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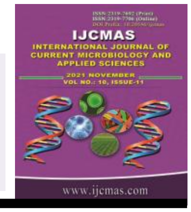

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Biotransformation of the Banana Pseudostem Scutchers into the Vermicompost by *Eisenia fetida*

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Vermicomposting is an ecofriendly technology to convert organic waste into organic fertilizer. An epigenic species *Eisenia fetida* was used for the biotransformation of the banana pseudostem scutchers and cow dung into the vermicompost. Three different combinations of the banana pseudostem scutcher and cow dung were evaluated in the vermicomposting process. The change in the physicochemical parameters were examined by the standard method at the end of the vermicomposting. The results showed that earthworms increased the EC and NPK content while total carbon, pH and C:N ratio was reduced significantly in vermicompost of banana pseudostem scutchers. The maximum significant change in NPK content and reduction of total carbon and C:N ratio was found in the mixture of (75% banana pseudostem scutcher + 25% cow dung). The highest growth and reproduction rate was also present in the T2 treatment. The seed germination test was performed to evaluate the maturity of the vermicompost. The prepared vermicompost shows the significant effect on the growth of the *Vigna radiata*. The germination of the *Vigna radiata* is comparatively higher in vermicompost of the T2 treatment. It is evident from the results that the banana pseudostem scutcher and cow dung are good substrates for the vermicomposting and *Eisenia fetida* able to convert this organic waste into the stable nutrient rich product. This nutrient rich vermicompost has no phytotoxicity and the ratio of carbon/nitrogen below 15 which indicates the acceptable maturity and it can be utilize as organic fertilizer.

Introduction

Management of the solid waste is an imperative challenge which require interventions at all levels of sustainable and healthy environment. There has been a continuous increase in solid waste and its disposal requires a large amount of land. Now a days biological technologies such as composting and vermicomposting are a best alternative to manage the solid waste (Sharma and Garg, 2020). Vermicomposting is the most acceptable, easy, low cost, green and an environment- friendly method. In vermicomposting process earthworms are used to enhance the conversion of organic waste into stabilized humus like structure called vermicompost (Alok *et al.,* 2008; Sharma and Garg, 2017; Negi and Suthar, 2018). Vermicomposting is a biological process which involves the interaction between the earthworm and microorganism. It convert different organic waste into nutrient rich value added products. The mutualistic approach alters the physical and chemical properties of waste material and convert it to vermicompost. During this process nitrogen, potassium, phosphorous and calcium are converted into more soluble form which are readily taken up by the plants. It also provides macro and micro nutrients which help to plant growth.

The selection of the earthworms is an important parameter in the preparation of vermicompost. The most common species of earthworms used in the vermicomposting are *Eisenia fetida, Eisenia andrei, Eudrilus eugeniae* and *Perionyx excavates.* Among these *Eisenia fetida* is an efficient epigenic species which is able to tolerate high moisture content, wide range of solid waste and temperature tolerant (Chaudhari and Bhattacharjee, 2002; Kibatu *et al.,* 2015). *Eisenia fetida* is also known as a red worm, red wriggler worm or tiger worm. In Comparison to other species of the earthworms, growth and reproduction rate of *Eisenia fetida* is faster.

Banana (*Musa*) is a second largest cultivated fruit in the world. Banana plant is a monocotyledonous herb, it produce only one bunch of the banana. After harvesting the bunch of banana, the stem of banana (pseudostem) is cut and left on the farmland which creates an environmental pollution. This environmental problem can be solved by the biotransformation of banana pseudostem in to valuable products. Banana pseudostem is a fibrous and lignocellulosic in nature. The fiber extraction from such rich cellulosic residues has attracted more interest due to its potential applications in composite material (Chauhan and Sharma, 2014). During the fiber extraction, a huge amount of waste (scutchers) is generated. This rich lignocellulosic waste can be used in the preparation of vermicompost (Patel *et al.,* 2012). The aim of this study is to prepare the nutrient rich vermicompost from the banana pseudostem scutchers and cow dung using *Eisenia fetida*. The vermicompost feedstock has been examined for physicochemical analysis and perform the seed germination assay. The growth and reproduction of the worm was also analyzed after vermicomposting.

