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Research Article

Vermi degradation of different dietary supplements mediated on the reproduction and metabolic profile of earthworm *Eudrilus eugeniae*

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Abstract

Earthworm growth and reproduction is influenced by the environmental variables such as temperature, moisture, substrate and nutrient content in vermibed materials. By using different kind of dietary supplements with the conventional vermibed materials raise the development and reproductive potential of the earthworm *Eudrilus eugeniae*. The current study evaluated on particular periods such as 10th, 45th, and 90th day in vermibins with dietary supplements in the ratio of 1:1 the 50% *Ficus religiosa* leaf litters + 50% cow dung (T1); 50% *Lawsonia inermis* leaf litters + 50% cow dung (T2); 50% sugarcane bagasse + 50% cow dung (T3) was used and 100% cow dung serves as control (C). Among these vermibins, T3 vermibin treated earthworms had the highest growth, reproduction, highest sperm count in the seminal vesicle, hatching success, and also increased the metabolic profile of earthworms such as protein, carbohydrate, and lipids in tissues and seminal vesicle. Compared to the other nutritional supplements, the present study result revealed that cow dung and sugarcane bagasse together have a significant positive impact on earthworm growth and reproduction. This study emphasized on the enhancement of the reproductive capacity of the earthworm *Eudrilus eugeniae* by incorporating different dietary supplements with the traditional vermicomposting methods. This also helps to analyse the influence of the metabolic profile in the growth and development and even on the reproductive index of the earthworm which is been observed on three different period: 10th, 45th and 90th day of experimentation. In the current research multi output can be ensured by integrating with production of crops, vermicompost, earthworm growth and waste management. This present study also represents that the open dumping of such a huge quantity of dung materials and plant and animal wastes will create environmental pollution and benefits for soil fertility improvement.

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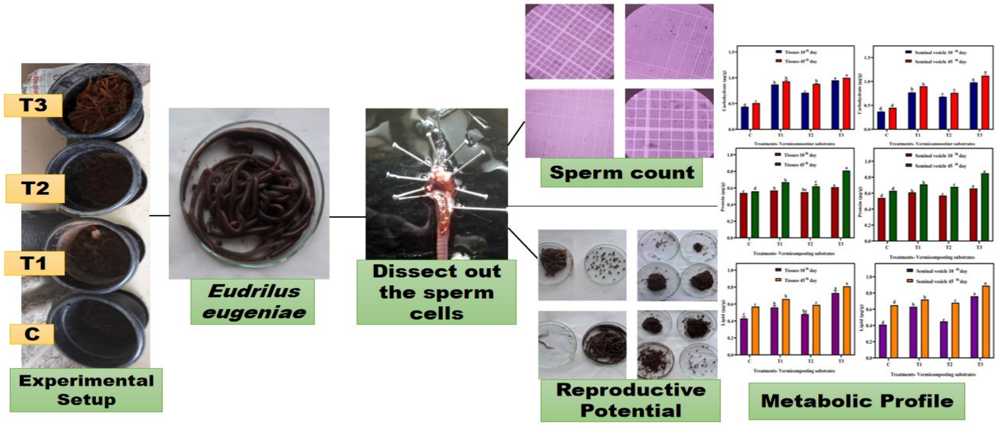


Graphical abstract

Keywords: *Ficus religiosa;* hatching success; *Lawsonia inermis*; reproductive capability; sperm count; sugarcane bagasse

Abbreviations: T1: 50% *Ficus religiosa* leaf litters + 50% cow dung; T2: 50% *Lawsonia inermis* leaf litters + 50% cow dung; T3: 50% sugarcane bagasse + 50% cow dung; C: 100% cow dung

Introduction

Earthworms are biologically significant soil-abiding organisms and decompose the organic matter and provide benefits for the soil (Deepthi *et al.,* 2021; Veena *et al.,* 2023). The environmental variables and nutrient content of substrates has been influence on earthworm growth and reproduction (George *et al.,* 2023; Preethee *et al.,* 2022). Earthworm *Eudrilus eugeniae* were widely used for vermicomposting because of high voracity, high reproductive potential and their ability to adapt adverse conditions (Preethee *et al.,* 2021). It has been selected as the experimental organism because it belongs to the epigeic groups and feeds on decomposing organic matter, including leaf litter, microflora, animal dung, and kitchen waste, among other things (Rombke *et al.,* 2005; Deepa *et al.,* 2022). These energy requirements will not only increase the growth of the exposed animals but also shows differentiation in the metabolic profile and reproductive capability of the earthworm (Givaudan *et al.,* 2014; Vaidhegi *et al.,* 2023).

According to Joseph and Kathireswari (2020) and Preethee *et al.* (2023), the quality and amount of organic wastes are very essential for lead the growth rate of earthworms. The influence of organic waste on the earthworm's biological parameters like growth, cocoon numbers, hatchling success, number of hatchlings per cocoon etc. Earthworms' life cycle parameters are direct relate to their fundamental five necessity, which are environment, temperature, wetness, population density, and substrate material quality (Suthar, 2007a). Thus the choosing of substrate material have a salient role in vermicomposting process (Preethee *et al.,* 2022). The beneficial effects of earthworm on the soil fertility may be due to several reasons apart from the presence of macronutrients and micronutrients in vermicast and in their secretions in considerable amount (Deepthi *et al.,* 2021). Certain metabolites produced by the earthworm may be responsible for stimulating soil fertility *i.e* induces the plant growth (Deepthi *et al.,* 2021), hence it becomes necessary to establish and examine the metabolic profile in earthworm. Differences observed in the metabolic profile can show a modifications of the energetic, reproduction and oxidative condition of the organism in response to the experimental treatment (Malmendal *et al.,* 2006; Vaidhegi *et al.,* 2023).

