Bio-stabilization of toxic weeds (Xanthium strumarium and Lantana camara) implementing mono- and polyculture of Eisenia fetida and Eudrilus eugeniae

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Abstract

The present study investigates the synergistic impact of earthworms (*Eisenia fetida, Eudrilus eugeniae*) and microbes during vermicomposting of invasive weed phytomass (*Xanthium strumarium* and *Lantana camara*). Evaluating both mono- and polyculture techniques, the research highlights the potential dual benefit of weed control and organic fertilizer production through vermitechnology. The study assesses vermicompost production, and physicochemical profiles such as pH, conductivity, total organic carbon, nitrogen, phosphorus, potassium, calcium, trace elements, and heavy metals in the end product. The Ash content, humification index, and C/N ratio analysis were conducted to assess the maturity of the vermicompost. The macronutrient enhancement in the vermicompost samples was recorded 1.5–2.47-fold for total N, 1.19–1.48-fold in available P, 1.1-1.2-fold in total K, and 1.1–1.18–-fold in total Ca. The germination index reveals a significant reduction in phytotoxicity, suggesting the production of mature and suitable vermicompost for agricultural use. Further, the earthworm population and biomass has significantly increased by the end of the 60-day experimental trial.

Translation of Scientific words

Bio-Stabilization- Bio-stabilization refers to a process or method that uses biological organisms, such as plants or microorganisms, to control or mitigate environmental issues.

Monoculture: Vermicomposting is carried out employing one earthworm species.

Polyculture: Vermicomposting is carried out employing more than one earthworm species.

1. INTRODUCTION

Invasive weeds and alien species represent a serious threat to biodiversity and the ecosystem at both the local and global levels. These plants have qualities including higher growth, high reproductive rates, and better survival and spread faster than the native plants, which contribute to their successful invasion. They are distinguished by their capacity to grow successfully under challenging habitats (Huang et al. 2023). A strong mutualistic interaction between invasive weeds is frequently present, enabling successful reproduction in a variety of environmental scenarios. In the period of establishment, they can also colonise and adapt to biotic and abiotic restrictions, developing into enduring populations. Their potential to persist and proliferate is a key factor in their long-term propagation and invasion process (Ramoneda et al. 2019). Anthropogenic activity helps the rapid spread of alien species across the different ecosystems across the globe, significantly harming local biodiversity and socioeconomic situations (Kumar Rai and Singh 2020). Weeds are the most significant and common of the many pests that lower agricultural yields. They have a negative effect on crop output that is worse than the total loss brought on by other pest categories. In terms of the reduction in yield in Indian agriculture, weeds, insects, diseases, and other problems are responsible for a relative 37%, 29%, 22%, and 12% of yield loss. Globally, however, weeds contribute around 45%, while insects, diseases, and other pests each account for about 30%, 20%, and 5% of the yield reduction, respectively (Sinchana and Raj; Kaur et al. 2018). Weeds are responsible for a large financial loss of $11 billion in India. The largest monetary loss among the major crops was suffered by rice, which lost 4.4 billion dollarswheat (3.3 billion dollars), green gram (0.16 billion dollars), whereas soybean suffered a loss estimated at 1.5 billion dollars among pulse crops. These findings highlight the significant financial impact that weeds have on India's agricultural productivity(Chauhan 2020a).

Several invasive weed species that pose risks to human health, the environment, and agriculture have been found in India. The main species that imperil crops involve *Lantana camara, Chromolaena odorata, Parthenium hysterophorus, Mikania micrantha, Xanthium strumarium, Eichhornia crassipes, Ludwigia adscendens, Persicaria hydropiper Hydrolea zeylanica* and others(Balachandar et al. 2021; Rai et al. 2021; Karimmojeni et al. 2021; Ganguly et al. 2022a; Haq et al. 2023). A terrestrial evergreen plant in the verbenaceae family, *Lantana camara* is also referred to as red sage or wild sage. Normal height is between one and two meters. The bushes have woody stems that are four-sided in shape and feature spines. The leaves have a rough texture and serrated edges, and when crushed, they give out a potent smell. The inflorescences are terminal, consisting of multi-colored flowers arranged in whorls on heads. The shrub produces hard green fruits in clusters, which mature into fleshy black drupes (Patel 2011). Due to its invasive nature, *L. camara* is regarded as one of the top ten worst noxious invasive weeds in the world. *L. camara* is cosmopolitan in distribution and is found in a variety of environments, primarily, a result of perturbations brought through anthropogenic and animal action. *L. camara's* successful invasion of vast geographic areas can be attributed to a number of morphological characteristics, including resilience to fire, potent reproductive ability, and competitiveness (Devi and Khwairakpam 2020a). *Xanthium strumarium*, an annual herb is commonly known as cocklebur within the asteraceae family. The genus *Xanthium* contains more than 20 species globally, it has a normal height of 20–90 cm and has upright, branching stems that are frequently speckled with purple. Short white hairs are sparingly scattered across the surface of the stems (Islam et al. 2009a). The exceedingly troublesome weed *X. strumarium* competes with newly sprouting crops for light, readily available water, and nutrients, causing losses in yield(Amini et al. 2020). *X. strumarium* contains a toxic compound, carboxyatrac-tyloside (CAT). CAT is present in the highest concentration in cocklebur seedlings but still present in mature plants. Consumption of CAT results in hypoglycemia and hepatic damage in grazing animals causing livestock loss. Additionally, *X. strumarium* includes sesquiterpene lactones, which in excessive concentrations can result in vomiting, weakness, trembling, and convulsions (Islam et al. 2009b).

There are plenty of methods used to prevent and control weeds like *L. camara, Parthenium hysterophorus, X. strumarium*, etc. with limited effectiveness, including various cultural, mechanical, biological, and chemical methods (Chauhan 2020b). Mulching, tillage, and drip irrigation are cultural practices that assist with managing weeds but are unable to entirely eradicate them. Using stale seedbed treatments, weeds are brought to the surface, given the chance to grow, and then eliminated with gentle tillage although some of the weed residues remain in the field. Cover crops are effective in eradicating about 70–90% of weeds. Weeds can be managed through changing methods of cultivation, fertilizer use, and herbicide treatment, but complete eradication remains improbable. Cost-benefit analysis is important in physical and mechanical techniques like cultivation, hand weeding, and flaming. Cultivation can also expose the naked ground and induce soil compaction(Hussain et al., 2008). Insects appear to be a promising biological control method. The risk for non-target species, however, requires frequent monitoring (Day & Neser, 2000). Chemical Herbicides used for weed management have an impact on the ecosystem, soil fertility, and the food chain is compromised. Ecosystems are seriously threatened by the presence of herbicides in the environment, particularly in aquatic systems. While there are many methods for managing weeds, obtaining complete eradication is difficult and necessitates careful consideration of the effects on the environment(Saha et al. 2018).

Adopting vermicompost technology, farmers can produce organic manure by composting biodegradable waste, including weeds, on-site, eliminating the need for additional raw material purchases. This stands as a sustainable alternative to chemical fertilizers, which have raised concerns regarding land productivity and cultivation costs. Problematic weeds can also be transformed into valuable resources through vermicomposting, as they grow naturally, providing a beneficial soil cover. By harnessing earthworms', the capacity to convert all biodegradable waste into nutrient-rich organic manure, this eco-friendly approach enhances soil health, fertility, and crop productivity, ensuring agricultural sustainability and reducing environmental impact (Das & Deka, 2021). Several publications have discussed the viability of vermicomposting with regard to various weeds, *Ageratum conyzoids* (Devi and Khwairakpam 2020b), *Chromolaena*odorata (Ganguly et al., 2022b), *Lantana camara*(Suthar and Sharma 2013), *Parthenium hysterophorus* (Yadav and Garg 2011), etc *X. strumarium*'s vermicomposting, however, has not yet been reported, and the aspects of and polyculture are yet inadequately characterized.

