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Research Article

Effective Microorganisms Growth Analysis by Various Estimation Methods such as pH, Salt, Moisture Content Organic Carbon, Nitrogen, Phosphorus, Zn, Fe, Mn, MH3PO4, CaMg and K in Rice and Black Gram Soil study

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Abstract

In the present study, the soil parameters such as pH, salt content, soil moisture, Organic carbon, N, P, K, Fe, Mg, Mn, Zn and Ca were estimated in control and other treated groups. The following were the experimental results obtained in the rice plant cultivated soil. There is a significant increase of pH (16th week) in all the experimental groups (G II = 6.7 ± 0.2, G III = 7.0 ± 0.4, G IV = 6.7 ± 0.4, G V = 7.3 ± 0.2 and G VI = 6.9 ± 0.4) when compared with control group 5.9 ± 0.5. There is a significant change in salt level of soil in G II = 0.15 ± 0.02mmhos/cm, G V = 0.15 ± 0.02 mmhos/cm and G VI = 0.15 ± 0.02 mmhos/cm when compared with control group plants (0.12 ± 0.02 mmhos/cm). Soil moisture content is increased significantly in G IV = 71.1 ± 3.7 (%), G V = 85.5 ± 2.8 % and G VI = 73.5 ± 3.7 % when compared with control group 57.8 ± 5.0%. The organic carbon level was increased significantly in all the treated groups (G II = 1.1 ± 0.36 %, G III = 1.4 ± 0.36 %, G IV = 1.7 ± 0.37%, G V = 2.3 ± 0.17% and G VI = 2.2 ± 0.38%) when compared with control group (0.8 ± 0.07%). The N level showed significant change in G IV = 83.0 ± 3.4 ppm, G V = 77.1 ± 3.4 ppm and G VI = 71.6 ± 3.7 ppm when compared with control group (53.3 ± 3.8 ppm). The P level was increased significantly in G V = 53.5 ± 4.2 ppm when compared with control group (27.0 ± 2.6 ppm). In the present study, the proper and regular addition of soil organic manure repeated at every 2 weeks interval. So, the samples may have such variations in SOC level. The drastic increase in GV may be due to the organic amendments. The K level was decreased significantly in G II = 18.0 ± 3.1ppm and G V = 19.1 ± 2.2 ppm when compared with control group (27.3 ± 2.3 ppm). The Fe level increased dramatically in G V = 406.5 ± 7.7 ppm and G VI = 383.1 ± 12.7 ppm while the control was 250.1 ± 13.9 ppm. The Mg and Mn levels were significantly increased in G V 211.0 ± 11.7 ppm and 10.8 ± 3.0 ppm, respectively. The Mg and Mn levels of control group were 95.6 ± 3.8 ppm and 2.0 ± 0.7 ppm respectively. The Zn level was significantly increased in G V = 5.0 ± 1.3 ppm and G VI = 4.5 ± 0.8 ppm. The Ca levels were increased significantly in G IV = 45.0 ± 3.9 meq/100gm, G V = 43.6 ± 2.5 meq/100gm and G VI = 40.8 ± 3.9 meq/100gm while the control group showed decreased level of Ca = 19.5 ± 3.2 meq/100gm.

Keywords: Effective microorganism, Soil parameters, Organic substances (Vermin compost, poultry manure and molasses).

Introduction

Application of synthetic products in the soil leads to environmental pollution. The agricultural products produced with the application of chemical fertilizers and pesticides induce many health hazards to the consuming community (Jeyarathan, 1985). The misuse and excessive use of chemical fertilizers and pesticides have often adversely affected the environment and created many problems associated with food safety and quality and human and animal health (Igbedioh, 1991). The green revolution potentially increased the quantity of the food produced but severely decreased its nutritional quality (De Brito Alvarez, 1995 and

Weltzien, 1989) and also the soil fertility over the years. Today, the organic farming is much popular among the farmers. Combinations of various crop residues, animal manures, green manures and municipal wastes are applied to soil during the organic farming. The application of beneficial microorganisms to soil can also help to define the structure and establishment of natural ecosystems (Conway and Barbier, 1990). It is very difficult to decompose the wastes generated by anthropogenic activities. The proper waste disposal without environmental pollution can be solved by using microbial methods and technologies in coordination with agricultural practices (Reganold *et al.*, 1990; Parr and Hornick, 1992). Most biological activities are influenced by the microorganisms. To improve the plant growth and yield, the beneficial interactions between plants and microorganisms should be encouraged. The application of beneficial microorganisms to soil can help to define the structure and establishment of natural ecosystems. The microbial formulations for agricultural practices currently available in the market are not much utilized by the farming community. Moreover the scientific studies also are very less in agricultural microbial formulations. It is the right time to evaluate the microbial role in plant growth and yield in Indian soils.

