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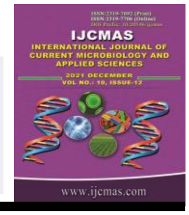

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Effect of Biochar Amendment on Vermicomposting of *Parthenium hysterophorus* Biomass

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A 15 day precomposted biomass of *Parthenium hysterophorus* in combination with cow dung (1:1, wt./wt.) was vermicomposted along with biochar amendment at different concentrations (0, 2, 4, 6 and 8%) using the earthworm, *Eudrilus eugeniae* for 50 days. The change of physicochemical parameters such as pH, electrical conductance (EC), total Kjeldahl nitrogen (TKN), total phosphorus (TP), total potassium (TK), total organic carbon (TOC), C/N ratio and C/P ratio for the initial substrates, control (final substrate maintained without earthworms) and vermicompost was analyzed. Besides, the level of total microbial population, *viz.*, bacteria, fungi and actinomycetes were performed using the plate dilution technique. The results revealed that the treatment with 4-6% biochar enhanced the EC, total NPK and total microbial populations considerably in comparison with control substrates after 50 days. The 4% biochar amendment showed a maximum of 2.46% TKN followed by the 6% biochar amendment (2.25% TKN). The pH, TOC, C/N and C/P ratios in the vermicomposts of all the treatments were lowered from the initial levels with a maximum decline in biochar amendments. The initial C/N ratio of the substrates was ranged from 34.27 to 58.28 while the final range was found between 12.48 and 17.33. The total microbial population of bacteria, fungi and actinomycetes in the final vermicompost was intensified in all the treatments. The findings of the present study reveal that the biochar amendment (4-6%) to vermicomposting of *Parthenium hysterophorus*+cow dung (1:1) improves the nutrient and total microbial population.

Introduction

The commercially and ecologically sustainable management of solid wastes is a great challenge worldwide. Industrial sludge disposal technologies adopted around the world include landfilling, land spreading, incineration, thermal drying, lime stabilization and vermicomposting (Singh *et al.*, 2020). Over the last few years, the problem of efficient disposal and management of organic solid wastes has become more rigorous due to rapidly increasing population, intensive agriculture and industrialization. Production of large quantities of organic waste all over the world poses major environmental (offensive odors, contamination of groundwater and soil) and disposal problems. Although various physical, chemical and microbiological methods of disposal of organic solid wastes are currently in use, these methods are time-consuming and involve high costs (Garg *et al*., 2006).

Each year, humans, livestock and crops produce approximately 38 billion metric tons of organic waste worldwide. The safe disposal and environmentally friendly management of these wastes have become a global priority. In India, the amount of waste generated per capita is estimated to increase at a rate of 1–1.33% annually. In such conditions, the total waste quantity generated in 2047 would be approximately above 260 million tons, more than five times the present level. This enormous increase in solid waste will have significant impacts in terms of the land required for disposing of this waste as well as on methane emission (Suthar, 2009).

Apart from these facts, the growth of weeds is posing a threat to the native biodiversity. Problematic weed can rather be used as a base material for composting to enhance crop growth and for environmental benefits like reducing water pollution, use of chemical fertilizers and greenhouse gas emissions (Chung *et al*., 2021; Ravindran *et al*., 2021; Thangamani *et al*., 2019). Several weeds have been successfully utilized as substrates for composting and vermicomposting, including *Ageratum conyzoides, Eichhornia crassipes, Lantana camara, Ipomoea carnea, Ipomoea staphylina, Salvinia molesta*, etc., employing epigeic earthworms (Balachandar *et al*., 2021; Thangamani *et al*., 2019). Recent studies on bioconversion of biomass wastes into benignant materials like compost and vermicompost incorporating biochar are given importance due to the enhanced compost qualities (Balachandar *et al*., 2021; Chung *et al*., 2021). However, the effect of the addition of biochar to weed biomass vermicomposting is least reported. Hence, the present study has been aimed to amend the biochar in different concentrations along with the vermicomposting substrates (*Parthenium hysterophorus* biomass + cow dung, 1:1) using the epigeic earthworm, *Eudrilus eugeniae.*