Bioconversion of varied animal dungs via vermicomposting demonstrates waste environmental sustainability by depleting, reprocessing, and reworking (Singh *et al.,* 2020). Cow dung plays a variety of roles and frequently utilized as manure as organic fertilizer to improve soil mineral status and plant growth. Recently, there has been gaining the research interest in developing nation with the use of cow dung in compost production (Gupta *et al.,* 2016; Yuvaraj *et al.,* 2021). *F. religiosa* leaf biomass combined with cow dung enhanced earthworm reproduction compared to *Coffea arabica, Erythrina variegata, Morus alba, Mangifera indica, Terminalia catappa, Azardirachta indica* and other substrates (Joseph and Kathireswari, 2020; Preethee *et al.,* 2022). *Lawsonia inermis* leaves were widely utilized for obtaining principle natural dye contained at 1.0-1.4 % (Kirkland and Marzin, 2003) called Lawsone ($C_{10}H_6O_3$) - dye molecule which is the chief constituent for coloration, apart from that it can also be used as an astringent, anti-hemorrhagic, cardioinhibitory, hypotensive and sedative (Kathireswari *et al.,* 2023). Sugarcane bagasse is an excellent biomass resource in sugar producing countries worldwide. The chemical analysis of the vermicompost derived from sugarcane trash showed that the quantity of organic carbon was reduced to the certain amount by earthworm (Saravanan *et al.,* 2022).

Sperm count demonstrates prospective, rapid-measurement endpoint biomarker for assessing the implication of inorganic and organic materials on earthworm reproduction (Cikutovic, 1993). In research laboratory studies with different earthworm species, biomarkers have been extensively used to analyse exposure (Spurgeon *et al.,* 2000). If there is a connection between the observed biomarker response and harmful changes to the organism and its reproductive output, then biomarkers may serve as early indicators at higher levels of organisation. The earthworm's testis is highly proliferative and contains progenitor cells, which give rise to mature sperm cells that are then stored in the seminal vesicle (Deepthi *et al.,* 2019; Preethee *et al.,* 2023).

Earthworms are continuous or semi-continuous breeders, producing ova throughout the year, and cocoon production (number of adults produced per year) varies depending on species and environmental conditions. Bhattacharjee and Chaudhuri (2002) analysing cocoon morphology, development, hatching success, cocoon production dynamics, and fecundity in earthworm species could be used to classify these earthworms into broad categories of reproductive strategies and allow for the selection of suitable species for vermiculture. The technique identified alterations in earthworm metabolic profiles caused by dietary supplements, implying that metabolomics is a powerful tool for assessing the impact of dietary supplements on earthworms (Bundy, 2004). The goal of the current study was to examine the diet related changes in the growth, reproduction, sperm count, hatching rate of cocoons on the metabolic profile of the earthworm *E. eugeniae* by incorporating different dietary supplements in the appropriate composition by the vermicomposting methods. The ideal purpose of the present research was focussed on examining the earthworm's reproductive index, metabolic profile in the tissues and seminal vesicle at three different time phases *viz.,* 10^{th}, 45^{th}, and 90^{th} days of the experiment. The current findings are thus immediately applicable and open for speculation among those who are interested in waste management and the composting process.

Materials and Methods

Collection of earthworms and raw materials

Epigeic earthworm *Eudrilus eugeniae* were collected from vermicomposting unit of Kongunadu Arts and Science College (KASC), Coimbatore, Tamil Nadu, India, and adapt the earthworms in the laboratory environment for one week and used for further studies. The dietary supplements of leaf litters such as *F. religiosa, L. inermis* were collected from the agriculture field and sugarcane bagasse waste were gathered from the road side juice shops.

Experimental layout

Vermibins were selected as round tubs with 12 cm height and 13 cm diameter and totally 12 bins were used with the different dietary supplements in the ratio of 1:1 *viz.,* 50% *F. religiosa* leaf litters + 50% cow dung (T1); 50% *L. inermis* leaf litters + 50% cow dung (T2); 50% sugarcane bagasse + 50% cow dung (T3) were used and 100% cow dung serving as the control. All these experiments were maintained in triplicates and in each vermibins 10 adult earthworms were introduced. Then these processes were carried out at the room temperature and moisture content were maintained around 70-80% moisture level. The earthworm growth and reproduction rate were assessed on 45th day and 90th day. For sperm count, hatching of cocoons and metabolic profile were observed in the particular periods such as 10th and 45th day.

