



Original Research Article

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## Effect of pre-composting on seed viability and subsequent vermicomposting of an invasive alien weed, *Alternanthera ficoidea* (L.) P. Beauv.

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### ABSTRACT

In the present study, the suitability of an invasive weed, *Alternanthera ficoidea* (L.) P. Beauv. (AF) biomass for vermicomposting has been tried by subjecting three different combinations of the weed and cow dung (CD) through 28 days pre-composting followed by 50 days vermicomposting with *Eudrilus eugeniae*. The maximum temperature recorded in P<sub>1</sub> (1CD:1AF), P<sub>2</sub> (2CD:1AF) and P<sub>3</sub> (1CD:2AF) during pre-composting was 66, 62 and 67°C respectively. The seed viability study using pre-composted substrates, for weed seeds if any, revealed that there were no seedlings appeared in P<sub>1</sub> and P<sub>3</sub> up to 60 days, while 41.57% germination was observed for P<sub>2</sub> compared to initial non pre-composted substrate. The C/N ratio in all vermicombed combinations was found to decline significantly (P<0.05) at the end of vermicomposting from the initial levels as well as from the substrate combinations without earthworms (control). The lowest C/N ratio of 14.56 was found in V<sub>2</sub> (2CD:1AF) followed by V<sub>1</sub> (1CD:1AF) and V<sub>3</sub> (1CD:2AF). The growth and reproduction of *Eudrilus eugeniae* was well supported by the combination of V<sub>1</sub> and V<sub>2</sub> which were significantly higher than V<sub>3</sub> (P<0.05). The tests on germination of *Alternanthera ficoidea* seeds in vermicompost confirmed that there were no viable seeds in V<sub>1</sub> and V<sub>3</sub> vermicomposts while V<sub>2</sub> vermicompost showed 16.77% viability in comparison with the control (non pre-composted). Based on germination of weed seeds in vermicompost, reduction of C/N ratio, growth and reproduction of *Eudrilus eugeniae* during vermicomposting, it is concluded that 28 days pre-composting followed by 50 days vermicomposting of 1:1 (CD+AF) ratio of substrate combination is suitable for utilizing the biomass of *Alternanthera ficoidea* for nutrient recovery and vermicompost production without any harm to the environment.

## Introduction

The population of invasive weeds is alarmingly increasing world-wide and is threatening the biodiversity, agriculture and economy (Early et al., 2016; Sudhakar Reddy et al., 2008). Various physical, chemical and biological weed control strategies have been adopted whereby they have their own limitations and adverse effects (Ani et al., 2018; Gianessi, 2008; Peerzada and Chauhan, 2018; Sudhakar Reddy et al., 2008). Alternatively, the large biomass of weeds can be used for compost and vermicompost production as a mean of nutrient recovery for agricultural use (Misra et al., 2003; Thangamani et al., 2019). Vermicomposting is an environmentally safe technology which makes use of the earthworms for the conversion of a variety of organic substrates into nutrient rich vermicompost (Singh et al., 2020). The organic substrates suitable for vermicomposting include agricultural and urban wastes (Biruntha et al., 2019), industrial wastes—sugar industry (Balachandar et al., 2020), paper industry (Yuvaraj et al., 2018a), textile industry (Yuvaraj et al., 2020), poultry litter (Yuvaraj et al., 2018b), weeds (Devi and Khwairakpam, 2020a), and others.

