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Growth and Reproduction of *Eudrilus eugeniae* in *Parthenium hysterophorus* Biomass and Mixed Leaf Litter Substrates Amended with Wood Biochar

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Abstract: Vermicomposting is one of the important sustainability technologies being popularized world-wide. During the process of vermiconversion, earthworms perform a vital role in mineralization of organic wastes without which the process of bioconversion is becoming low besides physico-chemical characteristics of final product. Hence, it is necessary to understand the growth and reproductive performance of earthworms during vermicomposting of organic substrates used for the vermiconversion. With this view point, the present study has been aimed to focus on the growth and reproduction of a vermicomposting earthworm species, *Eudrilus eugeniae* in vermicomposting substrates amended with different rates of wood biochar which has not been addressed so far. Therefore, in this study 1:1 proportion of the biomass of *Parthenium hysterophorus* and cow dung, and mixed leaf litter and cow dung were amended with wood biochar at the rate of 0, 2, 4, 6 and 8% in the vermibeds inoculated with *Eudrilus eugeniae* under controlled conditions for 50 days. The results revealed that a maximum of biomass was recorded in 2% biochar amendment followed by 6% biochar (2868.83 ± 74.59 mg/worm) where the biomass values were not significantly different ($P>0.05$). A maximum of 130.71 ± 3.40 worm numbers per vermibed was recorded in 2% biochar. This is similar for *Parthenium hysterophorus* biomass substrate also. The optimum use of 2-4% wood biochar is suitable for the maximum activity of earthworm in the vermicomposting substrates as concluded on the basis of the findings of the present study.

Keywords: Earthworm, *Eudrilus eugeniae*, Organic substrates, Vermicomposting, Cocoon production, Fecundity, *Parthenium hysterophorus*, Wood biochar, Biomass

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Introduction

Earthworms act as the main decomposing organisms during the natural process of vermicomposting, which turns bio-waste into nutrient-rich manure and humus (Yuvaraj *et al.*, 2021). Various epigeic earthworm species are employed in the vermicomposting treatment, including *Eudrilus eugeniae*, *Perionyx excavatus*, *Dendrobaena veneta*, *Dendrobaena andrei*, and *Eisenia fetida* (Domínguez, 2018; Singh *et al.*, 2020). Vermicomposting is thus an enhanced form of composting (i.e. bioaugumented composting) which is cheaper as it does not require expensive physical /mechanical unit process associated with traditional microbial composting process (Hand *et al.*, 1988). Composting alone is not enough to accelerate stabilization, vermicomposting can also accelerate stabilization (Ndegwa and Thompson, 2001; Balachandar *et al.*, 2020). However, the growth and fecundity of earthworms are significantly influenced by the nature of organic substrates and amendment materials provided as feedstocks during vermicomposting.

According to Shaaban *et al*. (2018), biochar is a fine-grained, porous material that resists degradation and is created by slowly pyrolyzing plant and animal waste at low to medium temperatures with little oxygen. It has been widely utilized in fields to minimize soil emissions of nitrogen dioxide (Zhang *et al.*, 2021). Applying biochar lowers N_2O, increases soil aeration, suppresses denitrifiers in the soil, and adsorbs mineralized N from the soil (Wu *et al.*, 2018; Biruntha *et al.*, 2020; Gao *et al.*, 2022; Wei *et al.*, 2022). Biochar added to the composting substrate before vermicomposting has a positive impact on the entire composting-vermicomposting process. When biochar is added to biowastes for vermicomposting, it may help earthworms grow, reproduce, and survive. With biochar amendments, biowaste could be converted efficiently into a useful resource and vermicompost quality could be improved (Malińska *et al.*, 2016; Khan *et al.*, 2019). Many management strategies have been employed to combat the *Parthenium hysterophorus* weed risk, but unfortunately, each has certain drawbacks. Vermitechnology is a useful method for controlling invasive *Parthenium hysterophorus* weed. The hazards resulting from *Parthenium hysterophorus* weed and vermicomposting using easily available epigeic earthworms in the local region can reduce these potential hazards (Sharma and Chowdhary, 2020). The leaf litter generated is most probably of mixed type and segregating plant-wise is time-consuming which is not required in the viewpoint of biomass utilization for value-added product and nutrient recovery.