Sperm count

Sperm count were determined by the protocol followed by (Cikutovic, 1993; Deepthi *et al.,* 2019; Preethee *et al.,* 2023) in the adult earthworms which separate from the vermibed after 10th and 45th day of exposure. The earthworms were then dissected, the seminal vesicles and testes were dissected out and weighed before being placed in a 20 ml glass vial containing 2ml of sperm counting fluid (5 g sodium carbonate:1 ml neutral formalin:100 ml distilled water). After gently teasing the seminal vesicle and filling it with 8ml of sperm counting fluid, the entire volume was filtered through a funnel lined with double gauze (375 m) into fresh 20 ml vials. Finally, the sperm count was performed using a Neubauer Haemocytometer chamber and the following formula:

$$\text{Number of sperm/cubic (mm3)} = \frac{\text{Number of sperm counted} \times \text{Dilution}}{\text{Number of square counted} \times \text{Depth of the chamber}}$$

Hatchling success and fecundity

For the hatchling experiments, ten fresh cocoons of each treatment were placed in Petri dish containing 200 g parental bedding materials (material in which cocoons were laid down originally) following by the method (Deepthi *et al.,* 2019; Preethee *et al.,* 2023). The Petri plates were kept in a wet and aphotic room at the temperature of (27.4–29.1 °C) was maintained during the whole hatchling experiment and observe regularly for cocoon hatchlings. The numbers of hatchlings produced from each cocoon was also recorded, until the last cocoon had hatched. Hatchling success was measured in percentage by counting the total number of hatched cocoons in each treatment from each Petri plate. During the hatchling observations, newly emerged earthworms (hatchlings) were separated from the plate and transferred to the separate containers with the same bedding material and the total earthworm production was calculated by the end of the experiment.

Growth and reproductive potential of earthworm

Reproductive potential was estimated by considering the numbers and weight of adults, sub adults, juveniles and cocoons of earthworm *E. eugeniae* on the different dietary substrates after 45th and 90th day of exposure. The reproductive potential of the earthworm is determined by the method of (Rini *et al.,* 2020; Preethee *et al.,* 2022) and after counting on 45th day, the earthworms were reintroduced into the respective vermibeds and the treatments were retained continuously until 90th day.

Metabolic profile

Based on the following standard protocols, the metabolic profile viz., total carbohydrate content (Manca *et al.,* 1996) by adding anthrone reagent; total protein content (Lowry *et al.,* 1951) using 0.5 N Na-OH and measured calorimetrically and total lipids (Fernandez *et al.,* 2011) were extracted with polar solvent mixtures (chloroform, methanol and water). These metabolic profiles were estimated from the tissue samples and seminal vesicles from the earthworm for all the treatments on 10th and 45th day of exposure with dietary supplements.

Statistical analysis

Data were determined by using the statistical analysis SPSS program (IBM, Version 20.0). The variables were all represented as Mean ± SE, and the statistical significance level ($p<0.05$) was determined using one-way ANOVA and the Duncan multiple range test (DMRT) a, b, c denotes the values of significant differences between the groups.

Results

Sperm count

Results observed for reproductive parameters viz., sperm count and weight of seminal vesicle in earthworm *E. eugeniae* on 10th and 45th day have been summarized in the Table 1and dissected out the seminal vesicle of the earthworm were depicted in (Figure 1). The results indicate a significant deterioration in the total biomass of seminal vesicles on the 10th day *i.e.,* T3 (0.25 ± 0.01) > T1 (0.22 ± 0.01) > T2 and C (0.20 ± 0.00) and on 45th day T3 (0.25 ± 0.00) > T1 (0.23 ± 0.01) > C (0.21 ± 0.00) > T2 (0.20 ± 0.00). The different dietary supplements influenced on the total biomass of the seminal vesicle and as well as on weight of the adult earthworm shows significant result obtained in the sperm count analysis. The increase in sperm count was observed on 10th day is T3 ($2.18 \times 10^7 \pm 4.40 \times 10^5$) followed by T2 ($1.75 \times 10^7 \pm 2.88 \times 10^5$), T1 ($1.18 \times 10^7 \pm 1.66 \times 10^5$), C ($1.05 \times 10^7 \pm 2.88 \times 10^5$) whereas on 45th day T3 treatment ($2.13 \times 10^7 \pm 7.26 \times 10^5$) followed by favourable trend in T1 ($1.36 \times 10^7 \pm 4.40 \times 10^5$), C ($1.20 \times 10^7 \pm 2.88 \times 10^5$) and T2 ($0.85 \times 10^7 \pm 5.77 \times 10^5$). The results clear that dietary supplements of sugarcane bagasse and cow dung (T3) highly enhanced the sperm count and weight of the seminal vesicle in earthworm *E. eugeniae*.

Figure 1. Dissection of seminal vesicle in earthworm *Eudrilus eugeniae* for sperm count analysis

Table 1. Sperm count of earthworm *Eudrilus eugeniae* on 10th and 45th day of experiment in different dietary supplements, i.e., C- Control, T1. Cowdung + *Ficus religiosa*, T2- Cowdung + *Lawsonia inermis*, T3- Cowdung + Sugarcane bagasse

Treatments		Weight of adult earthworm (gm)	Weight of seminal vesicle (gm)	Sperm cells per ml
C	10th day	0.85 ± 0.33^{c}	0.20 ± 0.00^{b}	$1.05x10^{7} \pm 2.88x10^{5d}$
	45th day	2.81 ± 0.01^{a}	0.21 ± 0.00^{bc}	$1.20x10^{7} \pm 2.88x10^{5b}$
T1	10th day	1.35 ± 0.04^{b}	0.22 ± 0.01^{ab}	$1.18x10^{7} \pm 1.66x10^{5c}$
	45th day	2.08 ± 0.04^{c}	0.23 ± 0.00^{ab}	$1.36x10^{7} \pm 4.40x10^{5b}$
T2	10th day	1.38 ± 0.02^{b}	0.20 ± 0.00^{b}	$1.75x10^{7} \pm 2.88x10^{5b}$
	45th day	2.03 ± 0.04^{c}	0.20 ± 0.00^{c}	$0.85x10^{7} \pm 5.77x10^{5c}$
T3	10th day	1.58 ± 0.04^{a}	0.25 ± 0.01^{a}	$2.18x10^{7} \pm 4.40x10^{5a}$
	45th day	2.36 ± 0.03^{b}	0.25 ± 0.00^{a}	$2.13x10^{7} \pm 7.26x10^{5a}$
F Value ANOVA $df_{(3,11)}$	10th day	68.261^{***}	6.102^{**}	282.952^{***}
	45th day	119.610^{***}	12.452^{***}	103.114^{***}

Values were expressed in mean±standard error. Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's (a,b,c) multiple range test and *** denotes $P<0.05$ levels were considered statistically significant.

