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Production of worm biomass (Vermiprotein) and vermicompost by using earthworm *Eisenia fetida* in different flower species

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Abstract
Earthworms *(Eisenia fetida)* are known to convert variety of organic wastes into useful products such as vermicompost, worm biomass and vermiwash. The present study was undertaken to know the potentiality of earthworms in bio-processing of organic waste flower species (*Rosa rubiginsosa*, *Tagetes marigold* and *Chrysanthermum*) for the production of worm biomass and vermicompost. Observations were made with respect to initial and final biomass of worms, biomass ratio, percent compost and vermicompost at various time intervals (15, 30 and 45 days) produced out of three species. The gross biomass, biomass ratio in worm number significantly ($P< 0.05$) varies with respect to Earthworm *(Eisenia fetida)*. As the days increases the gross biomass, biomass ratio, per cent compost and vermicompost were also increased over the time in all three species. The maximum worm biomass and percent vermicompost were noticed in all cultures of Earthworm. The percent vermicompost produced was directly proportional and positively correlated with gross biomass over the time from 15, 30 and 45 days in all three species. Hence, all three variety of flower species were effectively be used in the production of quantity and quality worm biomass and vermicompost for pharmaceutical use and sustainable agricultural practices respectively.

Keywords: Earthworms *(Eisenia fetida)*, worm biomass, flower species (*Rosa rubiginsosa*, *Tagetes marigold* and *Chrysanthermum*), vermicompost

Introduction

Earthworms contribute significantly in managing various organic wastes for the production of useful products like vermicompost and worm biomass. Effective recycling of different organic wastes is a novel work towards organic waste management and tackling many environmental problems. Earthworm transfers unavailable nutrients present in the organic matter of wastes into available forms necessary for plant growth through the process of mineralization by the help of saprophytic microorganisms present in the gut of earthworms (Ansari and Hanief, 2015) [2]. Vermitechnology is one of the valuable methods of converting organic wastes into useful products like vermicompost, worm biomass and vermiwash by using specialized earthworm species. The production of vermicompost and worm biomass varies with respect to different types of earthworms, food type and prevailing environmental conditions etc. Vermicomposting by different species of earthworms have been undertaken by various researchers using different organic wastes such as sewage sludge (Mitchell, 1977) [14], pig manure (Chan and Griffiths, 1988) [6], cotton industrial wastes (Albanell *et al*., 1988) [1], industrial and vegetable wastes (Bano *et al*., 1987) [3] and paper mill wastes (Butt, 1993) [4] etc. Vermicomposting is not only used as an alternative source of production of organic fertilizers, but it also provides economical animal feed protein in the form of worm biomass for fish and poultry industries (Edwards, 1985; Kale, 2000) [7, 13]. The organic wastes generated in agricultural fields and gardens are abundant that are creating serious disposal problems and are the major sources of environmental pollution (Inbar *et al*., 1993) [9]. All these wastes were utilized as raw materials in vermi-technology for the production of vermicompost as well as worm biomass. Hence, the present study was undertaken to know the potentiality of different flower species in *Eisenia fetida* for production of worm biomass and percent vermicompost cultured in cattle manure at various time intervals in uncontrolled room environmental conditions.

Material and Methods

- **Collection of different flower species:** Flower waste was collected from local flower market and temples at Ujjain. About 21kg was collected and the non-biodegradable part of flower waste was removed by hand sorting and the biodegradable waste were segregated and shredded into small pieces.
- **Collection of earthworms:** Earthworm species *Eisenia fetida* was procured from local suppliers at Ratlam, Madhya Pradesh, India.
- **Combinations of composting substrate:** For vermicomposting, *Rosa rubiginsosa*, *Tagetes marigolds* and *Chrysanthemum* flower waste and cow dung were mixed in different ratios. These different flower species floral waste substrates were filled in the plastic bins.

