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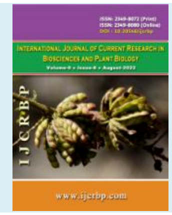

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Effect of biochar amended *Parthenium hysterophorus* vermicompost on germination and seedling growth of maize

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Abstract

Vermicompost is a soil conditioner which improves crop growth and soil physicochemical characteristics. Currently, the nutrient status of vermicompost has been revealed to be enhanced by the addition of biochar. In the present study, biomass of *Parthenium hysterophorus* (15 days predecomposed) + cow dung (1:1, wt./wt.) was vermicomposted with biochar amendment at the rates of 0, 2, 4, 6 and 8% utilizing *Eudrilus eugeniae* for 50 days. The resultant vermicompost was tested for its efficiency through seed germination assay, seedling growth, and chlorophyll content analysis. The germination percentage of maize was found to be higher in 4% biochar amendment followed by 6% biochar incorporated vermicompost ($P<0.05\%$). Overall maize seedling showed variation in shoot length, root length, fresh weight, and dry weight in the vermicompost amended with 0, 2, 4, 6 and 8% biochar; however, these attributes were higher in 4-6% biochar amended vermicompost of *Parthenium* biomass. Moreover, the chlorophyll content of maize seedlings showed increment proportionately with that of the biochar amendment.

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Introduction

Vermicomposting refers to the process of decomposing organic waste using earthworms to produce vermicompost. Earthworms convert decaying organic matter into worm castings during vermicomposting, also known as worm composting. Earthworms play a vital role in decomposing organic waste in this natural system. It is called vermicomposting or vermistablization when earthworms are used to manage organic wastes (Edwards and Bohlen, 1996). Various organic waste materials are collected from earthworms to produce vermicompost, which can be used as a fertilizer for crops (Jayakumar et al., 2011; Singh et al., 2020). Vermicomposting is a common practice of converting vast array of organic wastes ranging from domestic and municipal wastes to toxic industrial wastes (Jayakumar et al., 2022; Karmegam et al., 2019; Prakash and Karmegam, 2010). According to the nature of raw materials used for vermicomposting, the nutrient contents in vermicomposts show great variation. For vermicomposting complex organic wastes, cow dung is added as a bulking material which also serves as initial nutrients for microbial and earthworm growth resulting in nutrient rich vermicompost (Yuvaraj et al., 2021).

Moreover, different materials are incorporated to enrich the quality of final product of vermicomposting to be used as soil conditioner for crop growth and yield. The green manure plants such as *Gliricidia sepium*, *Tephrosia purpurea* and *Sesbania* species are amended along with the vermicomposting substrates for enhancing the quality of vermicompost (Balachandar et al., 2020; Jayakumar et al., 2022; Karmegam et al., 2021).

Biochar derived from different substrates is used as composting and vermicomposting amendment material due to its beneficial properties. Carbon and ashes remain after biomass is pyrolyzed to form biochar, a lightweight black residue (Brewer and Brown, 2012). Using biochar as a carbon storage and capture medium is based on its refractory properties, that is, it may be possible to mitigate climate change through the use of biochar for carbon sequestration (Yousaf et al., 2017). It is possible to increase agricultural productivity by using biochar on soils.

Interestingly, biochar amendment in composting substrates has improved the composting process and compost characteristics. For instance, microorganisms are reported to thrive in biochar, and biochar may directly or indirectly affect changes in microorganism populations, affecting heavy metal bioavailability and the addition of biochar along with microbial inoculum reduces the bioavailability of heavy metal in pig manure composting (Wu et al., 2022). By adding wood vinegar along with biochar, the composting process is faster, and greenhouse gas emissions are reduced by 61.9%. Nitrogen loss is also decreased by 35.8% and carbon loss is decreased by 15.5% when using biochar and wood vinegar (Wang et al., 2022). The amendment of 5% biochar, 0.5% lactic acid and 20% pond sediment was found to be optimum for green waste composting which increased the organic matter decomposition rate by 157% relative to the control (Feng and Zhang, 2022). The combination of potting medium with peat: perlite: biochar: vermicompost at 30:30:35:5 by volume had improved the growth of *Alpinia zurumbet* (Zulfiqar et al., 2021). However, the studies on the amendment of biochar along with vermicomposting substrates and the effect of biochar amended vermicompost on crop plants are very limited. Hence the present study has been undertaken to find out the influence of biochar amended *Parthenium* vermicompost on seed germination, growth and chlorophyll contents of maize seedlings.

