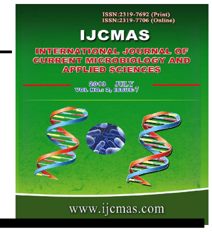

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Original Research Article

Dynamics of Microorganisms during Vermistabilization of organic substrates and enhances performance of Plant Growth Promoting Rhizobacteria on Black Gram

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Keywords

Paper mill sludge; Leaf litter; Press mud *Bacillus, Pseudomonas, Rhizobium; Azotobacter;* PGPR; *Vigna mungo* L. Hepper; Vermi-stabilization.

A B S T R A C T

The Quality of the, Paper mill sludge, Leaf litter and Press mud before and after 60 days of vermicomposting was investigated on Physico-chemical and microbiological methods. Vermistabilization of Paper mill sludge, Leaf litter and Press mud sludge by using Epigeic species of *Eisenia fetida* in three different vermibed substrates by using cow dung as the supporting material. The bulk density on 0th day in Paper mill sludge, Leaf litter and Press mud with cow dung incorporated sludge sample were higher than the control and compost obtained on 60th. The chemical analysis of N, P and K also showed increasing trend on 60th day composting than the initial 0th day and control sample in all the three organic substrates. The total bacterial count on the 60th day of composting in three vermibed substrates with cow dung incorporated Paper mill sludge; Leaf litter and Press mud were tremendously higher than the 0th day and control sample under investigation. The principal bacterial genera encountered in Paper mill sludge, Leaf litter and Press mud on the initial 0th day and 60th day comprised of 4 bacterial sps includes *Bacillus, Pseudomonas, Rhizobium, and Azotobacter*. Few PGPR tolerant to multiple heavy metals and exhibiting a couple of resistant activities mainly *Pseudomonas sps* were resistant to 400 μgml^{-1} of Hg. It was apparent that cultures of PGPR isolated from vermicompost were tolerant to elevated levels heavy metals. The plant growth parameters Shoot length, root length, number of roots hairs, Number of root nodules and number of branches and leaves were determined and also yield of Black gram (*Vigna mungo L. Hepper*) was determined for selecting the right organic input and plant growth promoting rhizobacteria.

Introduction

Vermiculture biotechnology is a dynamic process brought about by the earthworms with the aid of mixed microbial population with specific function. The worms maintain aerobic conditions in the mixture ingest solids, convert portion of the organic into worm biomass and to respiration products and they expel the remaining partially stabilized matter as discrete material (Castings).

Earthworms improve the aeration of soil by their burrowing activity. They also influence the porosity of the soil. Earthworms are observed to improve the nature of soil by breaking up organic matter and increasing the amount of microorganism made available to plants. The stability of biopores increases with pore diameter and with more nearly vertical orientation of pores.

Many of the microorganisms are transported by earthworms are those involved in the decomposition of organic materials. Earthworms also consume and transport other beneficial microbial group such as the plant associated mycorrhiza and other root symbiont, biocontrol agents and microbial antogonistics of plants pathogens. Dispersal of nitrogen-fixing bacteria that form mutalistic association with plant roots can also be enhanced by earthworm it also enhances the translocation of Bradyrhizobium japonium to greater soil depths (Madsen and Alexander, 1982). Earthworm while ingest organic waste and soil, consume heavy metals through their intestine as well as through their skin, therefore concentrating heavy metals in their body. The present study deals with vermiculturing and earthworm interaction microflora, physical, chemical parameters of vermicompost material and influence of plant growth.

An important waste that emerges out from a sugarcane industry in the form of filter mud or the press mud, there is a major disposal problem for the filter mud as thought it is fairly rich in organic nutrients, it finds little use as agricultural fertilizer. The primary reason for this is the insoluble and imbalanced nature of the nutrient content in it.

Filter mud (or) press mud is one of the major by products from sugar factory, constitutes 4-5 % of cane weight and 35 kg per tonnes of cane. According to recent reports in TamilNadu more than 4 Lakh tonnes of sugar cane is crushed by sugar factories leading to the production of 100 tonnes of press mud per day. Its importance lies in its fertilizer value as it contains 1.63%N, 2.52 %, P and 0.55% K on dry weight basis (Patil *et al.*, 1983). But wax content (8.15%) of press mud affects the soil property by direct application (Thopate *et al*., 1997) and reduces its nutritional value for plant and bacterial growth, it is high rate of direct application (up to 100 tonnes /acre) leads to soil sickness and ground water pollution (Bhawalkar and Bhawalkar, 1992), its organic fraction decomposition requires 5-7 months under dry natural conditions and it occupies large area of land and create fly menace at dumping areas. Hence it is not advisable to apply press mud directly to the land or crop. Due to the awareness of deleterious effects of chemical fertilizers on soil and environment, in recent years there is an increasing emphasis to develop technology to produce organic manures from agro-industrial and other organic wastes. The microbes and earthworms work as scavengers for agro-industrial wastes. Epigeic earthworms have the potential to convent organic wastes into valuable vermicompost for plant growth and vermiprotein for use as animal feed in poultry and aquaculture.