Materials and Methods

Collection of banana pseudostem

In the present study, banana pseudostem scutchers were used for the preparation of vermicompost. Banana pseudostem scutchers is a waste generated during the fiber extraction. The banana pseudostem was obtained from local farmers, Anand, Gujarat, India.

Collection of earthworm and cow dung

The biotransformation of banana pseudostem scutchers into valuable product (vermicompost) was done by using *Eisenia fetida.* The healthy species of *Eisenia fetida* was acquired from AAU (Anand Agricultural University), Anand, Gujarat, India. The fresh cow dung was procured from local dairy farm.

Experimental design for vermicomposting

The vermicomposting was carried out by preparing uniform feed material of banana pseudostem scutchers and cow dung in the plastic container having diameter (32 cm x 8 cm). Three different combination of banana pseudostem scutchers and cow dung were used in the preparation of vermicompost. (I): banana pseudostem scutchers and cow dung (50%: 50%), (II): banana pseudostem scutchers and cow dung (75%: 25%), (III): banana pseudostem scutchers and cow dung (100%: 0%). The soil was added in all the containers for enrichment. Prior to the experiment, all the bedding containers were kept for partial degradation and thermal stability for 15 days, because during the degradation process heat is generated and earthworms are very sensitive to the temperature change. The moisture level of all the container was maintained at 60-80% by sprinkling the water throughout the duration of the experiment. After the partial degradation 200 healthy *Eisenia fetida* were introduced in all the containers except the control. All the containers were kept in a dark room at optimum temperature 30°C. The moisture content was maintained between 60-80% throughout the experiment. All the containers were covered with jute clothes to prevent the moisture loss. The mixture was turned regularly at an interval of 15 days. After 115 days, a dark brown to black color, uniform, odorless vermicompost was formed. At the end of the process, the earthworms were removed from the container and growth and total number of *Eisenia fetida* were recorded.

Physicochemical analysis

The homogenous experimental samples of the beginning of the experiment and at the end of the experiment were analyzed for pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (TN), phosphorous (P) and potassium (K) using standard methods. The pH and EC were evaluated by the pH meter and Elico conductivity meter using the ratio of 1:10 (w/v) of different samples. Nitrogen and potassium was determined after the treatment of the sulfuric acid and perchloric acid (9:1, v/v). The phosphorous content was measured by the spectrophotometric method with molybdenum in sulphuric acid. The ratio of carbon and nitrogen was calculated by dividing the content of carbon present in the sample with the content of nitrogen.

Growth rate

The important biological parameters such as cocoon production, hatching and weight gain by *Eisenia fetida* were evaluated after the vermicomposting process. At the end of the process, earthworms and cocoons were separated from the mixture. The biomass of *Eisenia fetida* was estimated after washing with distilled water and drying on tissue paper.

The worms and cocoons were counted manually and weighed using digital weighing balance. The number of worms, cocoons and biomass of the *Eisenia fetida* was recorded. The final vermicompost was air dried and stored in airtight plastic bags.

Microbiological analysis

The initial and final vermicompost of the banana pseudostem scutchers were evaluated for the microbial analysis by standard plate count method (Subba Rao, 1995). 1 gram of sample was added to 9 ml of sterile distilled water, vortexed for 10 minutes and different dilutions were prepared up to 10-5. 100 ηl aliquot of appropriately diluted sample was poured on the plate containing three different media (nutrient agar for bacteria, glucose asparagine agar for actinomycetes and malt extract for fungi). All the plates were incubated at optimum temperature between the 28 and 37°C for 48 hrs. After the incubation, microbial colonies of bacteria, fungi and actinomycetes were expressed as a Colony Forming Unit (CFU)/g weight of the sample.

Seed germination assay

The efficiency of the vermicompost was also examined by the cultivation of *Vigna radiata*. During the study, fresh vermicompost was used for all the treatments. Four different treatments were used in the experimental study. (i) Soil (as a control), (ii) Soil + commercial vermicompost, (iii) Soil + experimental vermicompost (T1) and (iv) Soil + experimental vermicompost (T2). The experiment was carried out in the plastic container having a diameter (14cm x 7cm) by mixing the soil and vermicompost. Plants were grown under natural condition. The length of the root, stem, number of leaves and germination index was calculated. The higher germination index (GI) value represent the maximum percentage and rate of the germination.