Invasive weeds are the unwanted plants growing in agricultural fields, wastelands and natural habitats disturbing agro- and natural-biodiversity. The method of utilizing the biomass of weeds through vermicomposting technology is expanding due to its eco-friendliness, low cost and beneficial end products. The utilization of different locally available weed biomass and cow dung in 1:1 ratio with the earthworm, *Eudrilus eugeniae* had been reported for quality vermicompost production (Karmegam and Daniel, 2000). Several authors have studied the possibility of vermicomposting of noxious weeds like *Ageratum conyzoides*, *Eichhornia crassipes*, *Lantana camara* and *Parthenium hysterophorus* using anecic and epigeic group of earthworms viz., *Drawida willsi*, *Eudrilus eugeniae*, *Eisenia fetida*, *Perionyx excavatus* and *Lampito mauritii* (Devi and Khwairakpam, 2020a, 2020b; Gajalakshmi et al., 2001; Gupta et al., 2007; Rajiv et al., 2013; Suthar and Sharma, 2013). One of the major desirable qualities of the compost is the absence of viable seeds. To avoid the seeds of the weeds in vermicompost, researchers used the vegetative

phase of the weeds or collected the leaves separately avoiding the other parts of the weeds. These practices are helpful on the one hand in eliminating the contamination of weed seeds, and on the other hand separately collecting the leaves, weeds without flowers/fruits are difficult to follow when large scale vermicomposting is sought.

*Alternanthera ficoidea* (L.) P. Beauv. (Synonym: *Alternanthera tenella* Colla), is an invasive weed rapidly spreading new habitats due to the production of enormous number of seeds, high percentage of seed viability, high frequency of vegetative propagation, and high adoptability to environmental conditions (Patil and Kore, 2018; Thangamani et al., 2019). In case of collecting leaves alone or vegetative phase of *Alternanthera ficoidea* is extremely difficult as it produces inflorescences with flowers and fruits at every node (see Fig. 1a). The ecology and biology of *Alternanthera ficoidea* and its utility as medicinal and nutrient source have been reported by Patil and Kore (2018). However, its huge biomass productivity requires suitable alternative methods like vermicomposting for the management of the weed, nutrient recovery and vermicompost production, and more importantly conservation of indigenous biodiversity. Also the fate of weed seeds after vermicomposting is, however, not well addressed, requiring further insights. Keeping in view of the above points, the present preliminary study has been aimed to optimize the weed, *Alternanthera ficoidea* for vermicompost production with reference to pre-composting effect on seed viability and vermicomposting on carbon to nitrogen ratio, growth and reproduction of the earthworm, *Eudrilus eugeniae*.

## Materials and methods

### Collection, identification and processing of *Alternanthera ficoidea*

The invasive alien weed, *Alternanthera ficoidea* (Fig. 1a) belonging to the family Amaranthaceae was collected from Government Arts College (Autonomous) campus, Salem, south India. The identification of the plant was confirmed with the local floras and validated with the help of Dr. S. Karuppusamy, Centre for Botanical Research, Madura College, Madurai (TN), India. The herbarium specimens of the plant were

deposited in the Department of Botany, Government Arts College (Autonomous), Salem-7. The above ground portion of the weed was

collected, cut into small pieces and transported to the laboratory, dried for a week under shade and used for the experiments (Fig. 1b-c).



**Fig. 1:** (a) Thick mats of *Alternanthera ficoidea*; box figure: close-up of a flowering branch; (b) and (c) Collection and processing of weed; (d) Mixing of weed + cow dung; (e) Pre-composting; (f) *Eudrilus eugeniae*; box figure: pilot vermibeds; (g) cocoons of *Eudrilus eugeniae*.

## Earthworms and cow dung

The epigeic earthworm species, *Eudrilus eugeniae* procured from Vermitechnology and Toxicology Laboratory of the Department Zoology, Periyar University, Salem, Tamil Nadu was mass multiplied using cow dung and the second generation clitellate worms with average weight of  $721.45 \pm 35.15$  g were used for vermicomposting trials. Fresh cow dung for the study was collected from local cow shed, air dried and used for the study.

