3. RESULTS AND DISCUSSION

Soil microbial biomass carbon (MBC), the most active and dynamic pool of the soil organic matter, acts as transient nutrients sinks and is responsible for releasing nutrients from organic matter for use by plants. In the present study application of 50% recommended dose of NPK + Vermicompost at the rate of 2 t ha^{-1} (mixed with microbial consortium) resulted in the highest MBC (244.86 µg g^{-1}). This might be due to the INM plots have provided a steady source of organic carbon to support the microbial community compared to 100% NPK treated plots. Application of biofertilizers, besides showing their primary effect are also known to produce diverse growth promoting substances that might contribute intense proliferation of microbial growth and augmented MBC [12]. Improvement of MBC in the vermicompost treated plots might be largely due to the microbes contained in the organic residues and the addition of substrate carbon, which stimulates the indigenous soil microbiota.

Hydrolysis of FDA has been widely accepted as a measure of total microbial activity in soils and functions as a broad spectrum indicator of soil biological activity [7,13]. In the present study maximum increase in FDA hydrolysis (7.28 µg fluorescein g^{-1} h^{-1}) was recorded in the T_3 treatment which received 50% recommended dose of NPK + Vermicompost at the rate of 2 t ha^{-1} (mixed with microbial consortium). The large increase of FDA hydrolysis due to INM could be attributed to increased microbial biomass resulting from organic matter enrichment in the soil since the addition of good quality compost has a direct bearing on MBC and soil enzyme activities [14].

Extracellular phosphomonoesterase (PME) is an enzyme of agronomic value because it hydrolyzes compounds of organic phosphorus (P) and transforms them into inorganic P. In this experiment addition of vermicompost (2 t ha^{-1}) along with inorganic fertilizer and biofertilizer recorded maximum increase in PMEase activity (50.15 µg p-nitrophenol g^{-1} h^{-1}) of soil (T_3). It might be due to the release of more organically bound P, as the synthesis of the enzyme is stimulated by the presence of organic substrate [15]. The persistence of elevated and active PME in organic amended soils might be due to their adsorption and protection against proteolysis, once released as an extracellular enzyme or following cell death or during humification of organic residues [16].

Soil dehydrogenase (DH) involved in oxidative phosphorylation, and is an important indicator of microbial activity in the soil which has been found to increase significantly in soils applied in combination of organic, inorganic and biofertilizer. In the present study application of 50% recommended dose of NPK + Vermicompost at the rate of 2 t ha^{-1} (mixed with microbial consortium) resulted in the highest DH activity (136.90 µg TPF g^{-1} soil 24 h^{-1}). This might be due to increased microbial activity and MBC in the same treatment. Incorporation of bulky sources of potential beneficial microbes may provide microbial diversity and activity of

microorganisms accompanied by better DH activity [12]. Nayak et al. [17] also described a generalized short to medium term increase in DH activity following organic matter addition.

A similar trend was also followed in case of arylsulphatase and arylesterase activity. The highest arylsulphatase (14.16 µg p-nitrophenol g^{-1} h^{-1}) and arylesterase (113.92 µg p-nitrophenol g^{-1} h^{-1}) activities were recorded in T_3 [50% recommended dose of NPK + Vermicompost at the rate of 2 t ha^{-1} (mixed with microbial consortium)]. The enzyme arylsulphatase catalyzes the hydrolysis of aromatic sulfate esters and releasing sulfate for plants uptake. Nath et al. [12] reported that regular application of biofertilizers and organic manure with 50% NPK exhibited significantly higher activity levels of arylsulphatase. Albiach et al. [14] also reported that the presence of higher levels of soil enzymatic activity under organic residue application and specifically the arylsulphatase. Arylesterase activity increased in the same treatment might be due to increased microbial population and MBC.

In the present study, the highest microbial population of bacteria (8.24 log cfu g^{-1} soil) and fungi (3.89 log cfu g^{-1} soil) were recorded in T_3 [50% recommended dose of NPK + Vermicompost at the rate of 2 t ha^{-1} (mixed with microbial consortium)]. The results illustrated that the greater part of the favorable effects of elevated and reasonably stabilized specific populations of fungi and bacteria were related to the added microorganisms as well as the application of organic manure for a longer period [18]. The availability of carbonaceous materials and substrates such as sugar, amino acids and organic acids to the soil from the decomposing organic materials and decay of roots under the plant canopy are important for supplying energy for microbial population [19]. As a consequence, organic inputs generally enhanced the development of microflora and increased the global activity of soil.