Results and Discussion

Physicochemical parameters

Fig. 1 represent the conversion of the banana pseudostem scutchers and cow dung into the vermicompost. The physical, chemical, and biological properties of the organic feed stocks change during the process of the biotransformation by *Eisenia fetida*. In this experiment, the lower pH was observed in all the experimental vermicompost. The significant difference was observed in the pH change from initial feed stock to final. The pH of all the bed shifts from alkaline pH (9.7-9.8) to neutral pH (7.5-7.8) (Table 1). The maximum decline in the pH was observed in T2 treatment. (22.6%) followed by T1 (20%) and T3 (5%) (Table 2). Decline in the pH during the vermicomposting may be attributed to the mineralization of nitrogen, phosphorous and the production of different components like organic acid, ammonia, nitrates, production of CO_2, humic acid and fulvic acids (Badhwar *et al.,* 2020). The decrease in the pH may also be due to the metabolic activity of microorganisms and earthworm and nitrification of the NH_4^+, N and NH_3 into NO_3^- (Cáceres *et al.,* 2016).

Electrical conductivity (EC) increased significantly from the initial feed stock (Table 1 and 2). The level of the EC in the initial organic waste was in the range of 1.89-3.4 mS/cm. After the biotransformation, the level of EC increased by 75-128% and was found in the range of 2.75- 8.89mS/cm in vermicompost. Among all the treatments, the maximum increase in EC was observed in T2 treatment (8.89 mS/cm) followed by T1 and T3 (5.28 and 1.75 mS/cm) respectively. The increase in the EC may be attributed to the greater avaibiltiy of mineral salt from the feed stock by the action of *Eisenia fetida* during vermicomposting process (Bhat *et al.,* 2015). Many researchers reported that the level of EC in the final valuable product is higher than the initial organic waste (Khwairakpam and Bhargav, 2009; Sharma and Garg, 2017; Badhwar *et al.,* 2020). Sharma and Garg 2011 also reported the value of EC in the range of 6.70-8.41 mS/cm from the vermicompost of rice straw and paper waste by *Eisenia fetida.*

Table 1 and 2 shows the total organic carbon (TOC) of initial organic agricultural waste and final vermicompost product. The results reveals that the amount of total carbon decreased in all the treatments. The loss of the total carbon was observed in the range of 148.5-298.4 g/kg from the initial feed stock. The maximum decline (50.92%) in the total carbon was found in the T2 treatment followed by T1 > T3. The reduction in TOC can be attributed to the respiration and mineralization of organic compound by *Eisenia fetida* and other microorganism as they use carbon as an energy source (Rini *et al.,* 2020). Similar result was also reported by the Karmegam *et al.,* (2019) in the preparation of vermicompost from the paper industry sludge with cow dung and green manure plant using *Eisenia fetida.*

The NPK components are the main key nutrient content in any organic fertilizers. The suitability of any organic fertilizer was assessed by NPK value as it play an essential role in plant growth. The result shows that the concentration of total nitrogen (TN) significantly enhanced in all the vermicompost than initial organic waste. The initial organic waste contained nitrogen in the range of (7.0-7.9g/kg) (Table 1). After the biotransformation of organic waste by *Eisenia fetida* the level of nitrogen increased 15.71-43.24% and was found in the range of (8.1-11.6g/kg). The highest increase of N content was obtained in T2 treatment and minimum N content in T3 treatment (Table 2). The vermicompost of the T3 treatment is less effective because of the absence of cow dung. Cow dung also plays an essential role in vermicomposting as it enriches the vermibed with the important microbes and nutrients (Negi and Suthar, 2018). The increase in nitrogen may have been due to the action of *Eisenia fetida.* They release excretory products, mucus, hormones, enzymes and decaying of dead earthworms. The pH of the vermibed also affect the concentration of nitrogen as at the neutral pH the content of N remain in the feed stock. However, at the higher pH nitrogen is lost as an ammonia gas (Yadav and Garg, 2011). Pandit *et al.,* (2020) found an increase 2.2 to 4.5 fold nitrogen content in the vermicompost by *Eudrilus eugeniae.*