Materials and Methods

Source Materials

The *Oryza sativa* (rice) and *Vigna mungo* L. (Black gram) seeds, Effective Microorganisms and NPK fertilizers.

Soil Preparation

The pot culture experiments were conducted in the pot culture yard of the department of microbiology, Annamalai University with 36 pots. Each pot was filled with 5kg of soil.

EM Preparation

Stock EM solution (Trade name / Commercial name: EM 1, Mapple India Pvt Ltd) was diluted in the ratio of 1:1000 (EM: Distilled Water). ie 0.1% solution. The EM was applied by spraying to the targeted plant.

Seed Preparation

The seeds were soaked for overnight and sown directly on the culture pots the next day.

Source of Molasses

Required quantity of Molasses was obtained from the animal feed manufacturer.

Experimental Design

The cement pots were divided into six groups (G-I, G-II, G-III, G-IV, G-V and G-VI) and each group has six pots. These pots were filled with 5 kg soil per pot. The experiment was carried out for 16 weeks during samba season in case of rice (*Oryza sativa*, Variety – BPT-5204) and 18 weeks during thaladi season in case of Black gram (*Vigna mungo* L. Variety – Local variety).

Soil Study

1. pH (MAES, 1998).

1. The pH of the soil was potentiometrically measured in the supernatant suspension of a 1:2.5 soil: liquid mixture. The liquid is either water (pH-H_2O) or a 1 *M* KCl solution (pH-KCl). The pH meter was calibrated. 5 g of soil sample was measured into a paper cup. 5 mL of distilled or deionized water was added to the sample. Stirred vigorously for 5 seconds and let stood for 10 minutes. The electrode was placed in the slurry, swirl carefully and read the pH immediately.

2. **Salt (Saturated Paste Method)** (MAES, 1998).

The Saturated Paste Method had long been the recommended method for assessing soil salinity in relation to plant growth. The advantage of this method was that the saturation moisture percentages were directly related to the field moisture range. Conductivity by this method relates directly to plant response for all soils without adjustment for texture as with the 1:1 method. The disadvantages of this method were more expense and time.

A saturated soil extract was made. Distilled water was added to the soil while stirring it with a spatula. After mixing, the sample was allowed to stand for at least 1 hour and then rechecked for saturation. The saturated paste was transferred to the filter funnel and vacuum was applied. The conductivity was measured on extract.

3. Moisture Content (%) (Van Reeuwijk, 2002)

Calculation of the results of soil analysis was done on basis of "oven-dry" soil. The moisture content of the sample should be determined shortly before soil analysis.
5 g of fine earth was transferred to a tared moisture tin and weighed ('A'gram). Dried overnight at 105 ^{o}C (lid removed). The tin was removed from oven, closed with lid, cooled in desiccator and weighed ('B' gram).

4. Organic Carbon (Walkey-Black, 1934).

The Walkley-Black (1934) procedure was followed. This involves a wet combustion of the organic matter with a mixture of potassium dichromate and sulphuric acid at about 125 EC. The residual dichromates were titrated against ferrous sulphate. To compensate for incomplete destruction an empirical correction factor of 1.3 were applied in the calculation of the result.

5 g fine earth was ground well to pass a 0.25 mm sieve. 1 g of this material was taken into a 500 ml wide-mouth Erlenmeyer flask. Added 10.00 ml dichromate solution. Two blanks were included (Erlenmeyer flasks without soil) to determine the Molarity of the ferrous sulphate solution. Then added 20 ml sulphuric acid with a measuring cylinder, swirl the flask and allowed to stand on a pad for 30 minutes (in fume cupboard). 250 ml water and 10 ml phosphoric acid were added with a measuring cylinder and allowed to cool. Added 1 ml indicator solution and titrated with ferrous sulphate solution while the mixtures were being stirred. The brown colour became purple or violet-blue. At the end-point the colour was changed into green.