***Rosa rubiginsosa*:**
1. 30% Flower waste (600g Flower waste + 3kg Cowdung + Earthworm)
2. 40% Flower waste (800g Flower waste + 3kg Cowdung + Earthworm
3. 50% Flower waste (1500g Flower waste + 3kg Cowdung + Earthworm)

***Tagetes marigolds*:**
1. 30% Flower waste (600g Flower waste + 3kg Cowdung + Earthworm)
2. 40% Flower waste (800g Flower waste + 3kg Cowdung + Earthworm)

3. 50% Flower waste (1500g Flower waste + 3kg Cowdung + Earthworm)

***Chrysanthemum*:**

1. 30% Flower waste (600g Flower waste + 3kg Cowdung + Earthworm)
2. 40% Flower waste (800g Flower waste + 3kg Cowdun g+ Earthworm)
3. 50% Flower waste (1500g Flower waste + 3kg Cowdung + Earthworm)

Each combination was prepared in plastic bin. The bins with different combinations were left for 7 and 14 days prior to experimentation and watering was done on alternate days. Then they were mixed upside down for pre-composting, microbial degradation, softening of waste and for thermos stabilization.

Preparation of composting substrate: The experiment was performed in plastic containers (40cm length x 20cm width x 20cm depth) with uncovered top surface. The vermibed substrates (*Rosa rubiginsosa*, *Tagetes marigolds* and *Chrysanthemum*) consisted of flower waste and cow dung in different combinations by dry weight as described above. These wastes were used to provide bedding for the earthworms as well as a carbon supplement. Cow dung was used as inoculant.

Alternate two layers of floral waste and cow dung are placed one over another. Adult clitellate worms, *Eisenia foetida* ranging in length from 12cm to 17cm were added at the rate of 100 grams of earthworms per 1Kg of composting substrate. The moisture content was maintained throughout the period of the study by periodic sprinkling of water. Watering was stopped, when the vermicompost got ready as indicated by uniform dark brown to black coloured granular structure.

The vermincast was passed through 2-3 mm sieve and the earthworms were removed manually. The vermincast was air dried by spreading it in large trays. The ratios of bioconversion rate of flower waste into vermicompost for all the groups were calculated and after sufficient moisture was lost, samples were analysed.

Rosa rubiginisosa

Tagetes marigolds

Chrysanthermum

Inoculation of earthworms: Five sexually matured earthworms (*Eisenia fetida)* were isolated separately from stock culture and inoculated in each experimental culture pots after noting their weight and simultaneously, to know the role of earthworms in vermicomposting. All culture containers of composts and vermicomposts of three different earthworm species at 15, 30- and 45-days time intervals in triplicates were maintained with sufficient food with daily sprinkling of required amount of tap water to import 70-75% moisture in uncontrolled room environmental conditions.

Earthworm *(Eisenia fetida)*

Termination of experiments: All the experiments were terminated after 15, 30- and 45-days time intervals. Observations were made with respect to number of old and new adult worms, subclitellets, juveniles, cocoons and their weight to determine the worm biomass, biomass ratio and fold increase in worm number. Gross biomass was calculated by adding weight of all new individuals of different stages including cocoons at the end of experiment gained by initial five inoculated adult worms. Biomass ratio increased from initial weight to final weight and fold increase in worm number from initial worms were also calculated. Percent compost and vermicompost produced at the end of each experiments (15, 30 and 45 days) were also calculated by isolating degraded materials with the help of 0.2 mm sieve.

Results and Discussion: Floral waste generation occur largely during functions, worships, ceremonies and festivals. Biological processes such as composting followed by vermicomposting to convert floral waste in useful organic fertilizer would be of great benefit. In this process, energy rich and complex organic substances have been bio-oxidized and transformed into stabilized products by combined action of earthworms and microorganisms (Edward *et al*., 1992), hence earthworms play a considerable role by fragmenting and altering all biological activity of the waste. In the floral vermicompost prepared was dark black granular in appearance which indicated that the decomposition of

flower waste was occurred successfully, as the earthworms consume floral wastes organic matter very rapidly and fragment them into finer particles, by passing them through a grinding gizzard. They derive their nourishment from the microorganisms that grow upon the wastes; at the same time they promote further microbial activity in the wastes (Jadhav *et al*., 2013) [10]. It was proved from the study that flowers can be very well used as substrate for vermicomposting.

The results of the present study with respect to production of gross biomass, biomass ratio, per cent compost and vermicompost produced by three different flower species at 15, 30- and 45-days time intervals were represented in the (Table 1). Flowers can be used as substrate for vermicomposting. Vermicomposting is a suitable technology for bioconversion of flowers into value-added compost and reduction of Soil waste pollution. The organic fractions of flower waste vermicompost and microorganisms in the biofertilizers improve the growth of yield. The conversion of floral waste into vermicompost using floral wastes (Rose rubiginsosa, *Tagetes marigold* and *Chrysanthermum*) for the production of worm biomass and vermicompost.