Materials and Methods

Preparation of vermicompost

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% (V1), 2% (V2), 4% (V3), 6% (V4) and 8% (V5)].

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.

The 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.

Seed germination bioassay using soil test method

The seeds of maize (*Zea mays* L.) were selected for the present study. The seeds of *Zea mays* were individually soaked in 2% mercuric chloride solution and then immersed in distilled water for 24 h. The plastic cups (germination test container) of uniform size were used for seed germination tests. Red soil and sand (1:1, volume/volume) were mixed thoroughly and 60 g of this mixture was taken in plastic cups to test. The aqueous extract concentrations (15 ml each) of 0, 15, 30, 45 and 60% were and added to respective treatments, separately for each concentration. The seeds were sown in five replicates of ten seeds each. The experiments were conducted in five replicates.

Vermicompost treatments

The vermicompost for the present study was procured from the local market. The plastic cups were filled with Red soil:sand:vermicompost (1:1:1, volume/volume) were mixed thoroughly and 60 g of this mixture was taken in plastic cups to test the ameliorative effect of vermicompost on allelopathic activity of *Lantana camara* aqueous leaf extracts with maize. The plastic cups were watered and the seeds were sown in five replicates of ten seeds each.

All the treatment sets were kept in an environmentally controlled room and observed for germination every day up to seven days. After seven days, the seedlings were carefully removed from the culture container and gently washed to remove adhering soil. The root length, shoot length, fresh weight and dry weight of the seedlings were measured and recorded.

Germination percentage

The germination percentage of maize in all treatments was calculated as per the method described in UAF (UAF, 2010).

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds tested}} \times 100$$

Germination Index (GI)

The germination index for maize was determined using the following formula (AOSA, 1983).

$$\text{Germination Index (GI)} \quad GI = \Sigma\left(\frac{GT}{Tt}\right)$$

$$\text{Or} \quad \left[\frac{\text{No.of germinated seed}}{\text{Days of final or last count}}\right] + \ldots\ldots + \left[\frac{\text{No.of germinated seed}}{\text{Days of final or last count}}\right]$$

Chlorophyll content

The analysis of total chlorophyll content of 20 days old maize seedlings was carried out according to Arnon's (Arnon, 1949). One gram of fresh leaves were blended and then extracted with 10ml of 80% acetone and left for 15 minutes for thorough extraction. The liquid portion was decanted into another test tube and centrifuged at 2500 rpm for 3 minutes. The supernatant was then collected and the absorbance was taken at 670 nm and 690 nm using Systronics Spectrophotometer and the chlorophyll contents were calculated and expressed as µg/g.

