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Original Article

Degradation of spent tea waste by vermicomposting and its effect on growth of *Abelmoschus esculentus*

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Abstract

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Keywords:

Eisenia fetida, Vermicompost, Macronutrient, Enzymes Vermicompost is the stable, organic matter that remains after the earthworms feed on the waste materials that occurs due to its decomposition in the gut of the earthworm under controlled conditions. Compost is a rich source of vitamins, hormones, enzymes, macro and micro nutrients which when applied to plants help in efficient growth. Vermicompost was sought to be made by putting spent tea waste as the worm feed. The present study has been carried out to analyze enzymes, physicochemical characteristics, and micro and macro nutrients present in the compost at regular interval of time and also a comparative study was done on the effect of compost on growth parameters namely germination percentage, root length, shoot length and number of leaf count in *Abelmoschus esculentus* after 30th day of planting. The results of the study revealed that the enzyme activities (amylase, cellulase, protease and invertase) and total macronutrients (N, P, and K) and micronutrients (Mn and Cu) showed elevated levels in compost than control. The compost applied plant (*Abelmoschus esculentus*) showed increased germination percentage, root length; shoot length and number of leaves than the compost untreated plant.

1. Introduction

In the modern age of development the increasing quantity of solid waste is one of the growing problems in both developed and developing countries. The rapid increase in the volume of waste is one aspect of the environmental crisis, accompanying global development. Most common practices of waste processing are uncontrolled dumping which causes mainly water and soil pollution. Although various physical, chemical and microbiological methods of disposal of organic solid wastes are currently in use, these methods are time consuming and expensive [1].

Earthworm farming (vermiculture) is another biotechnique for converting solid organic waste into compost [2].Vermiculture biotechnology refers to the breeding and propagation of earthworms. The vermiculture provides for the use of earthworms as natural bioreactors for cost-effective and eco friendly waste management [3]. Earthworm fecundity is based on the rate of cocoon production, hatching success of cocoons and number of offspring's emerging from each cocoon. The success of the composting depends upon the fecundity of the earthworm. It has the efficiency to consume all types of organic rich waste material including vegetable wastes, spent tea waste, industrial, dairy farm wastes, garden waste, sugar mill residues, slaughter house waste, hatchery waste and municipal wastes [4].

Selection of earthworm species is very important factor because only few species are able to survive and adjust to a particular type of environment. The exotic earthworm species namely *Eisenia fetida* is commonly used for breaking down the organic waste like spent tea. The ability of the exotic composting species, *Eisenia fetida* to transform the spent tea into valuable compost is considerable. *Eisenia fetida* a tropical earthworm commonly called Red wriggler, is large in size, grows rapidly, breeds fast and is capable of decomposting large quantities of organic materials into usable vermicompost [5].

Vermicompost contain enzymes like amylase, lipase, cellulase and chitinase, which continue to break down organic matter in the soil (to release the nutrients and make it available to the plant roots). They also increase the levels of soil enzymes like dehydrogenase, acid and alkaline phosphatases and urease. Use of vermicompost over the years build up the soil's physical, chemical and biological properties restoring its natural fertility. Vermiculture biotechnology will bring in 'economic prosperity' for the farmers, 'ecological security' for the farms and 'food security' for the people [6].

Vermicompost contain nutrients that are changed to forms which are more readily taken up by plants, such as nitrate, exchangeable phosphorous and soluble potassium, calcium and magnesium. Use of vermicompost promotes soil aggregation and stabilizes soil structure. This improves the air, water relationships of soil, thus increasing the water retention capacity, encouraging extensive development of root system of plants and enhancing the mineralization of nutrients resulting in boosting up of crop productivity [7]. Vermicompost being available indigenously at lower cost, results in enhanced crop yield by creating and maintaining better physical and chemical environment for sustaining higher productivity [8].

Vermicompost is a rich source of vitamins, hormones, enzymes, macro and micronutrients which when applied to plants help in efficient growth. The growth rate is fast due to increased uptake of macro and micronutrients present in the vermicompost, which results in increased shoot length and number of leaves in vermicompost applied plants.

2. Materials and Methods 2.1 Collection of Waste

The spent tea waste (*Camellia assamica*) was collected from a restaurant in Thrissur, Kerala, India. The collected wastes were allowed to partial decomposition for 10 days. Then the wastes were mixed with cowdung and soil in the ratio 3:1:1.

