

## Enhancing growth in black gram and *Eudrilus eugeniae*: The impact of vermicompost derived from *Gliricidia sepium* and sugarcane bagasse

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### Abstract

This study examines the impact of vermicompost formulated with varying proportions of cow dung, *Gliricidia sepium*, and sugarcane bagasse on the growth dynamics of black gram (*Vigna mungo*) and the earthworm *Eudrilus eugeniae*. Five distinct substrate treatments combining cow dung with green manure and sugarcane bagasse were assessed to determine their effects on the biomass, length, and reproductive performance of *E. eugeniae*. Measurements were taken biweekly over a 50-day period, with growth rates calculated using Mazantseva's formula. Analysis of variance (ANOVA) was employed to compare the effects of different vermicompost doses under varying irrigation conditions. Significant growth and reproductive enhancement were observed in the VCT<sub>4</sub> vermicombed substrate, which demonstrated the highest growth metrics for *E. eugeniae*, reaching a maximum weight of  $91.88 \pm 2.44$  milligrams. Additionally, this group exhibited the fastest development of the clitellum, maturing in just about 2 days. The seed germination percentage was 78, 81, 84, 99, and 89 respectively in final substrate, VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub> and VCT<sub>5</sub> whereas, it was 70, 77, 71, 94, and 84 for the initial substrates. The results evidently showed that the vermicomposts were less phytotoxic, especially vermicompost of VCT<sub>4</sub> treatment, thereby enhancing plant health and productivity while offering a sustainable alternative to conventional agricultural inputs. This approach promises considerable environmental benefits, supporting a more sustainable and ecologically sound agricultural practice.

**Keywords:** *Eudrilus eugeniae*, Biomass, Reproductive performance, *Gliricidia sepium*, *Vigna mungo*, plant health.

### Introduction

In today's world, one of the predominant challenges confronting humanity is population growth, leading to an unprecedented demand for resources, particularly in agriculture (Carrasco-Bahamonde & Casellas, 2024) [8]. This necessitates the production of vast quantities of agricultural goods. However, the extensive use of weedicides, pesticides, and chemical fertilizers in modern farming practices has resulted in significant repercussions, affecting both agricultural land and the health of consumers (Sharma & Singhvi 2017 [40]; Prashar & Shah, 2016) [35]. Chemical fertilizers, synthesized from inorganic materials, are designed to provide essential nutrients to plants, promoting rapid growth and increasing crop yields (Kumar *et al.*, 2019) [24]. Typically, these fertilizers contain key nutrients such as nitrogen, phosphorus, and potassium, which are critical for plant development. However, their extensive use has raised environmental concerns. Over-application can lead to nutrient runoff into nearby waterways, causing eutrophication, a process that depletes oxygen in water bodies and leads to the death of aquatic life (Fetahi, 2019; Wato *et al.*, 2020) [14]. Additionally, excessive fertilizer use can alter soil chemistry, reducing its fertility and increasing its acidity. This not only impacts plant health and soil biodiversity but can also lead to increased greenhouse gas emissions, contributing to global warming (Pautasso *et al.*, 2010 [33]; Haj-Amor *et al.*, 2022 [20]; Singh *et al.*, 2011) [43]. Vermicompost is often considered superior to chemical fertilizers for several reasons (Sinha *et al.*, 2010 [44]; Shirkhani & Nasrolahzadeh, 2016) [41], particularly from an environmental and sustainable agriculture perspective. Vermicompost, produced through the breakdown of organic waste by earthworms, is highly valued in sustainable agriculture for its numerous benefits. This natural fertilizer