The amendment materials mixed with vermicomposting substrates are known to be significantly playing a role in improving vermicomposting process as well as worm growth and activity. Among the various amendment materials, cow dung is the most preferred amendment material which supports the initial microbial and earthworm growth which in turn enhances the vermicomposting process (Yuvaraj *et al*., 2021). However, the studies on growth and reproductive performance of the earthworms such as *Eudrilus eugeniae* during the vermiconversion of *Parthenium hysterophorus* biomass and mixed leaf litter substrates amended with biochar are reported scantily. Due to this fact, the present study was aimed to explore the growth and reproductive capacity of the epigeic earthworm, *Eudrilus eugeniae* in the organic vermibed substrates amended with biochar.

Materials and Methods

The plant, *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. Vermicomposting substrate was amended with powdered Prosopis wood biochar obtained from a local vendor. A 1:1 wt-to-wt ratio of plant biomass and cow dung was mixed for vermicomposting as suggested by Thangamani *et al*. (2020). When the substrate mixtures were pre-composting for 15 days, a moisture content of 40-50% was maintained. Different concentrations of biochar were added to the substrate mixtures (0, 2, 4, 6 and 8%) (Table 1) before pre-composting.

Table 1: Experimental design showing the proportion of verminbed substrates

Exp. No.	Treatment mixture (wt/wt)			Biochar (%)
	Organic substrates*	Cow dung	Ratio	
T1	50	50	1:1	0
T2	50	50	1:1	2
T3	50	50	1:1	4
T4	50	50	1:1	6
T5	50	50	1:1	8

* *Parthenium hysterophorus* biomass and mixed leaf litter substrates

Filled uniform plastic containers with pre-composted substrate mixtures and water to hold 70-80% moisture for 24 h. 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 vermibeds of the treatments, T1, T2, T3, T4 and T5 were separately maintained for assessing the growth and reproductive behaviour of the earthworm, *Eudrilus eugeniae* in biochar amended *Parthenium hysterophorus* biomass + cow dung and mixed leaf litter + cow dung substrates. The experiments were carried out in dark chamber with 27±1 ºC using triplicate treatments for each combination inoculated with 20 clitellate adult earthworms. This was done to avoid disturbance of vermicomposting beds that were used for physico-chemical, enzymatic and microbiological analyses. Initial biomass, final biomass, and worm numbers were counted periodically until the end of vermicomposting process.

Statistical analysis:

The results were expressed as mean ± standard error of mean (SE). The results obtained were computed with SPSS statistical software (version 20.1) to find out the statistical significance (P =0.05) between treatment by Tukey's HSD multiple comparison tests.

Results

Growth and fecundity of Eudrilus eugeniae in Parthenium hysterophorus biomass amended with biochar:

The growth and reproductive performance of earthworms during vermicomposting is considered vital for the bioconversion of organic substrates. If the worms are active, the vermicomposting process is also good. For this reason, the growth and reproductive performance of the earthworm, *Eudrilus eugeniae* in the vermicomposting substrates, *Parthenium hysterophorus* + cow dung (1:1) incorporated with biochar was assessed in replicative treatments alongside of vermicomposting treatments. The biomass change, population buildup, mean worm growth rate and cocoon production rate were systematically done and the results are shown in Tables 2 and 3.

The individual biomass of *Eudrilus eugeniae* ranged from 832.77 to 865.33 mg/worm at the time of inoculating them into the vermibed which increased to 2345.46 – 2923.83 mg/worm after 50 days of vermicomposting. A maximum of 2923.83 mg/worm biomass was recorded in T2 (2% biochar) followed by T3 (2912.52 mg/worm) amended with 4% biochar where the biomass values were not significantly different (P>0.05) indicating that T2 and T3 substrate combination is more favourable for the earthworms than the rest of the treatments. The individual worm biomass gain (mg) was higher in T2 followed by T3>T4>T5>T1. The individual worm biomass gain

Table 2: Biomass change of *Eudrilus eugeniae* during vermicomposting of *Parthenium hysterophorus* + cow dung (1:1) amended with biochar