Another essential nutrient component is phosphorous (P), as it play a vital role in the plant growth. The phosphorus content significantly increase in all the treatments at the end of the process. The content of the P in the initial organic waste found in the range of 1.5-2.8 g/km which increased by 1.91, 2 and 1.6 fold in T1, T2 and T3 treatment respectively at the end of the process. The difference of the phosphorous content from initial organic waste to vermicompost was higher in T2 treatment compared to other two treatments. The enhancement of the phosphorous in all the different treatment may be attributed to the phosphate solubilizing microflora and the two main enzymes i.e. phosphatase and phytase which are present in the gut of the earthworm (Perez- Godinez *et al.,* 2017). These two enzymes are known to play a major role in phosphorous augmentation. Badhwar *et al.,* (2020) reported the value of phosphorous content in the range of 0.86-0.89% in the vermicompost of paper mill sludge and tea waste. Sharma and Garg (2020) also reported phosphorous content increased 1.33-1.56 fold in vermicompost by *Eisenia fetida.*

The total potassium (TK) exhibited an upward trend in all the three different vermicompost bed. The concentration of total potassium in the initial organic waste was in the range of 4.5-5.9 g/kg which significantly increased to 22.03-51.11 % in the three different treatments. At the end of the process, the concentration of TK was found in the range of 5.9-7.0 g/kg. The maximum difference was observed in T2 treatment and minimum in T3 treatment. The enhancement of total potassium in the final product may be correlated to the activity of *Eisenia fetida,* insoluble potassium ion convert into the soluble form by promoting the organic acid. The K content also enhanced after the vermicomposting due to the reduction of organic feed stock during the process. Yadav and Garg (2011) reported 39.5-50% increase potassium content in vermicompost after the recycling of different organic waste.

The C:N ratio is an another important parameter which is used to evaluate the quality of the vermicompost. The ratio of C:N declined in all the treatment at the end of the process than the initial waste. The maximum reduction of carbon/nitrogen ratio was 14 in the T2 treatment followed by 20.52 in T1 treatment and 36.83 in T3 treatment (Table 2). T2 treatment shows 65% reduction from the T1 and T3 which is correlated to the higher degradation of organic waste in the T2 treatment. The decline in the carbon/nitrogen ratio is probably attributed to the respiration, production of CO2, stabilization, enzymatic activity and mineralization of organic matter in nutrient, in *Eisenia fetida.* Few researchers reported that C:N ratio below 20 indicates the acceptable maturity of the bed and the ratio below 15 is an appropriate vermicompost to be used as an organic fertilizer (Kavitha *et al.*, 2010). Abdollahinejad *et al.*, (2020) reported C/N ratio in the range of 17.60-26.20 after the bioremediation of diesel and gasoline contaminated soil by using *Eisenia fetida*.

Reproduction and growth of *Eisenia fetida*

The growth and reproduction of the earthworms during the biotransformation of agricultural waste is beneficial for successful vermicomposting. In this experiment, change in weight of individual *Eisenia fetida*, total number of cocoons, juveniles and adult earthworms were counted in all the treatments at the end of the vermicomposting. Table 3 shows the change in total number of worms, difference in initial and final biomass of earthworms, total number of cocoon production and total number of hatching. The data reveals that the significant difference is observed in all the three different treatments. A highest number of worms were obtained in T2 treatment (510) followed by T1 (412) > T3 (367). There was a 2.5 fold increase in total number of *Eisenia fetida* with 27 cocoon production at the end of the maturity of the vermibed of T2 treatment. Another important parameters such as change in biomass, cocoon production and hatching were also high in T2 treatment in compare to T1 and T3 treatments. This may be attributed to the favorable environment, nutrient content and C:N ratio. Many researchers have reported that the cow dung is the most suitable and essential for earthworms which provide the nutrients, ambient temperature and enhance the vermicomposting process (Balachandar *et al.*, 2020; Deka *et al.*, 2011; Sen and Chandra, 2007).