## Pre-composting trials

The substrates for the study, processed *Alternanthera ficoidea* and cow dung were mixed in three different proportions (P1, P2 and P3), volume/volume (Table 1 and Fig. 1d-e), moistened

to hold 50-60% moisture, and filled in  $19.24 \times 10^4$  cm<sup>3</sup> plastic bins in triplicates for each combination. The temperature of the pre-composting bin was measured using soil thermometer on daily basis up to 28 days. The samples were taken from each treatment on 0, 7, 14, 21 and 28 days, air dried and preserved in poly-bags for testing the viability of weed seeds contained in. Five gram of sample collected from each interval for individual treatments were laid over the earthen pots filled with sand, red soil and farm yard manure (1:1:1) and covered with a layer of garden soil, then moistened with enough water. Triplicates were maintained for each sample, watered on alternate days for the period of 60 days. The number of seeds germinated in all the treatments were recorded and compared with respective initial (non pre-composted) samples.

**Table 1.** Treatment combinations of pre-composting (P) and vermicomposting (V) substrates, *Alternanthera ficoidea* and cow dung.

Treatments	Cow dung	<i>Alternanthera ficoidea</i>	Ratio	No. of days
<b>Pre-composting</b>				
P1	50%	50%	1:1	28
P2	66.67%	33.33%	2:1	28
P3	33.33%	66.67%	1:2	28
<b>Vermicomposting</b>				
V1	50%	50%	1:1	50
V2	66.67%	33.33%	2:1	50
V3	33.33%	66.67%	1:2	50