Plant Growth Promoting Bacteria

Plant growth promoting bacteria are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. In last few decades a large array of bacteria including species of *Pseudomonas*, *Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Rhizobium, Flavobacteriaum, Bacillus* and *Serratia* have reported to enhance plant growth (Kloepper *et al*., 1989; Okon and Labandera-Gonzalez, 1994; Glick, 1995). The direct promotion by plant growth promoting bacteria entails either providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when plant growth promoting prevent deleterious effects of one or more phytopathogenic microorganisms.

Materials and Methods

Collection of Materials

The raw materials, Paper mill sludge (PMS) was collected from Wall's Paper Packaging Ltd., Chennai and transported to the laboratory.

Leaf litters of Teak tree (*Tectona grandis*) (LLT) were collected from Kancheepuram, Tamil Nadu and subjected to initial decomposition in rectangular draining cement tanks of 75cm×60cm×45cm size by sprinkling water, regular mixing and turning of the substrates for 15 days. The cowdung (CD) was collected from nearby cattle sheds in fresh form and allowed to stabilize for one week and used for the study. The stabilization of cowdung was done to make it acceptable by the worms.

The Pressmud (PM) was collected from The Sugar Mills Ltd., located in Thiruvannamalai District, TamilNadu.
The earthworm, *Eisenia fetida* for the study, originally collected from culture bank of the Department of Biology, Gandhigram Rural University, Tamilnadu was mass multiplied in cowdung and used for the study.

Vermistabilization of Paper mill sludge, Leaf litter, and Press mud

Vermicultural Tub Treatment Process

The Paper mill sludge, Leaf litter, and Press mud was mixed with standard bedding material and introduced into standard plastic tubs occupying about 3kg of the materials. The each pre-decomposed substrates were mixed with cow-dung in 1:1 ratio on dry weight basis in separate plastic trays of 45cm x 35cm x 15cm size with six replicates for a period of 2 months. Vermicomposting was carried out in an environmentally controlled experimental chamber at a temperature of 27±1°C and the vermibeds were maintained to contain a moisture level of (65-75%) by sprinkling water over the surface daily. Each tray containing vermibed substrate was introduced with 60 adult Epigeic species of Earthworm *Eisenia fetida* were inoculated manually in selected bedding materials in plastic tubs. The culture tubs were placed Indoor in the laboratory. The bedding material upper surface was covered with wire mesh to avoid entry of predators.

Sample collection and processing

The samples for analysis were taken out from the vermicomposting plastic tub at the start of the experiment, then after 15 days, 30 days, 45 days and lastly, after 60 days. That is, a time interval of fifteen days was taken as the standard for taking out the samples.

Nutrient analysis

Samples from vermibed substrates (LLM + CD) and vermicompost were dried, ground and sieved. The pH and electrical conductivity were determined by the method of Jackson (1973) in distilled water solution. Moisture was determined by heating a sample of known weight (5 g) placed in a moisture bottle, till constant weight in an electric oven at 70°C, and deducting the loss in weight. The determination of organic carbon was carried out as per the procedure of Walkley and Black (1934); total nitrogen, phosphorus, potassium, sodium, calcium, magnesium, sulphur, copper, zinc, iron and manganese were determined according to standard methods as described by Jackson (1973) and Tandon (1993); C/N ratio was calculated by dividing the percentage of carbon estimated for the sample by the percentage of nitrogen estimated for the same sample; the same procedure was used to estimate C/P ratio. The percent increase/decrease of various physico-chemical (nutrient) parameters over the worm-unworked substrates was calculated [(A – B/A) × 100; where A = values in the worm-worked substrate, B = values in the worm-unworked substrate].

Microbiological analysis

One gram of each sample was transferred to test tubes containing sterilised water and serial dilutions were made. This was used as inoculum and 1.0 ml was transferred to Petri plates containing Nutrient agar media, Rose Bengal agar and Kenknight's media respectively for the enumeration of bacteria, fungi and actinomycetes in triplicate and incubated for 24 hrs, 72 hrs and one week respectively. At intervals, colony-forming units (CFU) were determined by making total counts on the incubated plates using a colony counter.