Hatching success and fecundity

Rate of cocoon production indicates the earthworm reproductive capacity in the specific vermibed material (Banu *et al.,* 2008). The significant variation on 45th day is been observed in cocoon hatching success percentage and fecundity among the earthworm *E. eugeniae* in different dietary substrates which is presented in the (Table 2). Hatching percentage rate were observed maximum in T3 treatment (83.33 ± 3.33) and fecundity (3.00 ± 0.00) whereas the minimum rate of hatching seen in control (40.00 ± 5.77) and fecundity are also found decreased in C (1.66 ± 0.33). A total hatching success was also increased in the order T3 (17.66 ± 0.88) followed by T1 (12.33 ± 0.88) and C (7.00 ± 0.57). The production of cocoons was absent in the T2 treatment; in this dietary supplement, the earthworms were unable to reproduce and produce cocoons. Similar results were obtained as in the sperm count only differed in that T2 treatment, i.e., *Lawsonia inermis* is not an efficient medium for vermicomposting and large deposition of this leaves in the soil may affect the soil biotic organisms (Vaidhegi *et al.,* 2023). The hatching percentage 83.3 in the present study were higher than the reports of (Suthar, 2007b; S, 2018) with 73.19% and 75.3% respectively it has been correlated with the (Deepthi *et al.,* 2019; Preethee *et al.*, 2023) studies.

Table 2. Hatching of cocoons on 45th day of experiment in different dietary supplements, i.e., C- Control, T1. Cow dung + *Ficus religiosa*, T2- Cow dung + *Lawsonia inermis*, T3- Cow dung + Sugarcane bagasse

Treatments	Hatching success	Hatching percentage	Hatching per cocoon/Fecundity
C	7.00 ± 0.57^{c}	40.00 ± 5.77^{c}	1.66 ± 0.33^{b}
T1	12.33 ± 0.88^{b}	63.33 ± 3.33^{b}	2.33 ± 0.33^{ab}
T2	-	-	-
T3	17.66 ± 0.88^{a}	83.33 ± 3.33^{a}	3.00 ± 0.00^{a}
ANOVA F Value $df_{(2,8)}$	45.176^{***}	25.400^{***}	6.000^{*}

Values were expressed in mean±standard error, Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's (a,b,c) multiple range test and *** denotes $P<0.05$ levels were considered statistically significant.

Reproductive potential of earthworm

Throughout the experimental period of 90 days, the worms flourished well in all of the vermicomposting substrates and no mortality was observed. The reproductive potential in different stages of earthworms, adults, subadults, juveniles and cocoons were counted in the different dietary substrates on 45th day and 90th day and it is summarized in Table 3. From the results significant variations were observed in different substrates at all the stages, higher growth rate was recorded in vermibin T3 followed by T1>T2 and slowest growth rate is in control setup. In T2 vermibin i.e., *Lawsonia inermis* substrate found only the adult (15.33±0.33) earthworm at the end of the observation, whereas in control shows the slowest reproduction rate only the cocoon production is higher (50.00±057) on 90th day. Therefore, this shows that earthworm growth is directly proportional to the cow dung percentage in feed in addition to that sugarcane bagasse, *Ficus religiosa* but in *Lawsonia inermis* are not supported the earthworm growth and reproduction and straightaway connected to the chemical nature of organic waste material and feeds with a adequate amount of easily metabolized organic matter and not assimilated carbohydrates favour the growth and reproduction of earthworms.

Table 3. Reproductive potential of earthworm *Eudrilus eugeniae* on 45th and 90th day of experiment in different dietary supplements, i.e., C- Control, T1- Cow dung + *Ficus religiosa*, T2- Cow dung + *Lawsonia inermis*, T3- Cow dung + Sugarcane bagasse

Treatments		Adults	Sub-Adults	Juveniles	Cocoons
C	45th day	6.00 ± 0.57^{d}	0^{c}	0^{c}	31.66 ± 0.88^{a}
	90th day	11.00 ± 0.57^{c}	0.66 ± 0.33^{c}	0.66 ± 0.33^{c}	50.00 ± 057^{a}
T1	45th day	14.66 ± 0.88^{b}	72.33 ± 1.20^{b}	87.33 ± 0.88^{b}	11.66 ± 0.88^{b}
	90th day	20.00 ± 0.57^{a}	170.00 ± 0.57^{b}	150.00 ± 0.57^{b}	18.00 ± 0.57^{c}
T2	45th day	10.00 ± 0.57^{c}	0.66 ± 0.33^{c}	0^{c}	0^{d}
	90th day	15.33 ± 0.33^{b}	0^{c}	0^{c}	0^{d}
T3	45th day	17.00 ± 0.57^{a}	197.00 ± 0.57^{a}	181.00 ± 0.57^{a}	5.00 ± 0.57^{c}
	90th day	21.66 ± 0.88^{a}	211.00 ± 0.57^{a}	241.00 ± 0.57^{a}	34.00 ± 0.57^{b}
F Value ANOVA $df_{(3,11)}$	45th day	54.063***	18223.451***	26864.900***	409.314***
	90th day	59.619***	63435.571***	72395.571***	1838.667***

Values were expressed in mean±standard error, Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's (a,b,c) multiple range test and *** denotes $P<0.05$ levels were considered statistically significant.