The goal of the current study was to use vermitechnology to manage problematic and noxious weeds while assessing the characteristics of the mono- and polyculture techniques. It may provide a dual benefit of effectively controlling weeds while simultaneously producing a valuable product in the form of vermicompost.

2. Materials and method

2.1 Collection of Cowdung (CD), earthworm (EW), and weed phytomass (WP):

Fresh urine-free CD was obtained from a livestock farm located nearby and was stored in plastic containers at room temperature. Individuals of *Eisenia fetida* and *Eudrilus eugeniae* were procured from Krishi Vigyan Kendra (KVK), Guwahati, Assam, India. For the experiment, a stock culture was maintained in the laboratory condition taking CD as feedstock and moisturizing at 3 days intervals *X. strumarium* and *L. camara* were collected from the agricultural field.

2.2 Experimental design

Raw material	Treatments
Xanthium strumarium + Cowdung (R1)	C1(Compost)
	VC1(R1 + *Eisenia fetida*)
	VC2(R1 + *Eudrilus eugeniae*)
	VC3 (R1 + *E. fetida* + *E. eugeniae*)
Lantana camara + Cowdung (R2)	C2 (Compost)
	VC4 (R2 + *E. fetida*)
	VC5(R2 + *E. eugeniae*)S
	VC6(R2 + *E. fetida* + *E. eugeniae*)

Table I : Illustration of the treatments of different raw materials during the experiment.

Four treatments for each WP i.e., *X. strumarium* and *L. camara* were established having WP and CD in the proportion of 4:1. The experiment was conducted in a 5L volume (diameter 15.9 cm and height 25.3 cm) plastic pot with proper holes in the bottom. Earthworm bed (3cm) was prepared using small stones and bricks, layering them with a mixture of sand and CD(Das and Deka 2021a). The vermicompost (VC) experiment was conducted with three treatments each having three replicas. In VC1 & VC4 treatment, monoculture was carried out employing 60 individuals of EW, *E. fetida*. In VC2 & VC5 treatments, 60 individuals of EW, *E. eugeniae* were introduced. VC3 & VC6 treatments were polycultures of *E. fetida* and *E. eugeniae*, engaging 30 + 30 numbers of both EW species. In each treatment, an equal amount of feed mixture (500 gm dry weight basis) was taken and kept for 14 days for partial decomposition to mitigate the volatile toxic gases and adverse heat release during the initial phase of decomposition. Pre-decomposition helps the effective functioning of EW, microbial proliferation, and enzymatic activity during vermicomposting (Mago et al. 2021a). A similar control setup was maintained without EW for experimental comparisons. The moisture content of the vermibins was kept around 50–80 % by a periodic sprinkling of water. Wile the temperature of the vermibins was in ideal conditions throughout the period of trial i.e. 25 ± 3 ºC. The trial was carried out for 60 days considering the EW introduction day as 0, all the vermibins were covered with breathable paper to keep away pests and maintain excessive moisture loss.

2.3 Physico-chemical, nutrient, and microbial analysis

The characterization encompassed the physico-chemical, biological, and nutritional characteristics of vermicompost, compost, and raw material samples. The samples were pre-dried and underwent triplicate analysis. The pH and conductivity (EC) were determined in the substrate to water extract ratio 1:10 (w/v) using a digital pH meter (Biochem PM79) and a conductivity meter (Systronics 304) respectively. The ash content was derived using methods of Nelson & Sommers (1982). The estimation of total organic carbon (TOC) was carried out following the Walkey Black titration methodwith slight modification (Sahrawat, 1982). The micro Kjeldahl method was used for determining the total Kjeldahl nitrogen (TKN) (Jackson 1967). The C/N ratio was obtained from the available total organic carbon and nitrogen content. The available phosphorus was characterized spectrophotometrically (Shimadzu UV 1601) employing the Stannous chloride method (APHA, 1998). The total potassium (TK) was derived using a flame photometer by the acid digestion method (APHA 1998). The humification index was determined by utilizing the method characterized by Boruah et al. (2019). The micronutrient and heavy metal, Ca, Mg, Zn, Fe, Cu, Mn, Cd, Ni, Co, and Cr contents were assessed by atomic absorption spectrophotometer (Shimadzu AA7000) after acid digestion of the samples. The pour plate method and serial dilution techniques were used in deriving the total population of nitrogen-fixing, phosphorus, and potassium-solubilizing bacteria (Dubey and Maheshwari 2005;Das & Deka, 2021), and the results have been presented as the number of colony-forming units per gram (CFU g^{-1}).

2.4 Germination and phytotoxicity

Germination and phytotoxicity tests were conducted on *Solanum lycopersicum* seeds. To experiment phytotoxicity, aqueous mixture of the substrate and a uniform mixture of vermicompost was prepared. The aqueous mixture was prepared with 1gm each of the sample with 10 ml of distilled water. After filtering the sample slurry using Whatman No. 1 filter paper, extracts were added to Petri plates that had been lined with filter papers. As a control, distilled water without any samples was used. The experimental plants' seeds were placed in separate moistened plates and incubated at 25°C in the dark. Five milliliters of distilled water were continuously added to the Petri plates to maintain the filter paper's moisture levels throughout the duration of the investigation. Following formulas were used to determine the relative seed germination (RSG), relative root growth (RRG), and eventually the germination index (GI) after five days of dark incubation(Khatua et al. 2018).

$$RSG = \frac{Numberofseedsgerminatedinsampleextract}{Numberofseedsgerminatedincontrol} \times 100$$

$$RRG = \frac{\text{Meanrootlengthinsamplesextract}}{Meanrootlengthincontrol} \times 100$$

$$GI\,(\%) = \frac{RSG \times RRG}{100}$$

3. RESULTS AND DISCUSSION

The unified approach of EW and microbes during vermicomposting changed the physico-chemical and biological nature of substrate materials. The present study demonstrates the production and physico-chemical properties of vermicast and were dependent on the substrate material and employed EW.

3.1 Production

The study reported that there were significant variations between the vermi-conversion rates of the two EW, i.e *E. fetida* and *E. eugeniae*) in different substrates. The results are represented in Figure I. The monoculture technique of *E. fetida* outperformed *E. eugeniae* in the production of both mono- and polyculture techniques. Vermi-conversion rates of substrate of *X. strumarium* feed mixture were significantly higher than *L. camara*. The results showed highest in VC1 treatment with 76.73 ± 1.37, VC2 treatment with 65.03 ± 2.21 and VC3 with 65.76 ± 5.19. Similarly, in *L. camara*, it was highest in VC4 with 57.76 ± 3.14, VC5 with 45.16 ± 2.48 and VC6 with 51.47 ± 2.53. These results showed similarities with the previous findings of Ahmed & Deka (2022). Production and quality of vermicompost depend on the nature of the substrate mix, reproductive and metabolic activity of earthworm species, introduced EW number, initial physico-chemical characteristics of organic material, and duration of vermicomposting (Sharma and Garg 2018; Ananthavalli et al. 2019).

Figure I: Amount of vermicompost produced in all the treatments after 60 days of vermicomposting. Values are mean; n = 3; error bars are indicators of SD. VC1 (R1 + E. fetida), VC2 (R1 + E. eugeniae), VC3 (R1 + E. fetida + E. eugeniae), VC4 (R2 + E. fetida), VC5 (R2 + E. eugeniae), VC6 (R2 + E. fetida + E. eugeniae), Dissimilar letters in treatments indicate statistically significant differences (ANOVA, LSD test, p < 0.05).