2.2 Collection of Earthworm

The exotic earthworms *Eisenia fetida* were collected from Selvam Organics, Udumalpet, Pollachi District and cultured in laboratory conditions for proper growth and survival of earthworms. **2.3 Vermicomposting technique**

Pits of $0.75 \times 0.75 \times 0.75$ m size were dug and floor of the pit was covered with a lattice of wood strips to provide drainage. Totally 3 pits were maintained for the experimental purposes. The pit

 T_1 was maintained as control for spent tea waste (without earthworm). T_2 and T_3 (with earthworm) were taken for composting of spent tea waste. In each pit 60 kgs of spent tea waste was taken and in T_2 and T_3 pits the earthworm *Eisenia fetida* was released on the surface at the rate of 60 worms per square feet except control. Care was taken to avoid light and rainfall. In control as well as in experimental pits the compost sample was taken on 30th and 60thday respectively for the analysis of macro and micro nutrients, physicochemical analysis, enzymes, and its effect on growth parameters Lady's finger (*Abelmoschus esculentus*).

2.4 Nutrient Content

2.4.1 Macronutrients and Micronutrients

Many researchers highlighted the role of earthworms in breakdown of organic wastes. Earthworms can consume almost all kinds of organic wastes and convert it into vermicompost. Vermicompost have higher content of macro and micro nutrients like nitrogen, phosphorus, potassium, calcium, sodium, magnesium and micronutrients namely iron, copper, zinc and manganese respectively [8].

2.4.2 Estimation of Total Nitrogen [9]

Principle: The nitrogen in organic material is converted to ammonium sulphate by sulphuric acid during digestion. This salt, on steam-distillation, liberates ammonia which is collected in boric acid solution and titrated against standard acid.

2.4.3 Estimation of Total Phosphorus [9]

Principle: Inorganic phosphate reacts with ammonium molybdate in an acid solution to form phosphomolybdic acid. Addition of a reducing agent reduces the molybdenum in the phosphomolybdate to give a blue colour, but does not affect the uncombined molybdic acid. The blue colour produced is proportional to the amount of phosphorus present in the samples.

2.4.4 Estimation of Total Potassium [9]

Principle: In flame photometry, the solution under test is passed under carefully controlled conditions as a very fine spray in the air supply to a burner. In the flame, the solution evaporates and the salt dissociates to given neutral atoms. A very small proportion of this move into a higher energy state. When these excited atoms fall back to the ground state, the light emitted is of characteristic wavelength which is measured.

2.4.5 Estimation of Manganese And Copper [10]

Principle: The technique involves determination of concentration of a substance by the measurement of absorption of the characteristic radiation by the atomic vapour of an element. When radiation characteristic to a particular element passes through the atomic vapour of the same element, absorption of radiation occurs in proportion to the concentration of the atoms in the light path.

2.5 Enzymes involved in the degradation of complex organic material into simple compounds

Amylase, cellulase and invertase are the enzymes involved in the degradation of complex organic material into simple compounds

2.5.1 Amylase

Principle: Starch degrading enzymes act on glycogen and related polysaccharides. α -amylase causes endo-cleavage of substrates and hydrolyses α 1, 4 linkages in a random manner. It has the ability to by-pass α -1, 6 branch points. β -amylase hydrolyses alternate bonds from the non-reducing end of the substrate. The enzyme degrades amylose, amylopectin or glycogen in an exo or stepwise fashion by hydrolyzing alternate glycosidic bonds. The end product is β -maltose.

2.5.2 Cellulase

Principle: Hydrolysis of crystalline cellulose is a complex process. Initiation of hydrolysis of native cellulose is done by exo - β - 1, 4 glucanase (C₁-cellulase). This enzyme splits alternate bonds from the non-reducing end of cellulose chain yielding cellobiose. The endo-glucanase (C_x-cellulase) act on carboxy methylcellulose. This enzyme does not act on native cellulose. β -glucosidases (cellobiase) play an important function in the degradation of cellulose by hydrolysing cellobiose which is an inhibitor of exo-glucanase. Assay of cellulase enzyme was done according to the procedure described by Denison and Koehn (1977)[11].

2.5.3 Invertase

Principle: Invertase is an enzyme that catalyzes the hydrolysis of sucrose to fructose and glucose. Assay of invertase enzyme was done according to the procedure described by Miller (1959)[12].

2.5.4 Protease

Principle: The blue colour developed by the reduction of phosphomolybdic phoshotungustic components in the Folin-Ciocalteau reagent by the aminoacids tyrosine present in the protein plus the blue colour developed by biuret reaction of the protein with alkaline cupric tartarate were measured in the Lowry's method.

