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Comparative studies on physico-chemical, biological and enzymatic properties of soil amended with organic manure, vermicompost and indigenous microorganisms (IMOs)

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ABSTRACT

Comparative studies were conducted on physico-chemical, microbiological and enzymatic properties of soil supplemented with organic fertilizers, Organic manure, Vermicompost and Indigenous microorganism (IMO). Supplementation of organic and microbial amendments, improved physicochemical and biological parameters in soil. Higher water holding capacity, moisture content, and electrical conductivity, organic carbon content and bacterial and fungal populations are observed in test soil than control. Improved physical properties, EC, WHC contents were noticed in vermicompost soil. Whereas Higher biochemical properties like Total C, N,P contents observed in IMO treated soil. Nearly threefold higher bacterial and fungal population observed IMO treated soil than control. Soil enzymes, protease and cellulase were enhanced in IMO treated and Vermicompost soil than without treatments. With increasing the soil incubation period soil enzyme activities were enhanced up to 14th day interval and thereafter declined in both test and control soil. Improved physicochemical, microbial and enzymatic parameters in organic manure, vermicompost amended soils are an indication of improvement of soil health and fertility. © 2013 Trade Science Inc. - INDIA

KEYWORDS

Organic manure;
Vermicompost;
Indigenous organisms (IMO)
physico-chemical and biological properties;
Soil cellulase and protease activities.

INTRODUCTION

One of the major concerns in today's world is the pollution and contamination of the soil. The use of chemical fertilizers and pesticides has caused tremendous harm to the environment. Organic fertilizer differs from chemicals in that they feed plants while adding organic material in the form of biocompost or organic manure (tree/vegetative waste, municipal/ vegetable waste) to the soil. Organic farming or natural farming technology is necessary to support the developing organic, sustainable and non-pollution agriculture. These methods are cost effective and ecofreindly in nature Soil is an excellent natural medium and microbial population and their secondary metabolites and enzymes play key biochemical functions in the

FULL PAPER

overall process of organic matter decomposition in the soil system[1]. Supplementation of solid organic amendments, organic manure, vermicompost and microbial inoculums IMO, improves the soil physco-chemical properties. Soil microorganisms and their enzymes directly involved in the degradation of organic wastes (lignocellulosic agriculture wastes like tree waste, leaves,) matter and nutrient cycling. They catalyze several important reactions which are necessary for the life processes of microorganisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling[2]. The activities of these enzymes in soils undergo complex biochemical processes and play an important role in agriculture and particularly in nutrient cycling[3, 4]. In soil, cellulose is available primarily in the form of litter (dead plant leaf material) or lignocellulosic agricultural waste that is relatively recalcitrant due to the high lignin content of terrestrial plants. A lack of fixed nitrogen and other nutrients may secondarily limit microbial growth, and the low moisture content of soils[5]. The enzyme cellulase is a complex enzyme it play an important role in bioconversion of cellulose to simple soluble reducing sugars. The cellulose in soils are derived mainly from plant debris and incorporated into the soil, the limited amounts may also originate from fungi and bacteria in soils. Growth and survival of microorganisms important in most agricultural soils depends on the carbon source contained in the cellulose occurring in the soils whether in the form agricultural waste or cattle feed waste[7]. The protease enzyme The Organic phosphate is converted by an enzyme the phosphatase is extra cellular enzyme plays an important role between biologically unavailable phosphorus and available phosphorus[8]. The inorganic phosphorus availability was controlled by soil organic matter which is influence microbial activity. The phosphatase activity was correlated with organic phosphate and microbial populations[9]. It plays key role in soil ecosystem and it is good indicator of soil fertility[10]. The soil pH influences the release and stability of phosphatase[3,11]. An attempt was made in this study to observe the influence of organic manure, Vermicompost and Indigenous microorganism (IMO) on soil physco-chemical, biological and enzyme particularly Protease and cellulase the key enzymes for degradation of Portentous and cellulosic waste in soil.

MATERIALS AND METHODS

Collection of samples

The Organic amendments, Organic manure, Vermicompost and Indigenous microorganism IMOs are collected from Natural Agricultural Forming Station at Pulicherla, Chittor District Andhra Pradesh. The soil without organic manure, vermicompost and IMO was treated control It was collected from adjacent site natural forming station. The soil samples were air-dried and mixed thoroughly to increase homogeneity and shifted through < 2 mm sieve and used for further studies.