Isolation of Plant Growth Promoting Rhizobacteria

The Paper mill sludge, Leaf litter, and Press mud vermicompost was collected and subjected for the isolation of Plant growth promoting rhizobacteria. All the bacteria were isolated on their respective media; Rhizobium was isolated on yeast extract mannitol agar, Azotobacter on Jensen's medium, Pseudomonas on Cetrimide medium and Bacillus on nutrient agar. Bacterial cultures were maintained on the respective slants.

Biochemical characterization of rhizobacteria

Selected isolates of *Bacillus, Pseudomonas, Azotobacter* and *Rhizobium* were biochemically characterized by Gram's reaction, carbohydrate fermentation, oxidase test, O-F test, H_2S production, IMViC tests, NO_2 reduction, starch and gelatin hydrolysis as per the standard methods (Cappuccino and Sherman, 1992).

Characterization of rhizobacteria for PGPR

Production of Indole acetic acid

Indole acetic acid (IAA) production was detected as described by Brick *et al.*, (1991). Bacterial cultures were grown for 72 h (Azotobacter and Rhizobium) and 48 h (Pseudomonas and Bacillus) on their respective media at 36±2 °C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink colour indicates IAA production.

Production of ammonia

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48–72 h at 36±2 °C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

Production of HCN and Catalase

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lorck (1948). Briefly, nutrient broth was amended with 4.4 g glycine/l and bacteria were streaked on modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 36±2 °C for 4 days. Development of orange to red colour indicated HCN production. Bacterial cultures were grown in a nutrient agar medium for 18-24 h at 36±2 °C. The cultures were mixed with appropriate amount of H_2O_2 on a glass slide to observe the evolution of oxygen.

Siderophore production

Siderophore production was detected by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye chrom azole S (CAS). Orange halos around the colonies on blue were indicative for siderophore production.

Heavy metal tolerance

The selected bacterial strains were tested for their resistance to heavy metals by agar dilution method (Cervantes *et al.,* 1986). Freshly prepared agar plates were amended with various soluble heavy metal salts namely Hg, Cu, Pb, Zn and Cr, at various concentrations ranging from 100 to 400 μg ml^{-1} were inoculated with overnight grown cultures. Heavy metal tolerance was determined by the appearance of bacterial growth after incubating the plates at room temperature for 24-48h.

Results and Discussion

Physico-Chemical Characteristics of vermicomposting

Table 1 shows the Physico - chemical characteristic of paper mill sludge during 60 days at composting using *Eisenia fetida* with control as comparative sample. The pH of the sample gradually increased from 6.5 to 7.8. The bulk density was reduced from 0.66 to 0.50. The pore space, moisture content and electrical conductivity (EC) showed an increasing trend from 62.05 to 73.52%, from 56.4 to 62.3 and from 1.16 to 1.32 respectively. The chemical analysis of the paper mill sludge showed well marked increase in the levels of N, P, K, Ca, Mg, Fe, and Zn.

Table 1 indicates the physico - chemical characteristics of Leaf litter of Teak subjected to vermicomposting using *Eisenia fetida* with a comparative control sample. The pH increased from 7.02 to 7.42. The bulk density reduced from 0.72 to 0.60.

Table.1 Physico - chemical characteristic of Paper mil sludge, Leaf litter (Teak) and Press mud during the composting period using the *Eisenia fetida* on comparison with control sample

S.No	Parameter	Paper mil sludge			Leaf litter (Teak)			Press mud		
		Initial	Final	Control	Initial	Final	Control	Initial	Final	Control
1	pH	6.53	7.80	6.80	7.02	7.32	7.10	7.40	7.65	7.12
2	Electrical conductivity(ds/m)	1.16	1.32	1.10	1.22	1.40	1.20	1.28	1.42	1.20
3	Bulk density	0.66	0.50	0.60	0.72	0.60	0.70	0.76	0.58	0.74
4	Pore space (%)	62.05	73.52	63.20	68.13	79.22	67.20	60.10	81.24	60.20
5	Moisture content (%)	56.4	62.3	56.8	58.4	68.3	58.8	57.4	68.20	56.8
6	Organic Carbon	28.48	25.6	27.89	30.48	24.6	30.89	34.48	25.6	32.89
7	Nitrogen %	1.20	1.96	1.16	1.10	1.88	1.12	1.18	1.92	1.12
8	Phosphorus %	3.25	4.39	3.11	3.05	3.98	3.08	3.18	3.98	3.08
9	Potassium %	1.40	1.80	1.10	1.28	1.60	1.20	1.38	1.72	1.30
10	Calcium (%)	2.30	3.13	2.15	2.10	3.02	2.12	2.58	3.62	2.18
11	Magnesium (%)	0.46	0.67	0.44	0.34	0.60	0.32	0.38	0.72	0.36
12	Iron(ppm)	184.0	199.0	180.0	168.0	189.0	170.0	178.0	192.0	174.0
13	Copper(ppm)	17.0	19.0	12.0	14.0	18.0	13.0	16.0	21.0	14.0
14	Zinc(ppm)	61.0	63.0	56.0	58.0	60.0	56.0	60.0	64.0	58.0
15	C/N ratio	23.73	13.06	24.03	27.70	13.08	27.58	29.22	13.33	27.58
16	C/P ratio	8.76	5.83	9.00	9.99	6.18	10.02	10.84	6.43	10.02