2.6 Studies on the effect of vermicompost on growth parameters of Lady's finger (*Abelmoschus esculentus*)

The seeds were sowed in three different pots 1, 2 and 3

1		-	Vermicompost
2		-	Inorganic Fertilizer (NPK)
3		-	Control
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The following parameters were observed on 30^{th} day of planting.

1) Gemination percentage.

- 2) Root length
- 3) Shoot length
- 4) Number of Leaves
- 1) Germination percentage

Germination percentage is an estimate of the viability of a population of seeds. The equation to calculate germination percentage is: GP = seeds germinated/total seeds x 100. The germination rate provides an measure of the time course of seed germination.

2) Root length

The length of the root was measured from collar region to the growing tip of the root and expressed in cm.

3) Shoot length

The length of the shoot was measured from collar region upto tip of the shoot and expressed in cm.

4) Number of leaves

The total number of leaves per needles in each plant was counted and expressed as number of leaves per plant.

3. Results & Discussion

3.1 Enzymes present in vermicompost

3.1.1 Amylase

The activity of Amylase present in vermicompost on 30 and 45 days of vermicompost is depicted in Table-1

Table 1: The activity of Amylase present in vermicompost

Level of amylase present			
Control	Vermico	mpost	
CONTROL	30 days	45 days	
0.145±0.001**	0.255±0.01**	0.185±0.001**	
 The values of	data are expr	essed as mean ±	SI

**P<0.001.

The results revealed that higher amylase activity was noticed on 30th day of vermicomposting when compared to control. Then the amylase content was decreased after 30th day steadily. With increase in incubation time, the amylase activity had decreased steadily to 40th day of vermicomposting with tea wastes. Ageing processes greatly affected the activity of amylase; since 30th and 45th day old casts, there was a significant continuous decline in the substrate, namely starch present in spent tea wastes. Breaking down of starch present in the waste by amylase results in decreased level of starch as vermicomposting time increases and decrease in starch concentration might be the reason for low levels of amylase.

3.1.2 Cellulase

The activity of cellulase preset in vermicompost on 30 and 45 days of vermicompost is depicted in Table 2:

Table 2: The activity of cellulase preset in vermicompost

Leve				
Control	Vermicompost			
Control	30 days	45 days		
0.065±0.10** 0.112±0.10** 0.102±0.10**				
The values of data are expressed as mean ± SD. **P<0.001.				

In the present study, the cellulase activity was increased in vermicompost when compared to control. In the present study the cellulase activity was decreased on 45th day of vermicomposting. The cellulase activity was decreased due to the catabolism of carbohydrates during vermicomposting. The cellulase activity sharply decreased upto 60th day of vermicomposting. The decrease in enzyme activity could depend on the decrease of relative substrate concentration during transit through the guts [13]. 3.1.3 Invertase

The activity of invertase present in vermicompost on 30 and 45 days of vermicompost is depicted in Table-3

Table 3: Level of invertase present in 30 and 45 days in vermicomnost

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Level of Invertase present				
Control	Organic vermicompost			
Control	30 days	45 days		
0.145±0.001** 0.250±0.001** 0.195±0.01**				
The values of data are expressed as mean + SD **P<				

The values of data are expressed as mean ± SD. **P<0.001.

The activity of invertase on leaf litter waste vermicomposted is found to be increased. In the present study, the invertase activity was decreased as the vermicomposting period increases and maximum invertase activity was noticed with leaf litter on 30 days of inoculation and decreases significantly to 45 days. 3.1.4 Protease

The activity of protease preset in vermicompost on 30 and 45 days of vermicompost is depicted in Table-4

Level of protease present			
Control	Organic ver	micompost	
Control	30 days	45 days	
0.109±0.001**	0.235±0.001**	0.115±0.010**	

The values of data are expressed as mean \pm SD. **P<0.001.

The activity of tea waste vermicomposted is found to be increased. In the present study, the protease activity was decreased as the vermicomposting period increases and maximum protease activity was noticed with tea waste on 30 days of inoculation and decreases significantly to 45 days. The enzyme protease was found to be elevated in the vermicompost due to the presence of proteolytic enzyme producing microbes which enhanced the trypsin activity.

