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Comparative Analyses of *Eisenia Fetida*and Herbal Vermicompost Soil on Treatment with *Rhizobium* Culture

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ABSTRACT

Effort was made to produce vermicompost soil rich with available nutrient from medicinal plant- Neem (*Azadirachtaindica*), Guava (*Psidium guajava*) and liquid cultures of rhizhobium (*BradyRhizobium japonicum*). Potential *Eisenia fetida* was used to recycle the nutrient from leaves medicinal plants namely Neem (*Azadirachtaindica*) and Guava (*Psidium guajava*). Three different vermicompost beds were prepared – Rhizhobium with medicinal plant and cow dung (R-MP), Medicinal plant with cow dung (MP) and only cow dung (CD). The physico-chemical properties of the R-MP, MP and CD vermicompost soil were recorded at the end of 60^{th} day. Histological examination of *E. fetida* grown in 3 different vermicompost were studied. Biochemical analysis, growth rate and cocoon production of *E. fetida* in R-MP, MP and CD vermicompost soil were measured. Results clearly shows the vermicompost soil of R-MP and MP were rich with ideal parameters for a healthy plant growth.

Keywords: Vermicompost, medicinal plant,*rhizhobium*, *Eisenia fetida*, physico-chemical, soil fertility, cow dung.

1. INTRODUCTION

Due to increase in population and increase in levels of global warming, there is very high risk of pollution related diseases to occur and spread globally. There are many control and preventive measures to minimize the pollution, but to reduce the levels of contaminants in already affected areas is still under process. The process of vermicomposting has gained much importance and it is now been the trusted areas of research. The use of medicinal leaves in the process of vermicomposting has been increasing since they are effective plant growth promoting microbes in the compost (Vijayabharathi*et al.*, 2015).

Vermicast also known as worm castings is the end-product of the vermicomposting process where the organic matter is broken down by an earthworm to create a heterogenous mixture of nutrients. These castings show a decrease in the levels of contaminants and an increase in the levels of nutrients than do organic materials before vermicomposting. One of the possible measures to reduce the levels of contaminants in soil is to engage vermicomposting process where variety of species of earthworms are used which help in the breakdown of wastes. To reduce the levels of contaminants in soil, vermicomposting process is been aided where variety of species of earthworms are used which help in the breakdown of wastes. Medicinal plants such as Neem (*Azadirachtaindica*) and Guava (*Psidium guajava*)

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which are rich in phytochemicals are broken down by earthworms and the resulting vermicompost is used as carrier with other biofertilizers and are used to engage soil fertility. Neem and Guava are very essential medicinal plants known for the immense medicinal properties. Contradiction to the fact that neem leaves are a potential nematicide and highly unpalatable, earthworms are observed to voracious feeders of neem compost and increases the process of composting to 7% per day (Gajalakshmi and Abbasi, 2004).

In this study, equal weights of partially dried leaves of *Azadirachtaindica*and *Psidium guajava*are mixed proportionately with cowdung and allowed for vermicomposting. The vermicomposting was carried out using *Eisenia fetida* earthworms. For a comparative study, liquid cultures of *BradyRhizobium japonicum* was added to the mixed proportions (leaves and cowdung) and observed the comparative changes of the earthworms in terms of histopathology and vermicompost- soil parameters.

2. MATERIALS AND METHODS

2.1 Materials Needed

Earthen clay pots, leaves for compost (Neem and Guava), paper strips, soil, cow dung, gunny bag, *Eisenia fetida*(Red worms), *Rhizobium japonicum* culture, non-chlorinated water. The procedures were followed according to Adegunloye*et al.*,(2007) with modifications. The materials used in this experiment were completely sterilised according to the traditional method of sterilisation.

2.2 Preparation of Vermicompost Bed

Three earthern clay potswere taken and maintained as a Control (CD), Medicinal Plant (MP) and *Rhizobium*- Medicinal Plant(R-MP). In the holed earthen pots six layers of bed made (Fig.1) as follows:

SET 1- Control (CD): 2-3cm moistened soil → paper strips (2-3cm)→ moistened soil with earthworm Eisenia fetida 4-5 cm)→ cow dung (3 cm) → dry straw (3 cm) → wet jute cloth.