Materials and Methods

The weed *Parthenium hysterophorus* was collected from in and around the college campus and washed with tap water for excess moisture removal. The plant biomass was chopped into 3-4 cm pieces and shade dried for a week. Fresh cow dung was procured from adjacent cattle shed and shade dried for a week and used for the study. The biochar of *Prosopis* wood was obtained from the local vendor, powdered and used for the amendment to the vermicomposting substrate. The plant biomass and cow dung were mixed in a 1:1 ratio on a dry weight (wt./wt.) basis for vermibed preparation as recommended by Thangamani *et al*. (2020). The substrate mixtures were subjected to 15 days of precomposting by maintaining 40-50% moisture content. The substrate mixtures were added with different concentrations of biochar (0, 2, 4, 6 and 8%) as shown in Table 1, before precomposting.

The precomposted substrate mixtures were filled in uniform plastic containers and watered to hold 70-80% moisture and allowed for 24 h stabilization. Then, 20 mature clitellate earthworms (*Eudrilus eugeniae*) were added to each experimental set-up. For each experimental set-up, triplicates were maintained. The experimental sets were kept in a dark room with a temperature of 27 ± 2 °C. The moisture content in the substrates was maintained by adding water when needed until the end of the vermicomposting, i.e., 50 days. A separate experimental set for each treatment without earthworms was maintained as a control.

Table.1 Experimental design showing the proportion of vermibed substrates

Exp. No.	Treatment mixture (wt/wt)			Biochar (%)
	Parthenium hysterophorus	Cow dung	Ratio	
BP1	50	50	1:1	0
BP2	50	50	1:1	2
BP3	50	50	1:1	4
BP4	50	50	1:1	6
BP5	50	50	1:1	8

The initial (0 day), final control substrate and vermicompost were taken and processed for analyzing the physicochemical parameters, pH, electrical conductivity (EC), total Kjeldahl nitrogen (TKN), total organic carbon (TOC), total phosphorus (TP) and total potassium (TK). The analyses were performed in the laboratory adopting the standard procedures (Jackson, 1973; Tandon, 1993; Walkley and Black, 1934). The C/N ratio and C/P ratio were calculated with the values obtained for TKN and TP with that of the TOC content, respectively. The results obtained are expressed as mean ± standard error.

Results and Discussion

The physicochemical characteristics such as pH, TOC, C/N ratio, C/P ratio in the final substrates (50 days) of all the treatments showed a decline, while EC, TKN, TP, and TK contents were elevated. The level of pH in the initial substrates decreased where the decrease was found to be higher in earthworm introduced substrates in comparison to the substrates without biochar and control (Fig. 1A). The EC of the substrates elevated from the initial levels and attained a maximum of 2.20 dS/m in BP3 followed by BP5 (2.19 dS/m). Overall, the level of enhancement of EC was higher in earthworm introduced and biochar amended treatments than the treatments without biochar and *Eudrilus eugeniae* (Fig. 1B). Earlier reports on the changes in pH and EC are in line with the findings of the present study (Karmegam *et al*., 2021; Paul *et al*., 2020).

The TOC contents showed a reduction from the initial levels in control and vermicompost in most treatments where the reduction was maximum in biochar and earthworm introduced substrates with biochar (Fig. 1C). As can be seen from Fig. 1D and Fig. 1E, the ratios of C/N and C/P greatly lowered in biochar amended vermicomposts, indicating that the action of earthworms and biochar amendment played a major role in the biotransformation of *Parthenium hysterophorus* biomass. The increase of nutrients and the decrease of C/N and C/P ratios were due to the change in TOC status of vermicompost due to the combined activity of the earthworms and microorganisms (Biruntha *et al*., 2020; Karmegam *et al*., 2019).

