

Effect of INM on Soil Enzymes, Microbial Biomass Carbon and Microbial Population under Okra Cultivation

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 INM 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 MBC ($244.86 \mu\text{g g}^{-1}$), bacterial population ($8.24 \log \text{cfu g}^{-1} \text{soil}$) and fungal population ($3.89 \log \text{cfu g}^{-1} \text{soil}$) and soil enzymes like fluorescein di-acetate (FDA) ($7.28 \mu\text{g fluorescein g}^{-1} \text{soil hour}^{-1}$), phosphomonoesterase (PME) ($50.15 \mu\text{g p-nitrophenol g}^{-1} \text{hour}^{-1}$), Dehydrogenase (DH) ($136.90 \mu\text{g TPF g}^{-1} \text{soil 24 hour}^{-1}$), Arylsulphatase ($14.16 \mu\text{g p-nitrophenol g}^{-1} \text{hour}^{-1}$) and Arylesterase activity ($113.92 \mu\text{g p-nitrophenol g}^{-1} \text{hour}^{-1}$) was found in the treatment T₃ [50% RD of N,P,K + Vermicompost @ 2 t ha^{-1} (mixed with microbial 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 agro ecosystem (Trasar-Cepeda *et al.* 2008). 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 (Bendick and Dick 1999). 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 (Trasar-Cepeda *et al.* 2008). 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 (Dick 1997). 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 (Kanwar and Randhawa, 1978) and nutrient imbalance (Singh *et al.*, 1989), 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 (Schloter *et al.* 2003).

2. MATERIAL AND METHODS

A field experiment was conducted at the Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat during March to July 2016. The experiment was laid out with Randomized Block Design and replicated three times. There were seven treatments consisting of T₁ [RDF(50:50:50 kg NPK ha⁻¹ + FYM @ 10 t ha⁻¹)], T₂ [75% RD of NPK + Vermicompost @ 1 t ha⁻¹ (mixed with microbial consortium)], T₃ [50% RD of NPK + Vermicompost @ 2 t ha⁻¹ (mixed with microbial consortium)], T₄ [75% RD of NPK + Microbial consortium as seed coat + Vermicompost @ 1 t ha⁻¹], T₅ [50% RD of NPK + Microbial consortium as seed coat + Vermicompost @ 2 t ha⁻¹], T₆ [FYM @ 10 t ha⁻¹ (mixed with microbial consortium)] and T₇ [Microbial consortium as seed coat + FYM @ 10 t ha⁻¹]. Microbial consortium was the mixture of four different groups of biofertilizers (PSB, 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. FYM was applied @ 10 t ha⁻¹ and vermicompost was applied @ 1 t ha⁻¹ and 2 t ha⁻¹ after final land preparation. 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, SSP, and MOP were applied three days before sowing as a basal application. Half of Urea, full dose of SSP and MOP was applied as basal. The second half of Urea was applied at 30 days after sowing. Prior to estimation of soil enzymatic activities, microbial biomass carbon and microbial population the field moist samples were collected after final harvest and preserved in 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.* (1964). The method of Tabatabai and Bremner (1969) is followed to estimate the phosphomonoesterase activity. FDA hydrolysis activities were carried out following the method described by Adam and Duncan (2001). The assay for arylsulphatase activity was carried out by using *p*-nitrophenyl sulphate (*p*-NPS) as substrate (Tabatabai and Bremner, 1970). The assay for aryl esterase activity was carried out by following standard protocol given by Zornoja *et al.* (2009). Microbial biomass carbon was determined by chloroform fumigation- extraction technique following the method of Vance *et al.* (1987). The classical serial dilution technique was used for isolation of total bacterial (PSB, Azotobacter and Rhizobium and

77 fungal (*Azospirillum*) population from the soil sample by spread plate technique on appropriate media.
78 The data were subjected to statistical scrutiny.

79 **3. RESULTS AND DISCUSSION**

80 Soil microbial biomass carbon (MBC), the most active and dynamic pool of the soil organic matter,
81 acts as transient nutrients sinks and is responsible for releasing nutrients from organic matter for use
82 by plants. In the present study application of 50% RD of N,P,K + Vermicompost @ 2 t ha⁻¹ (mixed with
83 microbial consortium) resulted in the highest MBC (244.86 µg g⁻¹). This might be due to the INM plots
84 have provided a steady source of organic carbon to support the microbial community compared to
85 100% NPK treated plots. Application of biofertilizers, besides showing their primary effect are also
86 known to produce diverse growth promoting substances that might contribute intense proliferation of
87 microbial growth and augmented MBC (Nath *et al.*, 2015). Improvement of MBC in the vermicompost
88 treated plots might be largely due to the microbes contained in the organic residues and the addition
89 of substrate carbon, which stimulates the indigenous soil micro biota.