SET 2- Medicinal Plant Bed (MP): 2-3cm moistened soil → paper strips (2-3cm)→ moistened soil with earthworm Eisenia fetida (4-5 cm)→ Slurry containing mixture of Neem, Guava and cow dung ratio 1:1:1 (4-5 cm) → dry straw (3 cm) → wet jute cloth.

SET 3- Rhizobium - Medicinal Plant Bed (R-MP): 2-3cm moistened soil → paper strips (2-3cm)→ moistened soil with earthworm Eisenia fetida (4-5 cm)→ Slurry containing mixture of Neem, Guava and cow dung ratio 1:1:1 with 25g of *Rhizobium japonicum* (4-5 cm) → dry straw (3 cm) → wet jute cloth.

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Fig. 1 Pictorial representation of vermicompost beds1) Control (CD), 2) Medicinal Plant Bed (MP) and3) Rhizobium - Medicinal Plant Bed (R-MP)

The entire set up is kept in a cool, dark and airy place. The moisture content is monitored regularly. The water is sprinkled for every alternate days and feed is given for every 25th day. It is regularly checked for pests and predators.

2.3 Histological changes of compost earthworm – *Eisenia fetida*

Histopathological analysis of *Eisenia fetida*obtained from control, medicinal plant and medicinal plant-*Rhizobium* vermicompost bed by following method Gao *et al.*,(2013). After 60 days of compost the earthworms were separated from compost soil, rinsed with distilled water. The earthworms were set at 10% formalin, enclosed in paraffin and cut vertically at 4 mm thickness with a freezing microtome. Sections were placed on microscope glass slides using traditional histopathological techniques. Sections were stained with Hematoxylin-Eosin (HE) and analysed by an optical microscope.

2.4 Biochemical analysis of *Eisenia fetida*obtained from C, MP, R-MP compost

After 60 days of feeding, the biochemical analysis of red worm was studied. Biochemical parameters analysed in the present study were Total protein (Lowry *et al.*, 1951), Carbohydrates (Joseph and Roe, 1955) and Cholesterol (Zak, 1977).

2.5 Analysis of Vermicompost Soil parameters

2.5.1 Measuring the various vermicomposting coefficients of the obtained compost from C, MP, R-MP

Parameters - Total Nitrogen (%) by IS 14684-1999 and C/N ratio by CHNS Vario MACRO cube (ElementarAnalysensysteme GmbH, Germany) was done in conjunction with García-Sánchez *et al.* (2017) for composting soil from C, MP, R-MP.

2.5.2 Determination of Total salt (ppm)

To determine the total water soluble salts in the compost, 50 g of the compost soil from each pots were soaked in 250 ml deionized water separately, surged for 4 hrs at room

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temperature, and then filtered through quantitative filter paper. Then, 50 ml of the filtrate in a 100 ml beaker was evaporated in a water bath at 100°C. During evaporation, 10 ml of 30 percent H_2O_2 was applied to each beaker to extract organic matter. The residues in the beakers were dried at 105°C for 4 h and measured. The resulting figure was multiplied by 5 to determine the soluble salt content of the 50 g compost sample (Lu, 1999).

2.5.3 Determination of Saturation moisturepercentage (water holding capacity)

Crushed air-dried soil from respective pots were passed through 2 mm sieve placed in round filter paper and fixed to the internal perforated floor of the dish. The weight of the dish and filter paper is noted. The dish is then filled with soil by tapping the dish briskly & making even surface at top of soil and weighed. The set of perforated dishes in enamel tray was placed and water was poured at half of the height of dish. Water may rise in dish through perforated bottom and moistened the soil to its capacity by storing it for 5 to 6 hours in water. Then the dishes were placed on a filter paper sheet, so that the excess of water may drain away from the pores within half an hour. The dish containing moist soil is weighted.

