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Asian Journal of Science and Technology Vol. 10, Issue, 09, pp.10122-10127, September, 2019

# **RESEARCH ARTICLE**

## VERMIPROTEIN (WORM BIOMASS) AND VERMICOMPOST PRODUCTION BY DIFFERENT EPIGEIC EARTHWORMS CULTURED IN MIXED ORGANIC WASTE FOOD

## Soumya R. Patil, Shaif Afsha, Geeta B., Soumya C., Priyanka R.G. and \*Pulikeshi M. Biradar

Department of Zoology, Karnatak University, Dharwad-580003 (Karnataka), India

ARTICLE INFO	ABSTRACT
Article History: Received 28 <sup>th</sup> June, 2019 Received in revised form 24 <sup>th</sup> July, 2019 Accepted 26 <sup>th</sup> August, 2019 Published online 30 <sup>st</sup> September, 2019 <i>Key words:</i> Compost, Vermicompost, Epigeic Earthworms, Organic Wastes and Worm Biomass.	The disposal of organic wastes and its management has become a major environmental problem all over the world. Earthworms are playing very important role in converting the waste into useful product in the form of vermicompost and worm biomass (vermiprotein). Hence, the present work was undertaken to evaluate the production of vermiprotein as worm biomass and biofertilizer as vermicompost by different epigeic earthworms cultured in mixed organic waste food at uncontrolled room environmental conditions. Observations were made with respect to gross biomass, biomass ratio; fold increase in worm number and percent compost and vermicompost at different time intervals (30, 60 and 90 days). The gross biomass, biomass ratio and fold increase in worm number of all three species of increased significantly from 30, 60 and 90 days period. There is positive correlation with increase in gross biomass, biomass ratio and fold increase in worm number of days (30, 60 and 90 days). There is a significant variation in gross biomass, biomass ratio and fold increase in worm number among all three species at different time intervals 30, 60 and 90 days. The % vermicompost is comparatively more than that of normal compost may be because of presence of earthworm and their feeding activities. The percent vermicompost produced was positively correlated with the biomass of worms and fold increase in worm number over the time from 30, 60, and 90 day's periods.

*Citation: Soumya R. Patil, Shaif Afsha, Geeta B., Soumya C., Priyanka R.G. and Pulikeshi M. Biradar,* 2019. "Vermiprotein (worm biomass) and vermicompost production by different epigeic earthworms cultured in mixed organic waste food", *Asian Journal of Science and Technology*, 10, (09), 10122-10127.

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# INTRODUCTION

In recent years, disposal of organic wastes and its management has become a major environmental problem all over the world (Edwards and Bater, 1992; Senapati and Julka, 1993). To overcome this problem, various physical, chemical and biological methods are in use, but many of them are time consuming and costly one. Epigeic earthworms are the alternate animal source to tackle these organic wastes and possible environment problems. As we know earthworms are known to produce useful products like vermicompost in maintaining healthy environment by consuming huge amount of organic wastes. They also play a vital role in aggregation of soil, litter incorporation and soil organic dynamics, microbial activity and in maintaining soil fertility (Brionen et al., 1998). Vermicomposting is a technique for the solid waste management, is a viable and cost effective method for recycling of organic wastes (Hand et al., 1988; Raymond et al., 1988; Harris et al., 1990; Logsdon, 1994). Vermicomposting is also a alternate source of animal feed protein for fish and poultry industries (Edwards, 1985; Kale, 2000).

\*Corresponding author: Pulikeshi M. Biradar,

Department of Zoology, Karnatak University, Dharwad-580003 (Karnataka), India.

Epigeic earthworms such as Eudrilus eugeniae, Eisenia fetida and Perionyx excavatus are mainly used in vermicomposting for biodegradation of organic wastes as they have high survival, growth rate, reproductive capacity and low mortality rate. The growth and reproduction of all three species is high compared to other species in consuming wide variety of organic wastes as they grew rapidly and attain early sexual maturity and gives large amount of biomass in a short period (Sivasankeri, 2016). Vermicomposting of various organic wastes by different earthworms have been studied and undertaken by many researchers such as sewage sludge (Mitchell et al., 1977), pig manure (Chan and Griffiths, 1988), cotton industrial waste (Albanell et al., 1988), industrial and vegetable wastes (Bano et al., 1987) and paper mill wastes (Butt, 1993). The production of vermicompost and vermiprotein (worm biomass) by different epigeic earthworms varies with respect to type of organic waste food, life cycle and prevailing environmental conditions etc. Hence, the present work was undertaken to evaluate the production of vermiprotein as worm biomass and biofertilizer as vermicompost by different epigeic earthworms cultured in mixed organic waste food at uncontrolled room environmental conditions.