3.2 Physico-chemical profiles

3.2.1 pH and Ec

The pH of raw *X. strumarium* and *L. camara* were initially measured at 8.13 ± 0.01 and 7.86 ± 0.02 and it decreased towards the neutral level over the course of the composting and vermicomposting processes. The pH of *X. strumarium* compost was 7.78 ± 0.02 while the value of pH in the vermicompost treatment VC1, VC2, and VC3 were 7.22 ± 0.005, 7.45 ± 0.02 and 7.37 ± 0.02 respectively. Similarly, in *Lcamara* the pH of compost was recorded at 7.64 ± 0.023 and in vermicompost treatment VC4, VC5, and VC6, it was 7.25 ± 0.02, 7.27 ± 0.01, and 7.22 ± 0.01 respectively. Earlier investigations of different raw materials, such as, *Parthenium hysterophorus*(Devi and Khwairakpam 2020c), *L. camara*(Devi and Khwairakpam 2020c), banana crop waste (Mago et al. 2021b) also reported a decrease in pH. The change in pH is dynamic in nature and attributed to different substrate materials used. The reduction in pH values is linked to the humification of substrate material and mineralization of nitrogen and phosphorus as well as the formation of CO_2 and NH_3 during the vermicomposting process (Gusain and Suthar 2020a). The pH is an important factor for establishing the maturity of the end product, the neutral range of pH in compost and vermicompost indicated the stabilization of raw material during the process of the experiment(Kauser and Khwairakpam 2022). The values of EC in both compost and vermicompost samples have a substantial increase compared to the initial values of the raw materials (as shown in Table I). The EC is a crucial parameter in compost for plant health, the toxicity of the compost can be monitored by the value of EC (Karak et al. 2013). The initial value of the substrate *X. strumarium* and *L. camara* was 2.21 ± 0.13 ds/m and 2.12 ± 0.30 ds/m and it showed an increase in compost to the range of 2.3 ± 0.34 ds/m and 2.5 ± 0.50 ds/m. The vermicompost samples showed a 1.4- 1.7-fold increase in EC which fell below the advised value of 4 ds/m for applying vermicompost to the soil(Li et al., 2012). The spike in EC observed in the final vermicompost may be attributed to the generation of diverse soluble salts and the accumulation of ions during the process of mineralization. This increase in EC was is consistent with a pattern observed in previous studies (Das and Deka 2021a; Ruangjanda et al. 2022; Preethee et al. 2022).

3.2.2 Total organic carbon (TOC)

The TOC content decreased in all the vermicompost and compost units. Among all, the most significant decline, at 71.78%, was observed in the VC4, whereas other vermicompost samples showed reductions ranging from 67.75 to 60.21% (Table II). In the compost treatments, the TOC content was reduced in the *X. strumarium* and *L. camara* substrates by 24.64% and 30.05%, respectively. The evolution of CO_2, along with the mineralization and humification processes during the composting and vermicomposting process reflects the loss of TOC, releasing CO_2 into the atmosphere(Negi and Suthar 2018). During the process of substrate maturity, the rate of CO_2 evolution increases with the start of the treatment and then gradually declines with the maturity of the product. The vermicompost's maturity index can be assessed before it is used as a soil fertilizer using the rates of CO_2 evolution and the Carbon-to-Nitrogen (C/N) ratio as key metrics(Saidi et al., 2009). The present studies showed conformity with the previously published results(Gajalakshmi et al 2001.; Hussain et al. 2016; Gusain and Suthar 2020b; Das et al. 2022; Ganguly et al. 2022c) with a decrease of 30–75% percent of TOC content. The substrate material is chemically altered by earthworms, which serve as accelerators for the proliferation of microorganisms. The joint action of earthworms and microbes leads to the consumption of organic carbon, ultimately resulting in a reduction of TOC content(Boruah et al. 2019). The extent of this decline, however, is contingent upon the specific substrate conditions that influence both microbial and earthworm activities throughout the vermicomposting process.

3.2.3 Ash content

The analysis of ash content in both compost and vermicompost samples reveals (Table II) a notable augmentation, with the most substantial increase observed in the *X. strumarium* VC1 treatment over the raw material, with a 2.02-fold rise and it was closely followed by VC2 and VC3 with a 1.95 and 1.86-fold respectively. *L. camara*'s substrate shows similar trends with a 1.73-1.88-fold increase in the vermicompost sample. Similar findings were recorded byBoruah et al. (2019) in paper mill sludge,Devi & Khwairakpam (2021) in *Parthenium* andDevi & Khwairakpam (2020b) in *L. camara*. The observed elevation in ash content in the vermicomposting process implies a transformation involving mineralization, degradation of lignin, cellulose, hemicellulose, and release of organically bound nutrients into inorganic forms (Singh and Kumar 2017a; Jain and Kalamdhad 2018a; Devi and Khwairakpam 2020d).

3.3 Macronutrients (TKN, AP, TK, and Ca)

Macronutrient enrichment was consistent across all the vermicompost samples. Gradually, the TKN content was elevated in both compost and vermicompost in comparison with initial waste mixers. The range of TKN in the initial substrate (R1 & R2) was recorded as 19.33 ± 0.68, 6.01 ± 0.8g/kg and in compost (C1 & C2) it was 14.89 ± 0.35, 6.53 ± 0.35-g/kg. The maximum TKN (30.96 ± 0.37 g/kg) content was observed (Table-II) in the vermicompost sample produced in the VC1 vermireactors. The vermicompost sample derived from *X. strumarium* substrate recorded a 1.46-1.56-fold increase in TKN value and the *L.*

camara showed a 2.20-2.47-fold increase. The present study showed conformity with the findings of the previous author (Jain and Kalamdhad 2018b; Ganguly and Chakraborty 2019; Chen et al. 2023). The TKN augmentation hinges on the substrate material and earthworm activity throughout the vermicomposting process. Several variables contribute to the elevation of nitrogen content, such as a decrease in TOC, and nitrogen-rich secretions by earthworms including enzymes, mucus, growth hormones, and excretory products (Sharma and Garg 2019). All the vermireactors consistently showed elevated available phosphorus (AP) content compared to the compost and raw materials. The VC1 with *X. strumarium* substrate shows the highest (4.74 ± 0.82 g/kg) AP content followed by VC3 (4.38 ± 0.5 g/kg), VC2 (3.83 ± 0.31 g/kg), VC4 (2.66 ± 0.13 g/kg), VC6 (1.87 ± 0.35 g/kg) and VC5 (1.84 ± 0.23 g/kg) vermireactors. An increase in AP in the vermicompost sample was previously reported by various researchers (Jain and Kalamdhad 2018c; Esmaeili et al. 2020; Kauser et al. 2022). Earthworms and microbes accelerated mineralization which releases certain metabolites, the reduction of phosphorus was expedited in EW activity resulting in the availability of phosphorus in the vermi sample (Suthar and Singh 2008). Furthermore, the rise in AP levels in vermicompost may stem from phosphate-solubilizing bacteria and the EW gut microbe-produced faecal phosphatase enzyme (Das and Deka 2021a). The total potassium (TK) and Ca levels both experienced a rise in vermicompost and compost samples, with the most significant increase resulting in VC1, registering at 24.24% for TK and 19.23 % for Ca. (Table II). Both potassium and calcium are indispensable nutrients crucial for plant development. The application of calcium in the form of oxides, hydroxides, or carbonates to the soil enhances te accessibility of nutrients to plants (Singh and Kumar 2017b). Mago et al. (2021b) also reported an increase in TK content in the vermicompost of banana crop waste, both TK and Ca increase was reported in polyculture of *E. fetida* and *E. eugeniae* in substrate *P. hysterophorus* (Devi and Khwairakpam 2021). The rise in TK and Ca levels in vermicompost samples can be attributed to the physical breakdown of substrate materials and the concurrent release of endogenous and exogenous enzymes, primarily driven by the activities of earthworms and microbes (Bhattacharya et al. 2016). Furthermore, the mineralization process occurring during vermicomposting leads to the liberation of Ca from its bound form, subsequently elevating its concentration in the final product (Malińska et al. 2016)

Table II: Showing the changes in physicochemical characteristics and macronutrients concentration of the control and different treatments (Mean ± SD, n = 3).