Metabolic profile of earthworm tissues and seminal vesicles

From Tables 4, 5 and 6 showed that there was a significant influence of the diet on the carbohydrate, protein and lipid level in the seminal vesicle and tissues of the earthworm which have been analysed on 10th and 45th day of exposure. These outcome of results were correspondent to the studies that states that feeding of earthworm can significantly influence on the biochemical components of the animal dung material (Bourre, 2005).

Table 4. Estimation of carbohydrates in tissue and seminal vesicle of earthworm *Eudrilus eugeniae* on 10th and 45th day of experiment in different dietary supplements, i.e., C- Control, T1. Cow dung + *Ficus religiosa*, T2- Cow dung + *Lawsonia inermis,* T3- Cow dung + Sugarcane bagasse

Treatments	Tissues		Seminal Vesicle	
	10th Day	45th Day	10th Day	45th Day
C	0.44 ± 0.00^d	0.51 ± 0.00^c	0.37 ± 0.02^d	0.45 ± 0.00^d
T1	0.87 ± 0.00^b	0.93 ± 0.02^b	0.77 ± 0.02^b	0.90 ± 0.00^b
T2	0.71 ± 0.00^c	0.88 ± 0.01^b	0.68 ± 0.01^c	0.76 ± 0.02^c
T3	0.95 ± 0.00^a	1.00 ± 0.00^a	0.98 ± 0.02^a	1.12 ± 0.03^a
F Value ANOVA df$_{(3,11)}$	1136.062***	140.504***	141.889***	163.653***

Values were expressed in mean±standard error, Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's (a,b,c) multiple range test and *** denotes $P<0.05$ levels were considered statistically significant.

Table 5. Estimation of protein in tissue and seminal vesicle of earthworm *Eudrilus eugeniae* on 10th and 45th day of experiment in different dietary supplements, i.e., C- Control, T1. Cow dung + *Ficus religiosa*, T2- Cow dung + *Lawsonia inermis,* T3- Cow dung + Sugarcane bagasse

Treatments	Tissues		Seminal Vesicle	
	10th Day	45th Day	10th Day	45th Day
C	0.54 ± 0.00^c	0.56 ± 0.00^d	0.54 ± 0.00^d	0.63 ± 0.00^d
T1	0.57 ± 0.00^b	0.67 ± 0.00^b	0.61 ± 0.00^b	0.71 ± 0.00^b
T2	0.55 ± 0.00^{bc}	0.62 ± 0.02^c	0.57 ± 0.00^c	0.68 ± 0.00^c
T3	0.61 ± 0.00^a	0.81 ± 0.00^a	0.66 ± 0.00^a	0.85 ± 0.00^a
F Value ANOVA df$_{(3,11)}$	26.062***	67.312***	81.000***	266.756***

Values were expressed in mean±standard error, Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's (a,b,c) multiple range test and *** denotes $P<0.05$ levels were considered statistically significant.

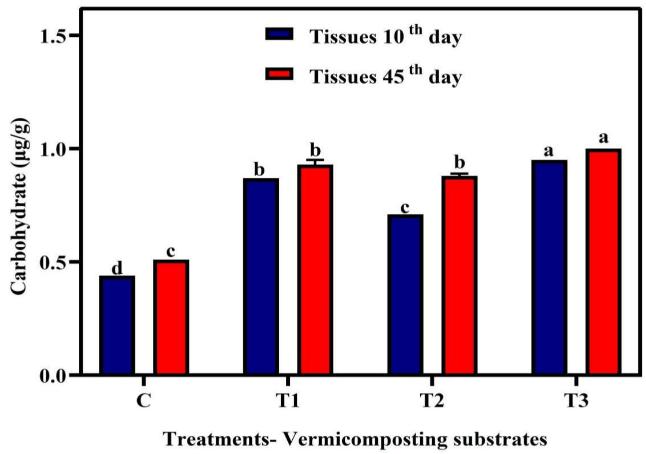
Table 6. Estimation of lipid in tissue and seminal vesicle of earthworm *Eudrilus eugeniae* on 10th and 45th day of experiment in different dietary supplements, i.e., C- Control, T1. Cow dung + *Ficus religiosa*, T2- Cow dung + *Lawsonia inermis,* T3- Cow dung + Sugarcane bagasse

Treatments	Tissues		Seminal Vesicle	
	10th Day	45th Day	10th Day	45th Day
C	0.43 ± 0.02^c	0.57 ± 0.00^c	0.41 ± 0.00^d	0.65 ± 0.00^d
T1	0.56 ± 0.02^b	0.66 ± 0.00^b	0.63 ± 0.00^b	0.72 ± 0.00^b
T2	0.48 ± 0.01^{bc}	0.59 ± 0.00^c	0.45 ± 0.00^c	0.68 ± 0.00^c
T3	0.73 ± 0.03^a	0.81 ± 0.00^a	0.76 ± 0.00^a	0.89 ± 0.00^a
F Value ANOVA df$_{(3,11)}$	26.991***	281.563***	794.750***	407.300***

