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Microbial Dynamics as Influenced by Bio-organics and Mineral Fertilizer in Alluvium Soil of Varanasi, India

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A B S T R A C T

The present investigation was carried out at the Vegetable Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh (India) during Rabi of the year 2009 and 2010 on alluvial soil to assess the effect of *Pseudomonas fluorescens* and humic acid in combination with three different levels of mineral fertilizer fertilizers on microbial dynemics in alluvium soil. All inoculated treatments in both years of experiment gave significantly greater microbial population and enzymetic activities compared to control at harvest of cabbage crop. The highest microbial population and enzymetic activities were caused by 100% fertilization with *Pseudomonas fluorescens* + humic acid which showed its significant superiority over rest of the treatments except with 75% RDF + *Pseudomonas fluorescens* + humic acid, 100 % RDF + *Pseudomonas fluorescens* and 100 % RDF + humic acid in both experiments. The lowest microbial population and enzymetic activity were found with 100 % RDF as control which was statistically at par with 50 % RDF + *Pseudomonas fluorescens* and 50 % RDF + humic acid in both year of experiments. Separately inoculation of *Pseudomonas fluorescens* and humic acid with fertilizer, humic acid gave higher microbial population, dehydrogenase and urease enzymetic activity and alkaline phosphatase activity with *P. fluorescens* inoculation in cabbage rhizospheric soil during both years of experiment at harvest.

Introduction

Modern agriculture production requires efficient, sustainable and environmentally sound management practices as the judicious application of bio-organic with mineral fertilizers to increase fertilizer use efficiency is need of an hour. The Use of effective bio-organics like biofertilizers, plant growth promoting microorganisms and humic substances for improved crop growth and soil health is one of the viable alternatives. The goal for the majority of organic substances research has been to identify the best growing conditions possible for best crop establishment and improves soil physical (Allison, 1962) and chemical and biological properties are important for plant health, soil environment (Yassen and Khalid, 2009). Organic matter is one of the most important components when evaluating the general fertility of a soil (Ding *et al*., 2002). In agricultural soils, organic matter consists mainly of plant biopolymer residues,

materials derived from them via the decomposition processes, microbial tissues and humic substances (Chefetz *et al*., 2000).

The microbial activity can be used as index of soil fertility and microbial functional diversity (Nannipieri *et al.,* 2002 and Meena *et al.,* 2014). Soil micro flora is biological indicator of soil quality because of the relationship to decomposition and nutrient cycling, ease and rapid response to changes in soil management (Dilly *et al.,* 2003). Microbial population as well as enzymatic activity and other biological properties (viz. SMBC, CO_2 evolution, C: N ratio) and phosphatase, indicate that incorporation of bio-organic material had contributed significantly to soil organic carbon, total nitrogen lead to greater microbial biomass and subsequently to greater enzyme synthesis and accumulation in the soil matrix (Dinesh *et al.,* 2000). Soil microbial biomass has been used as an index of soil fertility which depends on nutrient fluxes (Hassink, 1991). Enzymatic activities have been proposed as a tool to monitor changes in soil ecology resulting from the interactions between inoculants and indigenous microbial populations of soil (Doyle and Stotzky, 1993). Soil enzyme assay is an indirect indication on the activities of microbes which is directly correlated with soil microbial dynamics. Soil enzyme activity varies with soil type and is influenced by the texture and the content of organic matter of the soils (Tarafdar and Jungk, 1987). The objective of this study was to evaluate the microbial dynamics as influenced by bio-organics and mineral fertilizer in alluvium soil.