## **MATERIALS AND METHODS**

**Collection of organic wastes**: The dried organic wastes such as lawn grass (*Zoysiate nuifodia*), mango leaves (*Mangifera indica*) and teak leaves (*Tectona grandis*) generated at gardens, agricultural fields were collected in quantity enough as raw materials for experimental purpose. The collected wastes were chopped into small pieces and mixed in equal proportion. Simultaneously sufficient quantity of urine free cattle manure was also brought, sun dried and powdered for usage in experiments.

**Preparation of culture beds**: The powdered cattle manure (CM) was amended with mixed organic wastes such as lawn grass, mango leaves and teak leaves in the ratio of 1:10(v/v) for maintaining proper C: N ratio (25-30%), The mixed food was sprayed with tap water in order to get moisture content of about 75-80% and kept it for a week for initiation of microbial degradation and softening of organic waste food. Culture beds of both compost(without worms) and vermicompost with different epigeic earthworms ( EE,EF, PE) for 30, 60 and 90 days intervals (in triplicates) were prepared in separate culture containers and were kept it in uncontrolled room environmental conditions.

**Selection of earthworm species**: The selection of appropriate earthworm species for vermicomposting is very important for the production of worm biomass and vermicompost. The adaptability to waste, minimal gut transit time, fast growth rate and high reproductive potentiality are some of the important qualities must be there in the earthworm species. At present *Eudrilus eugeniae* (EE), *Eisenia fetida* (EF) and *Perionyx excavatus* (PE) were widely used as these earthworms are voracious feeder and breeder throughout the year widely used for biodegradation of organic wastes and solid waste management.

**Inoculation of worms:** Five sexually matured all three epigeic earthworm species (EE, EF and PE) were isolated from stock culture; they were inoculated in each experimental pots after noting their weight except compost experimental pots. Sufficient food was provided and moisture content in all the culture pots was maintained throughout the experimental periods. To know the role of earthworms in vermicomposting, another set without worms served as control were also maintained (in triplicates). All the experimental pots were terminated at different time intervals of 30, 60 and 90 days.

**Observations:** Observations were made with respect to number of old and new adult worms, cocoons, juveniles, subclitellates and their weights were noted to calculate gross biomass, biomass ratio and fold increase in worm number. Simultaneously, percent compost and vermicompost was also calculated at the end of 30, 60 and 90days experimental periods. Gross biomass was calculated by adding weight of all the individuals of various stages. Biomass ratio was obtained through initial weight and gross biomass (final) weight and fold increase in worm number was calculated by counting number of fresh individual of all the stages. Percent compost and vermicompost produced out of mixed organic waste food were calculated by isolating degraded material with the help of 0.2mm sieve at 30, 60 and 90 days time intervals.

**Statistical analysis:** Statistical analysis of the data and correlation co-efficient were carried out through ANOVA and Pearson's co-relation test respectively by SPSS programme.

## **RESULTS AND DISCUSSION**

The results of the present work is represented in Table-1 with respect to gross biomass, biomass ratio, fold increase in worm number and percent compost and vermicompost produced at different time intervals (30,60 and 90 days) by three different epigeic earthworm species (EE, EF and PE).

Worm biomass (vermiprotein) production: The gross biomass, biomass ratio and fold increase in worm number of all three earthworm species increased drastically from 30, 60 and 90 days (Table-1). There is a positive correlation with increase in gross biomass, biomass ratio and fold increase in worm number with number of days (30, 60 & 90 days) means as the days increases from 30 days to 90 days time intervals, the gross biomass, biomass ratio and fold increase in worm number also increases. The gross biomass of Eudrilus eugeniae, Eisenia fetida and Perionyx excavates were 7.11±0.22, 11.59±0.66 & 16.47±0.60; 3.46±0.33, 6.45±0.36 & 10.20±0.26 and 1.75±0.02, 2.45±0.06 & 4.76±0.39 during 30, 60 & 90 days respectively (Table-1). Similarly, the biomass ratio was  $1:1.62\pm0.15$ ,  $1:2.67\pm0.17$  &  $1:3.75\pm0.12$ ;  $1:1.65\pm0.02$ ,  $1:3.16\pm0.18$  &  $1:4.84\pm0.16$  and  $1:1.42\pm0.04$ , ratio 1:2.33±0.06 &1:4.55±0.00 from initial weight to final weight (Table-1). There is drastic increase in the fold increase in worm number from 30 to 60 and from 60 to 90 days i.e. 7.00±0.57, 18.33±0.33 & 26.66±1.20; 4.33±0.33, 10.33±0.33 & 21.00±0.57 and 1.66±0.02, 10.33±0.33 & 18.00±0.57 by Eudrilus eugeniae, Eisenia fetida and Perionyx excavates respectively (Table-1). There is a significant difference in gross biomass, biomass ratio and fold increase in worm number among all three species at different time intervals 30, 60 and 90 days (Table -1).