The pore space, moisture content, and Electrical conductivity showed an increasing trend from 68.13 to 79.22 and from 58.40 to 68.30 and 1.22 to 1.40. The chemical analysis of the leaf litter showed well marked increase in the levels of N, P, K, Ca, Mg, Fe, and Zn.

Table 1 shows the physico - chemical characteristics of press mud subjected to vermicomposting using *Eisenia fetida* with a comparative control sample. The pH increased from 7.40 to 7.65. The bulk density reduced from 0.76 to 0.58. The pore space, moisture content, and Electrical conductivity showed an increasing trend from 60.10 to 81.24 and from 57.40 to 68.20 and 1.28 to 1.42. The chemical analysis of the press mud showed well marked increase in the levels of N, P, K, Ca, Mg, Fe, and Zn. Table 4 shows the total microbial population count during the process of vermicomposting with reference to the control.

The microbial load showed on 0^{th}, 15^{th}, 30^{th}, 45^{th}, and 60^{th} day. The vermicompost of *E. fetida* worked compost showed maximum population of bacteria, fungi and actinomycetes whereas in control decreased number of microbial population (Figures .1,2 & 3). Total microbial population count in the Earthworm gut on the initial day i.e 0^{th} day and final day i.e 60^{th} day. The earthworm gut load was also found to be in an increasing order. Table 2, 3 indicates Morphological and characterization for Plant Growth Promoting Rhizobacteria. Table 4 shows the tolerance of metals Heavy Metals (Hg,Cu, Pb, Zn ,Cr) tolerance among PGPR isolated from Vermicompost (heavy metals range 100to 400μg ml-1).

Physiological parameters analysis

The results on the effect of inoculation of Plant growth promoting Rhizobacteria along with an uninoculated control in black gram (*Vigna mungo L. Hepper*) which were treated with organic inputs like vermicompost which were Vermistabilised of paper mill sludge, leaf litter and press mud have been presented the data in Table 5 and 6.

Root Length

All the treatments were significantly increasing their efficiency on the growth of roots. Here, this experiment showed the best performance of T3 (24.9 cm) followed by T1 (22.8cm) and T2 (22.5) than T0 as control having 22 cm in 90^{th} day of plant growth.

Shoot length

This simple experiment was conducted to find out the effect of plant growth promoting rhizobacteria which increased the shoot length over uninoculated control. In this experiment, the treatment T3 (41.8 cm) showed the best performance than the T1 (41.2 cm) followed by T2 (39) and treatment T0 as a control (32cm).

Number of Roots hairs

Plant growth promoting Rhizobacteria increased the number of root hairs growth over the control. In this experiment, the T3 (79) and T1 (79), showed the good performance than the T2 (76) and T1 as a control (70).

Table.2 Morphological and biochemical characterization for Plant Growth Promoting Rhizobacteria

Morphological and biochemical characterization	*Bacillus* sp	*Pseudomonas* sp	*Azotobacter* sp	*Rhizobium* sp
Grams reaction	G +ve	G -ve	G -ve	G -ve
Shape	rods	rods	rods	rods
Pigments	-	+	+	+
Lactose	+	-	+	+
Dextrose	+	+	+	-
Sucrose	+	+	+	-
Mannitol	+	-	+	+
Oxidase	-	+	+	+
OF test	-	+	+	-
H2S production	-	+	-	+
Indole	-	-	+	+
Methyl red	-	-	+	+
Vogues Proskauer	+	-	+	+
Citrate utilization	+	+	+	-
Nitrate reduction	+	+	+	+
Starch hydrolysis	+	+	+	+
Gelatin hydrolysis	+	-	-	-