enriches the soil with essential nutrients, improves soil structure, and enhances its water retention capacity, making it more fertile and robust for plant growth (Javed *et al.*, 2022) [21]. It also introduces beneficial microorganisms to the soil (Domínguez *et al.*, 2019) [13], which help suppress plant diseases and promote a healthier, more biologically active soil ecosystem. Additionally, the use of vermicompost contributes to waste reduction by recycling organic kitchen and garden waste into valuable plant nutrients (Ansari *et al.*, 2020; Theunissen *et al.*, 2010) [4, 47]. Vermicompost is safe to handle and does not pose health risks to humans or animals, unlike some chemical fertilizers that can contain harmful compounds and residues. Due to these benefits, vermicompost is often preferred in organic farming and by gardeners seeking to maintain long-term soil fertility and ecological balance. It aligns well with practices that support environmental health and sustainable agriculture. As of 2023, vermicomposting has gained significant recognition as a sustainable waste management and soil enhancement technique across various sectors, including agriculture, horticulture, and urban gardening (Hajam *et al.*, 2023 [19]; Oyege *et al.*, 2023 [31]; Mohite *et al.*, 2024) [29]. Studies focus on understanding how vermicompost improves soil health, plant growth, and disease resistance. Researchers are also exploring the use of vermicompost in various agronomic conditions and its potential for sequestering carbon in the soil. The integration of green manure and factory waste materials into vermicomposting processes represents a significant advancement in waste management and sustainable agriculture (Ganguly & Chakraborty, 2020 [15]; Gupta, 2019) [18]. Using crops that are especially cultivated as green manure offers a rich source of organic matter that earthworms can break down into high-quality compost

(Sinha *et al.*, 2009) <sup>[45]</sup>. Incorporating factory waste materials, especially organic waste from food processing and agricultural industries, into vermicomposting can similarly reduce the environmental burden of waste disposal while transforming it into valuable compost (Yadav & Garg, 2011 <sup>[52]</sup>; Lee *et al.*, 2018) <sup>[26]</sup>. This process helps in closing the loop of organic waste production by turning potential landfill material into a resource that improves crop yields and soil structure (Sharma *et al.*, 2019) <sup>[38]</sup>. The use of these materials in vermicomposting not only maximizes resource efficiency but also contributes to reducing greenhouse gas emissions associated with organic waste decomposition in landfills. Studying the growth of earthworms is vital for several reasons, particularly in the contexts of soil health and ecological research. Understanding the factors that influence their growth, such as soil quality, temperature, moisture, and available nutrients, helps in evaluating the health of ecosystems and the effectiveness of agricultural practices like vermicomposting (Ceritoğlu *et al.*, 2018; <sup>[9]</sup> Singh & Singh, 2017) <sup>[42]</sup>. Moreover, earthworm growth studies can provide insights into the broader environmental impacts of pollutants and soil contaminants (Velki&Ećimović, 2017) <sup>[48]</sup>, as these organisms are sensitive indicators of ecological changes and soil toxicity (Adeel *et al.*, 2021 <sup>[1]</sup>; Gong & Perkins, 2016) <sup>[17]</sup>. By monitoring earthworm populations and their growth, researchers can assess the sustainability of agricultural practices and make informed decisions to promote soil regeneration and biodiversity vermicompost applications on plant growth. The use of vermicompost in agricultural fields has been shown to significantly enhance plant growth and yield (Lazcano & Domínguez, 2011) <sup>[13]</sup>. Vermicompost, a rich, nutrient-packed organic fertilizer produced by the digestion of organic waste by earthworms, contains not only essential plant nutrients like nitrogen, phosphorus, and potassium, but also a variety of beneficial micro organisms (Kumar *et al.*, 2020) <sup>[23]</sup>. These microorganisms improve soil structure, increase soil aeration, and enhance water retention, which are crucial for healthy plant development (Lehman *et al.*, 2015) <sup>[27]</sup>. Additionally, the bioactive compounds and hormones in vermicompost, such as auxins and cytokinins, stimulate plant growth and root development more effectively than conventional composts or chemical fertilizers (Arancon & Edwards, 2005) <sup>[5]</sup>; Wong *et al.*, 2020) <sup>[50]</sup>. Plants grown in soil amended with vermicompost are typically healthier, more robust, and more

resistant to diseases and pests, leading to improved agricultural productivity and sustainability. The unique characteristics of *Gliricidia sepium*, sugarcane bagasse, and cow dung suggest significant potential when combined in vermicomposting. Our investigation aimed to determine how these growth parameters are influenced by various organic waste treatments. Earthworms can thrive temporarily on GM due to its palatability and rapid biodegradability, while sugarcane bagasse offers a consistent food source. Moreover, the overall organic content is significantly boosted by *Gliricidia sepium*, which contains essential trace metals like iron, nickel, and zinc that are critical for microbial activity during the decomposition process. By studying both earthworm and plant growth in vermicompost derived from these materials, deeper insights into soil functionality and health can be gained. Such research not only helps in understanding the ecosystem dynamics but also supports the development of biotechnological approaches crucial for enhancing agricultural productivity and environmental sustainability. The current study aims to investigate the growth dynamics of black gram and *Eudrilus eugeniae* using vermicompost formulated with varying proportions of cow dung, *Gliricidia sepium*, and sugarcane bagasse.