Treatments	Initial individual biomass (mg)	Final individual biomass (mg)	Individual biomass gain (mg)
T1	865.33 ± 20.50	2345.46 ± 11.22[c]	1480.13 ± 17.80[b]
T2	845.92 ± 15.26	2923.83 ± 18.15[a]	2077.91 ± 12.63[a]
T3	840.18 ± 18.21	2912.52 ± 21.56[a]	2072.34 ± 15.79[a]
T4	832.77 ± 15.12	2719.36 ± 23.09[b]	1886.59 ± 12.53[a]
T5	850.49 ± 19.67	2619.52 ± 19.33[b]	1769.03 ± 14.31[b]

Values are mean ± SE. The same alphabet between treatments did not differ significantly at P<0.05 by Tukey's HSD multiple comparison tests.

Table 3: Dynamics of *Eudrilus eugeniae* population during vermicomposting of *Parthenium hysterophorus* + cow dung (1:1) amended with biochar

Treatments	Initial worm population (no. inoculated per vermibed)	Final worm population (no./vermibed)	Growth rate (mg/worm/day)	Cocoon production rate (no./worm)
T1	20	93.43 ± 3.18 [b]	75.96 ± 2.58 [c]	5.51 ± 0.19 [b]
T2	20	127.01 ± 4.32 [a]	98.91 ± 3.36 [a]	7.08 ± 0.24 [a]
T3	20	132.70 ± 4.51[a]	90.76 ± 3.09 [b]	6.28 ± 0.21 [ab]
T4	20	98.30 ± 3.34 [b]	91.04 ± 3.10 [b]	5.51 ± 0.19 [b]
T5	20	84.70 ± 2.88 [c]	71.28 ± 2.42 [c]	3.72 ± 0.13 [c]

Values are mean ± SE. The same alphabet between treatments did not differ significantly at P<0.05 by Tukey's HSD multiple comparison tests

was 1480.13 ± 17.80, 2077.91 ± 12.63, 2072.34 ±15.79, 1886.59 ± 12.53, and 1769.03 ± 14.31 mg/worm, respectively in T1, T2, T3, T4 and T5 (Table 2).

The individual number of *Eudrilus eugeniae* at the time of inoculating them into the vermibed was 20 uniformly, which increased to 84.70 ± 2.88 - 132.70 ± 4.51 worms/vermibed after 50 days of vermicomposting. A maximum of 132.70 worm numbers per vermibed was recorded in T3 (4% biochar) followed by T2 (127.01 worm/vermibed) amended with 2% biochar where the biomass values were not significantly different (P>0.05) indicating that T3 and T2 substrate combination is more favourable for the earthworms than the rest of the treatments. The growth rate of earthworms (mg/worm/day) was higher in T2 followed by T4>T3>T1>T5. The cocoon production rate (no./worm) was 5.51 ± 0.19, 7.08 ± 0.24, 6.28 ± 0.21, 5.51 ± 0.19 and 3.72 ± 0.13, respectively in T1, T2, T3, T4 and T5 (Table 3).

The overall results on the growth and reproductive performance of the earthworm, *Eudrilus eugeniae* was well supported by the addition of biochar, especially in 2% and 4% biochar amended vermibed substrates, *Parthenium hysterophorus* + cow dung (1:1) combination.

Growth and fecundity of Eudrilus eugeniae in mixed leaf litter amended with biochar:

The biomass change, population buildup, mean worm growth rate and cocoon production rate in the vermicomposting substrates, mixed leaf litter + cow dung (1:1) incorporated with biochar were systematically done and the results are shown in Tables 4 and 5. The individual biomass of *Eudrilus eugeniae* ranged from 835.27 ± 21.72 to 867.93 ± 22.57 mg/worm at the time of inoculating them into the vermibed which increased to 2868.83 ± 74.59 - 2879.97 ± 74.88 mg/worm after 50 days of vermicomposting. A maximum of biomass was recorded in T2 (2% biochar) followed by T3

Table 4: Biomass change of *Eudrilus eugeniae* during vermicomposting of *Parthenium hysterophorus* + cow dung (1:1) amended with biochar