Figure.1 Total bacterial count on paper mill sludge, leaf litter and press mud

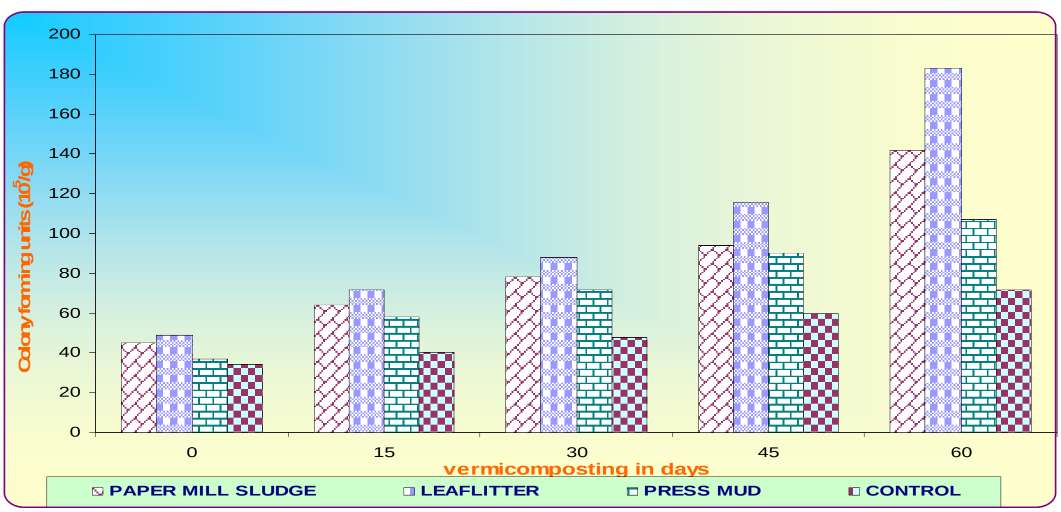

Figure.2 Total fungal count on paper mill sludge, leaf litter and press mud

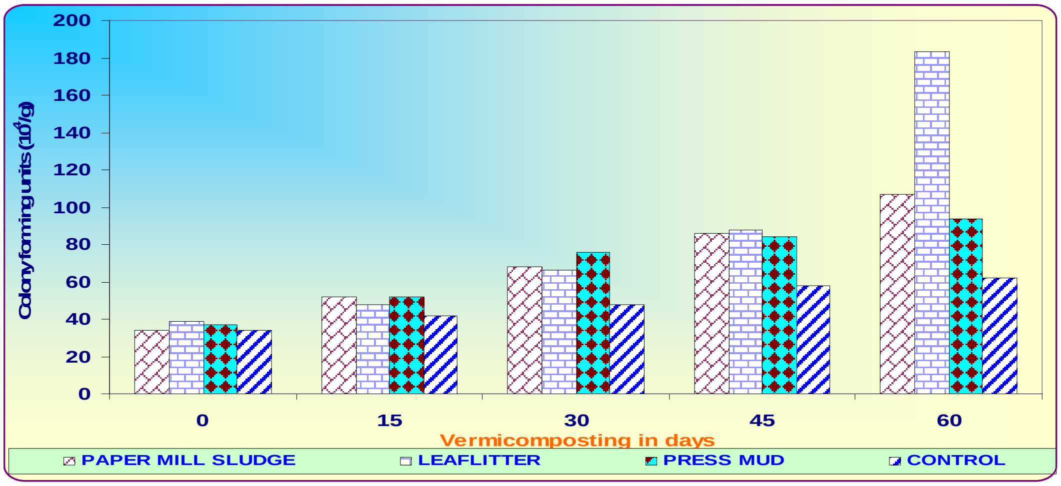

Chart.3 Total actinomycetes on paper mill sludge, leaf litter and press mud

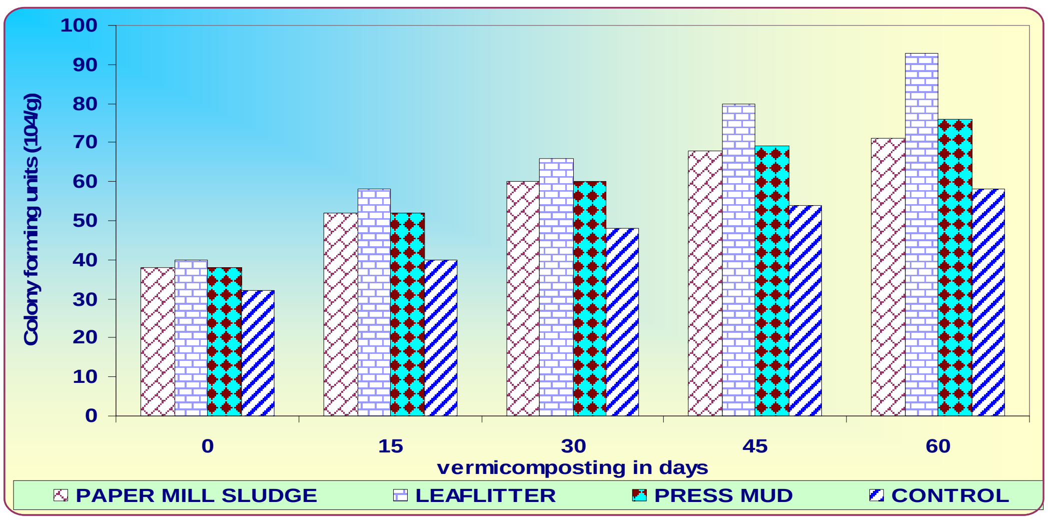

Table.3. PGPR Characterization

PGPR Characterization	***Bacillus***	***Pseudomonas***	***Azotobacter***	***Rhizobium***
IAA Production	+	+	+	+
Ammonia Production	+	+	-	+
Catalase Production	+	+	+	+
Siderophore Production	-	+	-	-

Table.4 Heavy Metals tolerance among PGPR isolated from Vermicompost

S.No	Bacterial genus	Heavy Metals (μg ml⁻¹)																			
		Hg				Cu				Pb				Zn				Cr			
		100	200	300	400	100	200	300	400	100	200	300	400	100	200	300	400	100	200	300	400
1	*Bacillus sps*	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	-	+	+	-	-
2	*Pseudomonas sps*	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-
3	*Azotobacter sps*	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	-
4	*Rhizobium sps*	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-

'+' indicates Positive; '-' indicates Negative

Number of Root nodules

All the treatments were significantly increasing their efficiency on the root nodules of black gram (*Vigna mungo L. Hepper*) plant. Here, this experiment showed the best performance of T1, T2 and T3 than T1 as control.

Number of branches and leaves

Plant growth promoting Rhizobacteria increased the number of branches and leaves on plant growth over the control. . Here, this experiment showed the best performance of T1, T2 and T3 than T1 as control.

Effect of vermicompost on the yield of Black gram (*Vigna mungo L. Hepper*)

The number of main parameters on the yield of black gram was significantly influenced by application of different treatments (Table.6). The number of flower per plant ranged form 2 to 12. The highest number (24) of pods produced per plant by the application of vermicompost T3 (Press mud) on the 90th day. The plant having no vermicompost produced the minimum number (16) of pods per plants on the 90th day. The highest number (8) of seeds present per pod of plant by the application of vermicompost T3 than T0 control. The fresh weight and dry weight of 100 seeds were higher in the treatments T1 to T3 than in T0 (control) (Table. 6).