The nutrient contents such as TKN, TP and TK are elevated during the vermicomposting process in general as a result of organic matter decomposition (Singh *et al*., 2020). In the present study also, the nutrient contents raised in all the treatments with earthworms and biochar considerably (Fig. 2). However, much more improvement in TKN (2.46%), TP (1.46%) and TK (2.23%) contents were confined to 4% biochar amendment. This indicates that the 4% biochar amendment to 1:1 vermicomposting substrates is suitable for the bioconversion of *Parthenium hysterophorus* biomass in combination with cow dung into nutrient-rich vermicompost using *Eudrilus eugeniae*. The addition of N in the form of mucous, excretory substances which were not initially present in feed substrates has been reported (Balachandar *et al*., 2021; Rini *et al*., 2020; Singh *et al*., 2021). The higher percent increase of NPK in vermicompost produced by *Eudrilus eugeniae* than in the control in this study might be attributed to the mineralization process caused by earthworm action, along with micro-organisms on organic materials due to the presence of cow dung and nutrient conservation in presence of biochar (Paul *et al*., 2020; Yuvaraj *et al*., 2021).

Fig.1 (A) pH, (B) EC, (C) TOC, (D) C/N ratio and (E) C/P ratio of initial substrate mixture, control (treatment without earthworms after 50 days), and vermicompost (treatment with earthworms after 50 days). Error bars indicate ± SEM (Refer Table 1 for treatment details).