Microbial analysis

The total microflora of initial and final vermicompost of banana pseudostem scutchers is shown in table 1 and 2. There was a significant increase observed in the total population of microflora after the 115 day of vermicomposting. The initial feed stock of the vermicompost is a very important parameter as it support the growth of earthworms and microorganisms. The treatment T2 containing the ratio 75% scutchers + 25% cow dung shows highest microbial population. The results indicates that *Eisenia fetida* supports the growth of the bacteria, fungi and actinomycetes which enhance the degradation and mineralization of organic agricultural waste. Pedersen and Hendriksen (1993) reported that the degradation of the agricultural waste during the vermicomposting process enhanced the availability of the nutrients which increase the number of germination spores and vegetative cell of the microbes in the gut of the earthworms. Similar results were also reported by the Raphel and Velmourougane (2011) during the vermicomposting of coffee pulp using *Eudrilus eugeniae* and *Perionyx ceylanesis*.

Table.1 Physicochemical and microbiological parameters at initial of the vermicompost from the banana pseudostem scutchers

Treatment	Chemical Parameters							Microbes (cfu x 10^4/g)		
	pH	EC	Total C	Total N	P	K	C:N	Bacteria	Fungi	Acitno mycetes
T1	9.8	2.40	248.2	7.2	2.4	5.1	31.45	28	8	7
T2	9.3	3.45	302.6	7.4	2.8	4.5	40.00	33	12	10
T3	7.8	1.89	318.2	7.0	1.5	5.9	45.42	15	5	6
SE	0.6	0.4	21.22	0.1	0.3	0.4	4.0	5.3	2.0	1.2
SD	1.0	0.7	36.75	0.2	0.6	0.7	7.0	9.2	3.5	2.0

All the values are given in the g/kg except pH, C:N ratio, microbial count and EC (mS/cm)
SE = Standard error, SD= Standard deviation
*Significant (P <0.005)

Table.2 Physicochemical and microbiological properties of final vermicompost

Treatment	Chemical Parameters							Microbes (cfu x 10^4/g)		
	pH	EC	Total C	Total N	P	K	C:N ratio	Bacteria	Fungi	Acitno mycetes
T1	7.8	4.28	188.8	9.2	4.6	7.0	20.52	39	10	11
T2	7.5	7.89	148.5	10.6	5.8	6.8	14.00	58	14	17
T3	7.6	2.75	298.4	8.1	2.5	7.2	36.83	17	8	9
SE	0.08	1.524	44.79	0.7	0.9	0.1	6.7	11.85	1.7	2.4
SD	0.1	2.6	77.57	1.2	1.6	0.2	11.76	20.52	3.0	4.1

All the values are given in the g/kg except pH, C:N ratio, microbial count and EC (mS/cm)
SE = Standard error, SD= Standard deviation
*Significant (P <0.005)

