

Rhizosphere Microflora of Potato as Affected by Organic Treatments

Bibhuti B. Das, Nagen Rajak, Neilhousano Nakhro and Mumtaz S. Dkhar
Microbial Ecology Laboratory, Department of Botany, North-Eastern Hill University,
Shillong-793022, Meghalaya, India

Abstract: The application of organic treatments has a favorable impact on the rhizosphere soil microbial population and on soil physico chemical properties. The present investigation was carried out at North-Eastern Hill University, Department of Botany to study the effect of different organic treatments combinations (soil+vermicompost, plant compost+vermicompost+soil in different ratio) on potato plant. Potato was planted in the pots with different types of treatment combinations in different ratios plant+soil compost (1:2) showed the highest fungal population and soil+vermicompost (1:2) recorded the highest bacterial population. A significant variation in bacterial population was observed according to Tukey's test at $p = 0.005$. Application of different organic treatment combinations favorably influenced the physicochemical properties of soil and exhibited a significant variation among various combination ratio according to Tukey's test at $p = 0.005$. Organic carbon and available phosphorus was found to be highest in soil+plant compost+vermicompost at 1:2:1 ratio. The study indicated that the application of organic amendment at different combinations favorably influence the microbial population and physicochemical properties of rhizosphere compared to soil where no organic amendments were applied.

Key words: Microbial population, plant compost, potato, rhizosphere, vermicompost, India

INTRODUCTION

The root system of higher plants is associated not only with inanimate environment composed of organic substances but also with a vast community of metabolically active micro-organisms. This unique environment under the influence of plant roots system is called the rhizosphere. The rhizosphere region is highly favorable habitat for the proliferation and metabolism of numerous microbial types. Because the micro flora is so intimately related with the root system, partially covering the root surface any beneficial or toxic substance produced can cause an immediate and profound response. The rhizosphere micro flora may, however favor plant development by producing growth stimulating substances, contributing to the formation of a stable soil structure, releasing elements in organic forms through the mineralization of organic complexes. The inseparable plant microorganism system is set up which undergoes short and long term fluctuation depending on plant development stage as well as agro ecological conditions (Balicka, 1983). In past, heavy doses of fertilizers and other agrochemicals are applied for higher productivity of crop plants. These practices even though increase yield, make the plant system more vulnerable to various stresses like infestation of pests, drought beside deleterious effect

on the environment. Because of the effect of synthetic chemicals and the associated quality, the demand for agricultural commodities produced in accordance with specialized system that is totally independent of any form of synthetic chemical influence has increased. This increase has led to the emergence of a movement toward specified farming concept based on the traditional farming philosophy which is popularly known as organic farming. Organic farming aims for efficient nutrient cycling through the maintenance of large and diverse population of soil organisms.

Organic treatments like compost, manures and uncomposted plant residues have widely different effects on the balance of soil microflora and plant pest and diseases depending on the nature of the residue concerned and its preparation method. The application of compost on the rhizobial population structure may affect the population structure of the indigenous rhizobial population (Cousin *et al.*, 2002). The effect of fertilizers on the soil microbes and rhizosphere microflora depends on the physico chemical characters and the type of vegetation. In recent years, the complex biology of the rhizosphere has emerged as fields of special interest for studies as the microorganisms make a close association with root systems with the result that either plant growth or yield are increased.

MATERIALS AND METHODS

Collection of samples: Samplings of rhizosphere soil was carried out from the potato plant with different organic treatment combinations; Soil (S)+Vermicompost (Vc) in the ratio of 1:1, 2:1, 1:2, Soil (S)+Plant compost (Pc) in the ratio of 1:1, 1:2, 2:1 and Plant compost (Pc)+Vermicompost (Vc)+ Soil (S) in the ratio of 1:1:1, 1:2:1, 2:1:1, 1:1:2 in pot culture at regular time interval (15 days) for a period of 3 months starting from February to May, 2009. Soil samples were kept in the fridge at 4°C until they were processed. Soil temperature was recorded using a soil thermometer at the time of collection.