2.5.4 Determination of Soil Moisture Content - Gravimetric Method (Direct Method)

The moist soil sample from respective pots was placed ina moisture box and weighed immediately. Placing the box without lid in an oven at 105^{o}C to dry the soil to a constant weight. The box was removed from the oven covered with the lid, and placed in the desiccators until it is cool. The box along with sample and the empty moisture box to determine the mass of the moisture.

2.5.5 pH of the compost soil

20 g of air dried soil was taken from the respective pots and 50 ml of distilled water were stirred with a glass rod thoroughly for about 5 minutes in a beaker and kept for half an hour. In the meantime turn the pH meter ON, allowed to warm up for 15 minutes. The glass electrode was standardised using standard buffer of pH 7 and calibrated with the buffer pH 4 or pH 9.2. The electrodes were dipped in the beakers containing the soil water suspension with constant stirring. The glass electrodes were standardized after every 10 determinations.

2.5.6 Determination of Organic Carbon in soil (Walkley and Black Method)

1 g sieved compost soil from respective pots taken in a 500 ml conical flask of 10 ml of 1N $K_2Cr_2O_7$. To this mixture, 20 ml of conc. H_2SO_4 from burette was gradually applied to the conical flask containing the compost soil, whirling gently until the soil and the reagents were vigorously mixed. 200 ml of distilled water, 10 ml of concentrated orthophosphoric acid and approximately 0.2 g NaF were slowly added and then incubated for 11/2 hours. Just before titration, 1 ml of the Ferroin indicator was added to the conical flask. The mixture is then titrated with 0.5 N ferrous ammonium sulfate until the colour flashes from yellowish green to green and eventually brownish red at the end stage. Simultaneously, a blank examination is carried out without soil.

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2.5.7 Determination of Phosphate in soil (Olsen's Method)

To 2.5 g of vermicompost soil sample taken from the respective pots, 3 g of phosphate free activated charcoal AR grade was added followed by 50 ml of Olsen's reagent and kept in shaker for 20 minutes at 180 rpm and filtered. 5 ml of aliquot of the resulting solution were transferred into 25 ml volumetric flask and 4 ml of the freshly prepared ascorbic acid and Ammonium molybdate solution was added, shaken and maintained as such for 30 minutes. The standard curve was prepared using 0, 1, 2, 3, 4 & 5 ml of 5 ppm standard P solution into 25 ml volumetric flask and the colour developed using the same procedure as above. The corresponding P concentration will be 0, 0.2, 0.4, 0.6, 0.8 & 1 ppm and absorbed at 882 nm and blank was used.

3. RESULTS AND DISCUSSION

Earthworms are standard research species for soil toxicity or soil stability testing. They have been commonly used to evaluate the environmental impact of heavy metal pollution; however, awareness of the toxic effects of pesticides on these species is still quite limited(Rodríguez and Hernandez, 2007). Previous studies have indicated that earthworm skin has close interaction with polluted soils and is known to be a major route to the absorption of toxicants (Saxe *et al.*, 2001; Jager *et al.*, 2003; Vijver*et al.*, 2003).

There are several instances where various carrier materials can be identified to enhance the growth and survival of biofertilizers. Nutrient complemented pumice containing a small percentage of peat and diatomaceous soil kept in sterile plastic bags to encourage good growth of the biofertilizer bacterium, *Rhizobiumlupine* (Einarsson*et al.*, 1993). The findings of the current study suggest that the vermicast alone or in higher proportions with different compost treatment supports the survival for long time. The R-MP vermicompost showed good growth parameter in earthworm and supplement made the earthworm to enrich in its biochemical parameter as well as soil parameter.

3.1 Growth performance and biomass production of *Eisenia fetida*

The changes in the survival rate, mass production and the average body weight of earthworms from R-MP, MP and CD are shown in Table 1. The weight gained by the earthworm was significantly higher than the CD. When fed a diet containing medicinal plant showed higher growth when compared to cow dung soil whereas there is a significantly higher growth of *Rhizobium* treated earthworm observed (2.15 g) when cross checked with both medicinal treated as well as CD.

