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Vermicomposting of leaf litter in E. eugeniae and its effects on epidermal and pharyngeal extract

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ABSTRACT

Vermicompost, when used as fertilizer, not only bears positive impact on soil quality, plant growth and yield but also enhances nutritional value of crops produced. *E. eugenia* earthworms are renowned for producing vermicompost quickly and effectively for cellular growth needs. This study analyzed the vermicompost of leaf litter, phytochemical analysis, and phytotoxicity of *E. eugeniae,* FT-IR, Brine shrimp analysis, and enzymatic activity. The Phytochemical analyses were recorded and the plant growth parameters were significantly higher than the control.FT-IR analysis, functional groups like alcohol, phenol, carboxylic acid can be found in *E. eugenia's* epidermis extract and pharynx extract. The brine shrimp assay reveals lower toxicity for both extracts. Pharynx extracts results are less toxic when compared to epidermal extract.

Key words: *E. Eugenia*, Epidermal fluid, pharyngeal fluid, phtyotoxicity

INTRODUCTION

Vermicomposting biotechnologies involve the bio-oxidation and stabilization of organic matter through the joint action of earthworms and microorganisms under aerobic and mesophilic conditions (Lorimor *et al*., 2001). The goals of vermicomposting are to continually increase the number and weight of worms and to convert the substrate material into vermicompost in the shortest time and highest recovery as possible (Rupani, *et al.,* 2013). The leaf litter biomass, which is one of the lignocellulose wastes generated in enormous quantity in hill towns in India, has always been a problem for municipal workers and city resource managers (Gairola and Suthar, 2014). Thermal composting or cool composting using earthworm (vermi-composting) are proposed as low-cost technologies to recover the valuable nutrients from leaf biomass under the anaerobic condition with the help of detritus micro- and macro communities under humid environmental conditions (Gairola and Suthar, 2014; Harindint wali *et al*., 2020). The reactors were started by introducing desired number of *Eudriluseugeniae* adults, which, in turn were randomly picked from a cowdung-fed culture of over 5000 animals. *E. eugeniae* Kinberg were used in this study as they had earlier proved more effective against water hyacinth, and nearly as effective against paper waste, as *Lampitomauritii* Kinberg (Gajalakshmi *et al*., 2001a, Gajalakshmi *et al.*,2001b).

Many metabolic processes occur in the coelom which contains coelomic fluid, which also serves as a site for temporary food storage, nitrogen-containing was tesex cretion, and gametes' maturation, and reproduction is either by both external and

internal types. Because annelids are hermaphroditic, development soccur without alarval stage (*Phillipsetal.*,2019). The bodily fluid that exists between the earthworm's body wall and intestine is called coelomic fluid. The coelomic fluid of the earthworm includes a variety of molecules and demonstrates significant biological characteristics like antimicrobials, hemagglutination, and anticoagulant agents. The main function of the earthworm's coelomic fluid is to demolish the cell membranes of foreign organisms (*Umamaheswari and Murugan,* 2021). Eisenia fetida is a typical and significant element of the earth ecosystem. Their physiological functions are aided by the coelomic fluid they produce. They release this liquid under stress, and it has a big impact on soil heath and ecotoxicity. The results of this research seek to determine the quantity of protein and carbohydrates found in E. eugenia (Demirkan *et al.*, 2022).

In this study, to evaluate the suitable leaf litter for vermicomposting, the physiochemical analysis; induce the germination on fenugreek; and the collected of epidermalandpharynx extract from *E. eugeniae* in FT-IR, Brine shrimp analysis and Enzymatic activity.

MATERIALS AND METHODS

Experimental design

Vermibed substrates were done in a circular plastic pot with a 12 cm height and 13 cm diameter. Precomposted various leaf litter was separately crushed and mixed with Cow dung, in the ratio of 1:1 and all the experimental setups were maintained in triplicates. Each vermibin was loaded with 2 kg of vermibed substrates and introduced to twenty mature earthworms. During this period, moisture content was monitored periodically and maintained at 70–80% kept at a temperature of 27 °C. Then the 0th day (initial mixture) of vermibed substrates were kept as control and also after 60th day (vermicompost) substrates were collected, dried, sieved and then stored in air-tight zip lock covers at cool condition for further analysis (Ramalingam and Thilagar, 2000).