90 Hydrolysis of FDA has been widely accepted as a measure of total microbial activity in soils and
91 functions as a broad spectrum indicator of soil biological activity (Adam and Duncan, 2001; Green *et al.*,
92 2006). In the present study maximum increase in FDA hydrolysis (7.28 µg fluorescein g⁻¹ hr⁻¹) was
93 recorded in the T₃ treatment which received 50% RD of N,P,K + Vermicompost @ 2 t ha⁻¹ (mixed with
94 microbial consortium). The large increase of FDA hydrolysis due to INM could be attributed to
95 increased microbial biomass resulting from organic matter enrichment in soil, since addition of good
96 quality compost has direct bearing on MBC and soil enzyme activities (Albiach *et al.*, 2000).

97 Extracellular phosphomonoesterase (PME) is an enzyme of agronomic value because it hydrolyzes
98 compounds of organic P and transforms them into inorganic P. In this experiment addition of
99 vermicompost (2 t ha⁻¹) along with inorganic fertilizer and biofertilizer recorded maximum increase in
100 PMEase activity of soil (T₃). It might be due to the release of more organically bound P, as synthesis
101 of the enzyme is stimulated by the presence of organic substrate (Biswas and Narayanasamy, 2006).
102 The persistence of elevated and active PME in organic amended soils might be due to their
103 adsorption and protection against proteolysis, once released as extracellular enzyme or following cell
104 death or during humification of organic residues (Nannipieri *et al.*, 1996).

105 Soil dehydrogenase (DH) involved in oxidative phosphorylation, and is an important indicator of
106 microbial activity in soil which has been found to increase significantly in soils applied in combination
107 of organic, inorganic and biofertilizer. In the present study application of 50% RD of N,P,K +
108 Vermicompost @ 2 t ha⁻¹ (mixed with microbial consortium) resulted in the highest DH activity. This
109 might be due to increased microbial activity and MBC in the same treatment. Incorporation of bulky
110 sources of potential beneficial microbes may possibly provide microbial diversity and activity of
111 microorganisms accompanied by better DH activity (Nath *et al.*, 2015). Nayak *et al.* (2007) also
112 described a generalized short to medium term increase in DH activity following organic matter
113 addition.

114 Similar trend was also followed in case of arylsulphatase and arylesterase activity. The highest
115 arylsulphatase and arylesterase activities were recorded in T₃ [50% RD of N,P,K + Vermicompost @ 2

t ha⁻¹ (mixed with microbial consortium)]. The enzyme arylsulphatase catalyzes the hydrolysis of aromatic sulphate esters and releasing sulphate for plants uptake. Nath *et al.* (2015) reported that regular application of biofertilizers and organic manure with 50% NPK exhibited significantly higher activity levels of arylsulphatase. Albiach *et al.* (2000) 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 and fungi were recorded in T₃[50% RD of N,P,K + Vermicompost @ 2 t ha⁻¹ (mixed with microbial consortium)]. The results illustrated that the greater part of the favourable effects of elevated and reasonably stabilized specific populations of fungi and bacteria were related to the added microorganisms as well as application of organic manure for longer period (Nath *et al.*, 2012). 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 (Mohammad *et al.*, 2017). As a consequence, organic inputs generally enhanced the development of micro flora and increased the global activity of soil.

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 good indicator of high quality soil. These biofertilizers provide good amount of nutrients and growth substances which is essential for good growth and development of plants. Although, inorganic fertilizers 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 longer period of time.

REFERENCES

- Adam, G. and Duncan, H. (2001). Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* **33**: 943-951.
- Albiach, R.; Canet, R.; Pomares, F. and Ingelmo, F. (2000). Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. *Biores. Technol.* **75**: 43-48.
- Bendick, A. K. and Dick, R. P. (1999) Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* **31**: 1471-1479.
- Biswas, D.R. and Narayanasamy, G. (2006). Rock phosphate enriched compost: An approach to improve low-grade Indian rock phosphate. *Biores. Technol.* **97**: 2243-2251.
- Casida, L.E.; Klein, D.A. and Santoro, R. (1964). Soil dehydrogenase activity. *Soil Sci.* **98**: 371-376.
- Dick, R.P. (1997). Soil enzyme activities as integrative indicators of soil health. In *Biological Indicators of Soil Health* (C. Pankhurst, B. Doube and V. Gupta, Eds.), pp 121-156. CAB International, Wallingford, UK.