Vermiculture has received considerable attention in recent years internationally for its immense potential in recycling biodegradable waste in popularizing organic farming. Certain species have been identified to be very useful in degradation of organic wastes, viz., *Eisenia fetida*, *Dendrobaena venata* and *Lumbricus rubellus* from temperate areas and *Eudrillus eugeniae* and *Perionyx excavatus* from the tropics (Edwards, 1998). The survival, growth, mortality and reproduction of these species have been studied thoroughly in the laboratory, using a wide range of organic wastes (Edwards, 1998). However, population dynamics, productivity and energy flow in earthworms cannot be fully understood unless the lifecycle of earthworm is known. Studies on the life cycle of earthworms are also necessary for effective vermiculturing (Bhattacharjee and Chaudhuri, 2002).

India's agro-industrial sector contributes huge potential resource of plant nutrients in the form of wastes, which is either thrown away or buried or burnt causing environmental pollution. Press mud (filter cake), a major-by product of sugarcane processing is produced 12 million tones annually. It is a spongy and dark brown material, contains sugar, fibre, cane wax, inorganic salts, soil particles and rich micro and macro nutrients, enzymes and microbes (Yadav, 1995; Parthasarathi and Ranganathan, 1999). Because of its bad smell, costs involved in transports and fear that its application might lead to crust formation, pH variation, pollution problems, farmers are reluctant to apply it to their land. Conventional composting of this press mud takes about six months, does not remove the bad smell completely, has less nutritive value and is compacted. We had already vermicomposted this pressmud by using *Lampito mauritii*, *Eudrilus eugeniae*, *Perionyx excavatus and Eisenia fetida* into an eco-friendly organic fertilizer/soil amendment and had found that this press mud vermicompost shows rich enzymatic microbial activities and nutrient contents in available form facilitating the easy uptake by the plants

(Parthasarathi and Ranganathan, 1999; 2001).