Phytochemical analysis of leaves

Litter leaves of plants consists of enormous organic chemicals. Lower organisms like invertebrtaes are feeding on decomposed plant products eg., earthworms . They procure food by intaking by decayed matter of plant byproducts and assimilate in their bodies, so plants play major role in the energy flow in the ecosystem. All the organisms directly or indirectly depending on (producers) plants Raman (2006).

Test used for qualitative analysis

Testforalkaloids

Mayer's test

The Mayers reagent was applied to a test tube containing 1 ml of leaf extract. The appearance of green precipitate indicates the presence of alkaloids.

Test Wagner

Wagner's reagent and 1 ml of the material were combined in a test tube. When a reddish-brown sediment forms, alkaloids are present.

Test of Dragendorff

A test tube containing 1 ml of material, 1 ml of Dragendorff reagent, and 2 ml of diluted HCl was used. Alkaloids are present when a variety of colored precipitates emerge.

Test for carbohydrates

Fehling's test

A minimum of 1 milliliter of the sample was combined with distilled water before being filtered through filter paper. Fehling's fluid was

diluted slightly and heated for a while. Brick red color formation suggests the presence of sugar.

Test for Benedict

To 2 milliliter of Benedict's solution, 1 ml of the sample extract was added. There are carbs present when a reddish-brown precipitate forms.

Test for flavonoids

Ferricchloride test

1ml of the sample was taken inatesttube and a few drops of neutralferricchloride was added. The appearance of a blackish-redprecipitatein dicates the presence of flavonoids.

Alkalinereagent test

1ml sample was treated with the aqueous NaOH and HCl. The appearance of yellow-orangecolourindicatesthepresenceofflavonoids.

Test for Glycosides

Keller killani test:

1ml of the sample was taken and 1ml of glacialacetic acid was added and then cooled. Then 2-3 drops of ferric chloride and 2ml of concentric sulphuric acid were added along the sides of the test tube. The appearance of a bluish-brown ring at the junction of two layer sindicates the presence of' Cardiac' glycosides.

Test for proteins Biuret test

1ml sample was mixed with 0.4ml of NaOH and 2 drops of Cu sulphate wereadded.The appearance of pink or purple colour indicates the presence of proteins.

Warming test

1ml sample was taken in a test tube and heated in a water bath for some time. The appearance of coagulation indicates the presence of proteins.

Test for Sterols

Salkowski's test

1ml sample was treated with a few drops of concentrated sulphuric acid and shaken well and the solution was allowed to stand for sometime. The appearance of red colour in the lower layer indicates the presence of sterols or the appearance of yellow colour in the lower layer indicates the presence of triterpenoids.

Liebermann-Burchard test

1ml sample was treated with chloroform, aceticanhydride, and drops of sulphuric acid were added. The formation of dark pink or red colour indicates the presence of sterols.

Test for Triterpenoids

Salkowski's test

1ml sample was treated with a few drops of concentrated sulphuric acid andshaken well and the solution was allowed to stand for some time. The appearance of red colour in the lower layer indicates the presence of sterols whereas the formation of yellow colour in the lower layer indicates the presence of triterpenoids.

Liebermann-Burchard test

1ml sample was boiled with the 2 drops of aceticanhydride and cooled then the concentrated sulphuric acid was added along the side of the test tube. The Appearance of the brown ring at the junction of two layers is taken as a reference, and the appearance of the brown ring at the junction of two layers is taken as a reference. If the upper layer turns green, sterols are present whereas the formation of deep red colour indicates the presence of triterpenoids.

Test for Tannins

Ferric chloride test

1ml of the sample was added with a few drops of 5% ferricchloride solution was added. The appearance of intense green or blue colour indicates the presence of tannins.

Lead acetate test

To the 5ml sample solution, a few drops of 10% lead acetate were added. The appearance of a yellow colour precipitate indicates the presence of tannins.