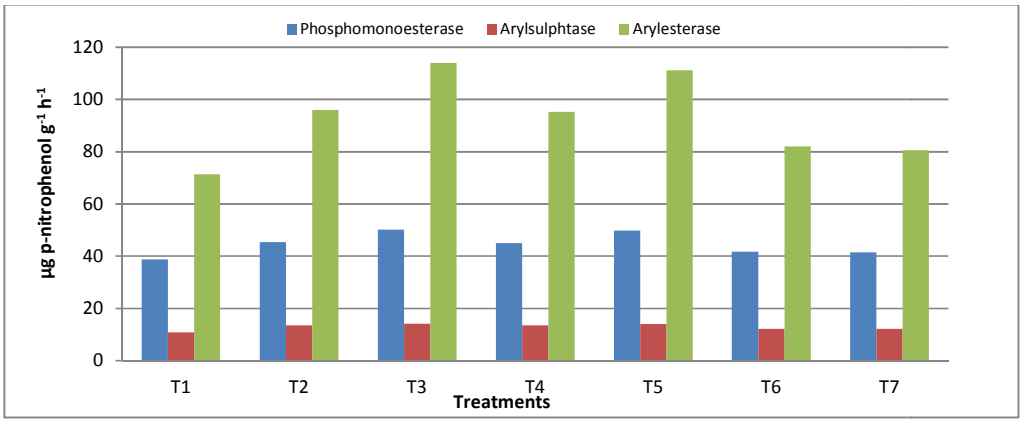

Fig. 1. Effect on different soil enzymes as influenced by INM

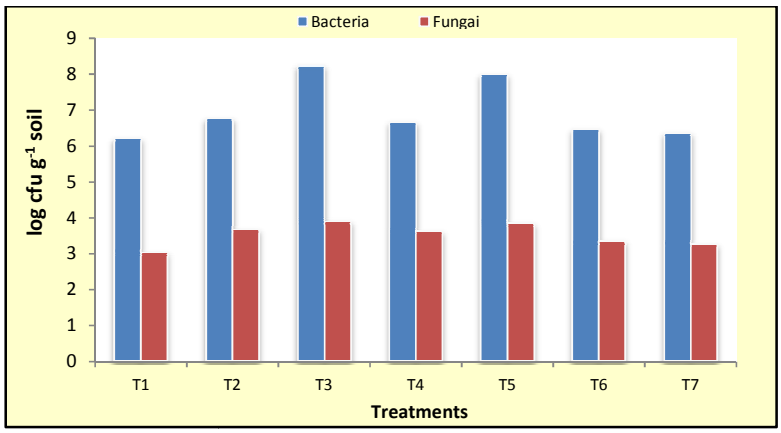

Fig. 2. Bacterial and fungal population as influenced by INM

Table 1. Effect of INM on soil biological properties

Treatment	MBC (µg g^{-1})	FDA (µg fluorescein g^{-1} soil h^{-1})	Phosphomonoesterase (µg *p*-NPS g^{-1} h^{-1})	Dehydrogenase (µg TPF g^{-1} soil 24 h^{-1})	Arylsulphatase activity (µg *p*-NPS g^{-1} h^{-1})	Arylesterase activity (µg *p*-NPS g^{-1} h^{-1})	Bacteria (log cfu g^{-1} soil)	Fungai (log cfu g^{-1} soil)
T_1	180.02	5.92	38.72	103.50	10.86	71.32	6.21	3.02
T_2	230.66	6.72	45.35	127.70	13.57	96.03	6.76	3.66
T_3	244.86	7.28	50.15	136.90	14.16	113.92	8.24	3.89
T_4	226.53	6.64	44.98	127.53	13.48	95.12	6.65	3.61
T_5	240.62	7.15	49.75	135.60	14.02	111.24	7.99	3.84
T_6	212.52	6.30	41.66	118.60	12.21	82.07	6.46	3.34
T_7	211.81	6.23	41.38	117.90	12.08	80.48	6.35	3.26
S.Ed(±)	2.01	0.13	1.10	2.52	0.18	2.25	0.03	0.03
CD at 5%	4.39	0.28	2.40	5.49	0.40	4.91	0.07	0.06
Intinal	160.76	5.65	32.46	86.20	7.46	62.82	5.66	2.92

