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Enzymatic Analysis and Effect of Vermicompost Production from Banana Leaves Waste Using Epigeic Earthworm *Eudrillus euginea*

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ABSTRACT

Recycling of organic waste with enormous nutrients gives soil fertility when added to the soil and reduce the usage of chemical fertilizers. Vermicomposting process of recycling the bio-organic waste by earthworms contains efficient nutrients with low levels of toxic contents. In the present study, banana leaves waste was mixed with cow dung in different ratios. The earthworm *Eudrillus euginea* was later added to the compost. Total organic matter (TOM), total organic carbon (TOC), pH, EC, total alkalinity and phosphorus were analysed and compared for different ratios of vermicompost at different day intervals. Microorganisms present in vermicompost were isolated and analysed for their enzymatic activities. Compost maturity was also observed by FTIR application. Results showed that the vermicompost quality was improved with positive catalase and protease enzymatic activity. FTIR analysis showed the proper maturation of vermicompost with reduced complex structures such as aromatic compounds, polysaccharides and polypeptides. It was concluded that composting of organic wastes by vermicomposting promote humification, increased microbial activity and enzyme production, which in turn increase the aggregate stability of soil particles resulting in better aeration with pollution-free technique.

INTRODUCTION

Organic wastes such as agricultural waste and household waste are useful raw materials when recycled. Even though these wastes are disposed off by landfill or incineration, the waste cannot be stabilized and could be pathogenic (Hill et al. 2013). When disposing of the waste through landfill, there may be a chance of landfill leachate often containing toxic compounds that can leach into groundwater contaminating drinking water. Incineration if done in an improper way can create air pollution and also produces a small amount of ash that should be again landfilled. Vermicomposting is the best-known process for biological stabilization of organic wastes (Domínguez et al. 2010). Vermicomposting gives a product without any waste, which in turn increase the yield of produce.

Vermicomposting is the simple biotechnological process that utilizes earthworms to give a finely divided, peat-like material with high porosity. It also has excellent structure and good moisture-holding capacity (Singh et al. 2010). Due to its numerous biological properties, vermicomposting is used to promote sustainable agriculture by enhancing the growth of plant growth promoting bacteria in the soil and controls the plant pathogen (Chauhan & Singh 2015). Vermicompost, therefore, can be effectively used as a carrier medium for *Azospirillum, Rhizobium* and phosphate solubilisers. Traditionally vermicompost is prepared with animal manure as the main substrate and when applied to the soil acts as a good soil conditioner and fertilizer. In recent years organic waste like banana leaves is found to be the good manure vermicompost (Raj et al. 2017). This highly nutritious and easily digestible fruit contains high levels of potassium and calcium content. Banana leaves were used as a primary writing surface in southeast Asia during ancient days. In countries like Malaysia, India and Vietnam, banana leaves are used to wrap food to seal its freshness. In terms of medicinal value, banana leaves contain polyphenols and polyphenol oxidase, an enzyme that produces L-DOPA, used in the treatment for Parkinson's disease (Ethiraj et al. 2018).

Earthworms processing the compost are naturally terrestrial soft bodied animals with uniform ring-like structures. During dry weather, earthworms aerate the soil and help in maintaining the moisture through supply of organic matter. Epigeics earthworm species are phytophagous that live above the mineral soil surface. They have greater potential in decaying the organic waste (Singh et al. 2010). During vermicompost production, studying the enzymatic activities are most important to check the soil fertility and ecosystem status. They are also used to describe the fast driven process of vermicomposting. Moreover, microbes are the primary decomposers in composting. Microbial enzymes play an important role in making the compost more effective (Lakshmanan & Muthunarayanan 2016). In considering all the factors, the present study was done to check the possibility of converting natural Banana leaves wastes *(Musa paradisiaca)* into value-added vermicompost using epigeic earthworm *Eudrillus euginea,* to check the nutrient value of the vermicompost in terms of total organic matter (TOM), total organic carbon (TOC), pH, EC, total alkalinity and phosphorus, to isolate and identify the bacteria present in the vermicompost, to qualitatively check the enzyme (protease and catalase) activity of the isolated bacterial strains and to detect the structural changes of the vermicompost produced from banana leaves using FTIR.