Phytotoxicity Assay

Experiments were carried out at the Laboratory condition, Different treatment of vermicompost, homogenized to a fine texture and removing inert matter before being used for seed sowing. Red soil and caste of tea dust powder 1:1 (75g: 75g=150g). 10 Fenugreek seeds of each treatment were sown in each pot for germination. Three replications of each treatment were arranged in a completely randomized design (CRD). Watering was done every evening until completion of the experiment (Annapoorani and Sindhu, 2019).

Preparation of epidermal and pharynx extract

Earthworms were collected from the vermicomposting pits and washed with distilled water. The coelomic fluid will ooze out to the epidermal region from epidermal surface the secretion was collected. They were centrifuged at 6000 rpm for 10 minutes. The pharynx is a white region that extends to the 4th segment of the body and were used to collect the pharynx and mixed with the 5ml of Distilled Water. Then it was ground well using a mortar and pestle. The obtained filtrate was centrifuged for 15 min at 6000 rpm and the samples were analyzed.

Fourier Transform-Infrared (FT-IR)

Spectrum Analysis

Fourier-transform infrared (FTIR) spectroscopy was applied for chemical structure analysis of Pharynx Extract and Epidermal Extract in a wave number range of 400–4000 cm−1 by spectrometer of Shimadzu (IRAffinity) with a resolution of 4 cm−1, and 64 scans.

Brine shrimp analysis

Brine shrimp analysis of Pharynx Extract and Epidermal Extract in different volumes (10, 50, 100, 250, and 500 l) is added to each beaker containing saline solution. This was repeated for both samples. 30 shrimp were introduced into the sample solution at various concentrations. The movement of shrimp is monitored at intervals of 2, 4, 6, 8, and 24 hours. In the blank solution, 30 shrimp were introduced into the brine solution. For positive control, potassium dichromate (100 g/ml) was taken. The mortality of shrimp was observed and calculated after 24 hours. For each of the samples, 30 shrimp were added to 25 ml of the brine solution. Shrimp mortality was tracked as a blank and positive control.

Method for enzyme activity test

To know the presence of different enzymes in the body of Earth wormanen zymatic test was done. The Earthworm was dissected into Foregut, Midgut, Hindgut and coelom or epidermal region for the enzymatic test. They were separately grounded using a motor and pistol with 2ml of Distilled Water. The nit was filtered using filter paper. The obtained sample was then centrifuge at 3000rpm for 10 minutes. Then the sediment was taken as a sample. This procedure is repeated for the Midgut, Hindgut and coelomor epidermal region crushed samples.

Test for Invertase

1ml of 5% sucrose was taken in the test tube along with the sample of 5 drops. This was kept at room temperature for half an hr. After half an hour 4 drops of DW were added. Into that 1 drop of Fehling's solution, A and B were added. Then the mixture was kept in the water bath for 5 minutes. The brown - reddish colour precipitate indicates the presence of the Invertase enzyme. This was repeated for three samples, Foregut, Midgut, Hindgut and coelom or epidermalregion.

Test for Amylase

1ml of starch along with 5-10 drops of the sample was taken in a porcelain tile. Then it was kept at room temperature for 20 minutes. At every 2 minutes, a drop of Iodine solution was added to the mixture. No colour change indicates the presence of maltose which is an automatic point. If there is a colour change, it indicates the presence of amylase. If the blue-black colour appears it indicates the presence of starch. After the blue-black colour, reddish violet appears indicating the starch gets hydrolyzed into dextrin. This was repeated separately for three samples, Foregut, Midgut, Hindgut and coelomore pidermal region.

5ml of Benedict solution was taken in the test tube and along with that 8 drops of the sample were taken in the test tube. To that few drops of 1%, starch was added and Boiled in the water bath for 2 minutes. The appearance of a red colour precipitate indicates that starch hydrolysis into monosaccharides. This was repeated separately for three samples, Foregut, Midgut, Hindgut and coelomor epidermal region.