Worm biomass: The gross biomass, biomass ratio and fold increase in worm number of all three species increased drastically from 15, 30 and 45days time intervals (Table 1 Fig. 1). There is a positive correlation with increase in gross biomass, biomass ratio and fold increase in worm with number of days (15,30 and 45 days) as the number of days increases, the gross biomass, also increases from 15 to 45 days periods. The mean gross biomass of *Eisenia fetida* in *Rosa rubiginsosa*, *Tagetes marigolds* and *Chrysanthermum* were 7.47±0.17, 14.55±0.06, 20.75±0.30; 8.15±0.00, 15.57±0.33, 18.20±0.48 and 10.59±1.29, 18.18±0.85 and 22.00±0.65 during 15, 30 and 45 days, respectively (Table 1; Fig. 1). Similarly, the biomass ratio is 1:1.79±0.30, 1:3.29±0.14 and 1:4.60±0.16; 1:1.97±0.00, 1:4.18±0.15 and 1:5.32±0.21 and 1:1.42±0.04, 1:2.97±0.02 and 1:5.13±0.38 from initial weight to final weight (Table 1; Fig. 2). There was a drastic increase from initially inoculated earthworm *Eisenia fetida* at 15, 30 and 45 days which were 16.06±0.05, 22.05±0.57 and 28.50±1.76; 15.06±0.33, 18.05±0.57 and 24.00±1.17 and 12.00±1.67, 20.07±1.25 and 25.00±0.05 respectively (Table 1; Fig. 3). The maximum gross biomass were noticed in *Rosa rubiginsosa* 50% flower waste (24.5.0±0.28 and 35.50±0.76) followed by *Tagetes marigold* 50% (24.50±0.28 and 40.50±0.78) and Chrysantermum 50% (32.50±1.01 and 44.05±0.88), whereas biomass ratio was more in *Eisenia fetida* increase *Tagetes marigold* (1:5.32±0.21) followed by Chrysantermum (1:5.13±0.38) and *Rosa rubiginsosa* (1: 4.60±0.16) cultured in flower waste + cow dung during 45 days period.

Table 1: Gross biomass, biomass ratio, and vermicompost produced by earthworm *Eisenia fetida* at various time intervals (15, 30 and 45 days) cultured in cowdung and their significant value ($p<0.05$) among different flower species

S. No.	Flower species	Periods (Days)	Parameters				
			Gross biomass	Biomass ratio (IW: GBW)	30% Flower waste	40% Flower waste	50% Flower waste
1.	*Rosa rubiginsosa*	15 Days	7.47±0.17	1:1.79±0.03	16.06 ± 0.05	20.05 ± 0.13	24.50 ± 0.28
		30 Days	14.55±0.06	1:3.29±0.14	22.05 ± 0.57	25.05 ± 1.16	30.75 ±1.01
		45 Days	20.75±0.30	1:4.60± 0.16	28.33±1.76	26.08 ± 1.10	35.50 ± 0.76
2.	*Tagetes marigold*	15 Days	8.15±0.00	1:1.97± 0.00	15.06±0.33	22.33± 1.66	24.50 ± 0.28
		30 Days	15.57± 0.33	1:4.18± 0.15	18.05± 0.57	25.33± 1.66	33.83± 1.01
		45 Days	18.20± 0.48	1:5.32± 0.21	24.00± 1.17	28.00± 1.74	40.50± 0.78
3.	*Chrysanthermum*	15 Days	10.59± 1.29	1:1.42± 0.04	12.00± 1.67	24.00± 0.65	32.50± 1.01
		30 Days	18.18± 0.85	1:2.97± 0.02	20.07± 1.25	30.05± 0.75	32.40± 0.75
		45 Days	22.00± 0.65	1:5.13± 0.38	25.35±0.05	32.55± 1.25	44.05±0.88
4.	F- value		F=274.14	F=73.19	F=97.18	F=57.88	F=89.55
5.	P- value		P=0.00	P=0.00	P=0.00	P=0.00	P=0.0