Treatments	pH	EC (mS/cm)	Ash contents (g/kg)	TOC (g/kg)	TKN (g/kg)	AP(g/kg)	TK (g/kg)	T Ca (g/kg)
R1	8.13 ± 0.017^{a}	2.21 ± 0.13^{a}	0.36 ± 0.005^{a}	56.55 ± 2.02^{a}	19.33 ± 0.64^{a}	3.20 ± 0.72^{a}	1.49 ± 0.2^{a}	4.99 ± 0.61^{a}
R2	7.86 ± 0.026^{b}	2.12 ± 0.30^{a}	0.29 ± 0.016^{b}	51.61 ± 1.47^{b}	6.01 ± 0.084^{b}	1.98 ± 0.53^{b}	1.21 ± 0.32^{a}	3.78 ± 0.52^{b}
C2	7.64 ± 0.023^{d}	2.5 ± 0.50^{a}	0.42 ± 0.423^{c}	36.01 ± 1.36^{d}	6.53 ± 0.35^{b}	2.10 ± 0.17^{c}	1.23 ± 0.08^{a}	3.76 ± 0.71^{b}
VC1	7.22 ± 0.005^{e}	4.26 ± 0.21^{b}	0.73 ± 0.028^{d}	20.41 ± 2.14^{e}	30.96 ± 0.37^{d}	4.74 ± 0.82^{d}	1.85 ± 0.12^{ab}	5.94 ± 0.65^{c}
VC2	7.45 ± 0.026^{f}	4.14 ± 0.20^{b}	0.71 ± 0.015^{d}	22.1 ± 1.19^{e}	29.09 ± 0.58^{e}	3.83 ± 0.31^{a}	1.81 ± 0.34^{ab}	5.65 ± 0.63^{c}
VC3	7.37 ± 0.020^{g}	3.92 ± 0.12^{b}	0.67 ± 0.027^{e}	20.93 ± 1.62^{e}	30.16 ± 0.56^{f}	4.38 ± 0.50^{d}	1.79 ± 0.31^{ab}	5.71 ± 0.47^{c}
VC4	7.25 ± 0.020^{h}	3.25 ± 0.02^{c}	0.74 ± 0.041^{d}	14.56 ± 0.59^{f}	14.89 ± 0.42^{c}	2.66 ± 0.13^{c}	1.48 ± 0.51^{a}	3.81 ± 0.73^{b}
VC5	7.27 ± 0.017^{h}	3.27 ± 0.36^{c}	0.69 ± 0.052^{d}	16.64 ± 1.84^{f}	13.4 ± 0.58^{g}	1.84 ± 0.23^{b}	1.45 ± 0.42^{a}	3.87 ± 0.54^{b}
VC6	7.22 ± 0.010^{e}	3.22 ± 0.01^{c}	0.72 ± 0.039d	17.68 ± 2.87^{g}	13.26 ± 0.29^{g}	1.87 ± 0.35^{b}	1.46 ± 0.19^{a}	3.86 ± 0.64^{b}

R1=*X.Strumarium* +Cowdung, R2=*L.camara* +Cowdung, C1=Compost −1, C2−Compost−2, VC1(R1+*E.fetida*), VC2(R1+*E.eugeniae*), VC3 (R1+*E. fetida*+*E. eugeniae*), VC4 (R2+E.fetida), VC5(R2+E. eugeniae), VC6(R2+E. fetida+E. eugeniae); Different letter in the same columns indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

3.4 C/N Ratio

The present study demonstrated a pronounced decline in the C/N ratio in each treatment of both mono- and polyculture techniques. The reduction in C/N ratio was also evident in the compost sample across all substrate types. The C/N ratio serves as a gauge for the maturity of compost and vermicompost material. A ratio below 20 is indicative of effective decomposition and nutrient-rich manure (Das & Deka, 2021). A substantial reduction of C/N value was observed in both compost and vermicompost samples of *X. strumarium*, the rate of decline in monoculture of *E. fetida* (VC1) was highest followed by VC3 and VC2. The monoculture treatment of *L. camara* registered a higher rate of decline in VC4 then closely followed by VC5 and VC6. Similar findings were reported by Wu et al. (2023) and Yasmin et al. (2022). The increased N level and accelerated breakdown of carbon matter during the vermicomposting process led to a more significant decrease in the C: N ratio of the mature product (Yatoo et al. 2022).Balachandar et al. (2021) illustrated that a low C/N ratio enhances earthworm and microbial activity. The C/N ratio can be lowered by a suitable bulking agent, notably cow manure, high C/N ratio inhibits earthworm efficiency in the vermicomposting process.

Figure II: C/N ratio of control, compost, and vermicompost treatments (after 60 days).

3.5 Humification Index (HIX)

The graphical representation of the HIX is illustrated in Figure III Regardless of the initial substrate material, the vermicompost showed a significant decrease in HIX values indicating effective composting both in mono and polyculture. The average HIX value for the initial substrate material *X. strumarium* was 8.25 ± 1.22 and *L.camara* was 7.03 ± 0.66 and were significantly decreased in compost to 6 ± 0.32 and 4.96 ± 0.05 respectively. Further, the vermicompost sample of *X. strumarium* monoculture reported 2.03 ± 0.11 (VC1) and 3.34 ± 0.12 (VC2). Similarly, in polyculture, the HIX value was 3.63 ± 0.54 (VC3). A similar trend was also seen in the product of *L. camara* substrate, the values were 3.12 ± 0.07 (VC4), 2.06 ± 0.03 (VC5), and 2.08 ± 0.12 (VC6). The HIXserves as an informative metric for assessing the stabilization of compost and vermicompost. The decomposition and stabilization of waste materials were probably aided by the cooperative efforts of microorganisms and earthworms, with the presence of cow dung accelerating the process. A HIX value below 5 denotes an elevated rate of organic matter humification within the substrate material(Ashok Kumar et al. 2023).

Figure III: Showing the HIX values of raw material, compost, and vermicompost treatments. Values are mean; n = 3; error bars are indicators of SD, Different letter in treatments indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

3.6 Bacterial population

The beneficial bacterial population in vermicompost samples shows a substantial increase over compost and raw material. The population of nitrogen-fixing, phosphate-solubilizing, and potassium-solubilizing bacteria was highest in the VC3 of *X. strumarium* substrate closely followed by VC1 > VC6 > VC2 > VC5 > VC4 > C1 > C2. The expanded results are plotted in the Figure IV. A significant increase in beneficial microbes found in substrates handled by earthworms has been extensively established in earlier investigations, including the one carried out by (Zhou et al. 2021). The earthworm's digestive system liberates nutrients from the materials it consumes, the earthworm's gut is a nutrient-rich habitat that fosters the development of bacterial communities, which ultimately results in an increase in the populations of helpful bacteria in the excreta or vermicompost. Furthermore, an increased stabilization of macro- and micronutrients was suggested by increase in beneficial bacterial populations in vermicompost (Kumar and Singh, 2000; Gómez-Brandón and Domínguez 2014). The precise types and combinations of the initial substrate materials employed have a significant impact on this outcome.