There is a significant difference in gross biomass, biomass ratio and fold increase in worm number was observed between three epigeic earthworm species at various time intervals except between EE-30 & EF-60; PE-60 & EF-30 ; PE-90 & EF-30 and PE-30 & PE-60 with respect to gross biomass (Table-2a), then between EE-30 & EF-30; EE-30 & PE-30; EF-30 & PE-30 and EF-90 & PE-90 with respect to Biomass ratio (Table-2b) and only between EE-60 & PE-90 and EF-60 & PE-60 in fold increase in worm number (Table 2c). There is a drastic variation in the gross biomass, biomass ratio and fold increase in worm number between different earthworm species at various time intervals (30, 60 and 90 days) may be due to difference in their feeding habit, growth and reproductive capability, life cycle, nature of food, time taken for conversion of organic wastes into worm biomass and adjustment to prevailing environmental conditions. Kale and Krishnamoorthy (1981) have reported that the nature of available food resources influences worm activity. Reinecke and Venter (1985) have also witnessed increase in worm biomass based on the feeding activities of the earthworms. Results of our study revealed that the earthworm EE have produced more amount of worm biomass as compared to EF and PE (Table-1), this may be because of its voracious feeding habit and breeding nature as that of other two species (EF and PE). Suthar (2011) expressed growth patterns in A.parva that there is a consistent trend of rapid increase in worm biomass up to 13<sup>th</sup> weeks, thereafter a gradual decline in worm biomass was noticed. Earthworms utilize microorganisms from their available substrates as a food source and can digest them selectively for their growth and reproduction (Edwards et al., 1998).



Table 1. Gross biomass, biomass ratio, fold increase in worm number, percent compost and vermicompost produced by different epigeic earthworm cultured in mixed organic waste food and their significant value (P<0.05) at various time intervals (30, 60 and 90 days)

SI.	Earthworm	Time intervals (days)	Parameters				
No.	species		Gross biomass (gm)	Biomass ratio (gm)	FIWN	Percent	Percent
				IW:GBW		Vermicompost	Compost
1	EE	30	07.11±0.22	1.62±0.15	07.00±0.57	40.00±0.00	21.00±0.57
		60	11.59±0.66	2.67±0.17	18.33±0.33	70.00±0.00	31.00±0.57
		90	16.47±0.60	3.75±0.12	26.66±1.20	95.00±0.00	40.00±0.00
2	EF	30	03.46±0.33	1.65±0.02	04.33±0.33	28.33±1.66	21.00±0.57
		60	06.45±0.36	3.16±0.18	10.33±0.33	41.66±1.66	31.00±0.57
		90	10.20±0.26	4.84±0.16	21.00±0.57	55.00±2.88	40.00±0.00
3	PE	30	01.75±0.02	1.42±0.04	01.66±0.02	25.00±0.00	21.00±0.57
		60	02.45±0.06	2.33±0.06	10.33±0.33	45.00±0.00	31.00±0.57
		90	04.76±0.39	4.55±0.00	18.00±0.57	60.00±0.00	40.00±0.00
4	F-value		F=206.56	F=126.06	F=201.00	F=310.05	F=406.50
5	P-value		P=0.00	P=0.00	P=0.00	P=0.00	P=0.00

EE-Eudrilus eugeniae, EF-Eisenia fetida, PE- Perionyx excavatus, IW: Initial Weight, GBW: Gross Biomass Weight, FIWN: Fold Increase in Worm Number.