**Materials and methods**

**Sample collection**

The study, which focused on vermicomposting with *Eudrilus eugeniae* earthworms from the Vermi Biotechnology Lab, Department of Zoology, Annamalai University in Tamilnadu, India. Cow dung was obtained from the university's dairy farm, sun-dried, powdered, and stored. It is necessary for the biodegradation of green manure and sugarcane bagasse. After being treated for a month to get rid of smells, sugarcane bagasse from M.R.K Co-operative Sugar Mill was used as bedding. Furthermore, leaves of *Gliricidia sepium* were gathered on campus in order to be ready for pre-composting, which is essential for lowering temperature and methane levels and preventing worm death.

**Experimental design**

Five substrate treatments were formulated by blending cow dung with green manure (*Gliricidia sepium*) and sugarcane bagasse (SCB) in varying proportions. The specific ratios for each treatment are provided below (table. 1)

**Table 1** Experimental Design for Prepared vermibed

Experimental groups	Substrate combination (%)		
	Sugarcane bagasse (SCB)	Cow dung (CD)	<i>Gliricidia sepium</i> (GM)
C	-	100%	-
VCT <sub>1</sub>	50%	50%	-
VCT <sub>2</sub>	70%	15%	15%
VCT <sub>3</sub>	60%	20%	20%
VCT <sub>4</sub>	50%	25%	25%
VCT <sub>5</sub>	50%	-	50%

C-Control, VCT-Vermicompost Treatments, and GM-Green manure

**Growth and fecundity of earthworms**

The vermibeds of the treatments, VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub>, and VCT<sub>5</sub> were separately maintained for assessing the growth and reproductive behavior of the earthworm, *Eudrilus eugeniae* in green manure amended sugarcane bagasse + cow dung substrates. The experiments were

carried out in a dark chamber with 27±1°C 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 physicochemical, enzymatic and microbiological analyses. Initial biomass, final biomass, and worm numbers were counted periodically

until the end of the vermicomposting process. Each week, a sample of substrate from each container was inspected to determine when cocoon production commenced. Upon the appearance of cocoons, they were carefully isolated and rinsed in distilled water. The total count of cocoons was recorded to assess fecundity. To evaluate hatching success, ten freshly laid cocoons from each container were transferred to miniature plastic boxes filled with the same bedding material as their parent's environment. The boxes were checked daily to monitor the emergence of hatchlings. Once a hatchling appeared, it was delicately separated using a fine paint brush, and the number of hatchlings that emerged was recorded.

**Seed germination assay**

The vermicompost maturity was also assessed by seed germination studies with black gram (*Vigna mungo*) seeds using substrates and vermicompost extracts obtained from each treatment. For this study, Each Petri dish contained 10 seeds of black gram and was subjected to testing across all experimental groups: C, VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub>, and VCT<sub>5</sub>. Initial and final readings were recorded throughout the test period, and the number of seeds germinated per day was recorded and analysed using the seed germination percentage assay. The germination percentage (GP) and germination index (GI) was calculated by adopting the method of UAF (2010) and Benech Arnold *et al.* (1991) respectively as detailed below. GI in VCs was calculated as it emphasizes on both the percentage of germination and its speed. A higher GI value denotes a higher percentage and rate of germination in different treatments (Kader, 2005).

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

$$GI = (10 \times n_1) + (9 \times n_2) + (8 \times n_3) \dots + (1 \times n_{10})$$

Where, n<sub>1</sub>, n<sub>2</sub> . . . n<sub>10</sub> = No. of seeds germinated on the first, second and subsequent days until the 10th day; 10, 9, and 1 are weights given to the number of seeds germinated on the first, second and subsequent days, respectively.