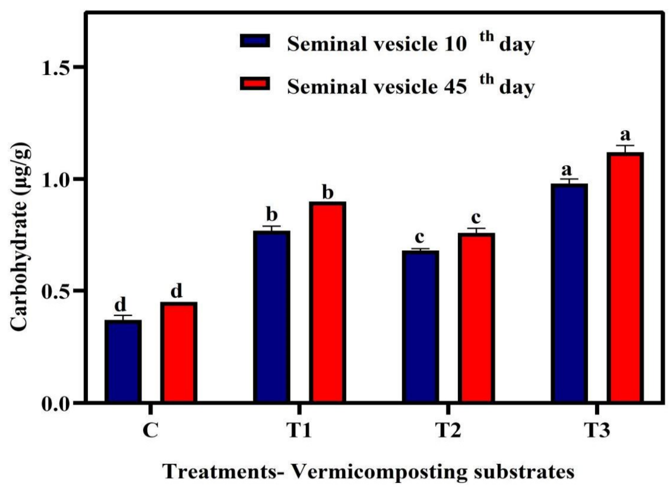
Values were expressed in mean±standard error, Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's (a,b,c) multiple range test and *** denotes $P<0.05$ levels were considered statistically significant.

The current study reveals that the metabolic profile i.e., carbohydrate content displayed in (Figure 2 a, b) where as protein (Figure 3 a, b) and lipid level (Figure 4 a, b) in the earthworm's seminal vesicle and tissues were higher in quality and quantity on 45th day of experiment. The metabolic profile of the treated earthworms of both seminal vesicles and tissues was higher in T3 treatment, when compared to all other vermibins. The earthworm seminal vesicle of T3 vermibin on 10th day contains 0.98±0.02, 0.66±0.00, 0.76 ± 0.00 and on 45th day increased into 1.12±0.03, 0.85±0.00, 0.89±0.00 of carbohydrate, protein and lipid contents respectively.

In the earthworm tissue samples amount of carbohydrate -0.95±0.00, protein-0.61 ± 0.00 and lipid-0.73±0.03were observed on 10th day and on 45th day it obtained about carbohydrate-1.00±0.00, protein-0.81±0.00 and lipid-0.81±0.00. The existence of dietary supplements in the gut tract could explain the increase in the nutrient content because in our study the gut tract of earthworms was not emptied of feed before analysis. This suggests the ability of earthworm to convert the nutrient with the diet into energy supplements in the seminal vesicle and tissues. Hence the attempt done to know the efficiency of diet on earthworm's seminal vesicle and tissues in which the biochemical components influence on the biomass and in the reproductive potential of the earthworm.



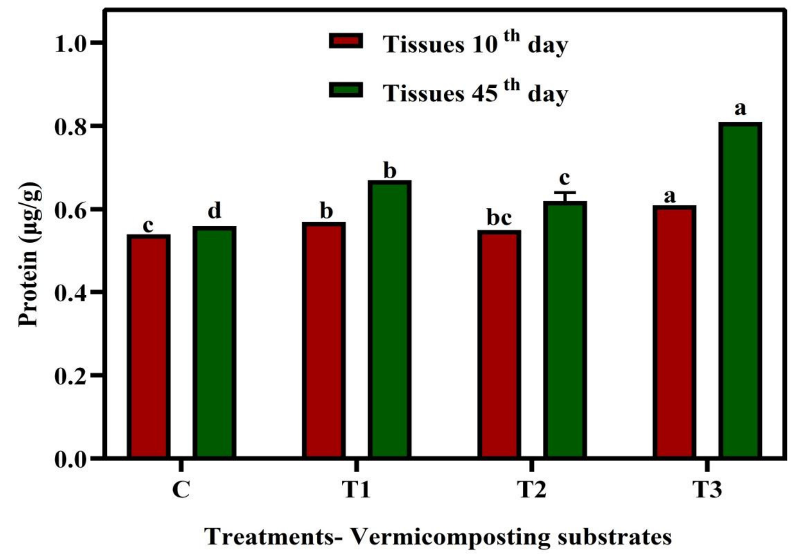
(A)



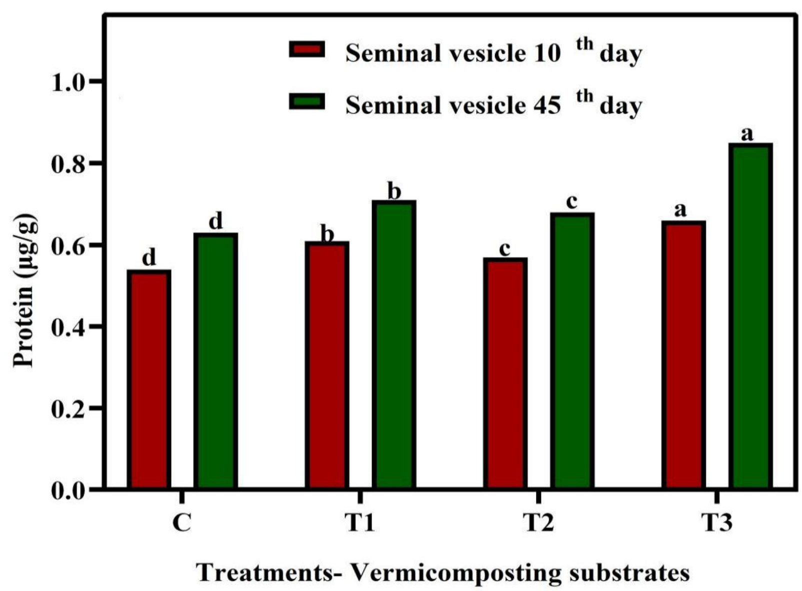
(B)