## Vermicomposting of *Alternanthera ficoidea*, growth and reproduction of *Eudrilus eugeniae*

Vermicomposting trials were conducted in cylindrical plastic troughs with perforated lid of  $47.13 \times 10^2$  cm<sup>3</sup> size as detailed in Table 1 and Fig. 1f. Three different treatments (V1, V2 and V3) were set-up with earthworms (vermicomposting) and without earthworms (control) in triplicates. The substrates were moistened to hold 65-70% moisture and left for 24 hours stabilization. After stabilization, 15 number of uniform size earthworms was introduced to each treatment and the experiments were maintained for 50 days. All the treatments were maintained in triplicate under laboratory conditions. Samples from each substrate combination, i.e., at the start of experiment (initial, 0-days), final control (substrate without earthworms after 50-days) and

final vermicompost (substrates with earthworms after 50-days) were collected, air dried and analyzed for total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) using the methods of Bremner and Mulvaney (1982) and Walkley and Black (1934) respectively. With the values of TOC and TKN, carbon to nitrogen ratio (C/N ratio) was derived. After 50-days, the worms and cocoons (Fig. 1f-g) were hand sorted, counted and the results were recorded. The worms were washed with tap water, blotted with filter paper and the biomass was recorded using digital electronic balance.

## Statistical analysis

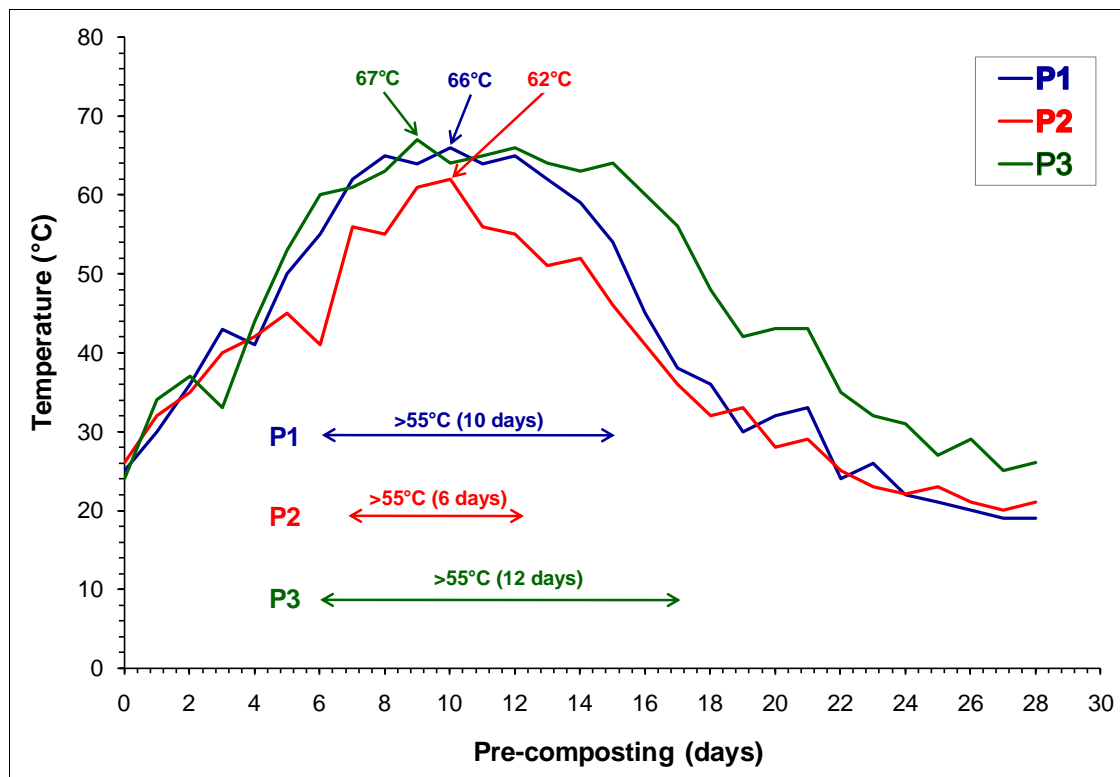
The results obtained in the present study were expressed as mean of three replicates  $\pm$  SEM. The number of seedlings germinated in pre-composted samples collected at different intervals was