**Results**

**Growth and reproductive performance of *Eudrilus eugeniae* in the vermibed substrates, sugarcane bagasse and cow dung amended with *Gliricidia sepium* (50 days)**

Earthworm biomass, worm number and number of cocoons recovered after 50 days of vermicomposting of sugarcane bagasse and cow dung amended with leafy green manure plant, *Gliricidia sepium* is shown in Table 2 whilst the fold increase of worm biomass and worm number is depicted in Fig. 1. The results of worm growth and reproductive studies indicated that the activities of earthworms, growth and reproduction of earthworms is substantially influenced by different vermicomposting substrate combinations. A maximum of 91.88 ± 2.44 mg worm biomass/vermibed was observed in VCT<sub>4</sub> vermibed substrate which contained a mixture of sugarcane bagasse, cow dung and *Gliricidia sepium* leaf biomass which was significantly higher than that of the other treatments (Table 2). Next to VCT<sub>4</sub>, VCT<sub>2</sub> recorded a worm biomass of 87.13 ± 2.28 g/vermibed while the least worm biomass was found in VCT<sub>1</sub>. The final worm numbers in different vermicomposting treatments, VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub> and VCT<sub>5</sub> were 80.27 ± 2.41, 85.55 ± 2.41, 89.04 ± 2.02, 98.98 ± 2.94, and 83.15 ± 1.92 g/vermibed, respectively (Table 2). The number of cocoons recovered per vermibed was ranged from 86.80 ± 2.91 - 144.09 ± 3.78 with a significantly higher number in VCT<sub>4</sub> followed by VCT<sub>3</sub>. The fold increase of worm biomass was 5.33, 6.01, 5.90, 6.25 and 5.49 in VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub> and VCT<sub>5</sub> respectively whilst it was 4.01, 4.28, 3.30, 4.95, and 4.16 folds for worm numbers in the respective treatments (Fig. 1). The results on the growth and reproductive performance of *Eudrilus eugeniae* revealed that the vermibed combination (sugarcane bagasse + cow dung + *Gliricidia sepium* biomass) is much favoured by the earthworms resulting in higher biomass gain, worm number increase and cocoon production. This phenomenon is important for efficient vermicomposting process.

**Table: 2** Worm biomass, worm number and number of cocoons recovered after 50 days of vermicomposting of sugarcane bagasse and cow dung amended with *Gliricidia sepium*

Treatment	Worm biomass/vermibed (g)		Worm no. /vermibed		No. of cocoons/ vermibed
	Initial	Final	Initial	Final	
C	14.37 ± 0.36	57.18 ± 2.46 <sup>a</sup>	20	61.49 ± 2.39 <sup>a</sup>	68.43 ± 2.83 <sup>a</sup>
VCT1	14.34 ± 0.38	76.40 ± 2.09 <sup>d</sup>	20	80.27 ± 2.41 <sup>bc</sup>	85.56 ± 3.00 <sup>c</sup>
VCT2	14.50 ± 0.38	87.13 ± 2.28 <sup>b</sup>	20	85.55 ± 2.41 <sup>b</sup>	88.38 ± 2.66 <sup>cd</sup>
VCT3	14.30 ± 0.37	84.31 ± 2.35 <sup>c</sup>	20	89.04 ± 2.02 <sup>d</sup>	119.80 ± 2.91 <sup>d</sup>
VCT4	14.70 ± 0.42	91.88 ± 2.44 <sup>a</sup>	20	98.98 ± 2.94 <sup>a</sup>	144.09 ± 3.78 <sup>a</sup>
VCT5	14.45 ± 0.36	79.28 ± 2.03 <sup>d</sup>	20	83.15 ± 1.92 <sup>bc</sup>	107.72 ± 3.27 <sup>b</sup>

Values are mean ± SE, n = 3. Based on Tukey's HSD test, the different alphabets between treatments within each group are significantly different at P < 0.05

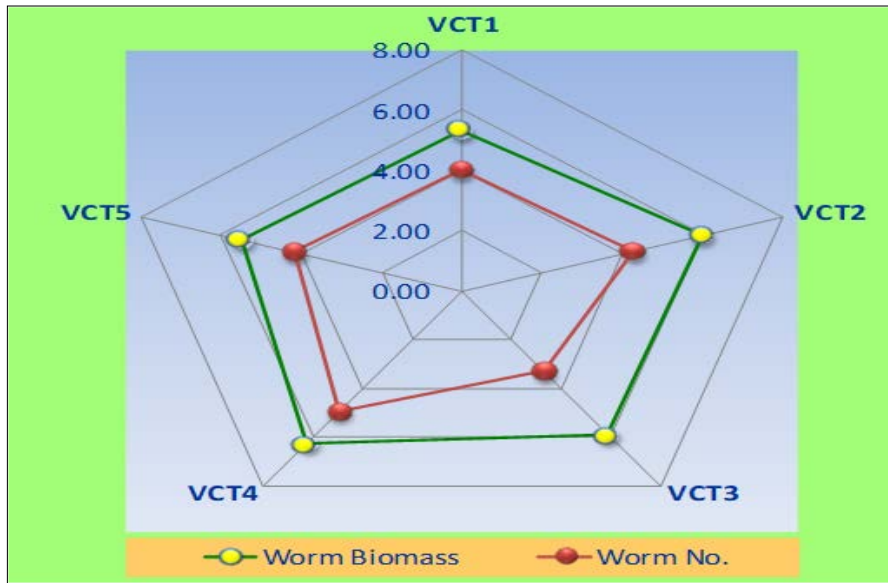
**Phytotoxicity assay through seed germination tests using the seeds of black gram**