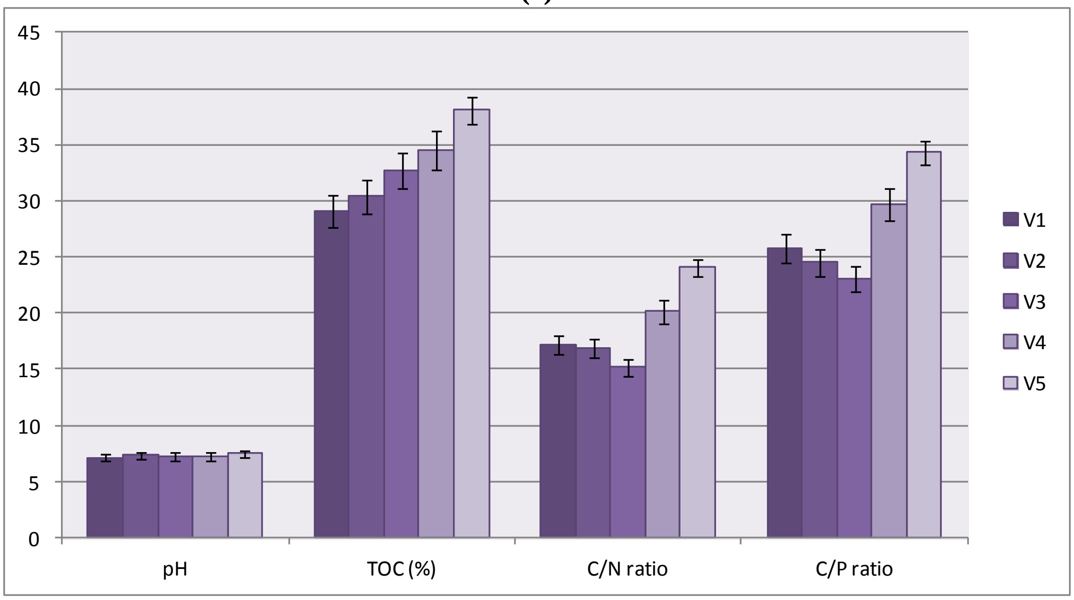
Results and discussion

The physicochemical characteristics of the vermicomposts of *Parthenium* weed with different proportions of biochar (0, 2, 4, 6 and 8%) are shown in Fig. 1. The values of pH, TOC, C/N ratio and C/P ratio of V3 vermicompost was 7.22, 32.71%, 15.21 and 23.04, respectively while it was 7.26, 34.45%, 20.15 and 29.70 in V4 respectively (Fig. 1a). The contents of TKN, TP and TK including EC showed variations with reference to the proportion of biochar amendment in vermicompost. The TKN content in V1, V2, V3, V4 and V5 respectively was 1.69, 1.8, 2.15, 1.71 and 1.58% while the TP contents were 1.13, 1.24, 1.42, 1.16 and 1.11% (Fig. 1b).

The vermicompost characteristics exhibited differences in nutrients based on the amount of biochar amended with vermicomposting substrates. The percentage germination of *Zea mays* showed variation with respect to the concentration of biochar amended with vermicompost (Fig. 2a). The germination of maize seeds ranged from 75.06% (V5) to 95.15% (V3) in the treatments with vermicompost-biochar amendments. The germination percentage of maize showed clear demarcation of variation which was biochar concentration dependent. However, the treatment with

6% biochar (V4) amended vermicompost of *Parthenium* did not differ significantly from that of the seed germination in V3, i.e., 4% biochar amendment. This has been clearly reflected in the germination index (Fig. 2b). The germination index was 4.4, 4.55, 4.82, 4.65 and 4.12 in V1, V2, V3, V4 and V5, respectively.

(a)



(b)