Material and Methods

The studies pertaining to the effect of *Pseudomonas fluorescens* and humic acid with mineral fertilizer on cabbage was conducted at Vegetable Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (25^o 18′ N latitude, 83^o 03′ E longitude and 128.93 m MSL). The experiment was laid out in Randomized Block Design with three replications. The experiment consist of ten treatment combinations *viz*., [(T_1) 100% RDF (control), (T_2) 50% RDF + *Pseudomonas fluorescens*, (T_3) 75% RDF + *Pseudomonas fluorescens*, (T_4) 100% RDF + *Pseudomonas fluorescens*, (T_5) 50% RDF + Humic acid, (T_6) 75% RDF + Humic acid, (T_7) 100% RDF + Humic acid, (T_8) 50% RDF + *Pseudomonas fluorescens* + Humic acid, (T_9) 75% RDF + *Pseudomonas fluorescens* + Humic acid (T_{10}) 100% RDF + *Pseudomonas fluorescens* + Humic acid. Cabbage seedlings were raised in seedbeds of 5 × 4 m size using seeds of cabbage *var*. Golden Acre F1 hybrid produced by Sakata Seed Corporation, Japan. Recommended dose of fertilizers were 120 N: 60 P_2O_5: 60 K_2O kg/ ha. Nursery raised transplanting, fertilization and crop cultivation practices according to Verma *et al.,* (2014).

Soil sampling

Initial soil samples were collected prior to the start of the experiment from surface soil (0-15 cm depth) and analyzed for physicochemical properties (Table 1). At harvest of cabbage, the rhizospheric soil collected from surrounding of cabbage plant roots from each plot at harvesting of crop and brought to laboratory. For microbial analysis soil samples were kept at 4 o C in plastic bags for a few days to stabilize the microbiological activity disturbed during soil sampling and handling, and then analysed. Total bacteria, fungi and actinomycetes were estimated by following the standard procedure of Rolf and Bakken (1987) and dehydrogenase and urease (Tabatabai, 1994) and alkaline phosphatase activity measured by Tabatabai and Bremner, (1986).

Statistical analysis

Statistical analysis of the data was done by using analysis of variance (ANOVA), assessed by Panse and Sukhatme (1985), with a probability, the treatment mean were compared at $P < 0.05$ by using the statistical computer programme MSTAT, version 5.

Results and Discussion

Microbial population: Microbial population was influenced by the application of mineral fertilizer levels and *P. fluorescens* with humic acid in both year of experimentation have been presented in table 2. Microbial population as bacteria, fungi and actinomycetes increased with increasing dose of mineral fertilizers with the combination of *Pseudomonas* and humic acid. The Pooled data showed that bacterial population varied 26 to 40 cfu X 10^5 g^{-1} soil. Maximum 40 cfu X 10^5 g^{-1} soil bacterial population was recorded with 100% RDF + *P. fluorescens* + humic acid showed its superiority over rest of the treatments. This treatment gave 57.3, 13.1 and 14.7% higher bacterial population than control, 100 % RDF + *P. fluorescens* and 100 % RDF + humic acid, respectively. Humic acid caused 1.42 % higher bacterial population as compared to *P. fluorescens* inoculation. Minimum 26 cfu X 10^5 g^{-1} soil bacterial population was recorded with 100% RDF which was statistically at par to 50% RDF + *P. fluorescens* and 50 % RDF + humic acid. With the application of mineral fertilizer, *P. fluorescens* and humic acid as nutrient source, bio stimulator and organic substrate, there was significant increase in total bacterial population which might be due to the increased microbial functional diversity of soil (Meena *et al.*, 2014). Manna *et al.* (1996) reported an increase in microbial growth, enzymatic activities and availability of nutrients with the addition of carbon substrate and bio-inoculants are positively related to bacterial population of soil. Similar results have been agreed with by Eissa *et al.*, (2007) and Awasthi *et al.*, (2011).

It is also apparent from the pooled data that mineral fertilizer levels with *P. fluorescens* and humic acid had significant increased of actinomycetes population of soil at harvest. The treatment 100% RDF + *P. fluorescens* + humic acid gave maximum 29 cfu X 10^{-4} g^{-1} soil actinomycetes population in soil which showed its significant superiority over rest of the treatments. This treatment caused 32.0, 13.9 and 15.65 greater actinomycetes population than 100% RDF, 50% + *P. fluorescens* and 50% RDF + humic acid, respectively. Minimum 22 cfu X 10^4 g^{-1} soil actinomycetes population in soil was recorded with 100% RDF which was statistically at par with 50% RDF + *P. fluorescens* and 50% RDF + humic acid. Each of *P. fluorescens* and humic acid with fertilizer combination showing highly significant increased actinomycetes population over the remaining treatments at harvest of cabbage might be due to addition of carbon substrate and bio sources (Manna *et al.*, 1996). Similar results augmented by Ismail *et al.*, (2007) and Timothy *et al.*, (2010).