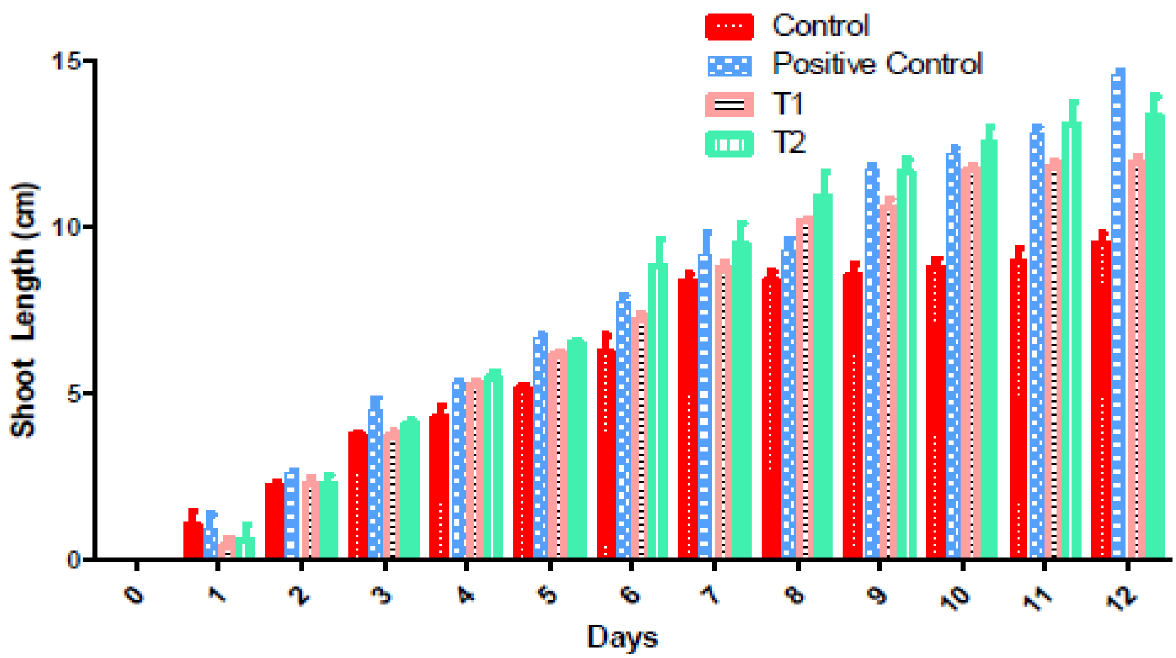
Table.3 Total number, growth, and reproduction of the *Eisenia fetida*

Treatment	Total number		Initial mass of *E. fetida* (mg)		Final mass of *E. fetida* (mg)		Total No. of hatching after 115 days	No. of Cocoon after 115 days
	Initial	Final	Juvenile	Adult	Juvenile	Adult		
T1	200	412	73±3	235±10	155±8	435±8	212	11
T2	200	510	75±3	246±9	181±5	494±4	310	27
T3	200	367	73±2	237±11	134±8	360±2	167	8

All the results are mean of two replicates

Fig.1 The conversion of banana pseudostem waste in to the vermicompost

Fig.2 The growth index of the *Vigna radiata*



Seed germination assay

The maturity and phytotoxicity of the vermicompost was examined by the seed germination assay. Four different treatments were used in seed germination test and germination index (GI) was calculated. Fig. 2 shows the growth of the *Vigna radiata* in different treatments. After the 12th day of the germination, the maximum height of the shoot was observed in the commercial vermicompost followed by vermicompost of T2 treatment > vermicompost of T1 treatment > control. In this study, growth index of vermicompost of T2 treatment is 1.25 time higher than the control. The length of the leaves from vermicompost of T2 was 1.2 time longer than the control. The dry weight of the leaves in vermicompost of T2 treatment was 2 and 1.4 time heavier than the control and T1 treatment. The maximum root length (12.9 cm) was observed in the T2 treatment which is 5.1 and 1.2 time longer than the control root length and T1 vermicompost root length. On the basis of the seed germination assay it can be concluded that the prepared vermicompost is effective and suitable for the agricultural use.

The present study was performed to explore the use of banana pseudostem waste (scutchers) into vermicompost. Present study proved that the banana pseudostem and cow dung are good substrate for the vermicomposting process. The growth and reproduction rate of the *Eisenia fetida* was vary with the different combination of the banana pseudostem scutcher and cow dung. The best result was found in T2 treatment (75% banana pseudostem scutcher + 25% cow dung) which clearly support the quality of the initial feed stocks. However greater concentration of the banana pseudostem scutcher shows the adverse effect on the growth and reproduction of the *Eisenia fetida*. The results indicate that the cow dung is an essential for the better growth, reproduction and biotransformation by the *Eisenia fetida* during the process. The higher NPK content and lower C:N content was observed in the final vermicompost than the initial feed stock. The maturity and phyto-toxicity of the vermicompost was proved by the seed germination test using *Vigna radiata*. There was no significant difference observed between the growth of the *Vigna radiata* in commercial vermicompost and vermicompost of T2 treatment. The physicochemical changes of the vermicompost prepared from the banana pseudostem scutchers indicates the enrichment in the nutrition value and quality of the compost and it enhanced the soil fertility and good source for agricultural use.

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