Figure IV: Total population of N2 fixing, P, and K solubilizing bacteria in the raw material, compost, and vermicompost treatments. Values are mean; n = 3; error bars are indicators of SD, Different letter in treatments indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

3.7 Earthworm population and biomass

The growth in the earthworm population and biomass is associated with the type of substrate, and microenvironment of vermireactors as well as the survival, growth rate, and reproductive capacity of earthworms. The palatability of the substrate mixture was established by the growth of EW. The present study reported the highest 3.1-fold increase in the population in treatment VC1 followed by VC3 > VC2 > VC6 > VC5 > VC4. The reproductive capacity and growth rate of *E. fetida* were found to be higher than *E. eugeniae*. The initial mean biomass of *E. fetida* was 0.26 ± 0.06 gm, which demonstrated a notable augmentation at the conclusion of the experiment to 0.44 ± 0.6 gm. Similarly, the initial and final biomass of *E. eugeniae* species was recorded at 0.13 ± 0.2 gm, and 0.25±0.04 gm respectively. This result shows profound conformity with (Biruntha et al. 2020). Mago et al. (2021c) reported that cow dung affects the growth and fecundity of earthworms. Earthworm growth was dependent on the C: N ratio of the feed mixture, a lower ratio promotes the activity of earthworms resulting in higher growth and reproduction.

Figure V: Total population of Earthworm in different vermicompost treatments. Values are mean; n = 3; The error band is the indicator of SD. Different letter in treatments indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

3.8 Micronutrients and heavy metals

Compost and vermicompost typically contain heavy metal necessitating precise reports to prevent soil contamination. Trace element (Fe, Zn, Mn, Cu) have some important functions in plants' metabolism but prolonged exposure to high concentrations of heavy metal (Cu, Co, Cr) leads to toxicity in plants as well as the microflora of soil (Khan et al. 2019). The study revealed a notable increase in the concentration of Fe, Zn, Mn, Ni, Co, and Cr across all treatments (Table III). However, the Cu and Cd concentration was slightly decreased in the VC1, VC2 and VC3 treatment. Similar findings are reported by Lv et al., (2016). Garcfa et al.,(1995) reported an upsurge in the concentration of Cd, Cr, Cu, Fe, Mn, Pb, and Zn in the compost of sewage sludge, city refuse, peat residue, and grape debris. Increased amounts of Cu, Cr, Fe, and Zn were observed in the vermicompost of sewage sludge and dung (Gupta and Garg 2008). Vermicompost treatment also reported a 10–50 % decline in the concentration of Fe, Cr, Cu, Ni, Pb, and Zn in the differen substrate materials (Jain et al., 2004; Suthar & Singh, 2009). Two divergent mechanisms can influence the concentrations of heavy metal constituents in the vermicomposting process: (i) The phenomenon of bioaccumulation within earthworms, leading to a decline in concentrations, and (ii) The volumetric reduction stemming from enhanced organic decomposition, contributing to elevated levels of heavy metals in the vermicompost (Zhu et al. 2014; Lv et al. 2016b). Nonetheless, the concentrations of metals detected in the vermicompost samples fell within the permissible limits outlined by both international standards and those specific to India for the application of compost in agricultural and horticultural practices (Haroun et al. 2009; Das and Deka 2021a).

Table III: Showing the changes in heavy metal and micronutrient concentration of the control and different treatments (Mean ± SD, n = 3).

Treatments	ZN(mg/g)	Cd(mg/kg)	Cu(mg/kg)	Ni(mg/kg)	Co(mg/kg)	Cr(mg/kg)	Fe(mg/kg)	Pb(mg/kg)
R1	1.3 ± 0.03^{a}	0.3 ± 0.02^{a}	0.76 ± 0.02^{a}	1.7 ± 0.05^{a}	1.13 ± 0.24^{a}	0.84 ± 0.03^{a}	165.32 ± 0.4^{a}	Bdl
R2	0.8 ± 0.04^{b}	0.45 ± 0.02^{b}	0.98 ± 0.44^{b}	1.32 ± 0.04^{b}	1.2 ± 0.22^{a}	0.32 ± 0.04^{b}	154 ± 0.3^{b}	Bdl
C1	1.42 ± 0.06^{a}	0.3 ± 0.01^{a}	0.73 ± 0.02^{a}	1.79 ± 0.08^{a}	1.12 ± 0.01^{a}	0.86 ± 0.02^{a}	153.34 ± 2.88^{b}	Bdl
C2	1.03 ± 0.37^{c}	0.4 ± 0.05^{c}	0.97 ± 0.06^{b}	1.36 ± 0.03^{b}	1.02 ± 0.02^{a}	0.56 ± 0.02^{c}	133.2 ± 1.71^{c}	Bdl
VC1	1.46 ± 0.09^{a}	0.21 ± 0.03^{d}	0.54 ± 0.24^{c}	2.4 ± 0.09^{d}	0.89 ± 0.06^{c}	1.21 ± 0.05^{d}	223.22 ± 3.1^{d}	Bdl
VC2	1.43 ± 0.1^{a}	0.22 ± 0.03^{d}	0.57 ± 0.25^{c}	2.37 ± 0.09^{d}	0.88 ± 0.06^{c}	1.24 ± 0.05^{d}	221.93 ± 2.98^{d}	Bdl
VC3	1.40 ± 0.09^{a}	0.25 ± 0.02^{e}	0.55 ± 0.27c	2.35 ± 0.08^{d}	0.85 ± 0.05^{c}	1.20 ± 0.04^{d}	219.10 ± 2.84^{e}	Bdl
VC4	1.21 ± 0.04^{a}	0.35 ± 0.02^{f}	0.87 ± 0.05^{d}	1.32 ± 0.03^{e}	0.98 ± 0.02^{c}	0.65 ± 0.04^{e}	198.99 ± 1.76^{f}	Bdl
VC5	1.18 ± 0.05^{a}	0.36 ± 0.02^{c}	0.85 ± 0.04^{d}	1.30 ± 0.03^{e}	0.99 ± 0.02^{c}	0.63 ± 0.04^{e}	200.25 ± 1.86^{f}	Bdl
VC6	1.22 ± 0.04^{a}	0.34 ± 0.03^{f}	0.82 ± 0.05^{d}	1.28 ± 0.02^{f}	1.02 ± 0.03^{c}	0.61 ± 0.03^{e}	198.57 ± 1.73^{f}	Bdl

R1=*X.Strumarium* +Cowdung, R2=*L.camara* +Cowdung, C1=Compost −1, C2−Compost−2, VC1(R1+*E.fetida*), VC2(R1+*E.eugeniae*), VC3 (R1+*E. fetida*+*E. eugeniae*), VC4 (R2+E.fetida), VC5(R2+E. eugeniae), VC6(R2+E. fetida+E. eugeniae), Bdl= Bellow detection level; Different letter in the same columns indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

3.9 Phytotoxicity and Germination

The germination index (GI) is determined by using relative seed germination (RSG) and relative root growth (RRG) of germinating seed. The GI index is used as a key parameter for evaluating the toxicity and maturity of the final products of compost and vermicompost. It is considered an accepted indicator in numerous national standards for compost maturity assessment (Yang et al. 2021). A GI value below 50% suggests a significant level of phytotoxicity, while a value exceeding 80% indicates the absence of phytotoxicity(Luo et al. 2018; Ahmed and Deka 2022). GI values surpassing 100% are a clear sign of compost maturity, making it suitable manure for use in agriculture. The present results showed a significant level of phytotoxicity in *Solanum lycopersicum* seeds by both substrates. The GI value of the *X. strumarium* (R1) substrate was 34.80 ± 3.3 followed by the *L. camara* (R2) 28.40 ± 2.5. The GI value was found to be higher in the vermicompost sample. Vermicompost extract shows effective stimulation in the germination of seeds. The most substantial enhancement in GI 2.32-fold compared to the control was observed aqueous extract of *X. strumarium* L. *camara* vermicompost (XSVC). Additionally, in the case of *L. camara* (LCVC), the GI was 2.26-fold higher than the control. The expanded results are demonstrated in Figure VI. The GI percentage exceeding 100% for both vermicompost sample extracts can be attributed to the significant activity of EW within the substrate mixture. This activity results in the complete decomposition and maturation of the products by the end of the 60-day experimental trial (Warman and AngLopez 2010; Ievinsh 2011). Earthworms play a crucial role in detoxifying initial bio waste, and the resulting vermicompost has a substantial positive impact on seed germination, plant growth, and development.