Phytotoxicity assay is commonly employed to know whether the organic composts are toxicity free and can be used for agronomic purposes. In the present study, the final vermicomposts' phytotoxicity was assessed using black gram seedlings. Seed germination germination index, seedling characteristics (fresh weight, dry weight, root length and shoot length) and chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll) of black gram seedlings were assessed using vermicompost extracts

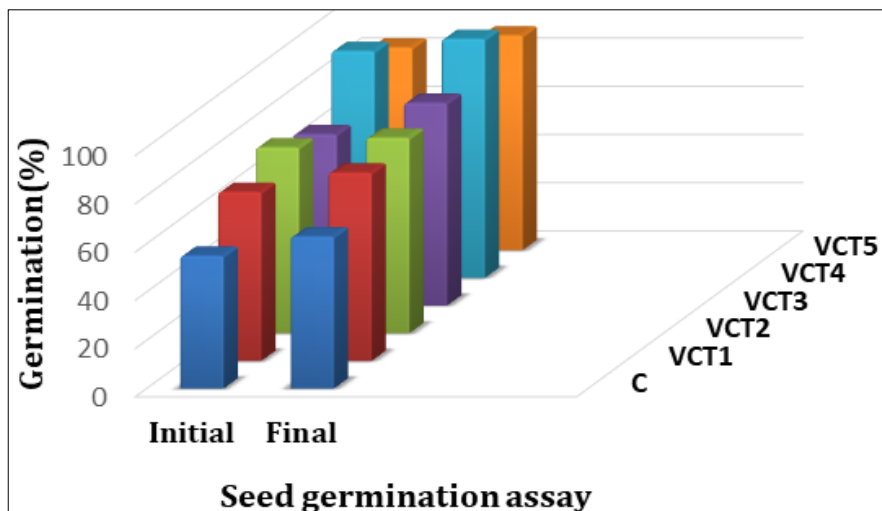
and the results obtained are provided in Tables 3, 4 and 5. In general, the germination of black gram seeds was higher in vermicomposts than that of the results obtained for initial substrates. However, the seed germination percentage varied between vermicomposts obtained from different treatments. The seed germination percentage was 78,81,84,99, and 89 % in vermicompost of VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub> and VCT<sub>5</sub>. The highest germination percentage was recorded in VCT<sub>4</sub>. However, the initial substrates showed the seed germination between 67.55% and 76.76% indicating that the substrates converted by earthworms into vermicompost are less phytotoxic in nature. The germination index percentage also

reflected the similar results to that of seed germination percentage. The seed germination index percentage was 79.51, 82.90, 84.83, 98.30, and 91.54 respectively in VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub> and VCT<sub>5</sub> whereas, it was 64.51, 66.53,

69.60, 71.65, and 67.55 for the initial substrates. The results evidently showed that the vermicomposts were less phytotoxic, especially vermicompost of VCT<sub>4</sub> treatment is equally good to that of distilled water.



**Fig 1:** Fold-increase of biomass and number of *Eudrilus eugeniae* after 50 days of vermicomposting of sugarcane bagasse and cow dung amended with *Gliricidia sepium*