The pooled data showed that fungal population was varied 17 to 25 cfu X 10^4 g^{-1} soil. Maximum 25 cfu X 10^4 g^{-1} soil fungal population was recorded with 100% RDF + *P. fluorescens* + humic acid which showed its significant superiority over to rest of treatments. This treatment gave 47.0, 15.1 and 16.8 % greater fungal population than control, 100 % RDF + *P. fluorescens* and 100 % RDF + humic acid, respectively. Humic acid caused 1.47 % greater fungal population as compared to *P. fluorescens* inoculation. Minimum 17 cfu X 10^4 g^{-1} soil fungal population was recorded with 100% RDF which was statistically at par with 50

% RDF + *P. fluorescens* and 50 % RDF + humic acid. This might be due to acidophilic nature of fungi. There was increase in fungal population from initial to post harvest soil. Variation of fungal population was also influenced by age of the plant (Kumari, 1961). The availability of nutrients and organic carbon is positively related with fungal population in soil Naseby and Lynch, (1998) and Awasthi *et al.,* (2011). Patil and Varade (1998) reported that populations of bacteria, actinomycetes and fungi significantly differed with rate of fertilizer treatments and proliferate under N, P, K and bio-inoculants with organic substances. Fertilizer and Bio-organics had a profound influence on the microbial population of the soil which in turn, affected the rate of assimilation of nutrients (John and Abraham, 1995).

Soil enzymatic activities

Enzymatic activities have been proposed as a tool to monitor changes in soil ecology resulting from the interactions between inoculants and indigenous microbial populations of soil (Doyle and Stotzky, 1993). In the present investigation, urease, dehydrogenase, and phosphatase activities significantly increased at harvest of cabbage rhizospheric soil due to application of levels of mineral fertilizer with *Pseudomonas fluorescens* and humic acid. The pooled data showed that the maximum dehydrogenase activity (142 µg TPF g^{-1} soil day^{-1}) was the treatment received with 100% RDF + *Pseudomonas fluorescens* + humic acid which showed its significant superiority over rest of the treatments. This treatment caused 55.5, 3.5 and 4.8% greater dehydrogenase activity than control, 100% RDF + *Pseudomonas fluorescens* and 100% RDF + humic acid, respectively. Humic acid gave 1.2% greater dehydrogenase activity of soil as compared to *P. fluorescens* inoculation. Minimum dehydrogenase activity (91 µg TPF g^{-1} soil day^{-1}) was observed with 100% RDF which was statistically at par with 50% RDF + *P. fluorescens* and 50% RDF + humic acid. This indicates that effect of *Pseudomonas fluorescens* and humic acid was similar in increasing dehydrogenase activity. Similar findings were also reported by Selamuthu and Govindaswamy (2003) in sugarcane, they observed increase in dehydrogenase activity with application of humic acid which might be attributed to organic carbon content of humic substance applied. It may be due to higher organic matter content and relatively higher organic carbon (Wlodarczyk *et al.,* 2002). Similar finding also were observed by Naseby and Lynch (1998); Eissa *et al.,* (2007); Timothy *et al*., (2010).