It is believed that certain active ingredients (flavonoids, steroids, saponins, alkaloids, etc.) in herbal plant leaves could be responsible for certain growth rates in the worms of this research, although more thorough studies are needed to support the hypothesis. The observed difference between vermibeds for weight gain may be due to difference in feed rate and/or growth rate in earthworms.

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The biomass produced in the various vermicomposts of the present study is a significant indicator of the suitability of the substrate for vermiculture. The findings of high levels of biomass in MP and R-MP bedding were unexpected. Garg and Gupta (2011) reported accumulation of biomass per unit waste by *E. fetida*, cultivated on industrial waste, spiked with cow dung and concluded that the quality of industrial waste affects the biomass gain in earthworms during the vermicomposting of industrial waste. Few scientists have also recorded losses in earthworm weight which may be due to the conversion of most of the substrate material to vermicompost, which could not further sustain the growth of earthworms. The weight loss was due to food shortages in vermibeds (Neuhauser*et al.,* 1988; Singh *et al.,* 2010). But in this study, the fresh healthy medicinal leaves and *Rhizobium* may have played a role in growth of earthworm by providing suitable nutrients and growth supplements.

As observed, the cocoon production was almost better in vermibeds with R-MP and MP suggesting that medicinal plant contains some reproduction stimulating chemical substances. The production of cocoon was around 3 times more in vermibeds containing R-MP than in CD beds. Few earlier research advocated the effect of substrate chemicals on the reproductive ability of composting earthworms (Suthar, 2007; Ganesh *et al.,* 2009). The increase in the biomass of earthworm may be due to a number of factors: temperature, substrate moisture, chemical constituents of culture media, etc. The results clearly support the idea that the viability of cocoon during the vermicomposting process is equally significant in the vermicomposting activity.

Table 1 Survival, biomass, and reproduction of earthworm in *Rhizobium* treated medicinal, medicinal plant and cow dung (mean ± SD)

Days	Survival rate (%)			Mean body weight (g)			Number of cocoons		
	R-MP	MP	C	R-MP	MP	C	R-MP	MP	C
0	100.00±0.0	100.00±0.0	100.00±0.0	0.37±13.2	0.39±8.2	0.38±32.5	0.00±0.00	0.00±0.00	0.00±0.00
10	100.00±0.0	100.00±0.0	94.91±70.3	0.71±5.02	0.52±16.7	0.41±13.2	21±0.28	16.2±26.9	15.7±3.97
20	100.00±0.0	94.12±37.97	90.02±69.7	0.94±8.1	0.74±14.3	0.76±0.97	179.0±2.15	157.9±8.64	99.1±27.2
30	100.00±0.0	95.77±63.72	87.17±26.9	1.39±6.9	1.09±17.4	0.83±2.8	213.1±13.4	193.1±0.33	142.7±31.5
40	100.00±0.0	92.49±38.13	83.51±15.9	1.70±32.1	1.33±20.8	0.90±0.63	185.7±15.7	144.4±1.20	121.3±16.9
50	100.00±0.0	90.17±12.72	77.73±32.4	2.00±0.26	1.76±11.14	1.08±10.9	146.1±7.35	92.5±3.9	73.8±0.89
60	100.00±0.0	88.02±52.87	69.66±7.50	2.10±10.91	1.91±20.3	1.20±0.04	98.3±0.73	67.12±16.7	39.3±40.8