Figure VI: GI (%) of Solanum lycopersicum seeds in different treatments. Values are mean; n = 3; The error band is the indicator of SD. Different letter in treatments indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

4. Conclusion

The present study reveals that earthworms can bio-stabilize the phytotoxicity of *X. strumarium* and *L. camara's* phytomass. The visible superiority of the EW species *E. fetida* over the *E. euguinae* was noticed in the monoculture treatment. Nevertheless, bacterial proliferation was very significant in the polyculture of *E. fetida* and *E. euguinae*. Vermicomposting has elevated levels of NPK while having lower levels of TOC and a lower C/N ratio. The final product contains minimal levels of heavy metals, well within recommended limits. Thus, vermicomposting effectively transforms the initial feedstock into a stabilized product suitable for enhancing crop productivity and soil health in agricultural fields. Notably, there was a visible increase in the size and population of earthworms by the conclusion of the study.

Declarations

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Figures

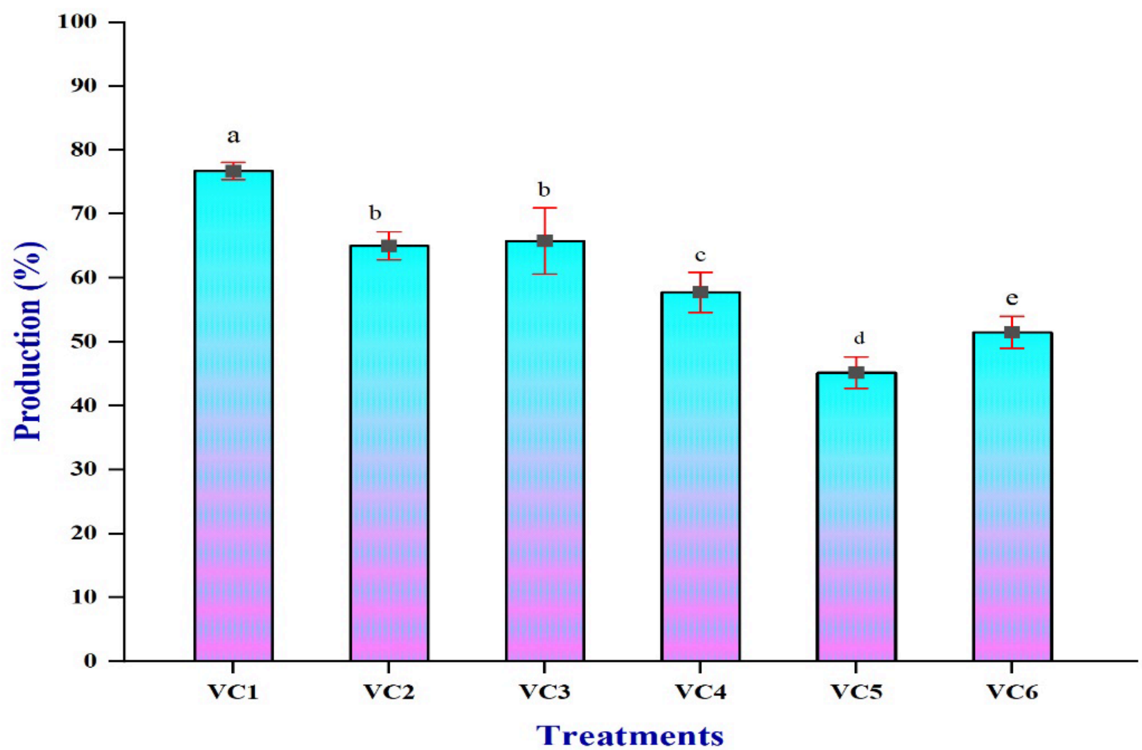

Figure 1

Amount of vermicompost produced in all the treatments after 60 days of vermicomposting. Values are mean; n = 3; error bars are indicators of SD. VC1 (R1 + *E. fetida*), VC2 (R1 + *E. eugeniae*), VC3 (R1 + *E. fetida* + *E. eugeniae*), VC4 (R2 + *E. fetida*), VC5 (R2 + *E. eugeniae*), VC6 (R2 + *E. fetida* + *E. eugeniae*), Dissimilar letters in treatments indicate statistically significant differences (ANOVA, LSD test, $p < 0.05$).

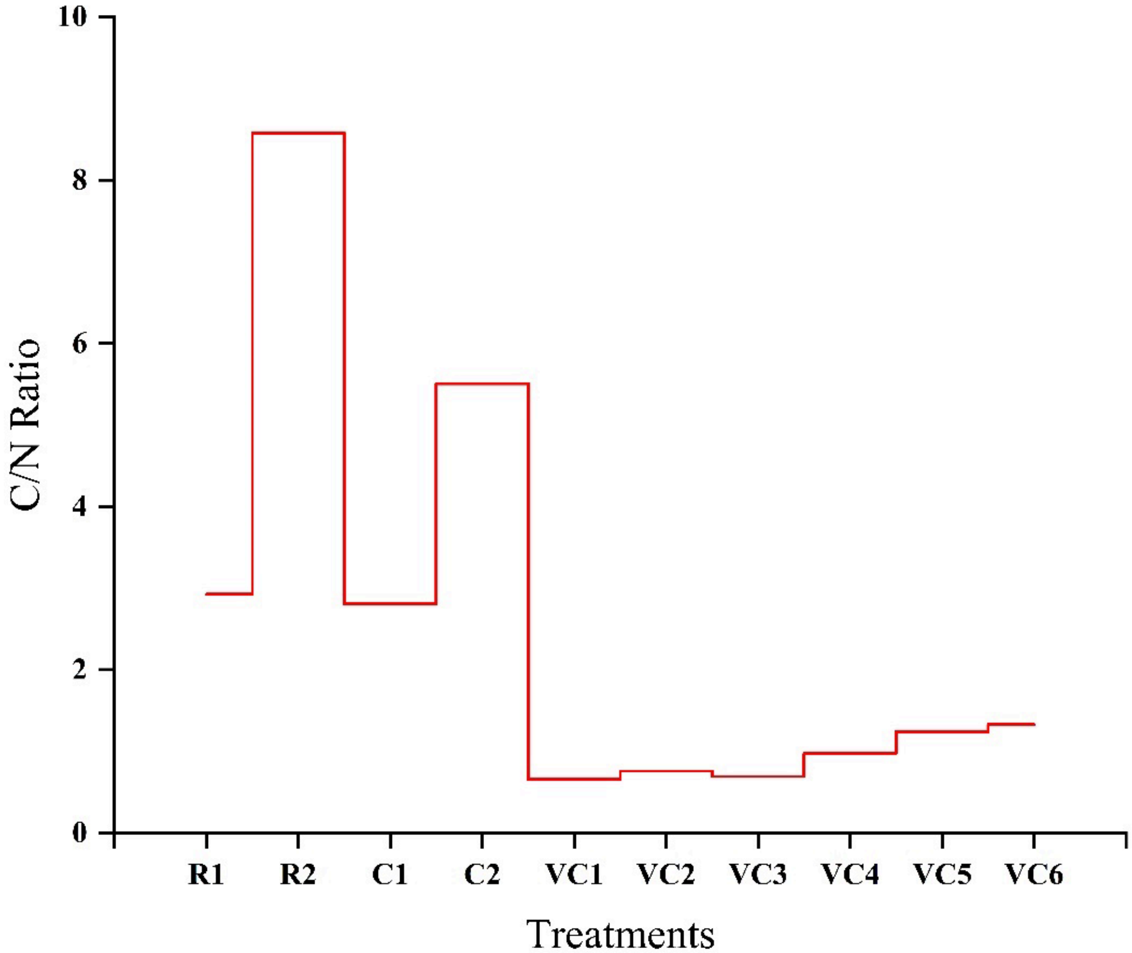

Figure 2

C/N ratio of control, compost, and vermicompost treatments (after 60 days).