MATERIALS AND METHODS

Collection of organic wastes and earthworm: Cow dung was collected from a dairy yard at an Agricultural field near Sooriyur, Trichy. Banana (*Musa paradisiaca*) leaves waste was collected from Trichy district near Woraiyur. The epigeic earthworms *Eudrillus euginea* were obtained from Periyar Maniyamai University, Tanjore.

Experimental set-up: Plastic tub containers suitable for aeration were taken. Banana leaves were chopped into small pieces and cow dung was then mixed with banana leaves in three different ratios (500g:500g), (400g:600g) and (600g:400g) respectively. Soil with banana leaves was taken as a control in the ratio of 500g:500g. After 15 days of decomposition, 50 non-ciliated earthworms were introduced into vermicompost plastic containers. The moisture content was maintained at 60-80% throughout the study period by periodic sprinkling of adequate quantities of water. The containers were kept in the dark under identical ambient conditions (room temperature 25 ± 3°C). The present study followed the method described by Gopal et al. (2009) with few modifications.

Physico-chemical analysis: The physico-chemical parameters such as pH, electrical conductivity (EC), total organic carbon (TOC), total organic matter (TOM) and phosphorous were analysed as per Boran et al. (2017).

Isolation of microorganisms from vermicompost samples: The isolation and identification of microorganisms from the manure sample was done by the spread plate technique. To 99mL of the sterile distilled water, 5g of the manure sample was added. From this, 1mL was transferred aseptically to 9mL of sterile distilled water to obtain 10^1-10^6 dilutions and 1mL from each dilution was transferred aseptically into sterile Petri plates. Nutrient agar medium was used to estimate the total heterotrophic bacteria. The plates were further subjected to incubation at 30°C ± 2°C for 24hrs for the isolation of bacterial colonies. After confirming the organism by the zone formation by qualitative analysis, the isolated colonies were subjected for catalase and protease enzymatic activity as it was mainly involved in the degradation of biological wastes. Proteolytic activity was identified by a clear zone around the cells as reported by Naik et al. (2008). To determine the catalase activity, the sample was inoculated with simple streaked and the plates incubated at 37ºC for 24hrs. Then hydrogen peroxide was added to each plate, which suddenly gets fumes in the plates (Iwase et al. 2013).

FTIR: The FTIR spectroscopy provides information about the chemical structure of the material analysis of the composting process. It is a reliable technique for compost maturity determination. Several indicator bands in the spectra such as functional groups represent the components or metabolic products present which indicate the degradation or stabilization process. The FTIR spectra of vermicompost obtained from banana leaves waste was compared for initial and final stage as described by (Bhat et al. 2017).

RESULTS AND DISCUSSION

The vermicompost has been prepared with cow dung and different quantities of banana leaves waste, along with the earthworm population. The compost was analysed for the parameters such as pH, EC, TOC, TOM and phosphorus.

Variation in pH: The pH is one of the most frequent parameters used to characterize the vermicompost quality. The pH range of 6.0-8.5 is found to be suitable for the soil in order to ensure compatibility with most plants (Cerozi & Fitzsimmons 2016). Slight changes in pH were noted in three different ratios with control as shown in Fig. 1. The pH shift was due to mineralization of nitrogen and phosphorus into nitrates and orthophosphate (Jalali et al. 2014).

Variation in EC: The EC values were increased significantly in final vermicompost as shown in Fig. 2. Initial decline was due to loss of organic matter and release of different mineral salts in available forms such as phosphate, ammonium, potassium, etc. (Mahaly et al. 2018).