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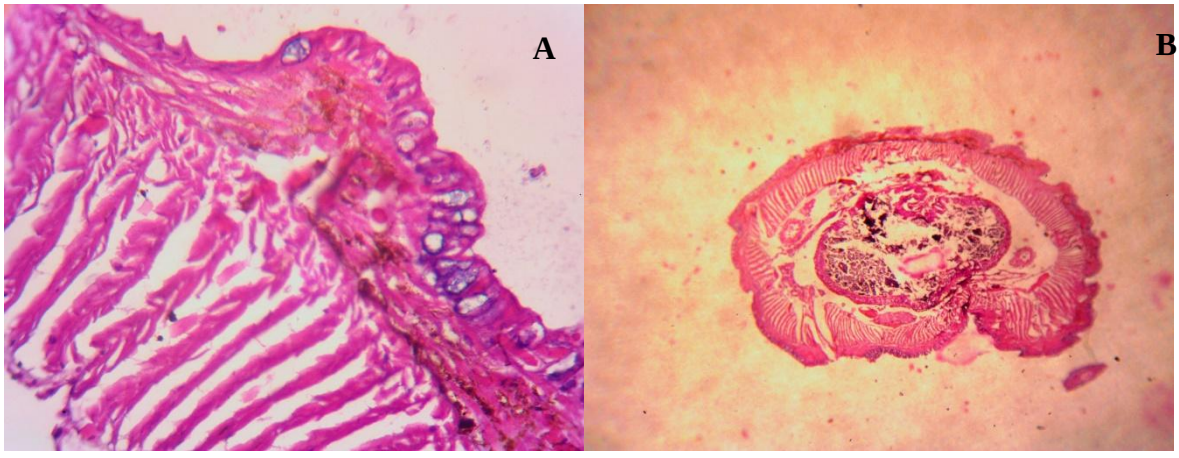
The number of cocoon of earthworms collected during the different stages of vermicomposting is shown in Table 1. The number of cocoon also showed an increase pattern initially followed by a time decline similar to Vig*et al*, (2011). From 10 days on, cocoon started to appear. At the 30 day, the number of cocoons reached the maximum of approximately 142 cocoons in CD, which was significantly less than the 213 and 193 cocoons found in R-MP and MP vermicompost respectively. Therefore, R-MP and MP were more conducive for cocoon production than CD. During the last 20 days, more cocoons were observed in R-MP and MP than in CD, which might be due to more adults and a nutrient surplus which could provide more durable nutrients for earthworm reproduction.

Sharma (2003) recorded a doubling of the earthworm number in 42 days during the vermicomposting of municipal solid waste and is due to a declining C/N ratio with a time span (Nedgwa and Thompson 2001). Organic waste, which provides soil worms with ample quantities of easily metabolizable organic matter and non-assimilated carbohydrates, encourages the growth and reproduction of earthworms (Edwards 1988). Domínguez and Edwards (2010) also reported *E. fetida* as tolerant to the environmental conditions (nature) of organic wastes and has been found to be very competitive in the vermicomposting process (Domínguez *et al.*, 2010).

3.2. Histopathology of earthworms of R-MP, MP and control

Internal organs are very clear, visible and growth of the tissue was normal without any ulceration and inflammation in all the compost worms. The growth rate and well-developed tissues and predominant development of internal organs seen in both R-MP and MP compost worms, but still the excellent growth was comparatively high in R-MP than Control and MP compost. (Fig 2 (A-F)).