Urease is an important enzyme to responsible for the hydrolysis of urea fertilizer applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH (Byrnes and Amberger, 1989. The pooled data showed that urease activity varied from 225 to 310 µg urea hydrolyzed g^{-1} soil h^{-1}. Maximum 310 µg urea hydrolyzed g^{-1} soil h^{-1} urease activity was recorded with 100%RDF + *P. fluorescens* + humic acid which showed its significant superiority over to rest of the treatments. This treatment gave 37.7, 0.72 and 5.6% greater urease activity than control, 100% RDF + *P. fluorescens* and 100% RDF + humic acid, respectively. Humic acid caused 4.9% greater urease activity of soil as compared to *P. fluorescens* inoculation. Minimum 225 µg urea hydrolyzed g^{-1} soil h^{-1} urease activity was recorded with 100% RDF which was statistically at par with 50% RDF + *P. fluorescens* and 50% RDF + humic acid. It could be attributed due to their higher N content and faster decomposition and release of NH_4-N. Crop growth stages also influenced the urease activity. Under field conditions, urease activity was highest at head formation

stage in compared to initial crop growth stage could be related to lower microbial biomass. Enzymatic activities of soils are usually correlated with their organic carbon and available N contents (Taylor *et al*., 2002).

Higher levels of organic carbon stimulate microbial activity, and therefore enzyme synthesis. Similar results were also reported by Naseby and Lynch, 1998; Ismail *et al*., 2007; Fliessbach *et al*., 2009. Organic phosphorus in soil can comprise 30 to 70% of the total phosphorus content.

Hydrolysis of these organic phosphorus compounds is essential for phosphorus nutrition of plants and microorganisms, which is carried out by the enzyme alkaline phosphatase (Tabatabai, 1994). Pooled data showed that alkaline phosphatase activity varied from 35.4 to 63.7 µg PNP g^{-1} Soil h^{-1} at harvest.

Maximum 63.7 µg PNP g^{-1} soil h^{-1} alkaline phosphatase activity was recorded with 100% RDF + *P. fluorescens* + humic acid which showed its significant superiority over to rest of the treatments. This treatment gave 80.3, 15.2 and 19.0% greater alkaline phosphatase activity than control, 100% RDF + *P. fluorescens* and 100% RDF + humic acid, respectively. Application of humic acid increased 3.2% alkaline phosphatase activity from *P. fluorescens* inoculation. Minimum 35.4 µg PNP g^{-1} soil h^{-1} alkaline phosphatase activity was recorded with 100% RDF which was statistically at par with 50% RDF + *P. fluorescens* and 50% RDF + humic acid.

Table.1 Initial physico-chemical soil properties of experimental field

Parameters	Value			Method
	2009	2010	Mean	
Physico-chemical Properties				
pH (1:2.5 soil water ratio)	7.81	7.69	7.75	Jackson (1973)
EC (dSm^{-1} at 25^0C)	0.221	0.217	0.219	
Organic carbon (%)	0.37	0.39	0.38	Walkley and Black
Available Nitrogen (kg ha^{-1})	192	196	194	Subbaiah and Asija
Available Phosphors (kg ha^{-1})	21.95	22.62	22.29	Olsen's (1954)
Available Potassium (kg ha^{-1})	213	217	215	Jackson (1973)
Biological Properties				
Bacteria (CFU $\times 10^5$ g^{-1} soil)	21.30	22.80	22.05	Rolf and Bakken (1987)
Actinomycetes (CFU $\times$ 10^4 g^{-1})	7.6	7.9	7.75	
Fungi (CFU $\times 10^4$ g^{-1} soil)	14.10	14.70	14.40	
Dehydrogenase activity (µg TPF g^{-1} soil day^{-1})	45.67	48.02	46.85	Tabatabai (1994)
Alkaline Phosphates activity (µg PNP g^{-1} soil h^{-1})	19.49	20.74	20.12	Tabatabai and Bremner1986)
Urease activity (µg UH g^{-1} soil h^{-1})	123.4	126.39	124.89	Douglas Bremner (1971)

Table.2 Effect of *P. fluorescens*, humic acid and level of mineral fertilizers on microbial population of cabbage rhizospheric soil at harvest

