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RESEARCH ARTICLE

GROWTH AND REPRODUCTION OF EARTHWORM *EUDRILUS EUGENIAE* IN *MURRAYA KOENIGII* LEAF LITTER

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ABSTRACT

Reproduction potential of vermicomposting earthworm *Eudrilus eugeniae* using *Murraya koenigii* leaf litter with cow dung and garden soil used as the mixed substrates. The growth and reproductive potential of earthworm *Eudrilus eugeniae* and the number of juveniles, cocoons and adults were higher in experimental setup T1 (100% cow dung alone). In curry leaf *Murraya koenigii* were suppressed the growth and reproduction of earthworm, it might be due to the higher concentration of chemical compounds Alpha-caryophyllene, 2-Phenyl-4-quinolinecarboxamide, Phenanthrene in the leaf and the physico chemical parameters were observed on the initial substrate, 45th day substrate and vermiwash. Compared to all experimental treatments the growth and reproduction potential of adult (21.82±2.17), juvenile (12.88±1.30) and cocoon (5.66±0.78) production increased in the T1 setup.

Keywords: *Eudrilus eugeniae,* Reproductive potential, *Murraya koenigii,* cow dung

1. INTRODUCTION

Earthworms play a vital role in agriculture and they are hermaphrodite and the reproduction is normally occurs through the copulation method and cross fertilization [1]. *Eudrilus eugeniae* is one of the most common and widely used earthworm in vermicomposting under tropical and sub-tropical conditions and it is a large worm that grows rapidly and is reasonably prolific, under optimum conditions it would be ideal for animal feed protein production [2]. It has been identified as a detritus feeder and can be reared in large number on organic waste [3]. Vermicompost is the organic manure produced as the vermicast by earthworm feeding on biological waste materials and plant sources [4], vermicompost is a microbiologically active organic materials formed from the interactions between the earthworm and different type of microorganisms [5].

Curry tree *Murraya koenigii* is a tropical, subtropical plant, the family is Rutaceae, which represents more than 150 genera and 1600 species [6]. *Murraya koenigii* is distributed from south and east to Australia. *M*. koenigii is widely used in Indian cookery for centuries and have been utilized by developing countries for primary and traditional healthcare system and it is used in ayurvedic and medicinal purposes. In several ancient systems of medicine *Murraya koenigii* used as a medically important herb has wide therapeutic applications such as a in bronchial abnormalities. *Murraya koenigii* is an aromatic leaf often used in indian cuisine [7] and cow dung is one of the good feeding material for earthworms and has an important role in protecting the environment also it is an important feed for earthworms in vermicompost production, gave rich nutrients to the earthworm and it helps in growth and reproduction, without cow dung the worms are suffer and the growth and compost quantity will affect.

The chemical compounds identified in the methanolic extract of the leaves of *Murraya koenigii* through GC-MS analysis revealed the presence of Alpha-caryophyllene, 2-Phenyl-4-quinolinecarboxamide, Phenanthrene, 10H-Phenoxaphosphine, 1,5-Diformyl-2,-anthracene [6,7]. GC/MS analysis of ethanol extract of *Murraya koenigii* revealed the existence of 1-Methyl-pyrrolidine-2-carboxylic acid, Ethyl a-d-glucopyranoside, n-Hexadecanoic acid, Ethyl ester, Oleic acid, Methyl ester, phytol, 9,12-Octadecadienoic acid(Z,Z), c-HIMACHALENE, 1,2-Benzenedicarboxylic acid, Diisooctyl ester, Isolongifolene.

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The vermicompost is beneficial to plant growth [8] and it is very good nutritional source to the plants also produce liquid fertilizer called vermiwash. The vermiwash have enzymes, secretions of earthworms which would stimulate the growth and agricultural crops and even develop resistance in crops receiving this spray [9] and the vermicompost is rich in macro and micronutrients. The vermiwash is rich in dissolved nutrients, amino acids which are easily available for plants and it is also a non-toxic and ecofriendly compound, which arrests the bacterial growth and forms a protective layer for their survival and growth also prevent from the soil born diseases [10]. Plants have played a major role in maintaining human health and civilizing the value of human life for thousands of years [11]. The green herb is also engaged for the preparation of either steam distilled essential oil or the solvent extracted oleoresin [12] [13].

The physico-chemical parameters of the substrate is an important factors in vermiculture process and it observed in the present study in both vermicompost and vermiwash that includes P^H, moisture, EC, TC, OC, nitrogen, phosphorus, potassium produced from *Murraya koenigii* leaf litter by using the earthworm *Eudrilus eugeniae* in the initial and 45th day substrate. The temperature level maintained at 25^{o} C and the moisture level at 70%-80% [3]. In the present study curry leaf used as the substrate and cow dung, garden soil were mixed in it and observed the reproduction of earthworm. The purpose of this study focused to analyse the survivability and reproduction of earthworm *Eudrilus eugeniae* in the *Murrya koengii* leaf substrate.