Isolation of fungi and bacteria from rhizosphere soil:

Serial dilution plate method (Johnson and Curl, 1972) was followed for the isolation of fungi and bacteria using rose Bengal agar medium (Martin, 1950) and nutrient agar medium, respectively. About 1 g of soil sample along with roots cut 1 cm was taken into 250 mL conical flask containing 100 mL of sterilized distilled water to give 1:100 dilution. To prepare homogenous suspension, the flask was swirled for 15 min. Then 10 mL of soil water suspension was transferred to another flask containing 90 mL of sterilized distilled water with the help of a sterilized pipette to get 1:1000 dilution, 10 mL of this suspension was again transferred to another flask containing 90 mL of sterilized distilled water to get 1:10,000 dilution.

For the isolation of fungi 1 mL of the soil dilution (1:1000) was transferred into sterilized Petri dishes containing sterilized rose Bengal agar medium which was then rotated gently to disperse the suspension uniformly. Three replicates were maintained for each sample. The Petri dishes were incubated upside down at 25±1°C for 5 days in the culture room. Colony Form Unit (CFU) of fungi per gram sample was calculated on the dry weight basis. For isolation of bacteria 1 mL of the soil dilution (1:10,000) was transferred to Petri dishes containing sterilized nutrient agar medium. The plates were gently swirled to disperse the suspension uniformly. Three replicates were maintained for each sample. The Petri dishes were incubated upside down at 30±1°C in the BOD incubator. Colony Form Unit (CFU) of bacteria was estimated by counting the number of bacterial colonies. The CFU of bacteria per gram sample was calculated on the dry weight basis.

Soil physico chemical properties: Soil temperature was noted using soil thermometer at the time of sample

collection. pH of the samples was read using an electronic digital pH meter. The moisture content was determined by drying the samples in hot air oven at 105°C for 24 h. Organic carbon was measured by the method given by Anderson and Ingram (1993). Available P molybdenum blue method (Allen *et al.*, 1974).

RESULTS AND DISCUSSION

Microbial population: A total of 20 fungal species were isolated from the rhizosphere soil of potato plant under different organic combinations. The highest fungal population (3.29×10^3 CFU g⁻¹ dry soil) from the rhizosphere of potato plant was found in the pot treated with a combination of soil+plant compost at a ratio of 1:2 and the least (1.99×10^3 CFU g⁻¹ dry soil) was found in soil where no organic amendments were applied. Fungal population did not exhibit significant variation between different treatments according to Tukey's test at $p \leq 0.05$. Most of the fungal species isolated were common to all the combinations whereas some were restricted to particular combinations (Table 1).

The highest bacterial population (3.8×10^5 CFU g⁻¹ dry soil) was found in the combination of soil + vermicompost at a ratio of 1:2 and the least (2.97×10^5 CFU g⁻¹ dry soil) was found in soil. Soil+plant compost, soil + vermicompost, soil + plant compost + vermicompost at a ratio of 1:2, 1:2 and 1:1:2 and 1:2:1 showed significant variation with other treatments according to Tukey's test at $p \leq 0.05$.

Soil physico-chemical properties: Application of organic amendment at different ratio had significantly influenced the physico-chemical properties of potato rhizosphere soil. Among the different organic combination soil+vermicompost at a ratio of 2:1 recorded the highest pH followed by other organic combination and the least value was found in soil where no organic treatment were applied (Table 2). pH of potato rhizosphere soil without any organic combination exhibited significant variation with those of treated potato rhizosphere soil according to Tukey's test at $p \leq 0.05$ (Fig. 1).

Addition of soil+plant compost at a ratio of 1:2 showed a significant increase in moisture content of potato rhizosphere soil. Whereas the combination of soil+vermicompost at a ratio of 1:2 showed decrease in moisture content then other combinations (Table 2) also a significant variation was observed between different treatments according to Tukey's test at $p \leq 0.05$ (Fig. 1). Organic carbon significantly increased due to the addition of soil+plant compost+vermicompost at a ratio of 1:2:1

Table1: Fungal species isolated from potato rhizosphere under different organic combinations