Analytical methods for characterization of samples

The physico-chemical properties of soil amended with organic manure/vermicompost and IMO and control were analyzed by standard methods APHA[12]. Water holding capacity of soil sample were measured by finding amount of distilled water added to soil sample to get saturation point and then sixty per cent water holding capacity of soil sample was calculated by the method[13].

Enumeration of bacterial population

Bacterial populations in control and test soils were enumerated from soil sample on nutrient agar medium with the following composition. (g/L):Peptone - 5.0, NaCl- 5.0,Beef extract- 3.0,Agar agar- 20.0, Distilled water- 1000 ml, PH- 7.2. After preparation of medium, 20 ml of sterile medium was aseptically transferred to sterile Petri plates and allowed for solidification. After solidification of the medium 100µl aliquots of soil suspension was speeded uniformly with the help of sterile glass spreader. The plates were incubated in an incubator at 37 °C for 2days. After incubation, bacterial colonies grown on plates were counted by Queby colony counter. Bacterial colonies are sub cultured on nutrient agar slants for further studies.

Enumeration of fungal populations

Fungal populations in both control and test soils were enumerated on Czapeck-Dox agar medium. After preparation of medium, 20 ml of sterile medium was aseptically transferred to sterile Petri plates and allowed for solidification. After solidification of the medium 0.1 ml aliquots of soil suspension was speeded uniformly with the help of sterile glass spreader. The plates were incubated at room temperature (28°C±30°C) for 7 days. After incubated, fungal colonies grown on plates were counted. The fungal colonies grown on the medium are sub cultured on the Czapeck-Dox agar slants for further studies.

Protease and cellulase assays

The protease and cellulase activities in soil samples were determined according to the method of Speir and Ross[14] and Pancholy and Rice[15] respectively. Duplicates of both test and control soil samples were drawn after 0, 7, 14, 21 and 28 days of incubation to determine protease activity. For assay of soil cellulase five grams of test sample individually treated (1%)with Organic manure,vermicompost and IMO and control were transferred to test tubes and maintained at 60% water holding capacity at room temperature in the laboratory (28±4°C) at regular intervals 0, 7, 14, 21, 28 days of incubation. Duplicate soil samples of each test and control were drawn with at periodic intervals to determine the cellulase enzyme. The effect of addition of to the soil organic manure to the soils studied by incubating the soil sample at 5, 10 percentages with control soil sample. The soil samples were transferred to 250 ml Erlenmeyer flasks and 1 ml of toluene was added. After 15 min,6ml of 0.2M acetate buffer containing carboxy methyl cellulose added to soil samples containing conical flasks were plugged with cotton and incubated for 30 min at 30^{0}C for cellulase activity. After desired incubation, soil extracts were passed through whattman filter paper and the filtrate was assessed by the method[15].

RESULTS AND DISCUSSIONS

Physico-chemical properties organic manure soil

Soil fertility mediated by microorganism is dependent on maintenance of physico-chemical and biological characteristics in soil. Analysis soil with bio-vermi compost and IMO underwent changes in all measured parameters than control soil. Soil composed with vermicompost exhibited improved physical and chemical properties. The compost imports black colour to soil. Higher water holding capacity from 0.26 - 0.44,0,79,,0.58ml/g, moisture content, and higher electrical conductivity 0.3.3 to 1.32, 1.84, and 1.48 μMhos/cm were observed in the control and organic manure, vermicompost and IMO treated soils respectively. These improvements in compost soil may be due to the deposition of organic manure and accumulation of major nutrients and microbial population in the form of organic/ green vegetation and direct inoculation of microbial cultures in terms of indigenous microorganism (IMO) to the soil. These results were confirmed by the previous studies[16,18,19-21] organic effluents had increased the electrical conductivity to the soil. Similar reports made Pradeep and Narasimha 2011 (Leather effluents)[23], Radha et al,(Abattoir)[24] disposal effluents from Leather and Abattoir wastes improved the physicochemical properties of soil. In contrast, soil polluted with cement industries had low water holding capacity and electrical conductivity[25]. Slightly improved condition of pH (7.11) in compost soil was recorded in the present study. Similarly, Lalithakumari *et al.*,[26] Sparling *et al.*,[17] and Nizamuddin, *et al.*,[22] reported that discharges of dairy products like milk residues from dairy industry improved the soil pH. In contrast, Zande *et al.*,[27] reported that the discharges of cane sugar residues from sugar cane industry reduced the soil pH. Higher organic matter content (8.9%) was measured in vermicompost soil than the control with 5.4. Higher organic content of soil may be due to the decomposed form of vermicompost in the soil. Zande *et al.*,[27], Dodor and Tabatabai,[28], Nizamuddin *et al.*,[22] made similar reports on the discharge of organic effluents like, dairy effluents, Improved organic content from 5.2 to 9.2 % was observed in organic manure treated soil, it could be supplementation of higher contents of carbaneous substrates like tree wastes decomposed leaf, litter to the soil.. Improvement in total contents of nitrogen (0.9-1.25, 0.72, 0.82) and phosphorous (35-178,233,384) and potassium (159-727,834,918 kg/h) observed in organic manure vermicompost and IMO treated soils respectively (TABLE.1). Higher contents of these chemical properties of organic manure or biocompost may be due to the decomposed form of organic manure in the soil. Similarly, Narasimha *et al.*, (cotton ginning industry)[18], Kaushik *et al.*, (Distillary)[29] made similar reports on the discharge effluents from agro based industries were improved the soil total phosphorous in contaminated soil. Nizamuddin[22], reported that discharge of effluents from sugar and dairy industry enhanced the potassium content and nitrogen content in the soil.