2. MATERIALS AND METHODS

2.1. *Collection of earthworm, cow dung, garden soil, and leaf litter*

In the present study, the epigeic earthworm *Eudrilus eugeniae* species were collected from ICAR-Krishi Vigyan Kendra (KVK) Vivekanandapuram, Thekkampatti, Karamadai, Coimbatore, Tamil Nadu. The curry leaf litter *Murraya koenigii* collected in Marudhur, Karamadai, Coimbatore, Tamil Nadu. Cow dung were collected from Kodadhasanoor village Karamadai, Coimbatore, Tamil Nadu. Garden soil were collected at college campus of Kongunadu Arts and Science College (Autonomous) Coimbatore, Tamil Nadu.

2.2. *Experimental design*

The collected leaf litters were chopped into small pieces and five different ratio of vermibin setup was prepared by using plastic bins i.e., 2 kg cow dung alone (T1) 100%; 1 kg cow dung+ 1 kg soil (T2) 50% + 50%; ½ kg soil + ½ kg cow dung +1 kg leaf litters (T3) 25%+25%+50%; 1 kg cow dung+ ½ kg soil + ½ kg leaf (T4) 50%+25%+25%; 670 gm cow dung+670 gm soil+670 gm leaf litters (T5) in equal proportion. The earthworms inoculated container treated as an experimental setup (T1, T2, T3, T4, T5) and without earthworms is used as control (C1, C2, C3, C4, C5).

The leaf litters, cow dung and soil were precomposted for fifteen days to remove the methane gas production and also it will help to the aeration of water content. Before the inoculation of earthworm 500g of initial substrate will be taken in each experimental bin to test the physio chemical parameters. Vermicomposting process was done in plastic container and the bins with substrates were maintained the moisture content by spraying water and temperature 25°C condition. Ten earthworms were introduced in each bins and collect the leakage water (vermiwash) from all the setup to analyse the macronutrients.

2.3. *Reproduction prospective survey*

Eudrilus eugeniae in all vermibins were examined by picking and hand sorting method from the culture media after 45th day of vermicomposting in all triplicate samples. The number of adult, juveniles and cocoons were separately counted and determined.

2.5. *Physico-chemical analysis*

Physico chemical parameter were analysed in initial day substrate compost and 45th day vermicompost. Also done the analysis of macronutrients in the *Murraya koenigii* leaf litter leakage water (vermiwash) samples and tap water. The pH was measured using digital pH, EC was determined with Elico conductivity meter, OC was analysed by the titration method. Macronutrients such as nitrogen by Kjeldahl method, phosphorus by Vanadomolybdate method and potassium content estimated using Flame photometer.

2.6. *Statistical analysis*

Duncan analysis and one way anova were performed using IBM SPSS 25 software to determine the significant difference between the experiments ($P<0.05$).

3. RESULTS

3.1. Reproduction prospective survey

In the present study earthworm reproduction potential carried out on 45 day and obtained the results in Table 1 and Figure 1. The population density of the earthworm *Eudrilus eugeniae* highly grown in 100% of cow dung (T1) and 50% cow dung + 50% soil (T2) of compared to the other leaf litter mixed samples, it is due to the rich nutrients on macro and micro nutrients in cow dung than the other experimental setups.

In this reproduction prospective survey the number of adult earthworms increased in T1 (21.82 ± 2.17) vermibin compared to other bins T2 (20.97 ± 1.69), T5 (7.58 ± 4.28), T4 (4.42 ± 0.461), T3 (3.53 ± 1.05). Maximum number of juvenile production present in T1 (12.88 ± 1.30) treatment followed by T2 (8.75 ± 0.25) and T5 (0.78 ± 0.40) but in T3 and T4 has no juveniles. Higher amount of cocoon production present in vermibin T1 (5.66 ± 0.78) but there is no cocoon production in other vermibins. The number of juveniles, and cocoons were found higher in the experiment T1 and T2. In this prospective survey the earthworms cannot survive in the Murraya koenigii leaf litter and obtained less result. The significant variations were calculated by using ANOVA and total number of adult, juveniles, and cocoons values are calculated by mean ± standard error.