**Fig 2:** seed germination tests using the seeds of black gram

**Seedling characteristics of black gram**

The fresh weight and dry weight of the black gram seedlings was higher in the vermicompost treatments when compared to the weight obtained for initial substrates with significant variation between treatments ( $F = 10.34689$ ;  $P < 0.05$ ) (Table 3). The range of fresh weight of the seedlings in initial substrates was  $1292.90 \pm 87.27 - 1411.85 \pm 95.30$  mg whereas, it was  $1418.25 \pm 95.73 - 1515.24 \pm 102.28$  mg in the final vermicompost. A maximum of  $134.51 \pm 9.08$  mg dry weight of the seedlings of black gram was recorded in VCT<sub>4</sub> followed by VCT<sub>3</sub> ( $128.10 \pm 8.65$  mg). In most of the treatments, the root length and shoot length of black gram seedlings were elevated in the vermicompost treatments in comparison with that of the initial substrates (Table 4). The overall results on the root and shoot length of black gram reflects enhancement than initial substrates and the difference between the initial and the final values was

statistically significant ( $F = 72.940$ ;  $P < 0.001$ ). The dry weight of black gram seedlings in vermicompost treated experiments was  $33.09 \pm 2.23$ ,  $33.87 \pm 2.29$ ,  $35.72 \pm 2.41$ ,  $36.60 \pm 2.47$  and  $31.63 \pm 2.14$  respectively for VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub> and VCT<sub>5</sub> which was significantly higher. The chlorophyll contents of the black gram seedlings was higher in the vermicompost treatments when compared to the weight obtained for initial substrates with statistically significant variation ( $P < 0.05$  for chlorophyll a;  $P < 0.001$  for chlorophyll b;  $P < 0.001$  for total chlorophyll) (Table 5). The range of total chlorophyll contents of the seedlings in initial substrates was  $17.24 \pm 1.16 - 21.90 \pm 1.48$   $\mu\text{g/g}$  whereas, it was  $24.27 \pm 1.64 - 27.73 \pm 1.87$   $\mu\text{g/g}$  in the final vermicompost. A maximum of  $134.51 \pm 9.08$  mg dry weight of the seedlings of black gram was recorded in VCT<sub>4</sub> followed by VCT<sub>3</sub> ( $128.10 \pm 8.65$  mg).

**Table 3:** Fresh weight and dry weight of black gram seedlings in Initial and final vermibed substrates, sugarcane bagasse and cow dung amended with *Gliricidia sepium*

Treatments	Fresh Weight (mg/seedling)	Dry Weight (mg/seedling)
<b>Initial Substrates</b>		
VCT1	1308.45 ± 88.32	85.10 ± 5.74
VCT2	1292.90 ± 87.27	87.84 ± 5.93
VCT3	1362.44 ± 91.96	88.76 ± 5.99
VCT4	1411.85 ± 95.30	86.93 ± 5.87
VCT5	1377.08 ± 92.95	89.67 ± 6.05
<b>Final Vermicompost</b>		
VCT1	1418.25 ± 95.73	116.21 ± 7.84
VCT2	1454.85 ± 98.20	122.61 ± 8.28
VCT3	1465.83 ± 98.94	128.10 ± 8.65
VCT4	1515.24 ± 102.28	134.51 ± 9.08
VCT5	1390.80 ± 93.88	126.27 ± 8.52
<b>One Way ANOVA (Initial × Final)</b>		
F-value	10.34689	146.7432
P-Value	0.0123	0.000002
Significant at	P<0.05	P<0.001

Values are mean ± SE, n = 3.

**Table 4:** Root length and shoot length of black gram seedlings in Initial and final vermibed substrates, sugarcane bagasse and cow dung amended with *Gliricidia sepium*

Treatments	Root length (mm/seedling)	Shoot length (mm/seedling)
<b>Initial Substrates</b>		
VCT1	17.52 ± 1.18	22.39 ± 1.51
VCT2	18.01 ± 1.22	23.36 ± 1.58
VCT3	18.49 ± 1.25	23.85 ± 1.61
VCT4	18.20 ± 1.23	22.87 ± 1.54
VCT5	18.10 ± 1.22	26.08 ± 1.76
<b>Final Vermicompost</b>		
VCT1	19.95 ± 1.35	33.09 ± 2.23
VCT2	20.73 ± 1.40	33.87 ± 2.29
VCT3	22.97 ± 1.55	35.72 ± 2.41
VCT4	25.70 ± 1.73	36.60 ± 2.47
VCT5	21.41 ± 1.45	31.63 ± 2.14
<b>One Way ANOVA (Initial × Final)</b>		
F-value	72.940	79.0733
P-Value	0.000027	0.0000202
Significant at	P<0.001	P<0.001

Values are mean ± SE, n = 3

**Table 5:** Chlorophyll contents of black gram seedlings in Initial and final vermibed substrates, sugarcane bagasse + cow dung amended with *Gliricidia sepium*