Data are in Mean ± SE

IW: Initial weight, GBW: Gross biomass weight,

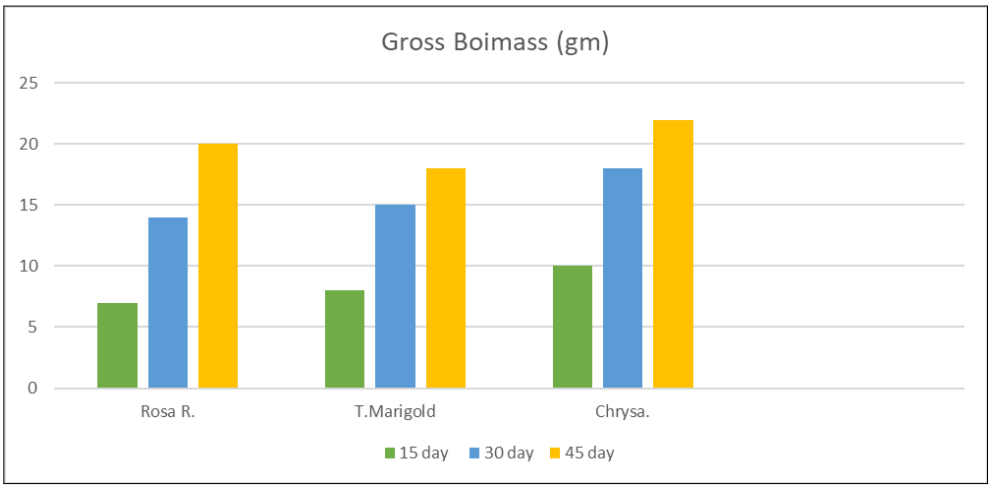

Fig 1: Gross biomass (Mean± SE) produced by different flower species in *Eisenia fetida* at various time intervals (15, 30 and 45 days)

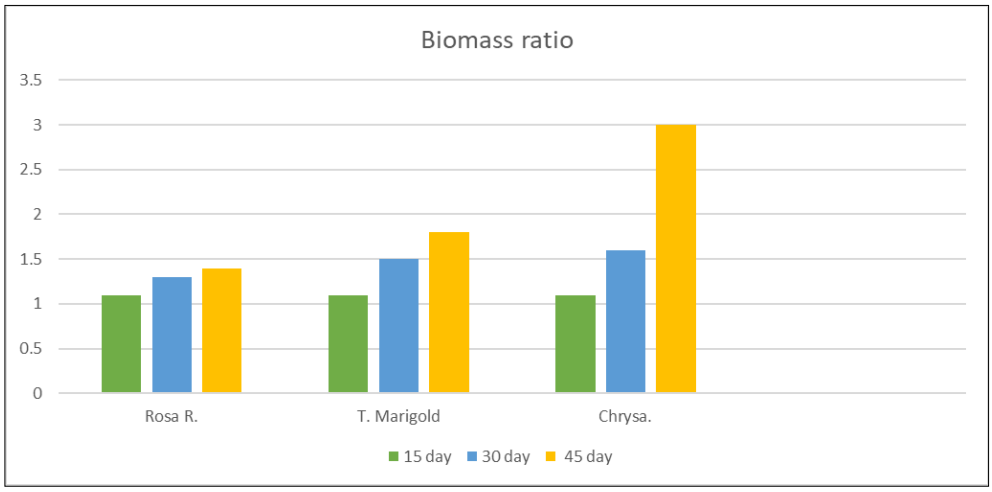

Fig 2: Biomass ratio (Mean± SE) observed by different flower species in *Eisenia foetida* at various time intervals (15, 30 and 45 days)

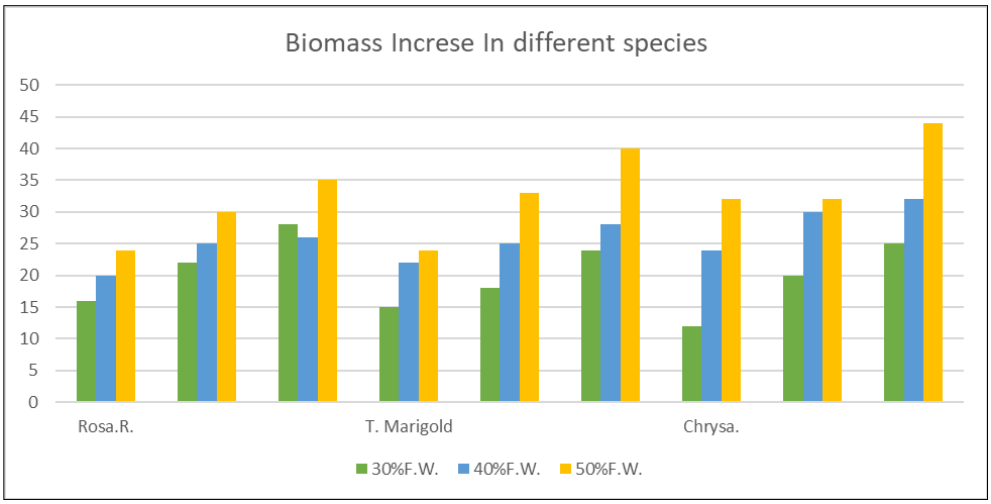

Fig 3: Biomass increase different combinations floral waste flower species in *Eisenia fetida* at various time intervals (15, 30 and 45 days)