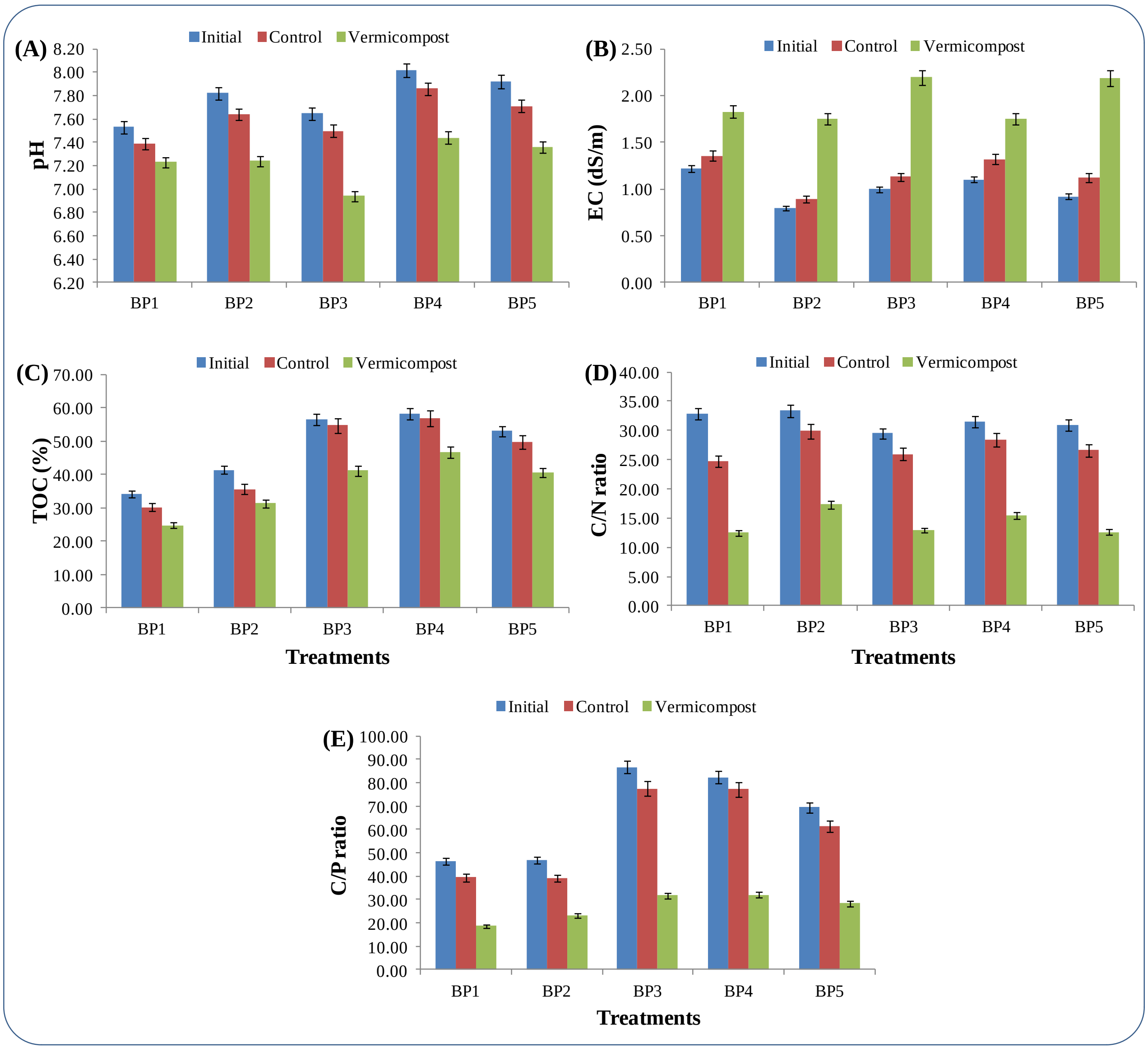


Fig.2 Nutrient contents in (A) initial substrate mixture, (B) control (treatment without earthworms after 50 days), and (C) vermicompost (treatment with earthworms after 50 days). Error bars indicate ± SEM (Refer Table 1 for treatment details).

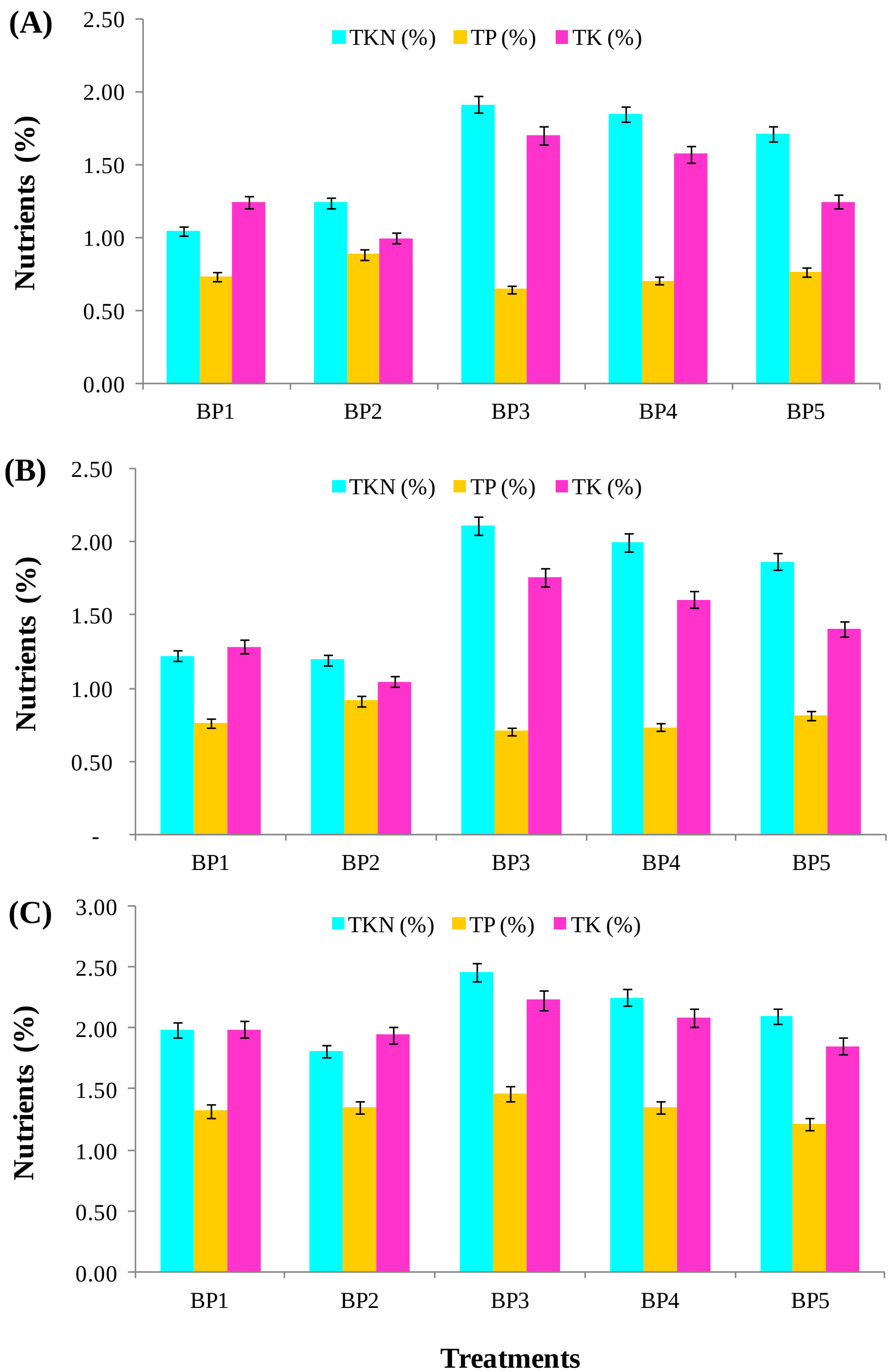
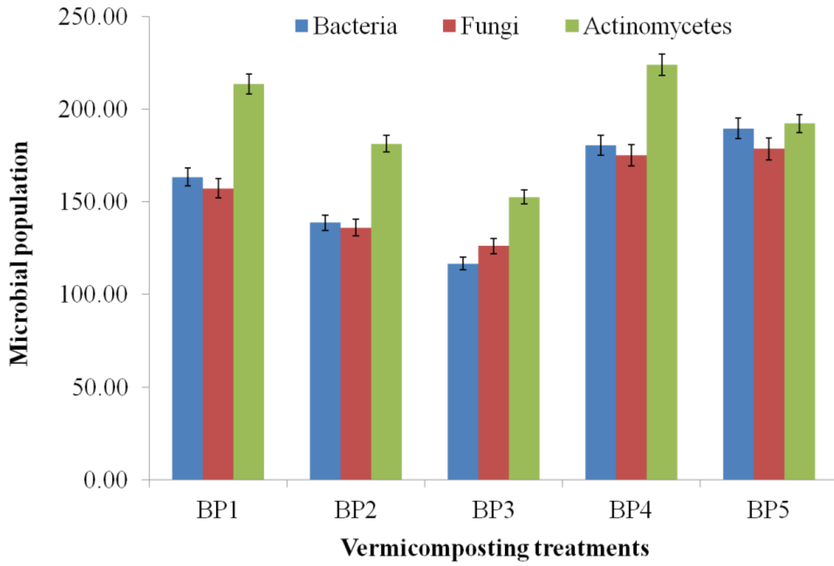