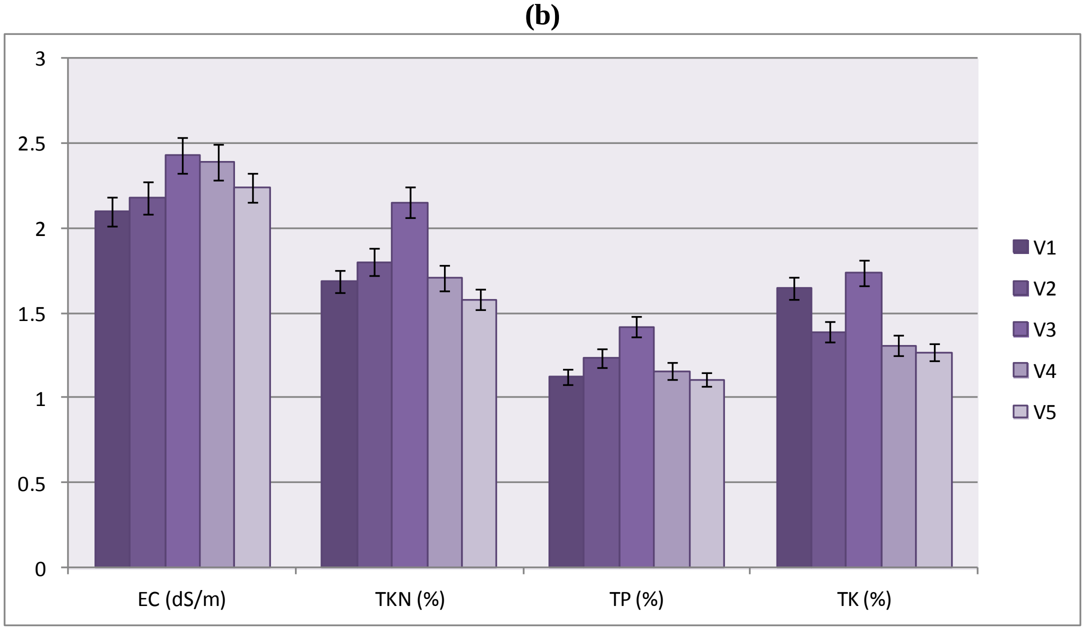


Fig. 1 (a & b): Physicochemical characteristics of vermicompost produced from *Parthenium* biomass amended with biochar.

As a measure of seedling growth, fresh weight, dry weight, root length and shoot length were recorded for different treatments of *Parthenium* vermicompost with various concentrations of biochar (Fig. 3 and Table 1). The growth parameters of maize seedlings were found to decrease in higher concentration in vermicompost (V5). Nonetheless, vermicompost treatments showed significant increase over control (without biochar). The root and shoot length of maize seedlings were higher in V3 followed by V4.