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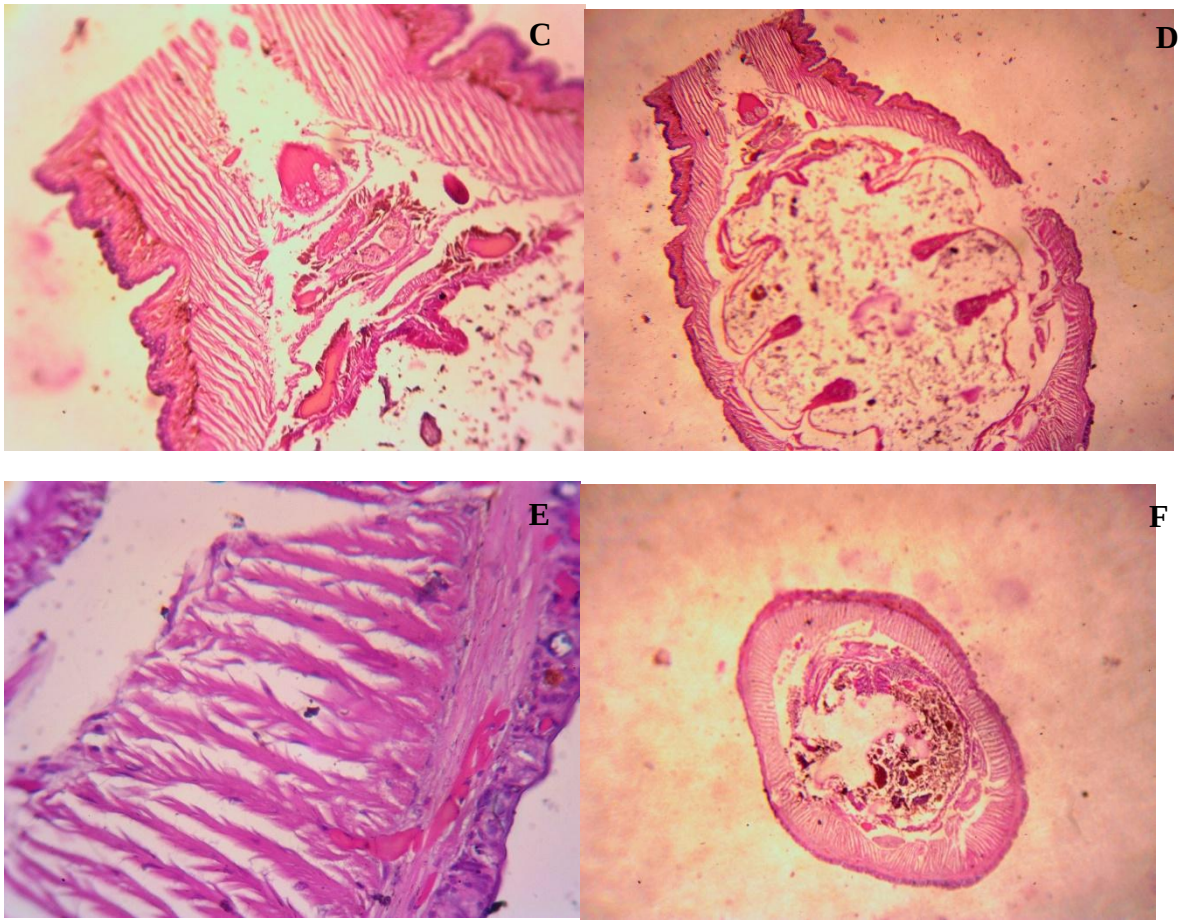

Fig 2. Histological examination of *E. fetida* fromdifferent culture pot (A & B shows the Histological examination of *E. fetida* at 400X and 40X magniciation from MP pot; C & D shows the Histological examination of *E. fetida* at 400X and 40X magniciation from R-MP pot; E& F shows the Histological examination of *E. fetida* at 400X and 40X magniciation from Control pot)

3.3 Biochemical analysis

Proximate analysis of the whole earthworm body (Table 2) revealed that the crude protein content was significantly high in *Rhizobium* treated earthworm (0.812 µg m^{-1}) than MP and control compost worms. Same way the significant value of carbohydrate was observed in R-MP (0.586µg m^{-1}) and MP (0.592µg m^{-1}) compost worms in which both of results showed more or less similar values and comparatively showed high value than the CD earthworm (0.284 µg m^{-1}).The lipid value of medicinal plant treated earthworm is low (0.058 µg m^{-1}) and the *Rhizobium* treated earthworm showed gradual increase (0.072 µg m^{-1}) whereas control value was (0.067 µg m^{-1}).

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Table 2 Biochemical estimation of *Eisenia fetida* cultured in different culture pot

Vermicompost pots	Protein ($\mu g\ m^{-1}$)	Carbohydrate ($\mu g\ m^{-1}$)	Lipid ($\mu g\ m^{-1}$)
Control	0.227	0.284	0.067
Medicinal plant	0.761	0.592	0.058
Medicinal plant-*Rhizobium*	0.812	0.586	0.072

3.4 Soil Parameters

The result on effect of vermicompost on physical properties of the soil observed in R-MP, MP and CD vermicompost is given in Table 3. The minimum pH 7.0 was observed in CD vermicompost and the maximum pH of 7.5 and 7.3 in MP and R-MP. Variation in pH between reactors is due to the difference in the chemical composition of macrophytes, because the recycling of different substrates could result in the development of different intermediate compounds resulting in different pH levels. Muthukumaravel*et al.* (2008) also reported a relative increase in pH over the vermicomposting era and attributed it to the decomposition of nitrogenous substrates. The temperature of all control, vermicompost both *Rhizobium* treated as well as medicinal plant treated showed room temperature between 32 - 35°C.