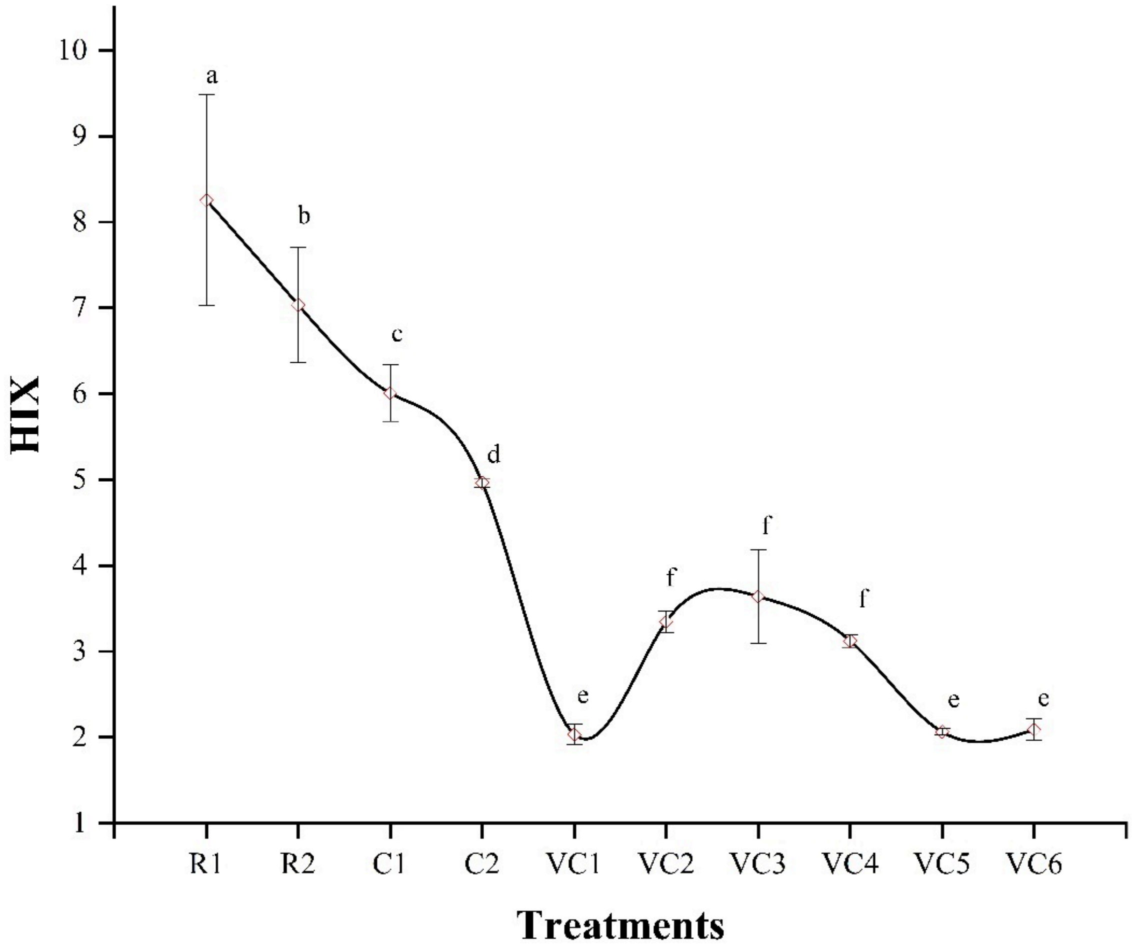

Figure 3

Showing the HIX values of raw material, compost, and vermicompost treatments. Values are mean; n = 3; error bars are indicators of SD, Different letter in treatments indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

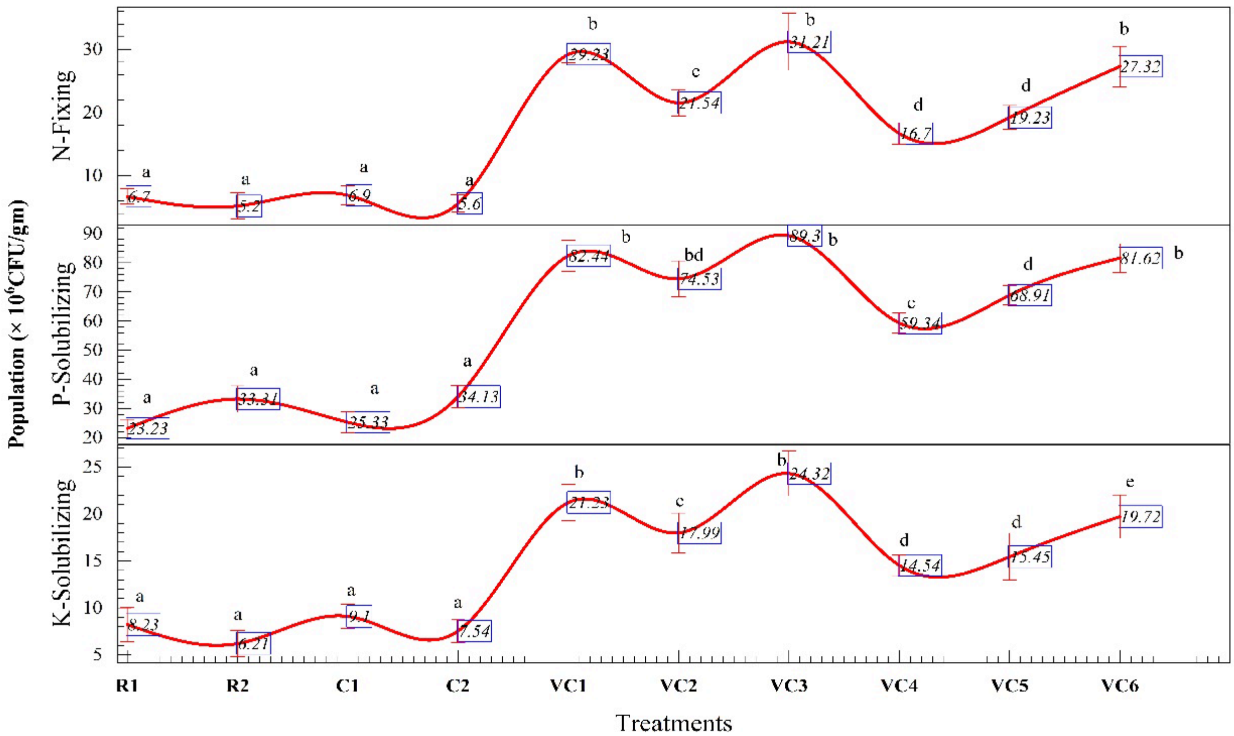

Figure 4

Total population of N2 fixing, P, and K solubilizing bacteria in the raw material, compost, and vermicompost treatments. Values are mean; n = 3; error bars are indicators of SD, Different letter in treatments indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