subjected to correlation analysis using SPSS statistical package (Version 18.0, SPSS Inc., Chicago, USA). The differences in C/N ratio between vermicomposting treatments and worm growth and fecundity between treatments were computed using One-way ANOVA with Tukey's honestly significant different (HSD) multiple comparison tests at  $P < 0.05$  significance level.

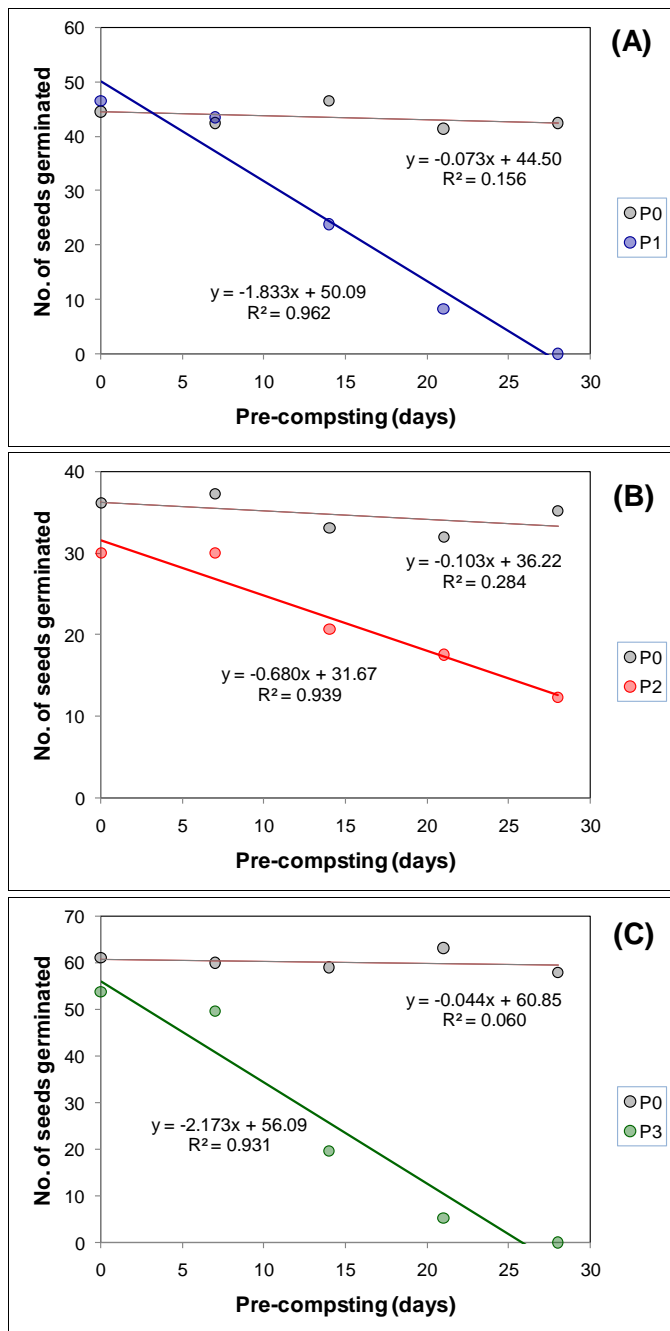
## Results and discussion

The pre-decomposition of organic materials is an important step in vermicomposting as it makes the substrates partially degraded, softer and reduces the temperature that elevate during initial phases of decomposition by microbes. This is the common phenomenon observed during aerobic composting piles which rapidly increase the temperature above  $65^{\circ}\text{C}$  and this phase is considered significant in terms of pathogen reduction and killing of weed seeds, and this too happens in anaerobic digestion (Johansen et al., 2013; Misra et al., 2003). In the present study, the temperature increased rapidly from  $24^{\circ}\text{C}$  to a maximum of  $67^{\circ}\text{C}$  in pre-composting bins. The maximum temperature of

$67^{\circ}\text{C}$  was recorded in P3 followed by P1 ( $66^{\circ}\text{C}$ ) and P2 ( $62^{\circ}\text{C}$ ) during pre-composting. The temperature of above  $55^{\circ}\text{C}$  was observed for 12, 10 and 6 days in P3, P1 and P2 respectively during pre-composting (Fig. 2). The germination rate of *Alternanthera ficoidea* seeds collected from pre-composting bins of different treatments at different intervals showed decline towards the progression of pre-composting days. The reduction of number of seeds germinated in P1 during pre-composting was 6.68, 48.89, 82.22 and 100% on 7, 14, 21 and 28 day samples respectively, in comparison with the number of seeds germinated in the initial untreated samples; it was 0.02, 31.05, 41.39 and 58.63% for P2, while it was 7.69, 63.46, 90.38 and 100% for P3 observed over a period of 60 days. The correlation of number of seeds germinated with that of the number of pre-composting days in all the treatments showed significant negative correlation, while no significant change was observed for untreated samples (Fig. 3). The present study fall in line with the report of Misra et al. (2003) which highlighted that the peak heating stage of pre-composting is vital as it kills majority of the weed seeds and pathogens.



**Fig. 2:** Changes in temperature during thermophilic pre-composting of *Alternanthera ficoidea* and cow dung combinations for 28 days. Refer Table 1 for substrate combinations.



**Fig. 3:** The effect of pre-composting days of *Alternanthera ficoidea* and cow dung combinations on the number of seeds viable seeds. P0: 0-day pre-composting (untreated) samples of respective treatments; (A) P1, (B) P2 and (C) P3. Refer Table 1 for substrate combinations.