Figure 2. Carbohydrate content on 10th and 45th day of experiment (A) Tissues of earthworm *Eudrilus eugeniae* and (B) Seminal vesicle of earthworm *Eudrilus eugeniae*

C- Control, T1- Cowdung + *Ficus religiosa*, T2- Cowdung + *Lawsonia inermis*, T3- Cowdung + Sugarcane bagasse



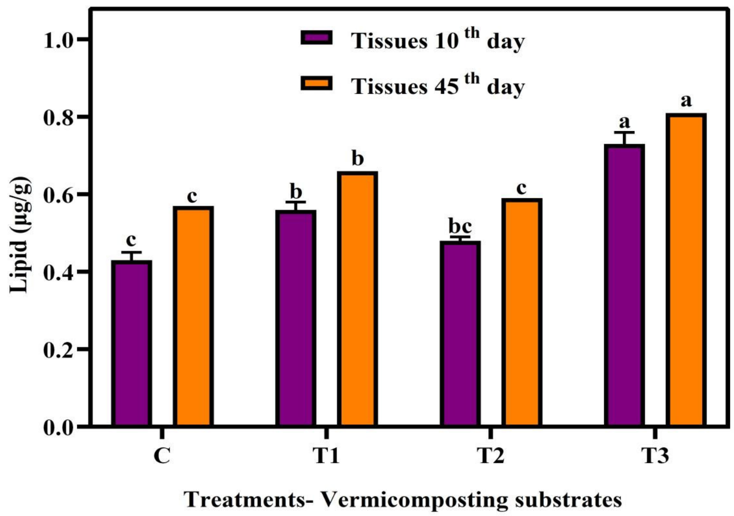
(A)



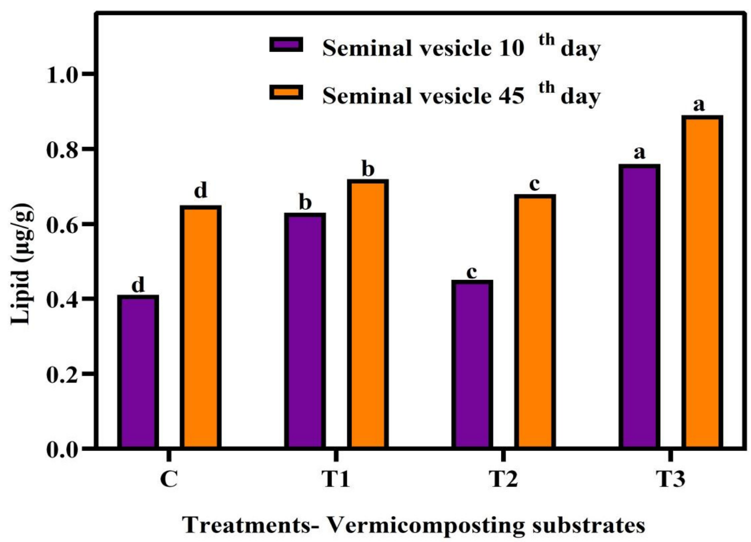
(B)

Figure 3. Protein content on 10th and 45th day of experiment (A) Tissues of earthworm *Eudrilus eugeniae* and (B) Seminal vesicle of earthworm *Eudrilus eugeniae*

C- Control, T1- Cowdung + *Ficus religiosa*, T2- Cowdung + *Lawsonia inermis*, T3- Cowdung + Sugarcane bagasse



(A)



(B)

Figure 4. Lipid content on 10th and 45th day of experiment (A) Tissues of earthworm *Eudrilus eugeniae* and (B) Seminal vesicle of earthworm *Eudrilus eugeniae*

C- Control, T1- Cow dung + *Ficus religiosa*, T2- Cow dung + *Lawsonia inermis,* T3- Cow dung + Sugarcane bagasse

Discussion

The current findings were consistent with the findings of Tripathi and Bhardwaj (2004) and Gajalakshmi *et al.* (2005) that the type, palatability, and quality of food can affect the organism's survival, growth rate, and reproductive potential. According to Preethee *et al.* (2021) and Kathireswari *et al.* (2023), the amount and type of chemical content in different leaf litters may influence the earthworm's health and reproductive potential. Kautz *et al.* (2013) hypothesized that increased microbial activity and polysaccharide production in casts resulted in higher organic matter concentrations than in bulk soil. Likewise, sugarcane bagasse is composed of cellulose, hemicellulose, lignin, and other natural polysaccharides and lipids that consequence the body mass and the fatty acid content of the seminal vesicle that aids in sperm counts and earthworm mass.

The present study hatching percentage was 83.33 was similar to that of (Deepthi *et al.,* 2019) and lower than that of Preethee *et al.* (2023) with 86.66% respectively, because of cow dung and sugarcane bagasse were combined and observed the cocoon hatchlings in the current study. Supportively the report of (Suthar and Ram, 2008) the cocoon production, cocoon weight and hatching success of earthworm will alter accordant to different substrates used in vermicomposting. The observed results on cocoon hatching and fecundity showed a close consistency with previous reports (Reinecke *et al.,* 1992; Dominguez *et al.,* 2001). Dramatically, there is a scarcity of literature regarding the impact of combination with substrates and animal dung quality on cocoon hatchling success and hatchling numbers. It can be hypothesized that the nutrient content of the feed stock, might be of primary importance in the growth and development of reproductive organs.