5. Nitrogen

The micro-Kjeldahl procedures were followed. The samples were digested in sulphuric acid and hydrogen peroxide with selenium as catalyst and whereby organic nitrogen was converted to ammonium sulphate. The solutions were then made alkaline and ammonia was distilled. The evolved ammonia was trapped in boric acid and titrated with standard acid. The procedure determines all soil nitrogen (including adsorbed NH_{4+}) except that in nitrates. . 5 g fine earth was ground well to pass a 0.25 mm sieve. Weighed 1 g of this material into a digestion tube. In each batch, two blanks and a control sample were included. Added 2.5 ml digestion mixture. Then added successively 3 aliquots of 1 ml hydrogen peroxide. The tubes were placed on the heater and heated for about 1 hour at moderate temperature (200 EC). Turned up the temperature to approx. 330 EC (just below boiling temp.) and continued the heated until mixture was transparent (about two hours). The tubes were removed from heater, allowed to cool and added approx. 10 ml water with a wash bottle while swirling.

Distillation

Added 20 ml boric acid-indicator solution to a 250 ml beaker and the beaker was placed to stand beneath the condenser tip. Added 20 ml NaOH 38% to digestion tube and distilled for about 7 minutes during which approx. 75 ml distillate were produced. The beaker was removed from distiller, rinsed the condenser tip, and titrated the distillate with 0.01 *M* HCl until colour changes from green to pink.

6. Phosphorus (Olsen, 1954)

The samples were extracted with a sodium bicarbonate solution of pH 8.5. Phosphate in the extract was determined colorimetrically with the blue ammonium molybdate method with ascorbic acid as reducing agent. The high pH of the extracting solution renders the method suitable for calcareous, alkaline or neutral soils containing Ca-phosphates because the Ca concentrations in solutions were suppressed by precipitation of $CaCO_3$. As a result, the phosphate concentration in solution can increase. The procedure can, in principle, also be applied to acid soils as the relatively high pH of the carbonate buffer suppresses the solubility of Al and Fe and thus allows the phosphate concentration to increase.

Weighed 5 g fine earth (accuracy 0.01 g) into a 250 ml polythene shaking bottle. Two blanks and a control samples were included. Added 100 ml of the extracting solution. Shaked for 30 minutes. Then filtered through a hardened filter (e.g. Whatman 42). Pipetted into (short) test tubes 3 ml of the standard series, the blanks and the sample extracts. Slowly added 3 ml of the mixed reagent by pipette and swirl. Allowed the solutions to stand for at least 1 hour for the blue colour to develop its maximum.Absorbance was measured on spectrophotometer at 882 or 720 nm.

DTPA Extraction Zn, Fe and Mn (Lindsay and Norwell, 1978).

The DTPA (diethylenetriaminepentaacetic acid) test, a non-equilibrium extraction developed for Zn, Fe and Mn.

The soil samples were air dried and crushed to pass a 10-mesh stainless steel sieve. 10 g of soil was scooped without pressing the soil against the side of the container. Firmly tapped the handle of the scoop three times with an 8 inch spatula and levelled off the soil by passing the spatula over the scoop. Added the measured volume of soil to a 50 mL Erlenmeyer flask.

Added 20 mL of extracting solution (1:2 soil-to solution ratio) to each flask and shaked at 180 or more ppm for 2 hours.Filtered through Whattman No. 42 filter paper or similar grade paper. The samples were read on the Atomic Absorption, ICP or DCP spectrometer unit using appropriate standards and instrument settings. The results were reported as ppm Zn, Fe, Mn in the soil: ppm in soil = ppm in extract x 2.