- 156 Green, V.S.; Stott, D.E. and Diack, M. (2006). Assay for fluorescein diacetate hydrolytic activity:
157 optimization for soil samples. *Soil Biol. Biochem.* **38**: 693-701.
- 158 Kanwar, J. S. and Randhawa, N. S. (1978). Micronutrient research in soil and plants in India (Annual
159 Review), Technical Bulletin 50, Indian Council of Agricultural Research, New Delhi.
- 160 Mohammad, I.; Yadav, B.L. and Ahamad, A. (2017). Effect of phosphorus and bio-organics on yield
161 and soil fertility status of Mungbean [*Vigna radiata* (L.) Wilczek under semi-arid
162 condition of Rajasthan, India. *Int. J. Curr. Microbiol. App. Sci.* **6**(3): 1545-1553.
- 163 Nannipieri, P.; Sastre, I.; Landi, L.; Lobo, M.C. and Pietramellara, G. (1996). Determination of
164 extracellular neutral phosphomonoesterase activity in soil. *Soil Biol. Biochem.* **28**:107-
165 112.
- 166 Nath, D.J.; Gogoi, D.; Buragohain, S.; Gayan, A.; Devi, Y.B. and Bhattacharyya, B. (2015). Effect of
167 integrated nutrient management on soil enzymes, microbial biomass carbon and soil
168 chemical properties after eight years of rice (*Oryza sativa*) cultivation in an Aeris
169 Endoaquept. *J. Indian Soc. Soil Sci.* **63**(4): 406-413.
- 170 Nath, D.J.; Ozah, B.; Baruah, R.; Borah, D.K. and Gupta, M. (2012). Soil enzymes and microbial
171 biomass carbon under rice-toria sequence as influenced by nutrient management. *J.*
172 *Indian Soc. Soil Sci.* **60**: 20-24.
- 173 Nayak, D.R.; Babu, Y.J. and Adhya, T.K. (2007). Long term application of compost influences
174 microbial biomass and enzyme activities in a tropical Aeris Endoaquept planted to
175 rice under flooded condition. *Soil Biol. Biochem.* **39**: 1897-190
- 176 Schloter, M.; Dilly, O. and Munch, J. C. (2003). Indicators for evaluating soil quality. *Agriculture,*
177 *Ecosystems and Environment* **98**: 255–262.
- 178 Singh, A. P.; Sakal, R. and Sinha, R. B. (1989). Effect of changing cropping pattern and fertility levels
179 on crop yields and micronutrient status of soil after five cycles of crop rotation. *Annals*
180 *of Agricultural Research* **10**(4): 361-367.
- 181 Tabatabai, M.A. and Bremner, J.M. (1970). Forms of sulphur and carbon, nitrogen and sulphur
182 relationships in Iowa soils. *Soil Sci.* **114**: 380-386.
- 183 Tabatabai, M.A. and Bremner, J.M. (1969). Use of P-nitrophenol phosphate for assay of soil
184 phosphatase activity. *Soil Biol. Biochem.* **1**: 301-307.
- 185 Trasar-Cepeda, C.; Leiros, M. C.; Seoane, S. and Gil-Sotres, F. (2008). Hydrolytic enzyme activities
186 in agricultural and forest soils. Some implications for their use as indicators of soil
187 quality. *Soil Biology and Biochemistry* **40**: 2146-2155
- 188 .Vance, E.D.; Brookes, P.C. and Jenkinson, D.S. (1987). An extraction method for measuring soil
189 microbial biomass C. *Soil Biol. Biochem.* **19**: 703-707.
- 190 Zornoza, R.; Landi, L.; Nannipieri, P. and Renella, G. (2009). A protocol for the assay of arylesterase
191 activity in soil. *Soil Biol. Biochem.* **41**(3): 659-662.

192 Table 1 Effect of INM on soil biological properties

Treatment	MBC ($\mu\text{g g}^{-1}$)	FDA ($\mu\text{g fluorescein g}^{-1}$ soil hour^{-1})	Phosphomonoesterase ($\mu\text{g p-NPSg}^{-1} \text{hour}^{-1}$)	Dhydrogenase ($\mu\text{g TPF g}^{-1}$ soil 24 hour^{-1})	Arylsulphatase activity ($\mu\text{g p-NPSg}^{-1} \text{hour}^{-1}$)	Arylesterase activity ($\mu\text{g p-NPSg}^{-1} \text{hour}^{-1}$)	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