Treatments	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)	Total chlorophyll (µg/g)
<b>Initial Substrates</b>			
VCT1	8.21 ± 0.55	9.12 ± 0.62	17.33 ± 1.17
VCT2	8.48 ± 0.57	10.04 ± 0.68	18.52 ± 1.25
VCT3	9.21 ± 0.62	10.49 ± 0.71	19.71 ± 1.33
VCT4	10.22 ± 0.69	11.68 ± 0.79	21.90 ± 1.48
VCT5	8.30 ± 0.56	8.94 ± 0.60	17.24 ± 1.16
<b>Final Vermicompost</b>			
VCT1	9.49 ± 0.64	15.33 ± 1.03	24.81 ± 1.67
VCT2	9.85 ± 0.67	15.69 ± 1.06	25.54 ± 1.72
VCT3	10.95 ± 0.74	16.33 ± 1.10	27.28 ± 1.84
VCT4	11.13 ± 0.75	16.60 ± 1.12	27.73 ± 1.87
VCT5	9.31 ± 0.63	14.96 ± 1.01	24.27 ± 1.64
<b>One Way ANOVA (Initial × Final)</b>			
F-value	7.586	96.568	40.38238
P-Value	0.0457	0.00000096	0.000219
Significant at	P<0.05	P<0.001	P<0.001

Values are mean ± SE, n = 3.

## Discussion

The growth and reproduction of earthworms during vermicomposting of organic substrates is very important as the worm activity and growth are the major factors for

hastening of decomposition of organic substrates fed to vermibed. If the substrates provided are acceptable by the earthworms, then they convert the organic materials at a faster rate so also reproduce and multiply quickly. Hence, it

is important to study the growth and multiplication of earthworms in vermicomposting systems. 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) <sup>[52]</sup>. The right amount of *Parthenium hysterophorus* mixing is necessary for earthworm survival without endangering them (Rajiv *et al.*, 2014). So, it is very essential to find out the correct proportion of vermibed substrate to vermicompost the organic materials into value added vermicompost. Preethee *et al.* (2023) reported the vermicomposting process using sugarcane bagasse as primary substrate with cow dung and elephant dung were taken to assess the quality of vermicomposting by using earthworm *Eudrilus eugeniae*. It has the capability to convert 50% of organic raw matter of sugarcane bagasse waste into effective nutrient rich vermicomposting reported by Bhandarkar *et al.* (2014). In the present study, the results of worm growth and reproductive studies indicated that the activities of earthworms, growth and reproduction of earthworms are substantially influenced by different vermicomposting substrate combinations. A maximum of  $91.88 \pm 2.44$  g worm biomass/vermibed was observed in VCT<sub>4</sub> vermibed substrate which contained mixture of sugarcane bagasse, cow dung and *Gliricidia sepium* leaf biomass which was significantly higher than that of the other treatments. Next to VCT<sub>4</sub>, VCT<sub>2</sub> recorded a worm biomass of  $87.13 \pm 2.28$  g/vermibed while the least worm biomass was found in VCT<sub>1</sub>. In a study, mean net worm biomass gain ( $1263 \pm 26$  mg worm<sup>-1</sup>) was also maximum in 100% of biogas plant slurry and minimum ( $874 \pm 15$  mg worm<sup>-1</sup>) in combination with 80% of biogas plant slurry 20% of *Parthenium hysterophorus* (Yadav and Garg, 2016) <sup>[52]</sup>. Vermicomposting was done using press-mud with cow dung and nitrogenous green manures were carried out using *Eudrilus eugeniae*. The pressmud and green manure substrates enhanced the biomass of *Eudrilus eugeniae*. However, the combination of cow dung with green manure highly favoured reproduction of earthworm (Balachandar *et al.*, 2020). The effect of sugarcane bagasse, cow dung, and elephant dung on the fatty acid profile in dietary supplements, which reveals the effect on the reproductive potential of *Eudrilus eugeniae* (Preethee *et al.*, 2023). The reproduction of earthworms is dependent on vermibed substrate composition. In this study, the final worm numbers in different vermicomposting treatments, VCT<sub>1</sub> VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub> and VCT<sub>5</sub> were  $80.27 \pm 2.41$ ,  $85.55 \pm 2.41$ ,  $66.04 \pm 2.02$ ,  $98.98 \pm 2.94$ , and  $83.15 \pm 1.92$  g/vermibed, respectively. The number of cocoons recovered per vermibed was ranged from  $86.80 \pm 2.91$  -  $144.09 \pm 3.78$  with a significantly higher number in VCT<sub>4</sub> followed by VCT<sub>1</sub>. The fold increase of worm biomass was 5.33, 6.01, 5.90, 6.25 and 5.49 in VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub>, VCT<sub>4</sub> and VCT<sub>5</sub> respectively whilst it was 4.01, 4.28, 3.30, 4.95, and 4.16 folds for worm numbers in the respective treatments. The results on the growth and reproductive performance of *Eudrilus eugeniae* revealed that the vermibed combination (sugarcane bagasse + cow dung + *Gliricidia sepium* biomass) is much favoured by the earthworms resulting in higher biomass gain, worm number increase and cocoon production. This phenomenon is important for efficient vermicomposting process. The composting process detoxifies the toxic substances present in the organic wastes