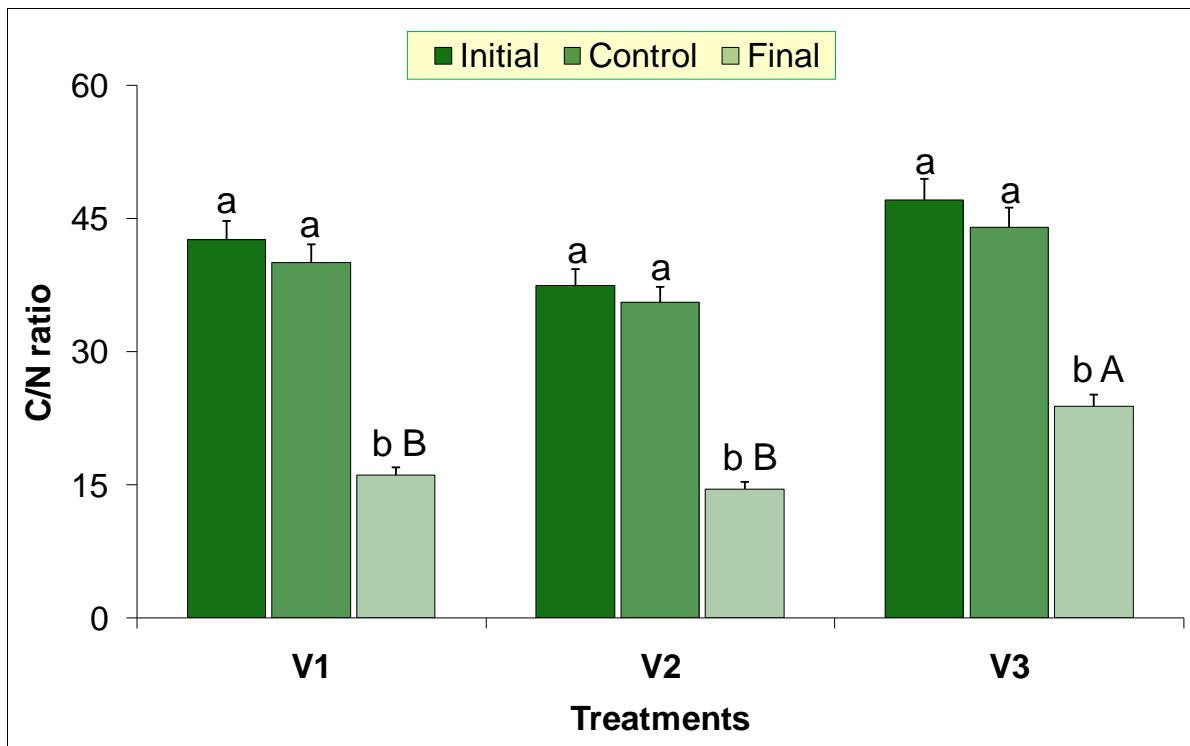
Among the various parameters of composting, C/N ratio is considered as important with regard to vermicompost maturity (Biruntha et al., 2020; Karmegam and Daniel, 2009), hence in the present

preliminary optimization study, C/N ratio has been taken into account to assess the maturity status of the final vermicompost. The C/N ratio recorded for all the treatments with earthworms after 50 days showed significant reduction over the initial and final control without earthworms (Fig. 3). The initial substrates of V1, V2 and V3 showed 42.63, 37.47 and 47.15 C/N ratio which was slightly reduced after 50 days in the control treatments without earthworms: V1 = 40.12, V2 = 35.56 and V3 = 44.06 where the values did not differ significantly ( $P > 0.05$ ) with initial levels. Whilst, the C/N ratio of 16.11, 14.56 and 23.88 respectively obtained for final vermicomposts of V1, V2 and V3 treatments significantly differ from initial and control values at  $P < 0.05$ . Also, the difference in C/N ratio between V1 and V3, and V2 and V3 statistically differ at 5% level of significance. The present study results on the reduction of C/N ratio during vermicomposting of the weed, *Alternanthera ficoidea* + cow dung combinations coincide with the observations of Biruntha et al. (2020), Devi and Khwairakpam (2020a, 2020b). According to CPHEEO (2016), a good quality compost requires to possess the C/N ratio below 20. The vermicompost recovered from V1 and V2 was well within the range.