4. CONCLUSION

From the present study, it can be concluded that addition of good quality organic matter along with biofertilizers helped in increasing soil biological properties which has been considered as a good indicator of high-quality soil. These biofertilizers provide a good amount of nutrients and growth substances which is essential for good growth and development of plants. Although, inorganic fertilizers not only provides good amount of nutrients to the plant which required by the plant for early growth and development but application of inorganic fertilizers also causing several hazards to the soil and environment. Therefore, INM practices should be adopted which helped in reduced application of inorganic fertilizers and also maintain the soil production potential for a longer period of time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Trasar-Cepeda C, Leiros MC, Seoane S, Gil-Sotres F. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. Soil Biology and Biochemistry. 2008;40:2146-2155.
2. Bendick AK, Dick RP. Field management effects on soil enzyme activities. Soil Biology and Biochemistry. 1999;31:1471-1479.
3. Dick RP. Soil enzyme activities as integrative indicators of soil health. In *Biological Indicators of Soil Health* (C. Pankhurst, B. Doube and V. Gupta, Eds.). CAB International, Wallingford, UK. 1997; 121-156.
4. Schloter M, Dilly O, Munch JC. Indicators for evaluating soil quality. Agriculture, Ecosystems and Environment. 2003;98: 255–262.
5. Casida LE, Klein DA, Santoro R. Soil dehydrogenase activity. Soil Sci. 1964;98: 371-376.
6. Tabatabi MA, Bremner JM. Use of P-*nitrophenol* phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1969;1:301-307.
7. Green VS, Stott DE, Diack M. Assay for fluoresce in diacetate hydrolytic activity: Optimization for soil samples. Soil Biol. Biochem. 2006;38:693-701.
8. Tabatabai MA, Bremner JM. Forms of sulphur and carbon, nitrogen and sulphur relationships in Iowa soils. Soil Sci. 1970; 114:380-386.
9. Zornoza R, Landi L, Nannipieri P, Renella G. A protocol for the assay of arylesterase activity in soil. Soil Biol. Biochem. 2009; 41(3):659-662.
10. Joergensen RG, Mueller T. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k EN value. Soil Biology and Biochemistry. 1996;28(1):33-37.
11. Fisher RA, Yates F. Statistical tables for Biological, Agricultural and Medical Research, Long Man Group Limited, London, Sixth Edition; 1963.
12. Nath DJ, Gogoi D, Buragohain S, Gayan A, Devi YB, Bhattacharyya B. Effect of integrated nutrient management on soil enzymes, microbial biomass carbon and soil chemical properties after eight years of rice (*Oryza sativa*) cultivation in an Aeric Endoaquept. J. Indian Soc. Soil Sci. 2015; 63(4):406-413.
13. Green VS, Stott DE, Diack M. Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. Soil Biol. Biochem. 2006;38:693-701.
14. Albiach R, Canet R, Pomares F, Ingelmo F. Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. Biores. Technol. 2000;75:43-48.
15. Biswas DR, Narayanasamy G. Rock phosphate enriched compost: An approach to improve low-grade Indian rock phosphate. Biores. Technol. 2006;97: 2243-2251.
16. Nannipieri P, Sastre I, Landi L, Lobo MC, Pietramellara G. Determination of extracellular neutral phosphomono-esterase activity in soil. Soil Biol. Biochem. 1996;28:107-112.
17. Nayak DR, Babu YJ, Adhya TK. Long term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. Soil Biol. Biochem. 2007;39:1897-190.

18. Nath DJ, Ozah B, Baruah R, Borah DK, Gupta M. Soil enzymes and microbial biomass carbon under rice-toria sequence as influenced by nutrient management. J. Indian Soc. Soil Sci. 2012;60:20-24.

19. Mohammad I, Yadav BL, Ahamad A. Effect of phosphorus and bio-organics on yield and soil fertility status of Mungbean [*Vigna radiata* (L.) Wilczek under semi-arid condition of Rajasthan, India. Int. J. Curr. Microbiol. App. Sci. 2017;6(3):1545-1553.

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