TABLE 1 : Physico-chemical properties of organic manure and control soil.

Properties	Control soil	Organic manure	Vermicompost	IMO
Color	Grey	Black	Black	Black
pH	7.2	6.9	7.21	6.82
Electrical conductivity (μ Mhos/cm)	0.33	1.32	1.84	1.48
Water holding capacity (ml/g of soil)	0.26	0.44	0.78	0.58
Organic matter (% soil)	5.2	9.2	7.96	8.54
Total nitrogen (Kg/h)	0.9	1.25	0.72	0.82
Phosphorus (kg/h)	35	178	233	384
Carbon (Kg/h)	5.7	12.6	8.75	8.5
Potassium (kg/h)	159	727	834	918

*Values represented in the figure are mean of two separately conducted experiments.

Microbial properties

The microorganisms play a vital role in nutrient cycling and soil fertility. Bacteria and fungi synthesize and secrete soil enzymes such as protease and cellulase enzymes. This enzyme constitutes an important part of the soil matrix as extra cellular enzymes[30]. Micro flora of organic soil amended soils and test was enumerated and listed in the TABLE 2. Higher microbial populations were observed in organic, vermicompost and IMO treated soil than control. The microbial population was counted in terms of colony forming units. In the present study, maximum count (3folds) of bacterial (120×10^4) and fungal (18×10^4) population were quantified IMO treated soil than the control. Similarly, Improved bacterial population from 50 (control) to 72(vermicompost), 90(organic manure), and fungi from 6, 10,12 X 106 observed in control vemicompost, Organic treated soils respectively. Nearly two fold higher bacterial and fungal populations were observed in organic amended and vermicompost soils than the control. (TABLE 2) Higher bacterial and fungal population in test soil could be suitable pH and accumulation of decomposed organic manure, vermicompost and direct inoculation of microorganisms

TABLE 2: Microbial population in organic manure and control soil

Parameter (Soil type)	Bacteria (CFU/g soil)	Fungi (CFU/g soil)
Organic manure	90×10^4	12×10^4
Vermicompost	72×10^4	10×10^4
Indigenerous microorganisms	120×10^4	18×10^4
Control	50×10^4	6×10^4

* Microbial population was counted in the form of CFU/g soil.
*Activity measured in liberation of micromole of tyrosine /g soil

(IMOs) to the soil. In contrast irrigation of soil contaminated with effluents from agro based industries such as dairy, sugar cane and cotton mill industries improved the soil microbial population[17,18,20,29].