3.2 Macronutrients and micronutrients present in vermicompost **3.2.1 Macronutrients**

a) Nitrogen

The Nitrogen content present in spent tea waste vermicompost is represented in Table-5.

l'able 5: The level of Nitrogen present in vermicompost	
Level of Nitrogen present	

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L	ever of with ogen presen	ll l	
Comtral	Vermicompost		
Control	30 days	45 days	
The values of data are expressed as mean ± SD. **P<0.00			

Increase in nitrogen content in the vermicompost is due to earthworm recycle nitrogen in very short time, addition of their

metabolic and excretory products (vermicast), mucus, body fluid, enzymes and decaying tissues of dead worms. The increase in nitrogen content was found in the final product in the form of mucus, nitrogenous excretory substances, growth simulating hormones and enzymes from earthworms [15].

b) Phosphorous

Table-6 depicts the Phosphorous content present in vermicompost.

Table	6: The leve	of Phosphorous	present in vermico	ompost

Level of Phosphorous present					
	Control	Vermicompost			
	Control	30 days	45 days		
	0.36±0.01**	0.6 ±0.005**	1.56±0.015**		

The values of data are expressed as mean \pm SD. **P<0.001.

The maximum level of phosphorous content was noticed on 60th day of vermicomposting. The spent tea waste was found to contain more available phosphorus after ingestion by earthworms, which may be due to the breakdown of the spent tea waste by worms [16].

c) Potassium

The Potassium content in vermicompost is represented in Table-7

An increase in potassium level during vermicomposting may be due to the microbes present in the gut of earthworms which might have played an important role in this process. The acid production by the microorganisms is the major mechanism for solubilizing insoluble potassium in the organic waste [17].

Table 7: Level of Potassium present in vermicompost

Level of Potassium present				
	Vermicompost			
Control	30 days	45 days		
0.502 ±0.01**	0.71 ±0.01**	1.98 ±0.01**		

The values of data are expressed as mean ± SD. **P<0.001.

3.2.2 Micronutrients

a) Copper

Table-8 depicts the Copper content present in vermicompost.

Table 8: Level of Copper present in the vermicompost

Level of Copper present				
	Control	Vermicompost		
	Control	30 days	45 days	
	0.097 ±0.01**	1.56 ±0.01**	1.99 ±0.01**	

The values of data are expressed as mean ± SD. **P<0.001.

Higher levels of copper content in vermicompost might be due to the presence of copper containing oxidizing enzymes. Copper is responsible for healthy, vigorous growth and strengthens stalks, stems and branches. It is also necessary for the production of plant proteins

b) Manganese

Table-9 represents the contents of Manganese in vermicompost.

Table 9: Level of Manganese present in vermicompost

Level of Manganese present			
Control	Vermicompost		
	30 th day	45 th day	
0.19 ±0.01**	0.48 ±0.01**	1.21 ±0.01**	

The values of data are expressed as mean ± SD. **P<0.001.

Higher manganese content was observed in vermicompost when compared to that of the control. Increase of manganese content in vermicompost is due to mineralization of this element by the earthworm activity. Manganese is a catalyst for many enzymes and also facilitates the photosynthesis and chlorophyll production. The application of vermicompost increased the available Mn concentration, almost 2-3 times as compared with the control.

3.3 Study on the effect of vermicompost on growth parameters of selected vegetable plant

The success of green revolution in recent decades has often marked significant externalities, affecting natural resources and human health as well as agriculture itself [18]. Soon the increased use of fertilizers and pesticides resulted in nitrate enrichment of ground waters, river waters, and estuaries, release of ammonia and nitrous oxide to the atmosphere. These drawbacks of modern agriculture forced the people to demand food grown without fertilizers and pesticides. This paved the way for use of vermicompost as a source of nutrients.

The vegetable plants play a vital role in maintaining health to control and cure certain diseases and hence an attempt was made to study the effect of vermicompost on growth parameters like germination percentage, shoot length and number of leaves of selected vegetable, Lady's finger (*Abelmoschus esculentus*).

3.3.1 Growth parameters

Table 10, 11, 12 and 13 elucidates the effect of vermicompost and inorganic fertilizer on growth parameters of selected vegetable plants.

3.3.2 Germination percentage

Table 10: Germination percentage of Abelmoschus esculentus

Samples	Germination percentage
Control	10±0.57**
Inorganic fertilizer	60±0.56**
Vermicompost	70±0.57**

The values of data are expressed as mean \pm SD. **P<0.001.

3.3.3 Root length

Table 11: Root length of Lady's finger treated with vermicompost

Control	Inorganic fertilizer(NPK)	Vermicompost
2.3±0.10**	3.1±0.05**	4.2±0.05**

The values of data are expressed as mean \pm SD. **P<0.001.