Samples	Adults	Juveniles	Cocoons
Experiment T1			
Experiment T2			-
Experiment T3		-	-
Experiment T4		-	-
Experiment T5			-

Fig. 1. Reproductive potential of earthworm Eudrilus eugeniae in *Murraya koenigii* leaf litter

Table 1. Reproduction potential of earthworm *Eudrilus eugeniae* in *Murraya koenigii* leaf litter by using Duncan analysis (Mean ± Standard error) on 45th day

Experimental treatments	Number of Adults	Number of Juveniles	Number of Cocoons
T1	21.82 ± 2.17[a]	12.88 ± 1.30[a]	5.66 ± 0.78[a]
T2	20.97 ± 1.69[a]	8.75 ± 0.25[a]	0.00 ± 0.00[b]
T3	3.53 ± 1.05[b]	0.00 ± 0.00[c]	0.00 ± 0.00[b]
T4	4.42 ± 0.46[b]	0.00 ± 0.00[c]	0.00 ± 0.00[b]
T5	7.58 ± 4.28[b]	0.78 ± 0.40[c]	0.00 ± 0.00[b]
F-Value	14.907***	92.467***	52.669***

3.2. Physico-chemical analysis

In the study macronutrients of *Murraya koenigii* leaf litter leakage water (vermiwash) samples and tap water were analysed and presented in Figure 3 and Table 2. Initial day compost substrates and 45th day vermicompost substrates were collected in both control and experimental treatments and physicochemical analysis obtained were presented in Figure 2 (a,b) and tabulated in Tables 3 and 4. The macronutrient content of *Murraya koenigii* leaf litter leakage water (vermiwash) samples were compared with the tap water, it revels that more NPK content in vermiwash samples than the tap water. The NPK content in vermiwash sample is (59.91 ± 2.82); (71.70 ± 1.14); (68.93 ± 3.48) whereas in tap water (5.96 ± 0.47); (0.06 ± 0.01); (0.70 ± 0.11).

Table 2. Macronutrient analysis in vermiwash of *Murraya koenigii* leaf substrate and tap water by using Duncan analysis (Mean ± Standard error)

WATER SAMPLES	Nitrogen	Phosphorus	Pottasium
Leaf litter vermiwash sample	59.91 ± 2.82	71.70 ± 1.14	68.93 ± 3.48
Tap water	5.96 ± 0.47	0.06 ± 0.01	0.70 ± 0.11
F-Value	317.48***	299.02***	11.46.432***

a) Control samples (without earthworm)

b) Experimental samples (with earthworm)

Fig.2. The macronutrients on 45th day substrates

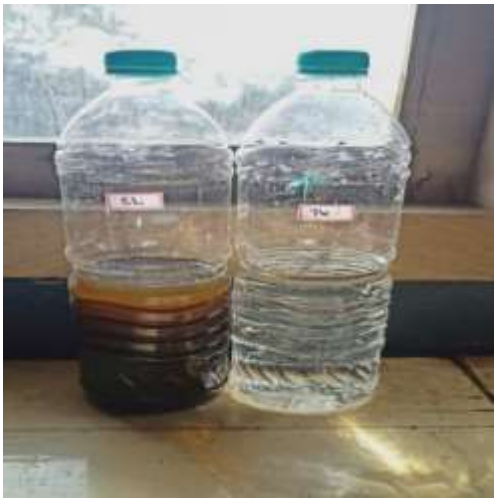

Fig. 3. Macronutrient analysis in vermiwash of *Murraya koenigii* leaf substrate and tap water

Table 3. Physico-chemical analysis in compost of initial day substrates by using Duncan analysis (Mean ± Standard error)

SAMPLES	PH	EC	OC	N (Nitrogen)	P (Phosphorus)	K (Pottasium)
T1	7.16±0.31^{a}	3.31±0.04^{a}	13.26±0.58^{b}	1.66±0.02^{a}	0.94±0.02^{a}	2.21±0.17^{a}
T2	7.06±0.31^{a}	3.20±0.18^{a}	12.73±0.66^{b}	1.71±0.03^{a}	1.02±0.05^{a}	2.56±0.17^{a}
T3	7.06±0.37^{a}	3.20±0.51^{a}	14.10±0.50^{b}	1.68±0.01^{a}	1.09±0.06^{a}	2.40±0.25^{a}
T4	7.20±0.60^{a}	3.32±0.67^{a}	15.21±0.13^{a}	1.57±0.02^{b}	0.87±0.02^{a}	2.56±0.28^{a}
T5	7.13±0.55^{a}	3.63±0.04^{b}	14.95±0.31^{a}	1.68±0.02^{a}	3.59±2.65^{a}	2.43±0.23^{a}
F-VALUE	0.032	14.334***	5.330*	4.160*	0.974*	0.401

Table 4. Physico-chemical analysis of control and experimental treatments on 45th day by using Duncan analysis (Mean ± Standard error)