Fungal species	1:1 Pc+S	1:2 Pc+S	2:1 Pc+S	1:1 Vc+S	1:2 Vc+S	2:1 Vc+S	1:1:1 Pc+Vc+S	1:1:2 Pc+Vc+S	1:2:1 Pc+Vc+S	2:1:1 Pc+Vc+S	S
<i>Absidia glauca</i>	-	-	-	-	-	+	-	-	+	-	+
<i>Aspergillus flavus</i>	+	+	+	-	+	+	+	+	+	+	+
<i>A. fumigatus</i>	+	+	+	+	+	+	+	+	+	+	+
<i>A. niger</i>	+	+	-	-	-	-	-	-	-	+	-
<i>Aspergillus</i> sp.	-	+	-	-	-	-	-	-	-	-	-
<i>Cladosporium cladosporoides</i>	+	+	+	+	-	-	+	+	+	+	-
<i>C. herbarum</i>	-	-	-	-	+	+	-	-	-	-	+
<i>C. macrocarpum</i>	+	+	+	+	+	+	+	+	+	+	+
<i>Fusarium oxysporium</i>	-	-	-	-	+	-	-	+	-	-	+
<i>Mucor piriformis</i>	+	+	+	+	+	+	-	+	+	-	-
<i>Penicillium atrovirens</i>	-	-	-	-	-	-	-	-	-	+	-
<i>P. canescens</i>	-	-	-	-	-	-	-	+	+	+	-
<i>P. jensenii</i>	+	-	-	-	-	-	-	+	+	+	-
<i>P. lanosum</i>	-	-	-	-	-	-	+	-	+	-	-
<i>P. rubrum</i>	+	+	+	+	+	+	+	+	+	+	+
<i>P. simplicissimum</i>	+	-	-	-	+	-	-	-	-	-	-
<i>Rhizopus stolonifer</i>	+	-	-	+	-	+	+	-	+	+	-
<i>Trichoderma koningii</i>	-	-	+	-	-	-	-	-	-	-	-
<i>Trichoderma</i> sp.	-	-	+	+	+	-	+	+	+	-	-
<i>T. viridi</i>	-	-	+	-	-	-	-	-	-	-	-

+ and - indicates presence and absence respectively, Pc = Plant compost, Vc = Vermicompost and S = Soil

Table 2: Range of soil physico-chemical properties of rhizosphere soil under different organic treatment combinations.

Combination ratio	ST°C	MC (%)	pH	OC (%)	P (%)
1:1 Pc + S	21.8-22.55 (22.09±0.04)	27.07-35.8 (32.67±0.97)	6.00-6.90 (6.56±0.18)	0.7-1.42 (1.09±0.05)	0.13-0.23 (0.17±0.02)
1:2 Pc + S	21.87-22.52 (22.11±0.01)	28.23-35.8 (30.41±0.95)	6.12-6.7 (6.41±0.08)	0.72-0.91 (0.79±0.03)	0.24-0.26 (0.25±0.01)
2:1 Pc + S	21.90-22.57 (22.14±0.01)	35.13-38.53 (36.47±0.45)	6.53-6.8 (6.67±0.1)	1.41-1.77 (1.6±0.77)	0.33-0.35 (0.34±0.01)
1:1 Vc + S	21.83-22.53 (22.12±0.02)	26.87-36.53 (29.92±1.03)	5.93-6.06 (6.22±0.18)	0.36-1.2 (0.74±0.05)	0.27-0.33 (0.30±0.01)
1:2 Vc + S	21.87-22.50 (22.11±0.02)	28.27-34.73 (30.49±0.93)	6.6-6.83 (6.71±0.07)	0.63-1.05 (0.83±0.01)	0.19-0.27 (0.23±0.02)
2:1 Vc + S	21.83-22.55 (22.12±0.02)	32.93-37.4 (34.92±1.23)	6.03-6.73 (6.33±0.07)	1.11-1.19 (1.15±0.04)	0.32-0.35 (0.33±0.01)
1:1:1 Pc + Vc + S	21.87-22.57 (22.12±0.04)	31.43-39.13 (32.87±0.55)	6.1-6.6 (6.31±0.07)	1.18-1.29 (1.24±0.04)	0.2-0.23 (0.21±0.01)
1:1:2 Pc + Vc + S	21.83-22.53 (22.09±0.03)	22.6-29.87 (26.32±0.78)	6.13-6.43 (6.33±0.05)	1.22-1.27 (1.25±0.06)	0.25-0.33 (0.28±0.01)
1:2:1 Pc + Vc + S	21.87-22.59 (22.13±0.03)	31.4-35.67 (32.31±0.42)	6.17-6.9 (6.53±0.14)	1.28-1.35 (1.35±0.05)	0.27-0.35 (0.31±0.01)
2:1:1 Pc + Vc + S	21.83-22.57 (22.11±0.02)	33.77-39.7 (35.44±1.34)	6.1-6.73 (6.33±0.13)	1.52-1.72 (1.62±0.04)	0.33-0.36 (0.35±0.01)