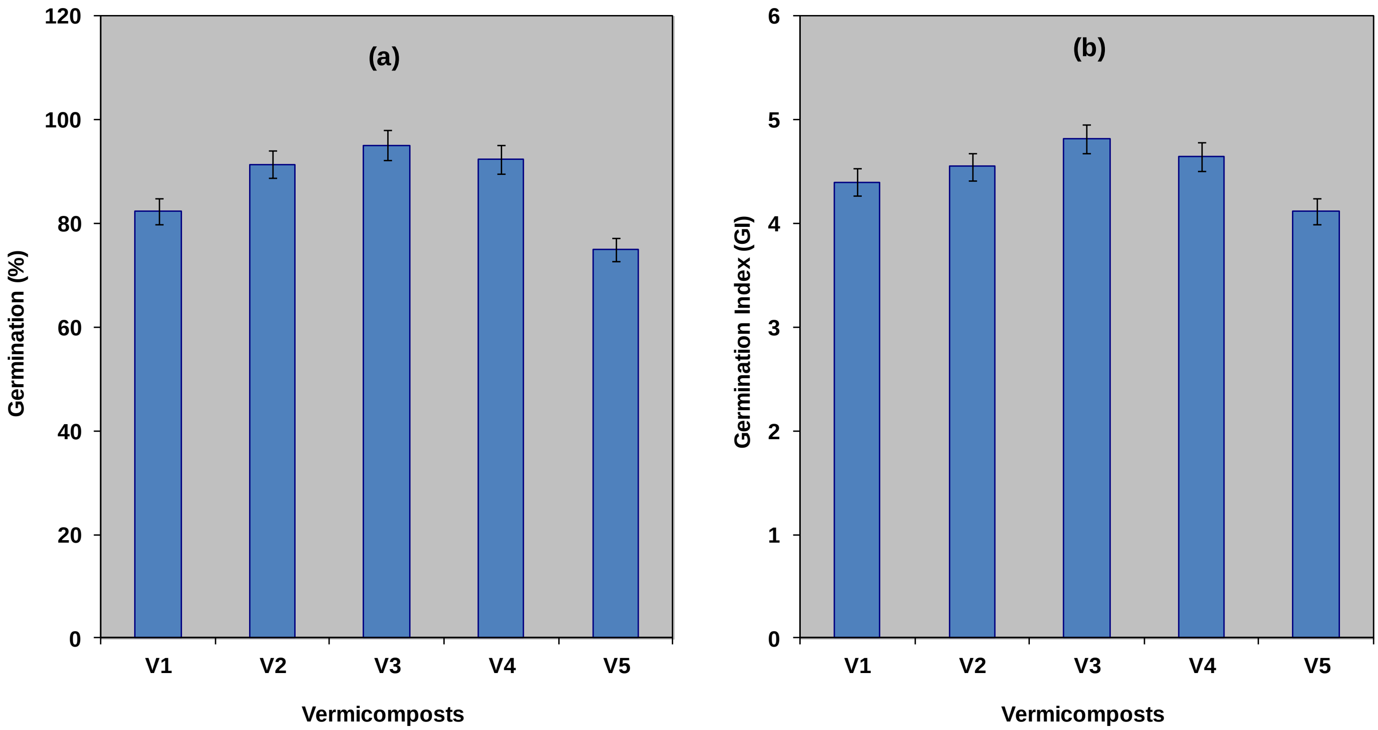


Fig. 2: Germination percentage (a) and germination index (GI) of maize seeds in vermicompost amended with biochar (Values are mean five replicates; Error bars indicate ±SE).

The chlorophyll contents in 20 days old maize seedlings showed pronounced improvement in vermicompost+biochar treatments (Table 1). Overall, the germination, seedling growth and chlorophyll contents of maize were higher in vermicompost treatments than the vermicompost without biochar (V1). The results of the present study reveal that the amendment of biochar with vermicomposting substrates and resultant vermicompost had considerable impact on the germination and growth of maize seedlings besides influencing chlorophyll contents.

Plant growth regulators, microbial activity, and total and available micronutrients have been shown to be higher in vermicompost as well as organic matter and carbon (Arancon and Edwards, 2010; Edwards and Bohlen, 1996; Tomati et al., 2000). They contain nutrients in forms that are taken up by the plants readily, such as nitrates, exchangeable phosphorus and soluble potassium, calcium and magnesium. Hence vermicompost had been shown to influence the growth and productivity of a variety of plants, cereals and legumes, ornamental flowering plants (Atiyeh et al., 2002; Jayakumar et al., 2011; Zaller, 2007). Arancon et al. (2008) reported the influence of vermicomposts, produced by earthworms and microorganisms from cattle manure, food waste and paper waste, on the germination, growth and flowering of petunias in the greenhouse.

Karmegam and Daniel (2009) reported the usefulness of earthworm casts as carrier (layering) material for an ornamental plant, *Codiaeum variegatum*. Moreover, it has been reported that chlorophyll a and b contents of *Alpinia zerumbet* plants grown in peat/perlite/biochar medium were reduced by more than 20% and 28%, respectively, compared to those grown in peat/perlite/biochar/vermicompost media (Zulfiqar et al., 2021), where the results were in coherence with the present findings. Vermicompost combined with biochar significantly increased rice yield by 26.5%–35.3% ($P<0.01$) compared with biochar alone (Wu et al., 2019). These studies confirm that the seed germination and plant growth were enhanced in vermicompost+ biochar combinations.

Table 1. Root length, shoot length, chlorophyll a and chlorophyll b contents of maize seedlings in *Parthenium* vermicompost amended with biochar (Values are mean five replicates ±SE).