Variation in phosphorus: Total phosphorus was greater in 60 days vermicompost than in the initial (20 days) as shown

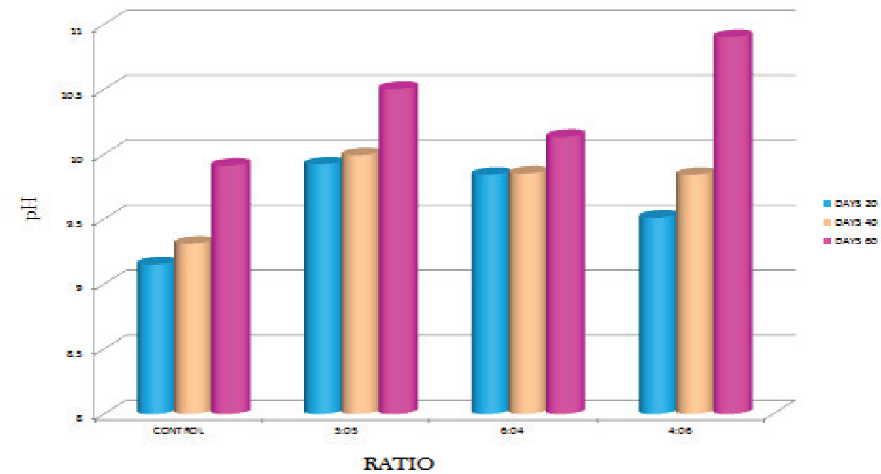

Fig. 1: Comparison of pH during different stages of vermicomposting.

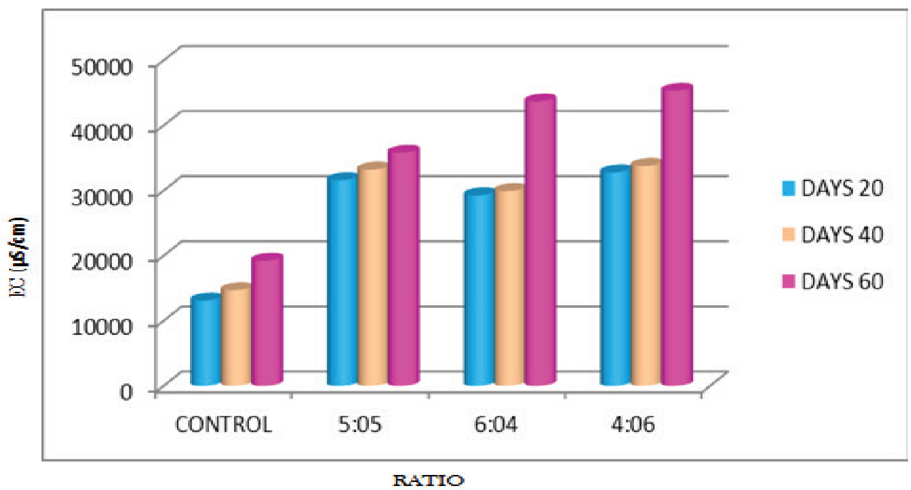

Fig. 2: Comparison of electrical conductivity during different stages of vermicomposting.

in Fig. 3. Total phosphorus content of banana leaf waste after earthworm activity was performed partly by earthworm gut phosphates and further release was attributed by the phosphate solubilizing microorganisms present in worm casts (Ramnarain et al. 2019).

Variation in TOC: The total organic carbon (TOC) in banana leaves waste reduced when compared to their initial levels as shown in Fig. 4. Loss of TOC was due to its removal in the form of CO_2 during vermicomposting. The earthworms are responsible for such modification to promote the carbon losses from the substrate through microbial respiration in the form of CO_2 (Aira et al. 2007).

Variation in TOM: The decomposition of organic matter reduces the amount of TOM as shown in Fig. 5 but leaves the compost enriched with nitrogen at 60^{th} day where cow dung was mixed in high ratio with banana leaves waste. Many such wastes were readily decomposed by soil microbes with release of end products such as carbon dioxide and water. The results are in accordance with the study done by Kaushik & Garg et al. (2004) with the solid textile mill sludge that acts as an excellent substrate when mixed with cow dung.