0.1 M HCl Extraction for Zinc (Nelson *et al.*, 1959)

This procedure was based on the assumption that all or a portion of the soil Zn that will become available for plant uptake during a growing season was acid soluble. The quantity of acid-soluble Zn extracted serves as an index of Zn availability. The method was primarily for determining acid-extractable Zn in neutral and acid soils. It was not suitable for alkaline soils with excess calcium carbonate because of the neutralization of the acid in the extracting solution, unless some adjustment in the interpretation of results was made for the excess lime. Nelson *et al.,* (1959) used "titratable alkalinity" as a correction. They also recommended repeated extractions on highly calcareous soils until the pH of the suspension was below 2.0. On calcareous soils, the DTPA tests were recommended over the 0.1 M HC1 procedure.

The 0.1 M HCl tests had been used quite successfully throughout the North Central Region and were presently used in Michigan, Ohio and Wisconsin. The 0.1 M HC1 test was developed with little coordination of procedures among states. Thus, procedural differences exist among laboratories. Sorensen *et al.* (1971) showed that soil properties, soil-to-solution ratio and length of extraction all affected the amount of Zn extracted. Variations in the method used must be taken into account when comparing Zn extracted and the interpretation of the results.

Air dry soil samples and crush to pass a 10-mesh sieve. Scoop 5 g of soil without pressing the soil against the side of the container. Firmly tap the handle of the scoop three times with an 8 inch spatula and level off the soil by passing the spatula over the scoop, holded the spatula at a 90° angle. Added the measured volume of soil to a 50 mL Erlenmeyer flask, tapping the scoop on the transfer funnel or flask to remove all of the soil from the scoop.Added 20 mL of the extracting solution to each flask, place on the shaker and shake at 180 ppm or more for 30 minutes. Filter through washed Whattman No. 2 filter paper (or equivalent) into 30 mL polypropylene beakers. Carry a blank through the entire procedure with each run. Determine Zn in the extracts with the AA unit using appropriate instrument settings and Zn standards.
Report results as ppm Zn in the soil:

ppm in soil = **ppm in extract x** 4.

0.33 M H_3PO_4 Extractions for Manganese

The method presented here was the procedure developed in the Ohio Agricultural Research and Development Center Research-Extension Analytical Laboratory, Ohio State University, Wooster.

Air dry soil samples and crush to pass a 10-mesh sieve. Scoop 1 g of soil without pressing the soil against the side of the container. Firmly tap the scoop handle three times with an 8 inch spatula and level off the soil by passed the spatula over the scoop, holded the spatula at a 90° angle.

Added the measured volume of soil to a 50 mL Erlenmeyer flask, tapping the scoop on the transfer funnel or flask to removed all of the soil from the scoop.Added 10 mL of the extracting solution to each flask, place on the shaker and shake for 10 minutes at 180 ppm. Filter through Whattman No. 1 filter paper or similar grade filter paper. Carry a blank through the entire procedure with each run.

Determine Mn in the extracts with the AA unit used appropriate instrument settings and Mn standards. For precise Mn readings on samples testing less than 1 ppm Mn, the AA should be recalibrated on lower standards than shown for the worked standards. Report results as ppm Mn in the soil:

ppm Mn in soil = ppm in extract x 10.

8. Ca, Mg and K (MAES, 1998)

Ca and Mg are measured by AAS and K and Na by FES in diluted extracts. Interferences in the measurements were suppressed by La and Cs additives respectively. A major problem was the uncertainty about the concentration of the ions in the extract before analysis. Therefore, measurements will often have to be repeated using a higher or lower dilution of the extract.

The 1000 mg/l Ca standard solution was diluted to 250 mg/l: pipetted 50 ml into a 200 ml volumetric flask, made to volume with water. Diluted the 1000 mg/l Mg standard solution to 25 mg/l: pipetted 25 ml into a 11 volumetric flask, made to volume with water. Of the 250 mg/l Ca solution and the 25 mg/l Mg solution pipetted a series of 0-5-10-15-20-25 ml into 250 ml volumetric flasks respectively. To each flask added 125 ml of La suppressant solution. The standard series were then 0-5-10-15-20-25 mg/l Ca and 0-0.5-1.0-1.5-2.0-2.5 mg/l Mg.

Results and Discussion

The soil pH affects the availability of plant nutrients (optimal pH is between 5.5-7.5). Low pH soils (<6.0) results in an increase in aluminum which is toxic to plants. Soil Organic Matter (SOM) stabilizes soil structure, improves water holding capacity and lowers bulk density; dark color may alter thermal properties. Microorganisms are the driving force for nutrient release to plants (Washington State University, 2004). In the present study, the soil organic manure was added at higher dose and also repeated at every 2 weeks interval. Similar results were reported by Maftoun and Moshiri (2008) and Ibrahim *et al.* (2010).