Treatments	Root length (cm/seedling)	Shoot length (cm/seedling)	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)
V1	10.45±1.15	14.12±1.03	9.56±0.33	13.78±2.04
V2	12.33±1.22	17.01±1.15	9.94±0.45	15.63±1.18
V3	14.80±1.51	18.85±1.06	11.17±0.61	17.33±1.76
V4	13.11±2.18	16.74±0.98	11.20±0.80	16.97±1.65
V5	12.29±1.04	15.58±1.83	10.02±0.56	14.18±1.27

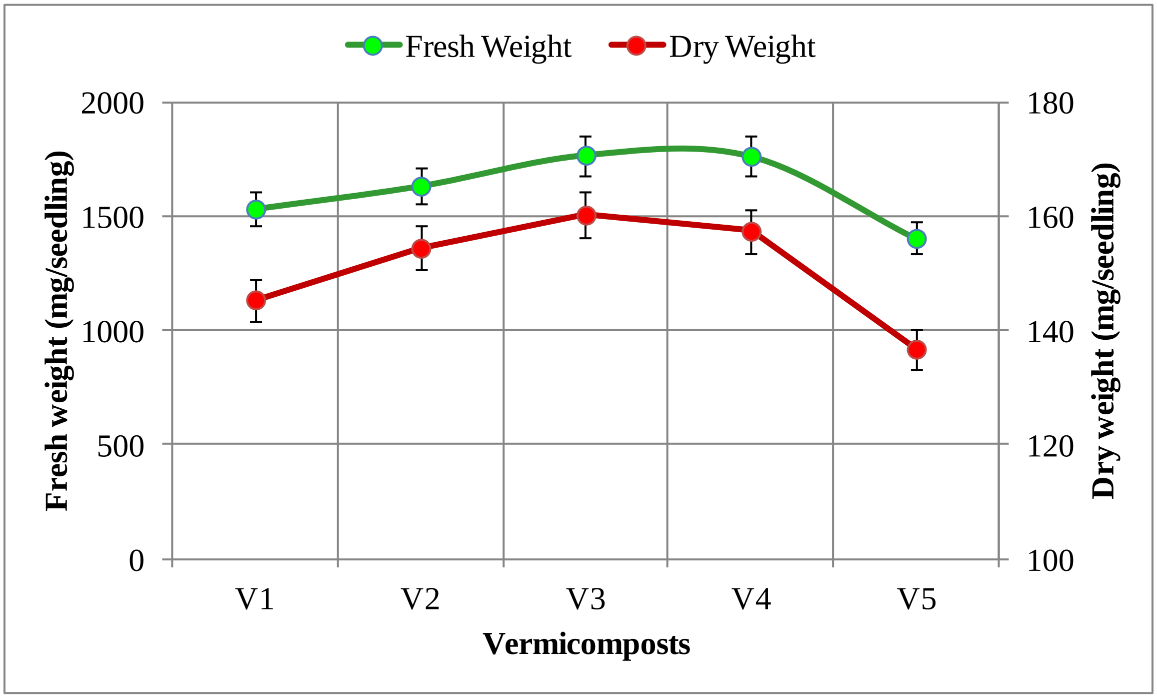


Fig. 3: Fresh and dry weight of maize seeds in *Parthenium* vermicompost amended with biochar (Values are mean five replicates; Error bars indicate ±SE).

Conclusions

In the present study, an attempt has been made to study the effect of *Parthenium* vermicompost containing biochar using germination and seedling growth studies with maize plant. The current study clearly indicated that the biochar concentration of 4-6% is able to influence the germination, germination index, seedling growth and chlorophyll content of maize significantly. In addition to providing plants with a natural organic input, vermicompost amended with biochar improves the growth of the plants. Conclusively, the vermicompost with biochar amendment is able to improve the germination and plant growth characteristics. Extended studies on plant growth and yield with the application of biochar amended vermicompost could provide further insights in support of biochar amendment in vermicomposting.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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