Test for Protease

Photographic film was taken and in that film 1 drop of the sample was placed. This setup was kept in the moist chamber for 2 hours. After 2 hours the photographic film was washed off. The drop digests the gelatin emulsion was noted. The disappearance of the Black colour of the film negative indicates the presence of enzyme-protease.This was repeated separately for three samples, Foregut, Midgut, Hindgut and coelomor epidermal region.

Test for Lipase

1ml of Methyl salicylate was taken in the test tube and kept at room temperature for half an hr. After half an hour the colour change to white precipitate indicates the presence of the Lipase enzyme. This was repeated separately for three samples, Foregut, Midgut, Hindgut and coelomor epidermal region.

RESULTS AND DISCUSSION

Vermicompost is a good soil fertilizer made up of worms digested excretory product known as worm caste which contains essential nutrients for plants to germinate, growth and development. It increase the soil richness, water holding, softening, aeration and porosity of the soil due to the movement of earthworms turns upside movements of top soil and sub-soil.

Phytochemical analysis

A phytochemical analysis was made in the leaflitter vermicompost, and the results were obtained by observing the colour changes in the alkaloid, carbohydrate, flavonoid, glycosides, proteins, sterols, triterpenoids, and tannins samples represented in (Table 1).Karmegam and Daniel, (2011) have reported that leaflitter and cowdungisa good source and has the ability to improve the quality of vermicomposting. Daniel and Karmegam, (1999) reported that vermicomposting prepared by *E. eugeniae* using specific three types of leaf litter *Azadirachta indica, Ceiba pentandra,* and *Morindatinctoria* with the cow dung in the ratio of 1:1 shows higher nutrients and micro-organisms and can be used for crop production and maintenance of soil fertility. The above results are similar to our present study as the different number of microbial colonies was developed.

Joseph and Kathireswari, (2020) stated that in addition to the animal dung, in some leaflitter substrates the reproductive potential and biomass got enhanced but in some leaf

litter, reproductive potential got reduced and leading to the death of Earthworms. This is because the variation in chemical contents of different leaf litters may affect health and reproductive potential. This result shows controversially with our present study whereas, in our vermicomposting pits all were active.

Table 1: Phytochemical analysis of dired Leaf extract

Phyto chemicals	Name of the Test	Result
Alkaloids	Mayer's	Negative
	Wagner's	Positive
	Dragen droff's	Negative
Carbohydrates	Benedict's	Positive
	Fehling's	Negative
Flavonoids	FeCl3	Positive
	Alkalinere agent	Negative
Glycosides	Keller Killani	Negative
Proteins	Biuret	Positive
	Warming	Positive
Sterols	Salkowski's	Positive
	Libermann-Buchard's	Negative
Triterpenoids	Salkowski's	Negative
	Libermann-Buchard's	Positive
Tannins	FeCl3	Positive
	Lead Acetate	Positive

Phtyotoxicity as say of Vermicompost

The present findings showed that fenugreek seeds were completely germinated. The effectively protected the ability of seeds to germinate capacity during the storage period, revealing non-phytotoxic nature. Thus, vermicompost of seeds may be further utilized for sowing or consumption. At concentration, leaf litter vermicompost were significantly increased up to 55.9% and 33.10% growth, plumule and radicle length of fenugreek seeds between the experiment and control groups of leaf litter vermicompost, there were no significant alterations in fenugreek seeds for germination or radicles.

The beneficial effect of vermicompost on these parameters might be due to its contribution in supplying additional plant nutrients and increasing availability of native soil nutrients with increased microbial activity. The results of the present investigation proved with that of (Khiriya *et al.,* 2001 and Jat *et al.*, 2006). The positive response of vemicompost may probably due to enhanced supply of macro as well as micro nutrients which led to high assimilation of food and its subsequent partitioning in sink. It improved yield components due to vegetative and reproductive growth led to higher seed and yields (Chaturvedi and Chandel, 2005 and Suman *et al*., 2007).