An important observation was showed in the physico-chemical characteristics of Pressmud+CD (1:1) substrates subjected to vermicomposting with *Eisenia fetida* for 60 days showed changes in nutrient contents. (Table 1). The physico-chemical characteristics such as E.C., NPK, Ca, Mg, Fe and Cu in Paper mill sludge, leaf litter and press mud (1:1) vermicompost showed increase over worm unworked substrates. Whereas pH, OC, Na, Mn, S, Zn, C/P and C/N in the vermicompost of Paper mill sludge, leaf litter and press mud (1:1) showed decrease over worm unworked substrates. The NPK contents in worm worked substrates were higher than worm unworked substrates. The C/N ratio of worm worked Press mud + CD showed decrease over worm unworked substrates and percent decrease was observed. The physico-chemical characteristics of different vermicomposts Paper mill sludge, leaf litter and press mud (1:1)) were higher than the values recorded on 0th day of experiment i.e., the initial values.

The parallel results have also been reported by Ramalingam and Ranganathan, (2001); Prakash, *et al*., (2008); Prakash and Karmegam (2010), that the N, P, K content showed an increasing trend, Nitrogen was excreted nearly as ammonia in the urine released in the gut it is mixed with cast. In earthworms, calciferous glands discharge amorphous calcium carbonate particles coated with mucus (Senapathi, 1980).

Leaf litter is the major pollutant of the water resource. Disposal of these by physical and chemical process is highly expensive (Gohil, 1985). Therefore biological treatment methods have received much attention and considered as efficient, low cost treatment for Leaf litter. The worms along with organic manure's can be utilized as an alternative to costly inorganic fertilizers (Senappathi, 1980).

The findings of the present study thus confirm the concept that the earthworm gut might be a specialized microhabitat of enhanced microbial activities in soils (Karsten and Drake 1995). Lee (1985) suggested that the increase in microbial population in the gut of earthworms might be due to the utilization of additional nutrients available as a result of enzymatic breakdown of ingested materials. In the present study, the high nitrogen and moisture contents recorded in the earthworms' guts may also have contributed to this by stimulating microbial activity.

Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals. This study observed few rhizobacteria tolerant to multiple heavy metals and exhibiting a couple of PGP activities Pseudomonas sps were resistant to 400 μgml-1. It was also apparent that more cultures of PGPR isolated from vermicompost were tolerant to elevated levels heavy metals. The selection of microorganisms both metal tolerant and efficient in producing PGP compounds can be useful to speed up the recolonization of the plant rhizosphere in polluted soils. Heavy metals, at higher concentration, are toxic to cells and may cause cell death by interacting with nucleic acids and enzymes active site. Azotobacter spp, when inoculated into heavy metal contaminated soil, inhibited N2-fixation. Rother and coworkers (1983) reported a reduction in nodule and plant size and in nitrogenase activity of clover at sites heavily contaminated with Cd and Pb. Chromium-resistant pseudomonads, isolated from paint industry effluents, were able to stimulate seed germination and growth of *Triticum aestivum* in the presence of potassium dichromate.

The growth of the Black gram (*Vigna mungo* L. Hepper) was enhanced due to the application of plant growth promoting rhizobacteria in combination with Vermicompost. The effect of vermicompost and plant growth promoting rhizobacteria in various Vermistabilised organic substrates is very scanty. Hence the present study has been carried out. In the present experiment, the results revealed a beneficial effect of selected organic substrates and Plant growth promoting Rhizobacteria on the black gram (*Vigna mungo*). Similar observations were made by Ramanathan (1986) clearly indicated that to obtain healthy seedlings.

The germination and seedling biomass of Green gram (*Vigna radiata*) was increased by organic inputs. The effect of organic manures integrated with NPK chemical and yield parameters of press mud with vermicompost recorded higher yield. The effect of organics and plant growth promoting rhizobacteria reflected in shoot, root length and root numbers in the investigation made in this study experiment. The highest shoot, root length, root hairs number, number of root nodules, number of branches and leaves were obtained in T3 (Press mud) in this experiment (Table.5 and 6).

The field trial has shown that vermicompost increased the plant growth when compared the control (T0) i.e., red soil (Table.5 and 6). All these observation showed that vermicompost can form a increased rate of plant growth, therby reducing the cost of crop production, improving soil fertility and saving the environment form the ill effects of chemical compounds. The nutrient uptake (NPK) by plants and seeds yield was higher with vermicompost application and decrease amount in normal soil application. Similar results for *A. hypogea* and *V.mungo* were reported by Parathasarathi and Ranganathan (2001).