Although the broad concept of ideal physicochemical requirements for composting earthworms in organic waste has been confirmed, the nutritional component of their needs is still unknown (Deepthi *et al.,* 2019; Preethee *et al.,* 2023). Additionally, there aren't many of these claims in the literature; therefore, a thorough study is still needed to fully comprehend this fact. Previous studies have suggested that the number of hatchlings produced per cocoon could be related to temperature (Kale *et al.,* 1992; Reinecke *et al.,* 1992). According to the results, earthworms produced more viable cocoons in nitrogen-rich culture media because their diets quickly supplied them with protein. This study attempted to evaluate the dietary effect on hatchling success and the number of hatchlings per cocoon in composting earthworms because observations on cocoon hatchling success in previous studies were correlated with some environmental variables, such as temperature, moisture, pH, and so on.

Earthworm growth and reproduction are directly correlated with the chemical composition of organic substrate materials used for the study, depicted by the results of cocoon hatchlings and sperm count. Growth rate is a good indicator for earthworm growth studies in various types of feedstock and allows for in-depth analysis (Gupta and Garg, 2009; Preethee *et al.,* 2022;). Previously, the reproductive potential of earthworms was studied using only dung substrates without the addition of leaf biomass. The growth and reproduction of vermicomposting earthworm species were determined during the vermicomposting of cow dung as well as when they were combined with agricultural wastes, industrially produced organic biosolids, weeds, and other materials (Rini *et al.,* 2020; Saravanan *et al.,* 2022). The study on reproductive potential of earthworm apparently related with the report of Suthar (2007a) on the growth parameters of earthworm depends upon the chemical quality of organic waste (Saravanan *et al.,* 2022) that is favoured by the supplementation of easily metabolize organic matter, non-assimilated carbohydrates and low concentration of growth retarding substances.

In accordance with the current study, toxic chemical compounds were identified in *Lawsonia inermis* leaf litters by GCMS analysis in the report of Kathireswari *et al.* (2023) and these were evidence of the observed suppression of earthworm growth and reproduction in T3 vermibin (*Lawsonia inermis*+ cow dung). Nonetheless, the study of Joseph and Kathireswari (2020) and Preethee *et al.* (2022) discovered that reproduction of earthworms was increased in the medium of *Ficus religiosa* leaf biomass compared to many other leaf litters. However, the current study discovered that sugarcane bagasse increased earthworm reproductive potential compared to *Ficus religiosa*. Current results relate to this hypothesis, since the number of earthworms were varied in each experimental bed materials and it was statistically significant. It is manifest (Sharma and Garg, 2017) that the forthcoming literature indicates that worms gain biomass if the feedstocks are palatable. Overall biomass of earthworm gain is determined by feed stock quality and environmental factors.

Nagar *et al.* (2017) reported that carbohydrates and other polysaccharides which are considered as the major source of carbon and digested rapidly by earthworm also some fractions of substances were then ingested into worm biomass. Also data accumulated in recent years have demonstrated that cholesterol or lipids is not only the structural component of the membrane but it is also actively involved in the cell signalling and reproduction (Jatwani *et al.,* 2016). The efficiency with which earthworms assimilate nutrients as energy from a variety of ingested materials varies depending on the species and the type of ingested materials. Earlier research (Curry and Schmidt, 2007) had noted variations in the vermicompost produced by *E. eugeniae* when given access to various leaf litters. The study by (Kathireswari et al., 2023) proved that *Lawsonia inermis* along with cow dung material in vermicomposting showed negative effects on the growth and reproductive parameters of earthworm *Eudrilus eugeniae* and that the decline in the earthworm biomass is caused by a few toxic phytochemical compounds. As a result, *L. inermis* is not an effective medium for vermicomposting, and the significant amount of leaf litter that is deposited in the soil affects soil biotic organisms.

The findings of the current study suggest that investigations into the potential use of earthworms with various substrates may provide more opportunities for the promotion of organic farming and sustainable agriculture. Singleton (1957) obtained evidence that collagen is stabilized by polysaccharide, with shrinkage temperature serving as his criterion of stability once more. It's tempting to speculate on the role of carbohydrate in earthworm cuticle in this context. Jensen and Holmstrup (1997) found that the earthworm *Dendrobaena octaedra* had a high concentration of long-chain unsaturated fatty acids (20:n and 18:n).

Conclusions

The current study came to the conclusion that dietary supplements containing traditional vermibed materials promote the growth and reproduction of the earthworm *Eudrilus eugeniae.* When compared to other supplements, sugarcane bagasse T3 treatment was found to be greater in efficacy at all the time periods. Furthermore, the research concluded that higher amounts of carbohydrate, protein, and lipid contents have been determined in the earthworm tissues and seminal vesicles, along with the highest reproductive parameters, such as sperm count and cocoon hatching in the sugarcane bagasse substrate. As a result, it is recommended that sugarcane bagasse is a good medium for the vermicomposting process, whereas *Lawsonia inermis* is not an efficient medium for vermicomposting, and the bulky deposition of these leaves in the soil may affect the soil biotic organisms. Multiple outputs can be ensured in the current research by integrating with the production of quality vermicompost, earthworms, and waste management.

Authors' Contributions

MA: Writing- Original draft; Conducted experiments; Methodology; Investigation; SP: Review and editing; Analysed data, Performed the statistical analysis; KS: Formal analysis; Conceptualization; Software; PK: Designed the study, Data curation; Supervision and Validation; SA: Editing; Visualization; Data analysis.

All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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