FT-IR Results for Epidermal Extract and Pharynx Extract of *E. Eugeniae*

The functional groups present in the epidermis extract and pharynx extract of E. eugeniae were analysed. The FTIR of epidermal extract displays peeks at (Fig. 1) 3317.56 cm^{1}, 1635.64 cm^{1}, 1211.30 cm^{1},

686.66 cm^1, 601.79 cm^1, 532.35 cm^1, and 478.35 cm^1, with it containing strong functional groups of alcohols, phenols, carboxylic acid, ether, and alkenes and medium functional groups of primary amine and alkyl halides. Similarly, 3317.56 cm^1, 1635.64 cm^1, 686 cm^1, 601.79 cm^1, 532.35 cm^1, 501.49 cm^1, 478.35 cm^1, and 455.20 cm^1 show peaks. It contains strong functional groups of alcohols and phenols, and medium functional groups of primary amines and alkyl halides were observed.

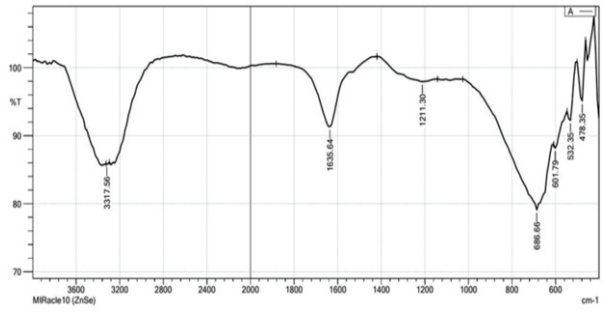

Figure1: FT-IR peaks recorded for Epidermal extract

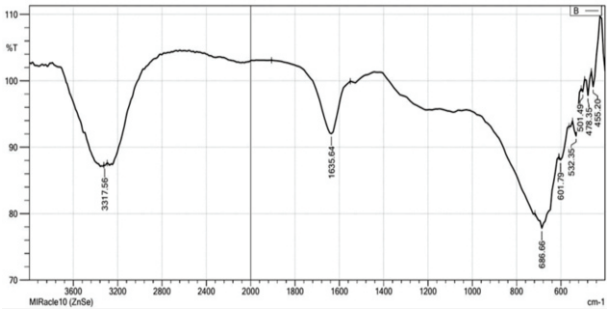

Figure 2: FT-IR peaks recorded for Pharynx extract

The peak responses of *E. eugeniae* epidermal extract and pharynx extract were similar, as observed at 3317.56 cm1, 1635.64 cm1, 686.66 cm1, 601.79 cm1, 532.35 cm1, and 478.35 cm^1 (Fig.2). Scullion *et al*., (2003) reported that the FT-IR spectroscopic technique is used todetermine the biomolecular profile of micro-samples of coelomic fluid. A few reports on the FT-IR studies relating to oligochaetes mainly focus on earthworm compost. Our study findings depicted as containing functional groups of alcohols, phenols, carboxylic acid, ether, and alkenes. Aja *et al.,* (2014) reported that the coelomic fluid of *Perionyx sansibaricus* showed characteristic peaks at 480 cm^{-1}, 566 cm^{-1}, 731 cm^{-1}, and 1663 (amide I) cm^{-1}, 2081 cm^{-1},2143cm^{-1},3122cm^{-1},and3504cm^{-1}.Inthe present study, the peaks observed in the ceolomic fluidof *E. eugeniae* was dissimilar to the above findings.

Brining shrimp assay result for epidermal extract:

The epidermal extract was taken in different concentrations for the analysis of the cytotoxicity of earthworms. They were taken in concentrations of 10, 50, 100, 250, and 500 g/ml. Less toxicity is seen in the brine shrimp assay at both extracts. Pharynx extracts results are less toxic when compared to epidermal extract.

A comparison assay of *E. euginae* pharynx and epidermal extract using brine shrimp is shown in (Table 2). Due to the organism's epidermal secretion, creates a slightly acidic level for the brine shrimps because of the acidic climatic conditions noted in samples 10, 50, 100 concentrati on results were recorded as the number of mortality recorded thirteen and 250 and mortality rate was increased as sixteen in 500 concentration. When compared to the control, this was more toxic.