In the present investigation there was a drastic variation in the gross biomass, biomass ratio between different flower species at various time intervals (15, 30 and 45 days) and was more in *Rosa rubiginsosa* followed by *Tagetes marigolds* and *Chrysanthermu*, these variations were due to difference in their feeding and breeding habit, growth pattern, life cycle, adjustment to available food materials, individual reproductive capability and congenial environmental conditions etc. The gross biomass, biomass ratio and incresase in worm number in all three species of flowers. The mean gross biomass of *Eisenia fetida* in *Rosa rubiginsosa*, *Tagetes marigolds* and *Chrysanthermum* also increased during 15 to 45 days of period. The increased gross biomass of *Eisenia foetida* during 45 days period different flower species (Rose rubiginsosa 20.75±0.30, *Tagetes marigold* 18.20±0.48 and *Chrysanthermum* 22.00±0.65) were observed. The results of present study corroborated well with the findings of Shouche *et al*., (2011) [18] observed that physical parameters plays important role for efficient waste degradation and rate of vermicomposting depends on the nature of vermicomposting mixture specially amount of cellulose.

Similar observation were reported by (Reinecke *et al*. 1992) [17] and (Viljoen and Reinecke 1994) [23] have noticed variations in growth rate and cocoon production with respect to earthworm species *Eisenia fetida* and also with different organic wastes, *Rosa rubiginsosa*, *Tagetes marigolds* and *Chrysanthermum* + cultured in cow dung for a total period increased at the rate of 24mg/worm/day, respectively. Kale and Krishnamoorthy (1981) [12] have reported that the nature of available food source influences worm activity. Likewise, Reinecke and Venter (1985) [15] have also noticed increase in worm biomass with the feeding activities of the worms. Suthar (2011) [21] studied biomass growth pattern in Allolobophora parva and noticed that there is a consistent trend biomass increase up to 7th weeks, thereafter a marked gradual decline in individual biomass.

The quality and palatability of food directly affect the survival, growth rate and reproductive potentiality of earthworms (Suthar, 2009, 2010) [19, 20]. Kale and Krishnamoorthy (1978) [11] have also reported that each species of earthworms have different preferences towards organic matter among various wastes. The cocoon production was affected by various food sources as the cumulative cocoon number was increased in Perionyx excavatus with the increase in the age of food substrates (Birundha *et al*., 2013) [5]. The present findings were also in agreement with Swatti and Vikram Reddy (2010) [22] have observed that the net individual weight and the total biomass gain were higher in the earthworm *Eisenia foetida* in different Floral waste. Ranganathan and Parthasarathi (1999) [15] reported that the kind and amount of food materials available will influence the size of the earthworm

population, species diversity, growth and fecundity. They also mentioned that earthworms require food rich in nitrogen, cellulose and microorganisms for their growth and reproduction.

Conclusion

The present study revealed that the flower wastes may be used as potential substrate for vermicomposting. Earthworm *(Eisenia fetida)* are known to convert variety of organic wastes into useful products such as vermicompost, worm biomass and biomass ratio. The present study was undertaken to know the potentiality of earthworms in bio-processing of organic waste Flower species (Rose rubiginsosa, *Tagetes marigold* and *Chrysanthermum*) for the production of worm biomass and vermicompost. Observations were made with respect to initial and final biomass of worms, biomass ratio, per cent compost and vermicompost at various time intervals (15, 30 and 45 days) produced out of three species. As the days increases (15, 30 and 45 days), the gross biomass, biomass ratio, percent compost and vermicompost were also increased over the time in all three-flower species. The maximum worm biomass and percent vermicompost were noticed in Earthworm with *Chrysanthermum* flower species. The floral waste vermicompost is an excellent soil additive made up of digested compost. Worm castings are much higher in nutrients and microbial life and therefore, are considered as a higher value product.

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