Value in the parenthesis indicates Mean±SE, Pc = Plant compost, Vc = Vermicompost, S = Soil, ST = Soil temperature, MC = Moisture Content, OC = Organic Carbon and P = available Phosphorus

and a decrease recorded in soil where no organic amendment was applied. A significant variation in according to Tukey's test at $p \leq 0.05$ (Fig. 1). Addition of organic carbon was observed between all the treatments organic source in the combination such as soil, plant compost, vermicompost in the combination of 1:2:1 showed increase in available phosphorus compared to soil where no organic amendments were applied. A significant variation was observed among the treatments according to Tukey's test at $p \leq 0.05$. It is known that organic matter introduced to soil stimulates soil microbial populations and soil biological activity (Parkinson and Paul, 1982). Compost used as a soil amendment or container media may improve the microbial diversity of the soil. Composts depending on the degree of maturity,

provides rich medium supporting high microbial activity (Chen *et al.*, 1988) and may also cause diverse microbial population (Mckinley and Vestal, 1984). The addition of compost to soil increased the incidence of bacteria in tomato rhizosphere (Alvarez *et al.*, 1995).

The number of colony forming units of bacteria and fungi increased when pig manure compost was added to the soil (Weon *et al.*, 1999). Increase in soil biological activity and microbiological growth were also reported when vermicompost sewage sludge was added (Dar, 1996; Marinari *et al.*, 2000). The organic fraction of compost was mostly comprised of the remains of fruits and vegetables with high carbohydrate content and it was used as carbon and energy source by microorganisms. The carbon and phosphorus in compost could be easily used as energy

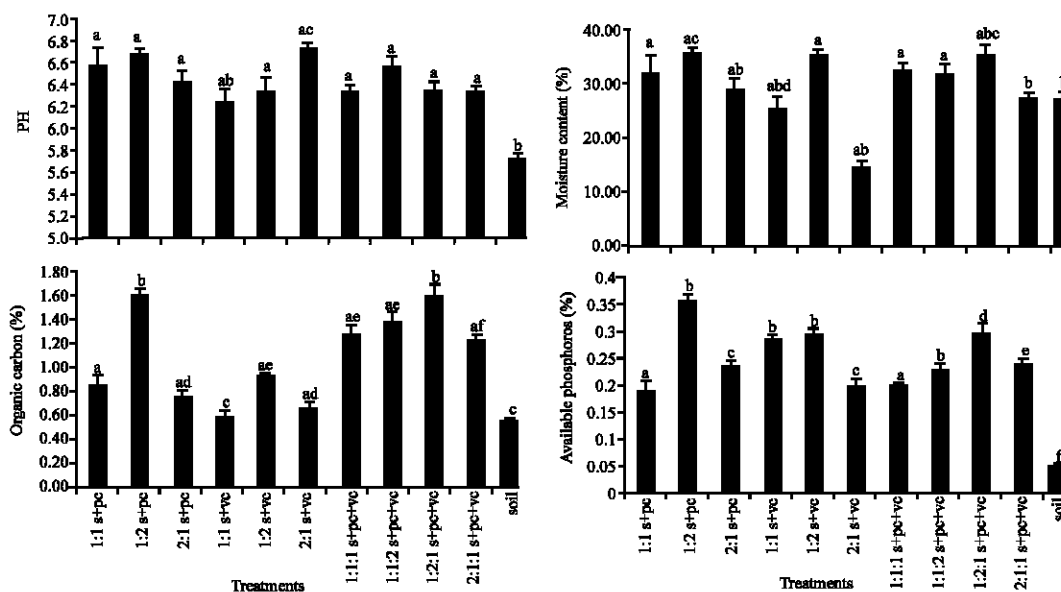


Fig. 1: Soil physico-chemical properties of rhizosphere soil under different organic treatment combinations. Significant difference is indicated by different letters according to Tukey's test at $p = 0.05$ (Pc = Plant compost, Vc = Vermicompost and S = Soil)