Enzyme activity: The isolated microorganisms were analysed for their enzyme activity. The organisms showed pro-

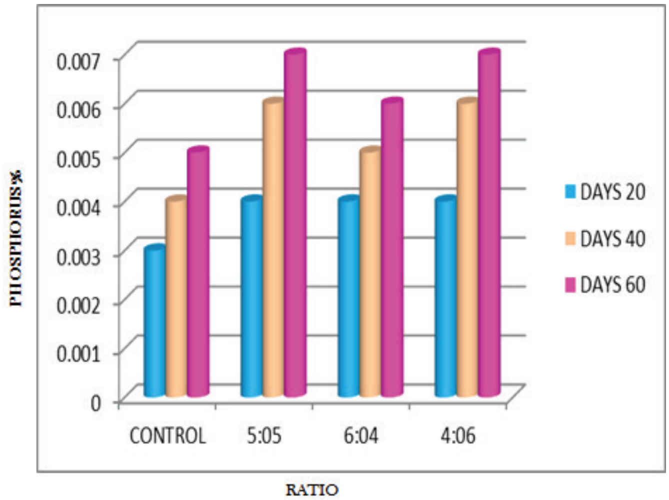

Fig. 3: Comparison of phosphorus during different stages of vermicomposting.

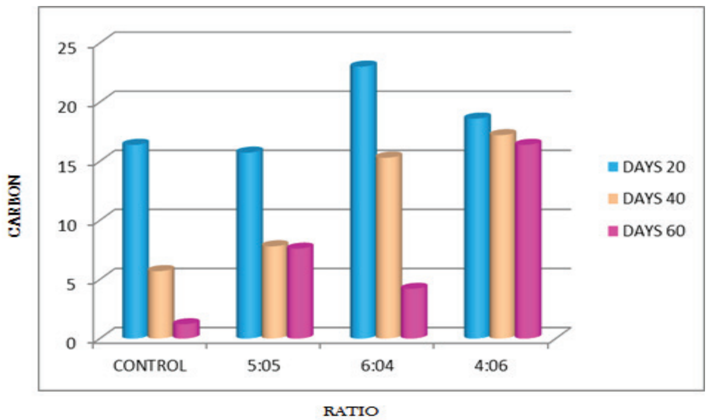

Fig. 4: Comparison of total organic carbon during different stages of vermicomposting.

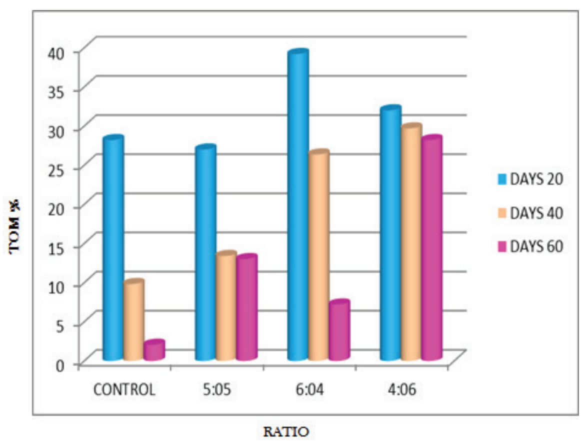

Fig. 5: Comparison of total organic matter during different stages of vermicomposting.

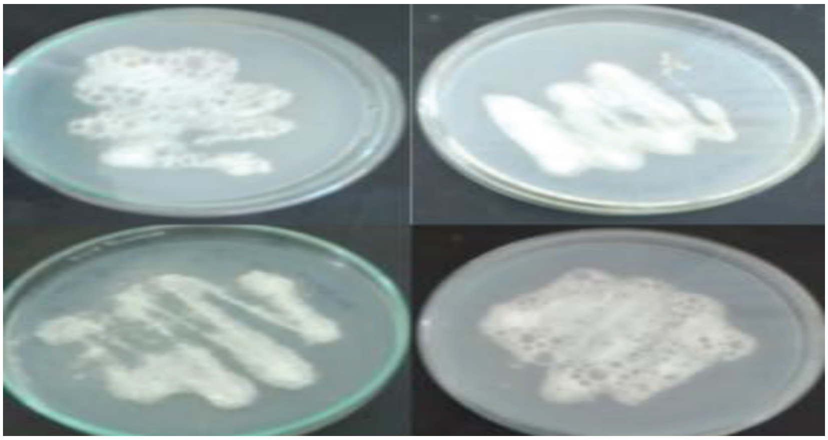

Fig. 6: Isolation of catalase producing bacteria from Vermicompost.