The essential micronutrients for plants required are boron, chlorine, sodium, copper, iron, manganese, zinc, vanadium and molybdenum. The essential macronutrients required for the plants are carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur, potassium, calcium and magnesium.

The previous investigator, Myint, (1991) proved that organic amendments and EM treatment may increase the favourable environment for the nitrogen fixing bacteria. Nitrogen may be obtained by some plants directly from atmosphere through nitrogen fixing bacteria. The nitrogen content in the soil has increased in the present study. There is an increase in both P, K levels in EM treated soil samples. Similar results were reported by Maftoun and Moshiri (2008) and Ibrahim *et al.* (2010).

Calcium deficiency in soil is due to calcium uptake by plants and leaching by carbonic acid in acidic soils and competitions with high levels of sodium, potassium and magnesium in alkaline soils. Unlike in the case of K^+, sulphate ions are not bound by ion-exchange binding and it would be available for assimilation by plant roots. Management of N and P cannot be accomplished without the cognizance of the transformations of the nutrients that occur in nature, represented conveniently by Nitrogen and Phosphorous cycles.

Manganese takes part in a number of important physiological and biological processes in the lower and higher plant organisms in the nitrogen metabolism, photosynthesis, breathing and maintaining the necessary oxidation-reduction conditions in the cell (Nason *et al.,* 1952; Udintseva *et al*., 1981; Sidorovich *et al.,* 1987). Manganese insufficiency leads to a considerable accumulation of nitrates, disturbs in the protein synthesis in plants and in some plants, illness (Nicholas, 1961; Heintze, 1966; Bergmann *et al.,* 1976), causes a decrease in Ca and Mg contents (Shkolnik and Smirnov, 1974).

In the present study, the soil parameters such as pH, salt content, soil moisture, Organic carbon, N, P, K, Fe, Mg, Mn, Zn and Ca were estimated in control and other treated groups.

Organic amendments have been shown to be useful in improving the soil properties of disturbed areas (Land Resources Network Ltd. 1993). Anaerobically treated sewage sludge (bio solids) has shown to increase vegetation production and promote soil formation with the hope of establishing a self-sustaining site (Seaker and Sopper 1988). Studies have indicated that the

organic amendments increase water holding capacity of soil. Additionally, vermicompost contain enzymes like amylase, lipase, cellulase and chitinase, which continue to break down organic matter in the soil (to release the nutrients and make it available to the plant roots) even after they have been excreted.

Phosphorus is also an important plant nutrient along with N, Phosphorus has significant effect on plant growth (Hargrove *et al.*, 1984). Only 10 to 20% of fertilizer phosphorus can be utilized by the plants, while the major part is deposited in the soil as Ca^{-}, Fe^{-}, or Al^{-} phosphates. Roemer and Scheffer, (1953) and Khaliq *et al.*, (2006) have reported that EM application in combination with organic matter or mineral NPK significantly increases cotton yield. Hussain *et al.* (1999) observed that the EM application with farmyard manure or mineral NPK increases the wheat and rice grains.

Cations such as Ca^{2+}, Mg^{2+} and K^{+} are produced during decomposition (Brady, 1990). Availability of phosphorus was sometimes much greater (Reganold, 1990). Martens and Frankenberger (1992) and Joost *et al.* (1987) have proved that organic amendments lead to an increase in soil carbon which in turn increased aggregate formation and improved soil structure.). Cultivation of high yielding crop varieties and multiple cropping is depleting the fertility of soils at a rapid pace as the soils, which were once well supplied with available nutrients, are now gradually becoming deficient (Zia *et al*., 1994).