Treatments	Initial individual biomass (mg)	Final individual biomass (mg)	Individual biomass gain (mg)
T1	867.93 ± 22.57	2310.28 ± 60.07 [d]	1438.69 ± 37.41 [d]
T2	848.46 ± 22.06	2879.97 ± 74.88 [a]	2019.73 ± 52.51 [a]
T3	842.70 ± 21.91	2868.83 ± 74.59 [a]	2014.31 ± 52.37 [a]
T4	835.27 ± 21.72	2678.57 ± 69.64 [b]	1833.77 ± 47.68 [b]
T5	853.04 ± 22.18	2580.23 ± 67.09 [c]	1719.50 ± 44.71 [c]

Values are mean ± SE. The same alphabet between treatments did not differ significantly at $P<0.05$ by Tukey's HSD multiple comparison tests

Table 5: Dynamics of *Eudrilus eugeniae* population during vermicomposting of *Parthenium hysterophorus* + cow dung (1:1) amended with biochar

Treatments	Initial worm population (no. inoculated per vermibed)	Final worm population (no./vermibed)	Growth rate (mg/worm/day)	Cocoon production rate (no./worm)
T1	20	92.03 ± 2.39 [b]	73.83 ± 1.92 [b]	5.36 ± 0.14 [b]
T2	20	125.10 ± 3.25 [a]	96.14 ± 2.50 [a]	6.88 ± 0.18 [a]
T3	20	130.71 ± 3.40 [a]	88.22 ± 2.29 [a]	6.10 ± 0.16 [a]
T4	20	96.83 ± 2.52 [b]	88.49 ± 2.30 [a]	5.36 ± 0.14 [b]
T5	20	83.43 ± 2.17 [c]	69.28 ± 1.80 [c]	3.62 ± 0.09 [c]

Values are mean ± SE. The same alphabet between treatments did not differ significantly at $P<0.05$ by Tukey's HSD multiple comparison tests

(2868.83 ± 74.59 mg/worm) amended with 4% biochar where the biomass values were not significantly different ($P>0.05$) indicating that T2 and T3 substrate combination is more favourable for the earthworms than the rest of the treatments. The individual worm biomass gain (mg/worm) was higher in T2 followed by T3>T4>T5>T1. The individual worm biomass gain was 1438.69 ± 37.41, 2019.73 ± 52.51, 2014.31 ± 52.37, 1833.77 ± 47.68 and 1719.50 ± 44.71 mg/worm, respectively in T1, T2, T3, T4 and T5 (Table 4).

The individual number of *Eudrilus eugeniae* at the time of inoculating them into the vermibed was 20 uniformly, which increased to 83.43 ± 2.17 - 130.71 ± 3.40 worms/vermibed after 50 days of vermicomposting. A maximum of 130.71 ± 3.40 worm numbers per vermibed was recorded in T2 (2% biochar) followed by T3 (125.10 ± 3.25 worm/vermibed) amended with 4% biochar where the biomass values were not significantly different ($P>0.05$) indicating that T2 and T3 substrate combination is more favourable for the earthworms than the rest of the treatments. The growth rate of earthworms (mg/worm/day) was higher in T2 followed by T4>T3>T1>T5. The cocoon production rate (no./worm) was 5.36 ± 0.14, 6.88 ± 0.18, 6.10 ± 0.16, 5.36 ± 0.14 and 3.62 ± 0.09, respectively in T1, T2, T3, T4 and T5 (Table 5).

Discussion

Vermicomposting using different concentrations of cow manure and the earthworm *Eisenia fetida* of *Parthenium hysterophorus* shows the largest cocoon production per earthworm in treatment CD (100%); however, there were significant differences between treatments ($P < 0.05$). The higher parthenium percentage in the treatments may have had an impact on the rate of cocoon production. The biochemical properties of the feed combinations, which can account for differences in cocoon production among treatments, are among the most significant factors influencing when cocoon formation starts (Yadav and Garg, 2011).

Using earthworms *Eudrilus eugeniae*, four various concentrations of *Parthenium hysterophorus* and cow manure combinations were vermicomposted. In the absence of cow dung, the earthworm development, biomass gain, cocoon creation, and antioxidant enzyme levels were all low in a high *Parthenium hysterophorus* concentration. The findings showed that the right amount of *Parthenium hysterophorus* mixing is necessary for earthworm survival without endangering them (Rajiv *et al*., 2014).