Treatments	Bacteria (cfu×10^5 g^{-1} soil)			Actinomycetes (cfu×10^4 g^{-1} soil)			Fungi (cfu×10^4 g^{-1} soil)		
	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
T_1-100% RDF	26	25	26	21	23	22	15	18	17
T_2-50% RDF+*P. fluorescens*	31	32	31	22	23	23	18	19	19
T_3-75% RDF+*P. fluorescens*	33	34	33	23	24	24	19	20	20
T_4-100% RDF+*P. fluorescens*	34	36	35	25	26	25	21	21	21
T_5-50% RDF+HA	31	32	31	22	23	22	18	19	19
T_6-75% RDF+HA	34	35	35	25	26	25	20	21	21
T_7-100% RDF+HA	35	36	36	25	26	26	21	22	21
T_8-50% RDF+*P. fluorescens*+HA	34	35	34	24	25	25	20	21	20
T_9-75% RDF+*P. fluorescens*+HA	36	37	37	26	27	27	22	22	22
T_{10}-100% RDF+*P.fluorescens*+HA	40	41	40	29	30	29	24	25	25
SEm±	1.20	1.24	1.21	0.91	0.95	0.93	0.76	0.89	0.78
CD (P=0.05)	3.57	3.68	3.59	2.71	2.82	2.75	2.25	2.63	2.31

Table.3 Effect of *P. fluorescens*, humic acid and level of mineral fertilizers on enzymetic activity of cabbage rhizosphere soil at harvest

Treatments	Dehydrogenase activity (µg TPF g^{-1} soil 24 h^{-1})			Urease activity (µg UH g^{-1}soil h^{-1})			Alkaline Phosphatase activity (µg PNP g^{-1} soil h^{-1})		
	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
T_1-100% RDF	92	90	91	227	223	225	35.0	35.7	35.4
T_2-50% RDF+*P. fluorescens*	93	105	99	229	233	231	37.6	43.0	40.3
T_3-75% RDF+*P. fluorescens*	111	118	115	259	262	261	45.0	49.5	47.2
T_4-100% RDF+*P. fluorescens*	132	138	135	292	295	294	54.6	56.0	55.3
T_5-50% RDF+HA	95	102	98	236	240	238	36.2	43.0	39.6
T_6-75% RDF+HA	112	119	116	263	266	264	43.4	49.0	46.2
T_7-100% RDF+HA	133	141	137	305	311	308	52.7	54.5	53.6
T_8-50% RDF+*P. fluorescens*+HA	97	105	101	243	256	249	39.2	43.6	41.4
T_9-75% RDF+*P. fluorescens*+HA	122	131	126	278	283	280	51.4	55.0	53.2
T_{10}-100% RDF+ *P.fluorescens*+HA	138	146	142	308	312	310	62.7	64.8	63.7
SEm±	1.24	3.86	1.85	1.63	7.41	3.92	1.43	2.45	1.31
CD (P=0.05)	3.68	11.45	5.49	4.83	22.01	11.63	4.25	7.28	3.88

The higher phosphatase activity in soil may be due to higher nitrogen content of the substrate, which in turn resulted in higher microbial activity. The increase in phosphatase activity following the addition of bio-organics could be due to the proliferation of phosphatase-producing microbes (Perucci *et al*., 1988). The phosphatase activity is directly related to content of organic carbon. The importance of organic carbon in nutrient cycling was evident from the fact that the enzyme activity quantified in the present study showed positive correlation with organic carbon.

This indicates that organic material significantly increases the enzymatic activity in soil. This finding is in accordance to Dick and Tabatabai (1984) they reported that the enzymes in the organics amended soils may also directly contribute to enzyme activities. Enzymatic activities of soils are usually correlated with their organic carbon and available nutrient contents (Taylor *et al*., 2002). Similar results were observed by Naseby and Lynch, 1998 and Ghaderi *et al*., (2008).

In conclusion, application of 100% RDF in combination with *Pseudomonas fluorescens* and humic acid appeared to be better microbial functional diversity in soil as microbial population and enzymatic activity. This treatment increased the nutrient uptake microbial population in rhizospheric soil and activities of urease, phosphatase and dehydrogenase in post harvest soil.

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