The soil obtained from three different vermicompost subjected to water holding capacity showed high water holding capability in medicinal plant treated vermicompost soil (51%) than the R-MP vermicompost (49%) and CD soil (43%). The water holding capacity of the vermicompost CD was close to that of the vermicomposed pig manure (Atiyeh *et al.*, 2001) but R-MP and MP vermicast are higher than the previous study. The total porosity of R-MP is higher than MP and CD but, even MP and CD showed better result compared to vermicompost treated samba cultivated soil.Greater porosity in the treated vermicompost soil was attributed to an increase in pore size (Marinari*et al.,* 2000). Medicinal plant and *Rhizobium* caused a significant increase of moisture content due to the more porosity (Bazzoffi*et al.,* 1998). The moisture content of R-MP vermicompost soil (49.13) was higher to that of MP vermicompost (43.20) whereas the control soil showed lower capacity hence the CD, which seems having a poor moisture content.

The Electric conductivity of vermicompost soil in this study was positively correlated with time period of vermicomposting process and the values are given in table 3. The electric conductivity of R-MP was lower than the MP and CD this may be due to low salt content. The increase in EC during the vermicomposting process is due to an increase in the soluble salt level resulting from the mineralization behaviour of the earthworms and microorganisms

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present in the organic material and in the intestines of the earthworms (Karmegam*et al.*, 2019) this study supports the EC value of R-MP and MP treated vermicompost. Similar results obtained with other species of earthworm on the vermicomposting of leaves, weeds and agricultural residues are reported (Yadav and Garg (2019)).

The soils treated with MP and R-MP exhibited a higher organic content than the CD. The organic content reached 15.1% and 13.9% in medicinal plant and *Rhizobium* treated soil. Both tested organic amendments had capacity to raise soil organic content and there is no significant difference between these amendments, in spite of higher large organic matter content in compost. The use of organic amendments increases the soil organic carbon and improves soil structure. Fortuna *et al.* (2003) argued that the vermicompost amendment could increase the carbon contents when compared to the original levels, and thus contribute to increase the soil structural stability, particularly that of the macroaggregates.

The Total Available Phosphorus(TAP) was normal range in all vermicompost soil generated in all vermicomposts (Table 3). The values showed maximum increase in the phosphate content of *Rhizobium* treated vermicompost i.e, 73.69 ppm whereas it showed 58.17 ppm in medicinal plant and 30.01 in control soil. Ghosh *et al.* (1999) reported vermicomposting as an effective method for converting unavailable forms of phosphorus into readily available forms for plants. Increased phosphorus content in vermicompost clearly shows soil worm-mediated phosphorus mineralisation (Suthar 2009).

The release of phosphorous in the usable type is partly induced by earthworm intestinal phosphatases and may be further released by phosphorous solubilizing microorganisms in casts (Ghosh *et al.* 1999). It is well known that microorganisms play an important role in the release of phosphorus from waste. Sterilized and Sterilized (2000) reported that microflora plays an important role in enhanced phosphatase activity in worm casts. It is therefore proposed that soil worm-processed waste material contains an excellent concentration of plant metabolites due to increased microbial activity during the vermicomposting process.

There is an increase in salt content of the medicinal plant treated soil which shows peak value of 1.27 % in MP when compared to CD. It states that the soil was having high electric conductivity was due to loss of organic material and release of high amount of salt like ammonium, phosphate, potassium etc. The *Rhizobium* treated soil contains 0.31 % of total salt which is similar to the CD soil (0.32 %) shows poor electric conductivity. The low salt content in soil always preferred for healthy plant growth because if the salt concentrations in the soil are higher than in the root cells, the soil will draw water from the root and the plant will wilt and die. In this present study the salt content of all the three vermicompost soil was observed to be normal range.