Protease activity

Protease activities of both the test and the control soil samples were determined with the amendment of substrate (1%casein) and the results. With increasing the soil incubation period the protease activity was raised nearly 3 folds at 14th day of interval and further ceased in both control and test soil samples at 28 day interval. Compare to the organic manure and vermicompost, the IMO treated soil exhibited maximum protease activity. For instance, protease activity of the IMO treated soil at initial day was 124 ug/g soil, and it was increased to 326u g/g at 14^{th} day, and later declined to 143 ug/g 42 at 21 and 28^{th} days respectively (TABLE 3). The protease activity in vermicompost treated soil was maximum at 14^{th} day intervals and declined in 21 and 28^{th} days of intervals. Protease activity in oganic manure treated soil exhibited medium level of activities between vermicompost and IMO treated soils. For instance protease activity in organic manure soil was increased from 116 to 297 at initial to 14 day intervals and lowered at further intervals. In the present assessment, increased proteolytic activity in the test soil is due to the organic substrates; nutrients applied and increased proteolytic microorganisms in the test soil sample. Similar reports were by other workers in different incidents, such as, soils treated with tomato processing waste[31] effluents of cotton ginning mills[32], dairy shed effluents[33] and pig slurry[34] improved the soil protease activity than the control soil. But the activity was declined with time, maximumat 14days; it is probably because of the exhaustion of the readily available substrates. Similarly, in soils treated with dairy shed effluents[35] the activity decreased with the time. In contrast, soils polluted with cement dust from cement industries[36] wastewater treatment plant discharge[37], herbicides[38], insecticides[39] and chlorothionil[40] ceased the soil protease activity. On the other hand, ammonium fertilizer application[35] did not result in any significant increases in protease activities due to the lack of carbonaceous materials in the ammoniumfertilizer. Increased photolytic activity by increasing the concentration of effluent is also correlated with the results reported[34] treatment of soil with pig slurry, higher protease activity was observed at higher concentration of this residue.

TABLE 3 : Protease activity in organic manure/ vermicompost/IMO treated and control soil

Incubation days	Control soil	Organic manure	Vermicompost	IMO
0	50	116	76	124
7	102	148	125	225
14	142	297	277	326
21	56	92	99	143
28	18	44	31	43

Activity measured in liberation of micromole/ug of tyrosine/g soil; *Values represented in TABLE are mean of Triplicates

Cellulase activity

The cellulase activity in soil amended with /without organic amendments and IMO was studied and listed in TABLE 4. Microorganisms and their enzymes are the indicators for the crop yield and soil fertility. The cellulase activity was higher in test soil than control. With increasing the soil incubation period cellulase activity was improved up to 14th day interval further the activities decreased at 21th to 28th day of interval in both inoculated and uninoculated organic amended soils. Compare to the vermicompost, organic amendments OMO treated soil shown highest cellulase activity (363 ug/gsoil) at 14 day and thereafter declined further intervals. Compared with uninoculated soil 2-3fold higher cellulase activity was observed in organic amended /treated soil than controls. The cellulase activity in IMO treated at 14th day interval was higher than remaining intervals in both treated and non-treated soil (TABLE 4). For instance the cellulase activity in vermicompost test soil initial (0) day interval was 76 µg/g of soil whereas at 14th day interval tremendously higher enzyme activity was observed that is 277µg/g of soil. Decreased cellulase activites were observed at 21 and 28 days of intervals. The cellulase activity in organic manure treated soil was 57 µg/g at initial day intervals and maximum activity was found at 14th day with 224 µg/g and declined at further intervals. In case of control soil this trend was reduced up to 40-80% at all intervals. Similar report was made others, Kannan and Oblisamy[41] (paper and Pulp) Rajasekhar Reddy[42] (Cotton ginning Industry) Jyothsna Devi[43] S (Dairy Industry Srilakshmi[44] forest soil Narasimha et al 2006[45] cotton ginning mill and Discharge of these agro based industrial effluents consisting of lignocellulosic organic waste improved the soil cellulase activity.

TABLE 4 : Cellulase activity in organic amendments and IMO treated and control soil

Incubation days	Control soil	Organic manure	Vermicompost	IMO
0	26	57	76	103
7	58	78	125	199
14	112	224	277	363
21	36	92	89	113
28	13.	44	51	69

Activity measured in liberation of micromole/ug of glucose/g soil; *Values represented in TABLE are mean of Triplicates

CONCLUSIONS

Analysis of soil amended with organic manure, vermicompost and indigenous micro organisms improved the physic-chemical, biological and parameters like water holding capacity moisture content, PH, electrical conductivity, organic contents and microbial populations including bacteria and fungi than the controls soil. Soil enzymes like protease and cellulase activities were improved in soil amendment with organic amendments and IMOs than control. With increasing the soil incubation period soil enzyme activities also improved in both control and test soil. Nearly threefold higher protease and cellulase activities were observed at 14th day of interval in test soil. Improved physico- chemical and microbial population and enzyme activities in organic amended/ Indigenous (IMOs) treated soil is an indication of improvement of soil health and fertility.

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