SAMPLES	PH	EC	OC	N (Nitrogen)	P (Phosphorus)	K (Pottasium)
C1	7.80±0.57^{a}	1.90±0.05^{a}	15.68±0.05^{d}	1.80±0.02^{a}	1.18±0.05^{e}	2.48±0.04^{c}
T1	7.56±0.33^{c}	1.91±0.02^{a}	16.51±0.18^{a}	1.70±0.04^{b}	1.39±0.05^{c}	2.38±0.05^{c}
C2	7.50±0.57^{d}	1.79±0.01^{c}	15.86±0.07^{c}	1.73±0.02^{a}	1.38±0.05^{c}	2.64±0.08^{b}
T2	7.60±0.57^{c}	1.89±0.00^{a}	15.54±0.08^{d}	1.69±0.04^{b}	1.67±0.03^{a}	2.80±0.05^{b}
C3	7.83±0.33^{a}	1.87±0.01^{a}	16.16±0.03^{b}	1.64±0.02^{d}	1.48±0.04^{b}	2.69±0.05^{a}
T3	7.86±0.33^{a}	1.86±0.01^{b}	15.86±0.08^{c}	1.73±0.02^{a}	1.16±0.05^{a}	2.65±0.03^{b}
C4	7.83±0.33^{a}	1.92±0.02^{a}	15.80±0.20^{c}	1.60±0.04^{d}	1.47±0.05^{b}	2.78±0.05^{a}
T4	7.70±0.57^{b}	1.90±0.04^{a}	16.30±0.02^{b}	1.83±0.07^{a}	1.75±0.03^{a}	2.84±0.02^{a}
C5	7.83±0.66^{a}	1.85±0.02^{b}	16.79±0.15^{a}	1.56±0.02^{d}	1.27±0.04^{d}	2.47±0.04^{c}
T5	7.86±0.33^{a}	1.86±0.00^{a}	16.76±0.12^{a}	1.82±0.02^{a}	1.67±0.04^{a}	2.79±0.05^{a}
F-Value	8.127***	3.487*	14.365***	5.178***	14.834***	9.821***

Physicochemical analysis of the substrates were compared with the initial, control and experimental treatments. pH of all the vermibin substrates ranged between (7.16-7.86) in all the setups initial day substrate is lower than the control and experimental treatment. Electrical conductivity level is enhanced in the initial substrates (3.31-3.63) compared with the control and experimental substrates. The content of organic carbon in all the vermibins gradually decreases in the initial substrates and in the control and experiment has an increasing of OC value ranges between (15.54-16.79). In most of the vermibins shows the macronutrient content of total nitrogen (1.60-1.83),

phosphorus (1.16-1.75) and potassium (2.38-2.84) nutrient contents has been increased in experimental samples compared with the initial and control substrates. It is due to the presence of earthworm and its action accelerate the nutrient content in the end products therefore it boost up the micro flora in vermicomposting substrates.

4. DISCUSSION

The growth and reproductive potential of earthworm *Eudrilus eugeniae* and the number of adults, juveniles, cocoons is higher in experimental setup T1 (100% cow dung). The curry leaf *Murraya koenigii* were suppressed the growth and reproduction of earthworm due to the chemical compounds identified in the methanoic extract of the leaves of *Murraya koenigii* are presented in GC-MS analysis revealed presence of Alpha-caryophyllene, 2-Phenyl-4-quinoline, Carboxamide, Phenanthrene, 10 H-Phenoxa phosphine, 1,5-diformyl-2,-anthracene [7]. GCMS analysis [14] of ethanol extract of *Murraya koenigii* revealed the existence of 1-Methyl-pyrrolidine-2-carboxylic acid, Ethyl a-d-glucopyranoside, n-Hexa decanoic acid, Ethyl ester, Oleic acid, Methyl ester, Phytol, 9,12-Octadecadienoic acid(Z,Z), c- Himachalene, 1,2-Benzenedicarboxylic acid, Diisooctyl ester, Isolongifolene

The physico chemical analysis shows the EC value is higher in initial substrate and OC value is increased in experimental substrates and more or less similar increase in the NPK values in the control and end substrates, comparing our results with the previous study reported by [14,15] shows inverse results and it indicates that substrates showing the negative aspect on the nutrient content. According to the literature study [6,7,16] the presence of these compounds in the leaf litter of *Murraya koenigii* and confirmed that the earthworms are not survived and less reproduction occurs. Further study on *Murraya koenigii* will reveal the ideal concentration for earthworm growth and reproduction.

5. CONCLUSION

Vermicompost is rich source of organic fertilizers that earthworms eats the waste materials and transformed into organic fertilizers. *Eudrilus eugeniae* were widely used for vermicomposting process because of high voracity, high reproductive capacity and their ability to adapt in adverse conditions. From this experiment it is known that earthworm survival rate is higher in the cow dung and lower in the *Murraya koenigii* leaf litter substrate. The physicochemical analysis study shows the inverse adverse effects of results in nutrient content and it's clearly denotes the correct concentration of *Murraya koenigii* leaf litter substrate is essential for earthworm reproduction. As we know that the cow dung is rich source for earthworm growth and resulted higher reproductive rate in adult, juveniles and cocoon stages.

Conflict of interest

The author declares no conflict of interest**.**

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