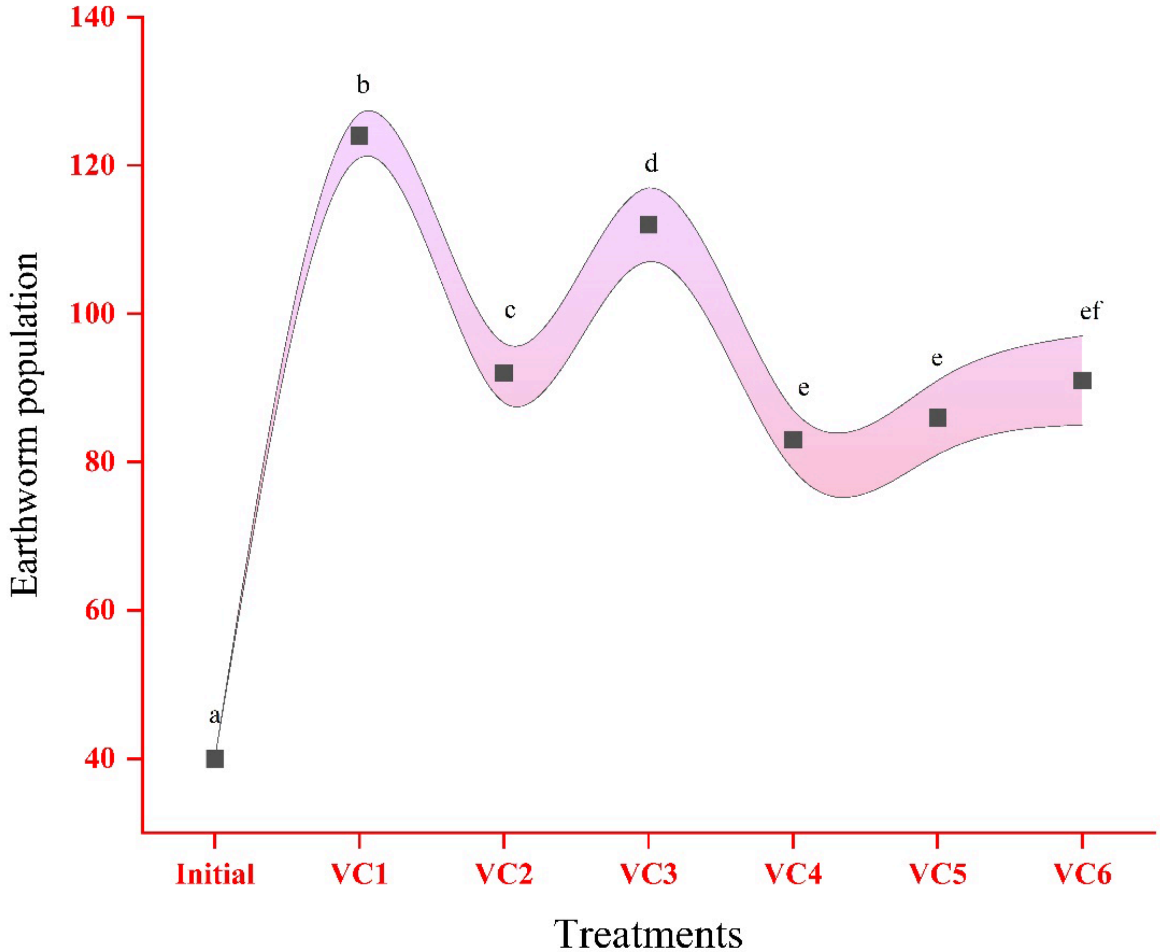

Figure 5

Total population of Earthworm in different vermicompost treatments. Values are mean; n = 3; The error band is the indicator of SD. Different letter in treatments indicates statistically different values (ANOVA, LSD test, $p < 0.05$).

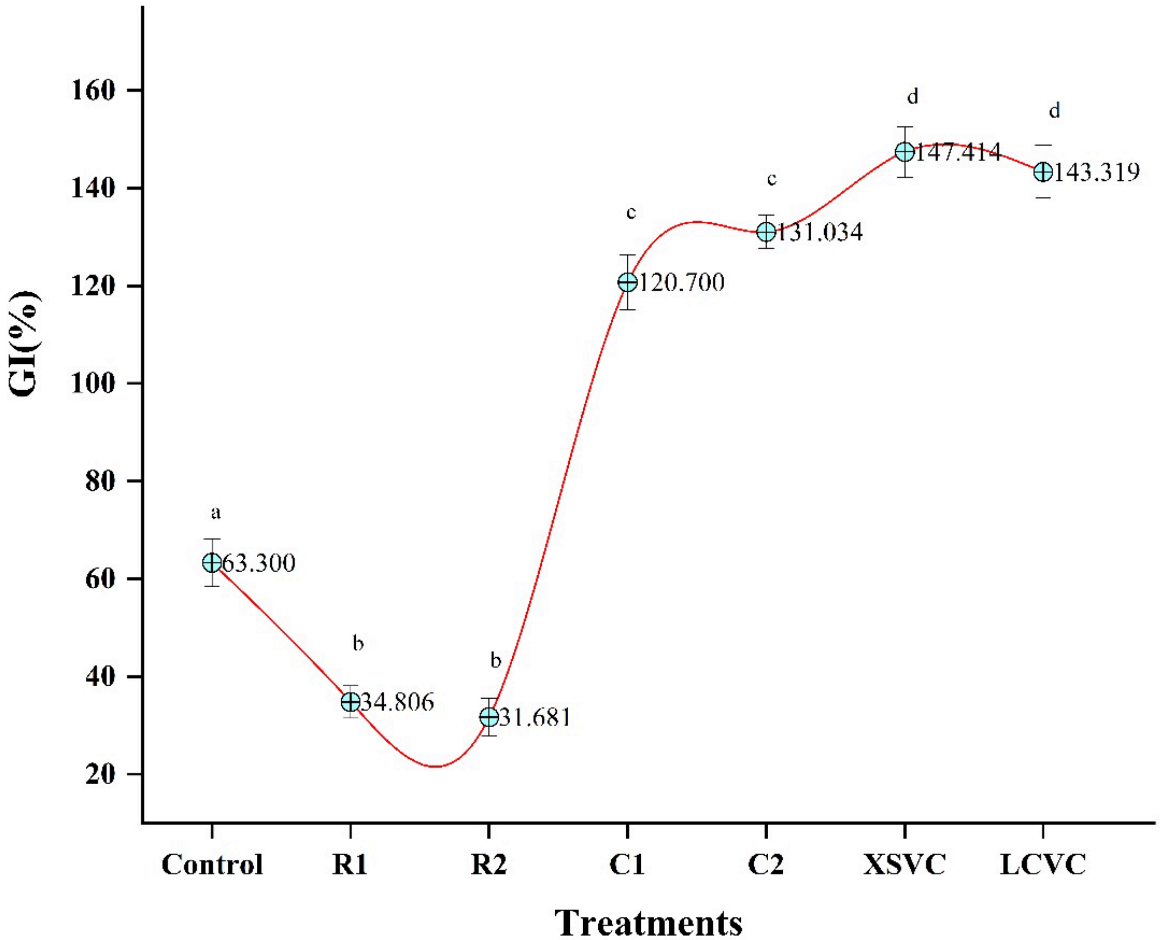

Figure 6

GI (%) of *Solanum lycopersicum* seeds in different treatments. Values are mean; n = 3; The error band is the indicator of SD. Different letter in treatments indicates statistically different values (ANOVA, LSD test, $p < 0.05$).