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Table 3 Soil parameters of different vermicompost soil from culture pots

Soil parameters	CD-control	MP-Vermicompost	R-MP Vermicompost
Water holding capacity (%)	43	51	49
Porosity (φ)	0.3	0.7	0.7
Moisture content **(θ_v)**	39.48	43.20	49.13
pH	7.0	7.5	7.3
Electric conductivity(mS/m)	126	314	121
Total organic carbon (%)	8.3	15.1	13.9
Available phosphorous (ppm)	30.01	58.17	73.69
Total salts (%)	0.32	1.27	0.31
Total nitrogen (ppm)	29.31	38.56	42.08
C:N ratio	7.5:1	12.17:1	19.1:1

The total nitrogen content of the vermicompost done with medicinal plant (38.56 ppm) showed higher value when compared to the CD soil (29.31 ppm) and the rhizhobium treated soil (42.08 ppm) showed very high value when compared with both medicinal as well as control nitrogen content. The increase in total nitrogen content was greater probably because of higher mineralization of organic matter. According to Viel*et al.* (1987), losses in organic carbon could be caused by the addition of nitrogen. Decomposition of organic material by earthworms accelerates the process of nitrogen mineralization and then adjusts the nitrogen profile of the substrate (Cynthia and Rajeshkumar, 2012; Suthar, 2007).

Earthworm activity improves the nitrogen profile of vermicompost by microbial mediated transformation of nitrogen and the rotting tissues of dead worms in vermicomposting systems result in further improvement of nitrogen (Suthar 2007). Satchell (1967) estimated that more than 70% of the nitrogen in dead earthworm tissue had been mineralized in less than 20 days. However, in general, the final nitrogen content of vermicompost depends on the initial nitrogen content of the waste and the degree of the decomposition (Chauhan and Joshi 2010).

The C:N ratio is important because plants cannot assimilate mineral nitrogen unless the ratio is 20:1 or lower (Edwards and Bohlen, 1996). The C:N ratio decreased over time in all feed mixtures. The R-MP, MP and CD vermicompost ratios obtained were 19.1:1, 12.17:1 and 7.5:1. The loss of carbon as carbon dioxide in the process of respiration and the production of nitrogen excreta increases the level of nitrogen which lowers the C:N ratio

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(Senapati *et al.*, 1980). Increasing the prevalence of heavy metals in organic waste, which may accumulate in earthworms, may pose a problem when considering worm fertility (Flechenstein and Graff, 1983). The study found that the C/N ratio of macrophytes, one of the most commonly used indices for compost maturation, stabilises during the vermicomposting process.

The C/N ratio of the substrate represents the mineralization and stabilisation of organic waste during the vermicomposting process. The loss of carbon dioxide by microbial respiration and at the same time the addition of nitrogen by worms in the form of mucus and nitrogenous excretory content reduces the C/N ratio of the Suthar substrate (2008). The importance of the C/N ratio is based on the fact that a reduction in the ratio means an improvement in the degree of humidification of organic matter (Singh *et al.* 2011). Decline of the C/N ratio to less than 20 suggests an advanced degree of stabilisation of organic matter and represents a satisfactory degree of maturity of organic waste Hayawin*et al.* (2010).

4. CONCLUSION

The above observations concluded that, the biofertilizer (Microbial Inoculums of *Rhizobium* sp.) enriched vermicompost (*Neem and Guava leaf*) has enhanced the various physiological properties in earthworm as well as treated soil. It enhanced the healthy growth of earthworm which was concluded by the biochemical analysis were amount of protein, carbohydrate and lipid content confirms the earthworm is supplemented with higher nutritional value. The earthworm productivity measured in terms of total worm biomass, population built-up and cocoon numbers etc. also indicates the suitability of medicinal plant and soil friendly bacteria in compost obtained through vermitechnology operation. It can save the farmers from the problem posed by high cost of fertilizers by offering a comparatively inexpensive sustainable agriculture and both the methods are strongly recommended to practice in agricultural field. It also proves that the method highly useful to overcome the environmental soil pollution.

CONFICTS OF INTEREST

The authors have claimedno conflicts of opinion

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