and nutrient source for soil microorganisms and this resulted in increased soil microbial populations. Also Cohen reported that excessive compost use in high value cropping systems bring about an overall increase in microbial activity. The highest bacterial population in Vermicompost treatment may be due to the ratio of gram +ive to gram -ive bacteria and of bacteria to fungi as determined signature phospholipids fatty acids were higher in organic treatment than in inorganic treatments (Marschner *et al.*, 2003). They also concluded that organic amendment increased the C_{org} of the soil whereas C_{org} and C/N ratio significantly affect bacteria and eukaryotic community structure. Improve in soil physico chemical properties by the application of organic matter have been supported by many researchers. Various agricultural management practices such as cropping systems, fertilisers applications, cultivation practices, soil organic amendments and pesticides alters the microbial dynamics of agro ecosystem. Addition of compost and other partially degraded materials improve the soil organic matter content and thereby increase the stability of soil structure. Incorporation of organic fertilisers in the form of plant compost enhances the organic carbon level of the soil which has direct and indirect effect on soil physical properties and processes. Dick and Crist (1995) reported that adding organic wastes to soil can increase total N, organic matter, microbial population, enzyme activity, moisture retention, pH buffering capacity and crop yield.

ACKNOWLEDGEMENTS

The researchers are grateful to the Department of Botany, North-Eastern Hill University, Shillong-793022, Meghalaya, India for providing laboratory facilities to carry out the experimental research. The first researchers is grateful to Rajiv Gandhi National Fellowship for SC/ST-University Grants Commission, India providing financial support.

REFERENCES

- Allen, S.E., H.M. Grimshaw, J.A. Parkinson, C. Quarmby and J.D. Roberts, 1974. Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford, London, pp: 565.
- Alvarez, M.A., S. Gagne and H. Antoun, 1995. Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. Applied Environ. Microbiol., 61: 194-199.
- Anderson, J.M. and J.S.I. Ingram, 1993. Tropical Soil Biology and Fertility: A Handbook of Methods. 2nd Edn., CAB International, Wallingford, UK.
- Balicka, N., 1983. Niektore aspekty wzajemnego oddziaływania roślin i drobnoustrojów [Some aspects of interaction between plants and microorganisms]. Post. Mikrobiol., 22: 87-94.

- Chen, W., H.A.J. Hoitink, A.F. Schmitthenner and O.H. Tuovinen, 1988. The role of microbial activity on the suppression of damping off caused by *Pythium ultimum*. *Phytopathology*, 78: 314-322.
- Cousin, C., J. Grant, F. Dixon, D. Beyene and P. van Berkum, 2002. Influence of biosoils compost on *Bradyrhizobial genotype* recovered from cowpea and soybean nodules. *Arch. Microbiol.*, 177: 427-430.
- Dar, G.H., 1996. Effects of cadmium and sewage sludge on soil microbial biomass and enzyme activities. *Bioresour. Technol.*, 56: 141-145.
- Dick, R.P. and R.A. Crist, 1995. Effect of long term waste management and nitrogen fertilisation on availability and profile distribution of nitrogen. *Soil Sci.*, 159: 402-408.
- Johnson, L.F. and A.E. Curl, 1972. Method for the Research on Ecology of Soil Borne Plant Pathogens. Burgess Publishing Company, Minneapolis, pp: 247.
- Marinari, S., G. Masciandaro, B. Ceccanti and S. Grero, 2000. Influence of organic and mineral fertilizers on soil biological and physical properties. *Bioresour. Technol.*, 72: 9-17.
- Marschner, P., E. Kandeler and B. Marschner, 2003. Structure and function of the soil microbial community in a long term fertilizer experiment. *Soil Biol. Biochem.*, 35: 453-461.
- Martin, J.P., 1950. Use of acid, rosebengal and streptomycin in plate method for estimation of soil fungi. *Soil Sci.*, 69: 215-233.
- Mckinley, V.L. and J.R. Vestal, 1984. Biokinetic analyses of adaptation and succession: microbial activity in composting municipal sewage sludge. *Applied Environ. Microbiol.*, 47: 933-941.
- Parkinson, D. and E.A. Paul, 1982. Microbial Biomass. In: *Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties*, Page, A.L., R.H. Miler and D.R. Kenney (Eds.). American Society of Agronomy, Inc. Madison, Wisconsin, pp: 821-830.
- Weon, H.Y., J.S. Kwon, J.S. Suh and W.Y. Choi, 1999. Soil microbial flora and chemical properties as influenced by the application of pig manure compost. *Kor. J. Soil. Sci. Fert.*, 32: 76-83.