Table 1 Estimation of soil physico-chemical parameters on the initial stage (0 day) of experiment

SOIL PARAMETERS	ESTIMATED
pH	4.7 ± 0.3
Salt (mmhos/cm)	0.12 ± 0.03
Soil Moisture (%)	55.5 ± 4.7
Organic carbon (%)	0.62 ± 0.16
N (ppm)	44.1 ± 6.0
P (ppm)	21.0 ± 4.0
K (ppm)	32.1 ± 3.4
Fe (ppm)	217.0 ± 15.4
Mg(ppm)	85.5 ± 3.6
Mn (ppm)	1.3 ± 0.5
Zn (ppm)	1.1 ± 2.7
Ca (meq/100 gm)	11.8 ± 2.7

Table2 Estimation of soil physico-chemical parameters on the 1st week of experiment (Black Gram)

Soil Parameters	pH	Salt (mmhos/cm)	Soil Moisture (%)	Organic Carbon (%)	N (ppm)	P (ppm)
G I (control)	5.6 ± 0.1^{d}	0.036 ± 0.01^{d}	49.6 ± 3.0^{d}	0.61 ± 0.1^{d}	40.6 ± 2.8^{c}	19.6 ± 2.5^{b}
G II (chemical fertilizer)	6.4 ± 0.2^{bc}	0.056 ± 0.01^{abc}	53.5 ± 3.6^{c}	0.76 ± 0.2^{c}	50.1 ± 2.7^{a}	25.6 ± 1.5^{a}
G III (EM 0.1 % alone)	6.2 ± 0.2^{c}	0.043 ± 0.01^{cd}	53.6 ± 2.7^{c}	0.91 ± 0.1^{bc}	44.6 ± 3.2^{b}	19.6 ± 3.1^{b}
G IV (EM 0.1% + poultry manure)	6.6 ± 0.2^{ab}	0.053 ± 0.01^{bc}	57.5 ± 2.3^{b}	0.80 ± 0.1^{c}	51.5 ± 3.7^{a}	21.1 ± 2.6^{b}
G V (EM 0. 1% + vermin compost)	6.8 ± 0.3^{a}	0.068 ± 0.01^{a}	65.1 ± 2.9^{a}	1.20 ± 0.1^{a}	48.6 ± 3.0^{a}	26.0 ± 2.5^{a}
G VI (EM 0. 1% + molasses)	6.4 ± 0.2^{bc}	0.060 ± 0.01^{ab}	63.6 ± 3.1^{a}	1.01 ± 0.2^{ab}	44.0 ± 3.2^{bc}	26.5 ± 2.0^{a}

Values are mean ± Standard deviation of six individual observations.

Values that are not sharing a common superscript letter in the same column differ significantly at p<0.05

Table 2 Estimation of soil physico-chemical parameters on the 1st week of experiment (Rice)

Soil Parameters	pH	Salt (mmhos/cm)	Soil Moisture (%)	Organic Carbon (%)	N (ppm)	P (ppm)
G I (control)	5.0 ± 0.2^{c}	0.038 ± 0.01	45.6 ± 5.6^{d}	0.62 ± 0.04^{d}	39.3 ± 3.5	19.1 ± 2.3^{d}
G II (chemical fertilizer)	6.2 ± 0.4^{a}	0.035 ± 0.01	49.1 ± 3.3^{cd}	0.67 ± 0.06^{bc}	42.0 ± 3.6	20.1 ± 3.2^{d}
G III (EM 0.1% alone)	5.8 ± 0.2^{ab}	0.036 ± 0.008	53.8 ± 2.9^{b}	0.78 ± 0.03^{a}	44.3 ± 3.5	20.5 ± 3.1^{d}
G IV (EM 0.1% + poultry manure)	5.5 ± 0.3^{b}	0.045 ± 0.01	52.5 ± 2.8^{bc}	0.78 ± 0.06^{a}	45.3 ± 5.0	22.8 ± 1.9^{bc}
G V (EM 0.1% + vermin compost)	5.9 ± 0.4^{ab}	0.048 ± 0.01	59.8 ± 3.0^{a}	0.72 ± 0.06^{ab}	43.0 ± 2.7	25.3 ± 3.6^{a}
G VI (EM 0.1% + molasses)	5.7 ± 0.2^{b}	0.041 ± 0.01	56.1 ± 2.6^{ab}	0.61 ± 0.03^{b}	42.6 ± 4.3	24.8 ± 3.2^{ab}

Values are mean ± Standard deviation of six individual observations.
Values that are not sharing a common superscript letter in the same column differ significantly at $p<0.05$

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