The growth and fecundity of the earthworms are good indicators of successful vermicomposting process of organic substrates. The acceptability of different types of vermibed substrates by earthworms depends on the physico-chemical characteristics and the nature of organic materials. When the substrates are acceptable, the worms along with microorganisms quickly act upon and accelerate the process of decomposition as well as multiply at faster rates. This has been reported while vermicomposting of weeds like, *Parthenium hysterophorus*, *Lantana camara*, *Eichhornia crassipes*, seaweeds and others (Ananthavalli et al., 2019; Rajiv et al., 2013; Suthar and Sharma, 2013; Varma et al., 2016). The number of worms, biomass and cocoons recovered from three different combinations of *Alternanthera ficoidea* and cow dung after 50 days of vermicomposting is shown in Table 2. The results clearly indicate that the number and biomass of the worms were found to be higher in V2 than in V1, while the number of cocoons produced was higher in V1. However, the difference in growth and reproduction between V1 and V2 did not differ significantly; whereas the number and

biomass of worms, and the number of cocoons recovered per vermibed for V3 was significantly lower than that of V1 and V2 indicating that the earthworms preferred to feed on V1 and V2 substrate combinations than V3 pointing out that 1:2 combination of cow dung and *Alternanthera ficoidea* least supported the growth and fecundity of *Eudrilus eugeniae*. Moreover, 16.99% viable seeds of *Alternanthera ficoidea* were present in the vermicompost derived from V2 (2:1 combination of cow dung and *Alternanthera*

*ficoidea*), in comparison with respective control. The presence of viable seeds in V2 vermicompost indicates that the thermophilic phase of pre-composting was not sufficient enough to kill the seeds of the weed or otherwise, the seeds of the weed could be resistant; the subsequent vermicomposting for 50 days did not reduce the viability of the weed fully, so the seed germination was observed in the vermicompost of V2 treatment, posing the danger of seed dispersal of the invasive weed through vermicompost application.



**Fig. 4:** C/N ratio of *Alternanthera ficoidea* and cow dung combinations before and after vermicomposting (50 days). Values are mean ± SEM; n=3; Error bars indicate SEM; Initial: 28 days pre-composted samples of respective treatments at the start of vermicomposting; Control: 28 days pre-composted samples of respective treatments at the end of 50 days without earthworms; Final: Vermicompost. The same superscript small letter within treatments, and superscript capital letters between vermicomposts, did not differ significantly by Tukey’s HSD at 5% level. Refer Table 1 for substrate combinations.

**Table 2.** Growth and reproduction of *Eudrilus eugeniae* in *Alternanthera ficoidea* (AF) and cow dung (CD) substrate combinations (50 days).

Treatments	No. of cocoons recovered/vermibed	No. of worms recovered/vermibed	Biomass of worms recovered (g)/vermibed
V1	59.17 ± 2.07 <sup>a</sup>	42.79 ± 1.97 <sup>a</sup>	29.96 ± 1.35 <sup>a</sup>
V2	56.09 ± 2.08 <sup>a</sup>	45.03 ± 1.87 <sup>a</sup>	31.52 ± 1.23 <sup>a</sup>
V3	35.28 ± 1.38 <sup>b</sup>	31.03 ± 1.55 <sup>b</sup>	21.72 ± 0.93 <sup>b</sup>

The different superscript letters between treatments differ significantly by Tukey’s HSD at 5% level.

## Conclusion

The findings of the preliminary optimization study show that the proper combination of cow dung and the invasive weed, *Alternanthera ficoidea*, the effect of pre-composting on seed viability and subsequent acceptability of the substrates by the earthworm-*Eudrilus eugeniae*, reduction of C/N ratio, growth and reproduction of the earthworm are the major criteria required for the vermicomposition of weed biomass into vermicompost. The study suggests that 28 days pre-composting followed by 50 days vermicomposting of 1:1 ratio of cow dung and *Alternanthera ficoidea* is helpful for utilizing weed biomass for vermicompost production in an eco-friendly manner. Further, the studies on physico-chemical, enzymatic and microbiological changes accompanying the vermicomposition of *Alternanthera ficoidea* might provide further insights on the utilization of large biomass for vermicompost production. Also, the mechanism of loss or retaining the viability of weed seeds after vermicomposting is required to be investigated in detail to ensure environmental and agricultural sustainability.

## Conflict of interest statement

Authors declare that they have no conflict of interest.

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