fed to earthworms in the vermibed. This analysis is one of the compost maturity indicators. Hence in the present research, phytotoxicity of the vermibed substrates has been carried out using the seeds of a common pulse crop, black gram (*Vigna mungo*). The final results revealed that the final vermicomposts are less phytotoxic than the initial substrates in almost all treatments. However, among the vermicomposts of different treatments, the vermicompost obtained from VCT<sub>4</sub>, showed greater performance in seed germination percentage, germination index, fresh, dry weight, root length and shoot length of black gram seedlings, whereas, other treatments, VCT<sub>1</sub>, VCT<sub>2</sub>, VCT<sub>3</sub> and VCT<sub>5</sub> showed significantly higher phytotoxicity than VCT<sub>4</sub>. *Eudrilus eugeniae* was employed to vermicompost cow manure blends with five various proportions of *Parthenium hysterophorus* when applied in the concentration range of 0.75–20%, *Parthenium hysterophorus* vermicompost increased seed germination success compared to the control. Ladies finger, cucumber, and green gram showed maximum germination rates of 97, 98, and 98% with 2, 0.75, and 1.5% vermicompost treatments, respectively. When applied in the concentration range of 0.75-4%, *Parthenium hysterophorus* vermicompost improved growth in green gram in terms of length and dry weight of shoot and root, when compared to that of the control treatment (Rajiv *et al.*, 2013). When compared with control, ladies finger seeds had significantly higher germination success with vermicompost made from *Parthenium hysterophorus*. A germination success rate of 85.71% was reached when compared to 62.29% for the controls. A higher concentration of nitrate and ammonium in vermicompost-amended soils showed an increase in seed germination. Nitrates and ammonium are now widely recognized as effective dormancy breakers that facilitate germination of seeds (Hussain *et al.*, 2017). Sugarcane bagasse 25% added to green waste and vermicomposted with *Eisenia fetida* decreased the toxicity to germinating seeds. It reveals the vermicomposting is a possible way to degrade green waste into the value-added product (Cai *et al.*, 2020). The results obtained in the present study indicate that the vermicompost obtained from VCT<sub>4</sub> vermibed combination is toxicity free and can be suitably used for enriching the soil and crop growth.

## Conclusion

The present research has been aimed to bioconvert the sugar industrial waste, sugarcane bagasse with cow dung through the amendment of leafy green manure plant, *Gliricidia sepium* employing the epigeic earthworm, *Eudrilus eugeniae* to produce enriched vermicompost. For this, different combination of vermibed materials were prepared and predecomposed using mushroom spawn (*Pleurotus sajor caju*) for 15 days and then the earthworms were inoculated. The vermicomposting setup was maintained for 50 days. The results of worm growth and reproductive studies indicated that the activities of earthworms, growth and reproduction of earthworms are substantially influenced by different vermicomposting substrate combinations. A maximum of  $91.88 \pm 2.44$  g worm biomass/vermibed was observed in VCT<sub>4</sub> vermibed substrate which contained mixture of sugarcane bagasse, cow dung and *Gliricidia sepium* leaf biomass which was significantly higher than that of the other treatments. The results the phytotoxicity studies on seed germination assay indicate that the

vermicompost obtained from VCT<sub>4</sub> vermibed combination is toxicity free and can be suitably used for enriching the soil and crop growth. Based on the present study findings, it is recommended that the sugarcane bagasse+cow dung combination amended with the green manure plant, *Gliricidia sepium* is suitable for producing nutrient rich vermicomposting utilizing the epigeic earthworm, *Eudrilus eugeniae*.

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