Bioconversion of *Parthenium hysterophorus* spiked with biogas plant slurry using epigeic worm *Eisenia fetida* was investigated. The biomass gain in different vermibins was inconsistent. Maximum worm biomass was observed in 100% of biogas plant slurry and minimum worm biomass was in the combination of 80 % of biogas plant slurry and 20 % of *Parthenium hysterophorus* (Suthar, 2010). The results also showed that maximum worm biomass was attained on the 36th day in 100% of the biogas plant slurry. Mean net biomass gain (1263±26 mg worm^{-1}) was also maximum in 100% of biogas plant slurry and minimum (874±15 mg worm^{-1}) in combination with 80 % of biogas plant slurry 20 % of *Parthenium hysterophorus* (Yadav and Garg, 2016).

Parthenium hysterophorus was vermicomposted using a polyculture involving two epigeic earthworm species. The highest worm population was reached in week seven of 100% of cow dung. A combination of 25% of cow dung and 75% of *Parthenium hysterophorus* had the lowest average live biomass of *Eisenia fetida* (908 ± 87 mg), while 100% of cow dung had the highest average (1150 ± 104 mg). An increase in feed parthenium concentration caused the growth rate to drop significantly ($p < 0.05$). The increase in growth rates was noticeably different from other treatments. The earthworm biomass increase with the largest percentage increment was 46.25% in the combination of 4% of *Parthenium hysterophorus* and 6% of cow dung (Devi and Khwairakpam, 2021)

In comparison to the initial number of worms, the total number of worms produced significantly. It shows which substrates were suitable for the growth of the organisms. The substrates provide the necessary amount of nutrients for worm development and reproduction. Palatability determines the earthworm activity's capacity to multiply and convert the substrate components into nutrient-rich organic manure. The breakdown of the organic waste increases the nutritious content of the final products (Yadav, 2015).

Eudrilus eugeniae was employed to vermicompost cow manure blends with five different concentrations of *Parthenium hysterophorus*. Earthworms in 100% of *Parthenium hysterophorus* treatment had the lowest living weight (86.53 ± 1.13 mg), while in the mixture of 30% of *Parthenium hysterophorus* and 70% of cow dung treatment, the maximum individual living mass of *Eudrilus eugeniae* was 111.60 ± 8.48 mg. Because of the high concentration of allergic chemicals that may be present in 100% of *Parthenium hysterophorus* plants, earthworm weight did not rise during the 100% of *Parthenium hysterophorus* treatment. Individual earthworm development and biomass decreased throughout the later stages of the composting process (Rajiv *et al.*, 2013).

The evaluation of *Parthenium hysterophorus* spiked with biogas plant slurry using epigeic worm *Eisenia fetida.* Cocoon production was started between the 18th and 24th day of vermicomposting in different vermibins. After 60 days maximum cocoons (383 ± 25) were produced in the ratio of 90% biogas plant slurry and 10 % of *Parthenium hysterophorus* (Yadav and Garg, 2013). Cocoon production was affected by the percentage of substrates in the vermibins (Yadav and Garg, 2016). All these studies revealed that the earthworm growth and reproduction is very important during vermicomposting of organic substrates and the findings fall in line with the results of the present study.

Conclusion

The growth and reproductive performance of earthworms during vermicomposting is considered vital for the bioconversion of organic substrates such as mixed leaf litter. If the worms are active, the vermicomposting process is also good. For this reason, the growth and reproductive performance of the earthworm, *Eudrilus eugeniae* in the vermicomposting substrates, mixed leaf litter + cow dung (1:1) incorporated with biochar was assessed in replicative treatments alongside of vermicomposting treatments. The biomass change, population buildup, mean worm growth rate and cocoon production rate were systematically done. The overall results on the growth and reproductive performance of the earthworm, *Eudrilus eugeniae* was well supported by the addition of biochar, especially in 2% and 4% biochar amended vermibed substrates, *Parthenium hysterophorus* + cow dung (1:1) combination and mixed leaf litter + cow dung (1:1) substrate.

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