Table 2. Significant variation (P<0.05) between gross biomass, biomass ratio, fold increase in worm number, percent compost and vermicompost produced by different epigeic earthworms cultured in mixed organic waste at various time intervals (30, 60 and 90 days)

#### a) Gross Biomass

Earthworm species	Time (in days)	EE			EF			PE		
		30	60	90	30	60	90	30	60	90
EE	30	-	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00
	60	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	90	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00
	30	0.00	0.00	0.00	-	0.00	0.00	0.00	0.49	0.13
EF	60	0.18	0.00	0.00	0.00	-	0.00	0.00	0.00	0.00
	90	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00	0.00
	30	0.00	0.00	0.00	0.00	0.00	0.00	-	0.15	0.00
PE	60	0.00	0.00	0.00	0.49	0.00	0.00	0.15	-	0.00
	90	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	-

EE-Eudrilus eugeniae, EF-Eisenia fetida, PE- Perionyx excavatus

## b) Biomass ratio (IW:GBW):

Earthworm species	Time (in days)	EE			EF			PE		
		30	60	90	30	60	90	30	60	90
EE	30	-	0.00	0.00	0.82	0.00	0.00	0.78	0.00	0.00
	60	0.00	-	0.00	0.00	0.00	0.00	0.00	0.04	0.00
	90	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00
	30	0.82	0.00	0.00	-	0.00	0.00	0.95	0.00	0.00
EF	60	0.00	0.00	0.00	0.00	-	0.00	0.00	0.00	0.00
	90	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00	0.08
	30	0.78	0.00	0.00	0.95	0.00	0.00	-	0.00	0.00
PE	60	0.00	0.04	0.00	0.00	0.00	0.00	0.00	-	0.00
	90	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	-

#### c) Fold increase in worm number (FIWN):

Earthworm species	Time (in days)	EE			EF			PE		
		30	60	90	30	60	90	30	60	90
EE	30	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	60	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.68
	90	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00
	30	0.00	0.00	0.00	-	0.00	0.00	0.05	0.00	0.00
EF	60	0.00	0.00	0.00	0.00	-	0.00	0.00	1.00	0.00
	90	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00	0.00
	30	0.00	0.00	0.00	0.05	0.00	0.00	-	0.00	0.00
PE	60	0.00	0.00	0.00	0.00	1.00	0.00	0.00	-	0.00
	90	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.00	-

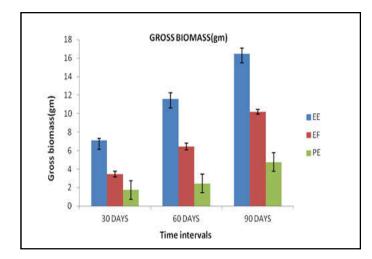
#### d) Percent vermicompost and compost:

Earthworm species	Time (days)	VC-E	E		VC-E	F		VC-PI	Ξ		Compo	st	
		30	60	90	30	60	90	30	60	90	30	60	90
VC-EE	30	-	0.00	0.00	0.00	0.35	0.00	0.00	0.01	0.00	0.00	0.00	1.00
	60	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	90	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	30	0.00	0.00	0.00	-	0.00	0.00	0.74	0.00	0.00	0.00	0.09	0.00
VC-EF	60	0.35	0.00	0.00	0.00	-	0.00	0.00	0.07	0.00	0.00	0.00	0.28
	90	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00	0.01	0.00	0.00	0.00
	30	0.00	0.00	0.00	0.74	0.00	0.00	-	0.00	0.00	0.01	0.00	0.00
VC-PE	60	0.01	0.00	0.00	0.00	0.07	0.00	0.00	-	0.00	0.00	0.00	0.00
	90	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	-	0.00	0.00	0.00

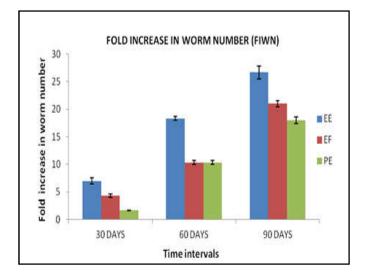
EE-Eudrilus eugeniae, EF-Eisenia fetida, PE- Perionyx excavatus, VC-Vermicompost

 Table 3. Pearson's correlation co-efficient between percent vermicompost (VC) with gross biomass (GB) and fold increase in worm number (FIWN) produced by different epigeic earthworms

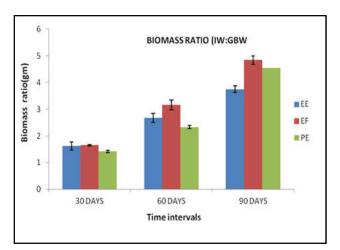
Sl. No.	Particulars	EE-VC	EF-VC	PE-VC
1	EE-GB	0.997	-	-
2	EF-GB	-	0.998	-
3	PE-GB	-	-	0.928
4	EE-FIWN	0.958	-	-
5	EF-FIWN	-	0.987	-
6	PE-FIWN	-	-	0.999