It has been assumed that inoculation with bacteria like Bacillus, Pseudomonas, Rhizobium, and Azotobacter may enhance the plant growth as a result of their ability to fix nitrogen. All the bacterial isolates in the present study were able to produce catalase. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical, and chemical stress. Some of the above-tested isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically. Similar to our findings of multiple PGP activities among PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored (Gupta *et al*., 1998). The excessive use of chemical fertilizers characteristic of modern farming could also have contributed to the low microbial loads observed in this location. Most changes in agricultural technology have ecological effects on soil organisms that can affect higher plants and animals, including man.

Table.5. Effect of vermicompost and PGPR isolated from vermistabilised organic substrates on plant growth parameters* of Black gram *(Vigna mungo L. Hepper)*

Treatment	No. of days	Shoot length (cm)	Root length (cm)	No.of root hairs	No.of root nodules	No. of branches	No of leaves
T0		7.2	3.5	5	0	2	3
T1		9.6	5.9	14	0	3	4
T2		8.8	4.8	7	0	3	4
T3	15	9.8	5.9	11	0	3	4
T0		14.5	8	13	13	5	8
T1		17	9.5	28	16	6	8
T2		14.8	9	20	15	6	7
T3	30	17.2	9.2	28	15	6	8
T0		18.8	11.9	30	14	7	12
T1		21	16.4	48	19	8	14
T2		21.2	13.5	45	22	10	12
T3	45	22.4	16.5	48	16	8	14
T0		21.8	16.2	48	14	8	18
T1		23.8	18.2	58	19	9	21
T2		24	18	56	22	11	20
T3	60	24.5	19.9	58	19	10	24
T0		25.8	19.6	60	15	9	28
T1		35.8	21.2	72	19	10	29
T2		32	20.4	65	22	12	24
T3	75	37	22.5	72	19	11	29
T0		32	22	70	15	10	28
T1		41.2	22.8	79	20	11	34
T2		39	22.5	76	22	13	30
T3	90	41.8	24.9	79	19	14	36

*Mean of Three replications, T0 = Red Soil; T1 = Paper Mil Sludge; T2 = Leaf Litter; T3 = Press Mud

Table.6 Effect of vermicompost on the yield of Black gram (*Vigna mungo*)

Treatment	No. Of days	No of Flowers	No of pods Per plant	No of seeds Per pod	Weight of 100 seeds (fresh wt., g)	Weight of 100 seeds (dry wt., g)
T0		-	-	-	-	-
T1		-	-	-	-	-
T2		-	-	-	-	-
T3	**15**	-	-	-	-	-
T0		-	-	-	-	-
T1		-	-	-	-	-
T2		-	-	-	-	-
T3	**30**	-	-	-	-	-
T0		0	0	0	-	-
T1		2	0	0	-	-
T2		2	0	0	-	-
T3	**45**	2	0	0	-	-
T0		6	2	5	6	4
T1		6	4	6	6.8	4.5
T2		10	6	8	7	4.8
T3	**60**	8	12	8	7.2	4.8
T0		7	6	6	6.8	4
T1		8	16	8	7.8	5
T2		8	12	8	7.8	5
T3	**75**	8	16	8	7.8	5
T0		6	16	8	7	5
T1		8	21	8	7.8	6
T2		8	22	8	8.2	6
T3	**90**	12	24	8	8.2	6

*Mean of Three replications, T0 = Red Soil; T1 = Paper Mil Sludge; T2 = Leaf Litter; T3 = Press Mud

The findings of the present study reinforce the general concept that the gut and casts of earthworms tend to be much more microbiologically active than the surrounding soil. Enhancing the growth of these soil organisms can serve as a basis for the development of 'living soils' by optimizing the potentials of the beneficial biotic populations identified in this work.

In conclusion, vermicomposting technology involves harnessing earthworms as versatile natural bioreactors playing a vital role in the decomposition of organic matter, maintaining soil fertility and in bringing out efficient nutrient recycling and enhanced plants growth. In this study Paper mill sludge, leaf litter and press mud were vermicomposted by using *Eisenia fetida* in laboratory conditions. Hence, mass rearing and maintaining the earthworm, *Eisenia fetida* and can be used for composting the Paper mill sludge, leaf litter and press mud. Then the vermicompost is used as an organic fertilizer since it has appropriate macronutrients, micronutrients and microorganisms that may support the plant growth.

The good evidence produced through plant growth experiments carried out in the present study shows that vermicompost can promote plant growth and retain major nutrients in soil. The study clearly indicates that the vermicompost in respect of organic substrates and earthworm species utilized had increased amount of plant growth when compared control (T0) i.e red soil. The long run effect of similar application trials are required to completely justify the improvement of soil fertility status.

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