In the digestive system, the pharynx plays a vital role in the process of taking in food and transforming it into energy. It is a tube-like structure located between the mouth and oesophagus that serves as a passageway for both food and air. The pharynx also has an alkaline climate, as seen in 10, 50, and three mortality were recorded in concentration 100. There is no mortality rate in 250; in 500, the mortality rate increased to ten, which was toxic compared to the control.

Table 2: comparative brine shrimp assay of epidermal extract and pharynx extract of *E.euginae.*

SAMPLE	CONCENTRATION (µg/ml)	Mortality of Brineshrimps (no of shrimps dead)(h)					
		2	4	6	8	24	% Mortality (at 24 h)
A	10	0	0	0	0	4	13
	50	0	0	0	0	4	13
	100	0	0	0	0	4	13
	250	0	0	0	0	5	16
	500	0	0	0	0	5	16
B	10	0	0	0	0	1	3
	50	0	0	0	0	1	3
	100	0	0	0	0	1	3
	250	0	0	0	0	0	0
	500	0	0	0	0	3	10
Control	100	0	0	12	21	30	100
Blank	SW	0	0	0	0	0	0

A - Epidermal extract ; B - Pharynx extract; SW – Saline Water

The brine shrimp lethality test, which is a brine shrimp assay, was conducted to determine the cytotoxic nature obtained from *E. eugeniae*. Because of its high survival rate, shrimp were used to test the cytotoxic nature. Shrimp in vinegar a micro crustacean called Artemia is well suited to surviving in and reproducing in habitats that are extremely hypersaline (*Gajardo and Beardmore, 2012*). Chanda, (2011) states that brine shrimp assay gives the cytotoxicity effects of a certain sample and gives the result of EW has fewer cytotoxicity effects comparing some other Oligochaeta. In our present study brine shrimp assay was used to test the cytotoxicity of earth worm parts. Using epidermal and pharynx extract gives the result of the pharynx extract is less toxicthan epidermal because the mortality rate of shrimps in the pharynx is less after 24 hrs of observation compared to the mortality rate in the epidermal extract.

Enzymatic test

The enzymatic test was done separately for three samples: foregut, midgut, hindgut, and coelom, or epidermal region. The lipase enzyme was absent in the foregut, hindgut, and coelom, or epidermal region, but present in the midgut. Amylase, invertase, and protease enzymes were present in all three gut and coelomic or epidermal regions (Table 3) shows enzymes present in different parts of intestine present in *E. euginae.*

The gelatin observation was washed away from the photographic film. The appearance of a brown or reddish precipitate suggests that invertase. Invertase is present in all three guts and the coelom, or epidermal, regions. Colour changes or slight precipitate formation indicate the presence of amylase. The blue coloration indicates the presence of starch. The presence of the lipase enzyme is indicated by the formation of a thick blue colour. The lipase enzyme is present in the colon, epidermis, and midgut. Earthworms secreted body fluids through epidermal dorsal pores onto the exposed body surfaces. The epidermal mucus layers protected body surfaces from contacting soil directly while earthworms move in soil. The living environment of earthworms is rich in pathogens and other potential threats (Wang *et al*., 2007).

Table 3: Enzymes present in different part of *E.euginae*

Extract	Enzymes			
	Invertase	Amylase	Protease	Lipase
Foregut	+	+	+	_
Midgut	+	+	+	+
Hindgut	+	+	+	_
Coelomic or epidermal region	+	+	+	_

CONCLUSION

The vermicompost produced can be of significant value to the end users like farmers for replacement of chemical fertilizers and procuring better prices for the organic produce using such composting material locally available at much lower cost. The earthworm *E. eugeniae* is known as a soil engineer. Because they travel from the topsoil and subsoil and penetrate deep soil layers, they rotate the soil again and again for feeding and also to make the soil smooth. These organisms feed on dead and decaying matter, absorb the nutrients, and disperse the remaining material as casts and its impact on seed germination and plant growth parameters. The pharynx of an earthworm has a pharyngeal gland that secretes pharyngeal fluid, which contains more microbial colonies that help the digestion of decayed parts and break them down into simple substances known as secondary metabolites.

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