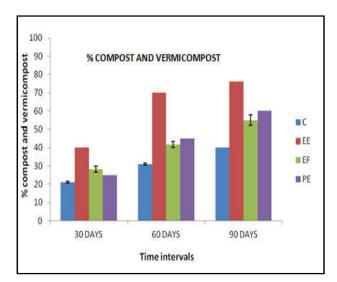
Graph 1. Gross biomass (Mean ± SE) produced by different epigeic earthworms at various time intervals (30, 60 and 90 days)



Graph 3. Fold increase in worm number by different epigeic earthworms at various time intervals (30, 60 and 90 days)



Graph 2. Biomass ratio (Mean ± SE) observed by different epigeic earthworms at various time intervals (30, 60 and 90 days)



Graph 4. Percent compost and vermicompost produced by different epigeic earthworms at various time intervals (30, 60 and 90 days)

The increase or decrease in earthworm growth or biomass may also be attributed the C:N ratio present in the food substrate (Ndegwa and Thompson, 2000). The quality and palatability of food substrate directly or indirectly affect the survival, growth rate and reproductive potentiality of earthworms (Suthar, 2009; Suthar, 2010). The cocoon production was also affected by the type of food source as the cumulative cocoon number increased in Perionyx excavatus with the increase in the age of food substrates (Birundha et al., 2013). Kale and Krishnamoorthy (1978) have reported that different species of earthworms have different preferences towards organic matter or nutrients present in the organic wastes. Life activities of earthworms also influenced by prevailing environmental factors as their optimum parameters differ from species to species and it also depends on their individual intrinsic property. There was a significant correlation between the production of worm biomass, fold increase in worm number with time intervals from 30-90 days, this may be due to increased biodegradation of organic waste with time period, that might increased more availability of nutrients and microorganisms in the food (Pulikeshi et al., 2003).

**Compost and vermicompost production:** The quantity percent compost and vermicompost produced were also gradually increased from 30, 60 and 90 days time intervals. The percent compost produced at 30, 60 and 90 days period was 21%,31% and 40% respectively, likewise percent vermicompost produced by *Eudrilus eugeniae, Eisenia fetida* and *Perionyx excavatus* were 40%, 70% & 95%; 28.33%, 41.66% & 55% and 25%, 45% & 60% at 30, 60 and 90 days periods. Here also there is a significant variation was noticed among percent compost and vermicompost produced at different time intervals (Table-1).

As per the Table- 2(d), there is a significant difference was observed in the production of percent compost and vermicompost produced by different earthworm species at various time intervals except between EE-30 & EF-60; EF-30 & PE-30 and EF-60 & PE-60 with respect to percent vermicompost and also no significant variation was noticed between EE-30 & C-90 ; EF-30 & C-90 and EF-60 & C-90 (Table- 2d). The percent vermicompost produced was positively correlated with the biomass of worms and fold increase in worm number over the time from 30, 60, and 90 days periods (Table -3). Vermicomposting involves the joint action of earthworms and microorganisms, although microbes are responsible for biochemical degradation and mineralization of organic matter but earthworms are the important drivers of this process by conditioning the substrate and altering the biological activity (Aira et al., 2002). Production of percent vermicompost is comparatively more than that of normal compost may be due to the presence of earthworms and their feeding activities. Feeding activity of earthworms may decrease the time of stabilization of organic wastes. Vermicomposting through earthworms is an ecobiotechnological process that transforms energy rich complex organic waste substance into a stabilized product called vermicompost as biofertilizer and worm biomass as vermiprotein (Benitez et al., 2000). Gosh et al., (1999) have reported the usage of epigeic earthworms in composting process and has witnessed the increased production of a better quality of vermicompost as compared with those produced through traditional composting methods.

#### Conclusion

The gross biomass, biomass ratio, fold increase in worm number and percent compost and vermicompost varies drastically with different time intervals 30, 60 and 90 days and also with respect to different epigeic earthworm species. All the parameters were more in *Eudrilus eugeniae* followed by *Eisenia fetida and Perionyx excavatus*. The gross biomass, biomass ratio and fold increase in worm number of all three species were directly proportional or positive correlation with the production of percent vermicompost. Based on the results, it can be concluded that the earthworms, *Eudrilus eugeniae* and *Eisenia fetida* can be best utilized in vermicomposting than that of Perionyx excavatus for the biodegradation and conversion and recycling of various organic wastes into a valuable resources like worm biomass as vermiprotein and vermicompost as biofertilizer within short period.

#### Acknowledgement

The authors are thankful to the authorities of Karnatak University, Dharwad for providing necessary facilities to carry out this work. We are also thankful to UGC-New Delhi for providing financial support under the programme UGC-SAP-DSA-I at Department of Zoology, Karnatak University, Dharwad.

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