3.3.4 Shoot length

Table 12: Shoot length of (*Abelmoschus esculentus*) Lady's finger treated with vermicompost

	Control	Inorganic fertilizer(NPK)	Vermicompost	
	10.3±0.10**	11.7±0.10**	13.8±0.10**	
Tł	The values of data are expressed as mean ± SD. **P<0.01.			

3.3.5 Number of leaves

Table 13: Number of leaves of (Abelmoschus esculentus) Lady's finger treated with vermicompost

	Control	Inorganic fertilizer(NPK)	Vermicompost
	5±0.57**	9±0.577**	16±0.57**
-		-	

The values of data are expressed as mean \pm SD. **P<0.001.

The effect of vermicompost on growth parameters of *Abelmoschus esculentus* was observed. The plant treated with vermicompost shows higher effects in germination percentage, shoot length, root length and leaves count.

Reference

- [1] Zirbes, L., Renard, Q., Dufey, J., Tu, K.P., Duyet, H.N., Lebailly, P., Francis, F. and Hanbruge, E. Valorisation of water hyacinth in vermicomposting using epigeic earthworm *Perionyx excavatus* in central Vietnam. Biotechnology, *Agronomy, Society and Environment*. 2011; 15: 85-93.
- [2] Ghosh. C. Integrated Vermi- Pisciculture an alternative option for recycling of solid municipal waste in rural India. *Bioresource Technology*. 2004; 93: 71-75.
- [3] Aalok, A., Tripathi A.K. and Soni. P. Vermicomposting: A better option for organic solid waste management. *Journal of Human Ecology*. 2008; 24: 59-64.
- [4] Zambare, V.P., Padul, M.V., Yadav, A.A. and Shete. T.B. Biochemical and Microbiological approach as ecofriendly soil conditioner. *Journal of Agricultural and Biological Sciences*. 2008; 3: 1-4.
- [5] Sajnanath, R. and Sushama, P.K. Recycling of bio-wastes through vermicomposting. *Agrobios Newsletter*. 2004; 3: 33-35.
- [6] Sinha, R.K. Earthworms: Charles Darwin's 'Unheralded soldiers of mankind: protective and productive for man and environment *Journal of Environmental Protection*. 2010; 1: 251 – 260.
- [7] Gunadi, B., Edwards, C.A. and Arancon, N.Q. Changes in trophic structure of soil arthropods after the application of vermicomposts. *European Journal of Soil Biology*. 2002; 38: 161-165.
- [8] Theunissen, J., Ndakidemi, P.A. and Laubscher, C.P. Potential of vermicompost produced from plant waste on the growth and nutrient status in vegetable production. *International Journal of the Physical Sciences* 2010; 5:1964-1973.
- [9] Pellett, L.P. and Young, V.R. Nutritional Evaluation of Protein Foods. United Nations University Publications. 1980.
- [10] Jackson, M.L. Soil Chemical Analysis, Prentice Hall India Pvt Ltd, New Delhi, India. 1973: 498-516.
- [11] Lindsay, W. and Norwell, W. A. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal*. 1978; 42: 421- 428.
- [12] Denison, D.A. and Koehn R.D. 1977, *Mycologia*, LXIX, 592 596.
- [13] Miller, G.L. Use of dinitrosalicyclic acid reagent for determination of reducing sugars. *Journal of Analytical Chemistry* 1959; 31: 426-429.
- [14] Aira, M. and Dominguez, J. Microbial and nutrient stabilization of two animal manures after the transit through the gut of the earthworm *Eisenia fetida* (Savigny, 1826). *Journal of Hazardous Materials* 2009; 161: 1234-1238.
- [15] Tripathi, G. and Bhardwaj, P. Decomposition of kitchen waste amended with cow manure using an epigeic species (*Eisenia fetida*) and an anecic species (*Lampito mauritii*). Bioresource Technology. 2004; 92: 215-218.
- [16] Parthasarathi, K. and Ranganathan, L.S. Aging effect on enzyme activities in pressmud vermicasts of *Lampito mauritti* (Kinberg) and *Eudrilus eugeniae* (Kinberg). *Biology and Fertility of Soils*. 2000; 30: 347-350.
- [17] Premuzic, Z., Bargiela, M., Garcia, A., Rendina, A. and Iorio, A. Calcium, iron, potassium, phosphorus and vitamin C content of organic and hydroponic tomatoes. *Journal of Horticultural Science* 1998; 33: 255-257.
- [18] Rao, S.I.V. Soil and environment pollution a threat to sustainable agriculture. *Journal of Indian Society of Soil Science* 1999; 47: 611 - 633.