Fig. 7: Isolation of protease producing bacteria from vermicompost.

tease and catalase activity as shown in Figs. 6 and 7. Microorganisms are the primary decomposers to favouring the composting, which has increased enzymatic activity and results in the digestion of soil organic matter. Humic like substances present in the vermicompost are usually rich in microbes with high enzymatic activity (Arslan Topal et al. 2008).

FTIR: FTIR spectra for initial and final stages of banana leaves waste vermicompost samples is shown in Fig. 8. By comparing the initial and final stage there was a strong hydrogen bond due to -OH stretch was observed at 3423.70 cm^{-1} and 3417.90 cm^{-1}. There was the partial mineralization of cellulose material. The phenolic compounds were observed at 1091.90 cm^{-1} and 1093.22 cm^{-1} respectively. Several other bonds at peak range of 790.08 cm^{-1}, 791.46 cm^{-1}, 691.72 cm^{-1}, 693.02 cm^{-1}, 538.44 cm^{-1}, 539.96 cm^{-1}, 466.12 cm^{-1} and 466.26 cm^{-1} were observed. While comparing the initial and final vermicompost produced by *Eudrillus euginea,* slight increase in bands were observed when initial vermicompost was subjected to FTIR. Hence, the result

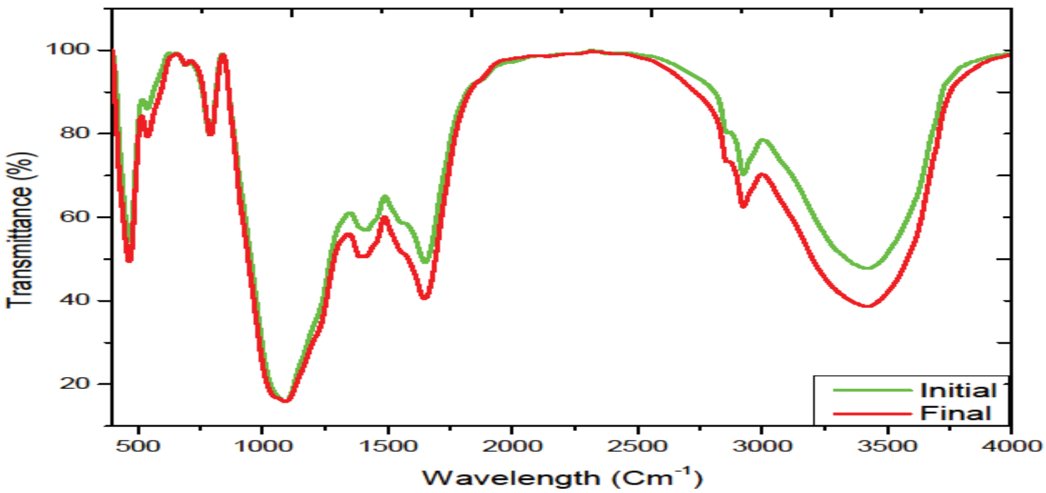

Fig. 8: FTIR Spectrum for initial and final stage of banana leaves waste vermicompost.

confirms the reduction of aromatic structure, polypeptides and polysaccharides in the vermicompost prepared from banana (*M. paradisiaca*) leaf waste and proves the maturity of compost. Results are aligning with study report by Sentil Kumar et al. (2013), where the vermicompost showed maturity with increased nitrogen and reduced mineral activities. They studied the vermicompost produced from flower waste inoculated with biofertilizers.

CONCLUSION

The present study demonstrates the role of earthworms and microorganisms during the degradation of banana leaves waste. The role of bacterial species in terms of their enzyme activity was highlighted. Cow dung mix with banana leaves waste in ratio of 6:5 yields good results when compared to control and other samples. FTIR results show the reduction of both aliphatic and aromatic structures in the vermicompost which is associated with extensive organic matter mineralization. Genomic DNA isolation for identification of bacterial species that possess enzymatic activity has to be done for future research. Hence, the present study concludes that the banana leaves waste generated could be managed by converting the waste into a good quality manure using earthworms by the process of vermicomposting.

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