Fig.3 Total microbial population in vermicomposts of *P. hysterophorus* and biochar combinations (60 days). Bacteria = cfu x 10^6; Fungi = cfu x 10^4; Bacteria = cfu x 10^3; Error bars indicate ± SEM (Refer Table 1 for treatment details).



The results on the total microbial population in the vermicompost analyzed in initial substrates and vermicompost showed an increase at the end of the vermicomposting process (Fig. 3). The total microbial population (bacteria, fungi and actinomycetes) present at the end of the experiment was considerably higher than the microbial population present at the start of the experiment. This observation is supported by Prakash and Karmegam (2010). While studying the vermicomposting of mango leaf litter using the same earthworm species, *Perionux ceylanensis*, Prakash and Karmegam (2010) reported that the total bacterial population recorded during vermicomposting were 51, 64, 99, 116 and 132 × 10^6 CFU g^{-1} on 0, 15, 30, 45 and 60 days, respectively. A similar trend of increase of fungi and actinomycetes was observed during vermicomposting of a weed, *Ipomoea staphylina* (Balachandar *et al.*, 2021). These studies well corroborated the findings of the present study results on microbial status.

In conclusion, the amendment of biochar to vermicomposting of the substrates, *Parthenium hysterophorus*+cowdung (1:1) showed beneficial traits of the end product, the vermicompost. The C/N ratio reduction to agronomically useful organic compost obtained in the present indicates the maturity of the final vermicompost. The data regarding the analyses of nutrients and microbial population obtained in the present study reveals that the action of earthworms enhances the nutrients and total microbial population which was higher in vermicompost than recorded in the control (worm un-inoculated). The elevated levels of TKN, TP and TK in vermicompost in presence of biochar at the rate of 4-6% demonstrate that the addition of biochar at such proportion is helpful to produce nutrient-rich vermicompost. The mechanisms of physicochemical and microbial population enrichment are needed to be explored for further application of biochar in vermicomposting of all kinds of biowastes.

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