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Impact of Biofertilizers on Growth and Reproductive Performance of *Eisenia fetida* (Savigny 1926) During Flower Waste Vermicomposting Process

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Authors' contributions

This work was carried out in collaboration between all authors. Author DSK designed the study, wrote the protocol, carried out the laboratory work and wrote the first draft of the manuscript. Authors PSK and VUK performed the statistical analysis and managed the literature searches. Author GA supervised the overall work. All authors read and approved the final manuscript

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ABSTRACT

The growth and reproductive performance of epigeic earthworm *Eisenia fetida* in biofertilizers enriched flower waste vermicomposting was studied for 80 days in the laboratory. Six feeding compositions – Co-Flower waste (FW) alone; A- FW + Earthworms (E. fetida); B- FW+ EW + Azospirillum (Azos); C- FW + EW+ Phosphobacteria (PB); D- FW+EW+ Blue green algae (BGA); E- FW + EW + Rhizobium (Rhizo). Maximum number of earthworms was recorded in C and minimum number in A, highest average body weight was observed in E and least in A. Maximum average body length was noted in B and minimum in A. Higher reproductive performance of earthworms under the influence of biofertilizers were clearly visible in C and E than A. The microbial populations and availability of food materials from biofertilizers enriched flower waste increases the biomass, average body weight, average body length and also increases the reproductive

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performance of earthworms in the vermicomposting process.

Keywords: Eisenia fetida; vermicompost; biofertilizers; reproductive performance.

ABBREVIATIONS

Azos - Azospirillum; PB – Phosphobacteria; BGA – Blue green algae; Rhizo – Rhizobium; Fw – Flower waste; TOC – Total Organic Carbon; TN – Total Nitrogen; VC – Vermicomposting Coefficient; Nrr – Net reproductive rate; RP – Reproductive performance.

1. INTRODUCTION

Every year approximately 40 MTY^{-1} of organic waste produces worldwide. Disposal and environmental friendly management of these waste has become one of the serious global problems. Earthworms are one of the major soil macro invertebrates and are known for their contributions to soil formation and turn over with their widespread global distribution [1] Recycling of waste through vermicomposting, reduces the problem of non- utilization of live stock excreta [2]. Vermicomposting is one of the ways to reduce this organic waste and it has been adopted all over the world. Earthworms utilize the rich complex organic substances and to give humus like product [3]. There are several reports regarding the potential utilization of epigeic earthworms for successful degradation of organic wastes, generated from different industries such as paper and pulp industry [4,5] dairy industry [6]; winery and distillery industry [7]; wood and chips industry [8]; flower waste [9] and textile mill [10,11]. The results of these studies have shown that environmental conditions and earthworm population density affect earthworm growth and reproduction.

World-wide spread *E. fetida* was and still remains a favoured earthworm species for vermicomposting operations due to their wide range of tolerance for environmental variables. The growth patterns *E. fetida* in number of different organic waste resources have been investigated by various authors in laboratory culture [11,12]. *E. fetida* (Savigny) has been studied extensively, in particular for its potential in vermiculture [13]. Various aspects of the general biology and ecology of this species are well known now. The life cycle has been thoroughly investigated by [14], the moisture [15] and temperature requirements [16] are documented [17,18] and [19] also studied the reproduction of this species intensively.

In vermicomposting process the earthworms converting the organic wastes into valuable fertilizers and it is essential to know the influence of prevailing environmental factors on growth and reproduction of earthworms. The activities of earthworms are known to be influenced by quality of food [20] and moisture [21]. Among these food and environmental factors play an important role in the biology of earthworms. The moisture content of organic waste used in vermicomposting is an important parameter influencing the growth of the surface-feeding (epigeic) earthworm species *E. fetida*, since the earthworm's body contains about 80% water. This species gained weight maximally and survived best at temperatures between 20ºC to 29ºC and moisture content between 70% and 85% in horse manure and activated sludge [22]. According to Edwards [23], the optimum growth of *E. fetida* in different animal and vegetable waste was at temperatures of 25–30ºC and at a moisture content range of 75–90%, but these units could vary in different substrates. *E. fetida* is an iteroparous earthworm, with continuous and high reproduction rates, and it should respond to adverse environmental conditions modifying those rates.

The aim of the present work was to know whether biofertilizers such as Azospirillum (Azos), Phosphobacteria (PB), Blue green algae (BGA) and Rhizobium (Rhizo) mixed in the flower waste have any influence on the growth and reproduction of earthworm *E. fetida* population during the vermicomposting process of 80 days.

2. MATERIALS AND METHODS

Healthy adult *E. fetida* were procured from Agricultural research and training centre, Namakkal, Tamil Nadu, India. The worms were brought to the laboratory and reared in plastic containers with partially decomposed organic matter (cow dung).

2.1 Preparation of Vermibeds

Flower waste were collected from gardens, shops, temples etc., were dried under shade for about 10 days and then mixed with dry cow dung at 1:1 ratio (w/w). Plastic circular containers of 28 cm diameter and 30 cm depth with pierced lid for aeration were used for vermicomposting experiment. The flower waste, cow dung mixture was served as bedding as well as food for the earthworms. The bedding substrates was kept for 4 weeks prior to experimentation and watering was done on alternate days for pre-decomposting, microbial degradation and softening of waste. The pre-decomposted substrate was divided into six combination of feed mix

Control (Co) - Substrate alone without Earthworms: A- Substrate with Earthworms; B- Substrate + Earthworms + Azospirillum; C- Substrate + Earthworms + Phosphobacteria (PB);D-Substrate + Earthworms + Blue green algae (BGA); E- Substrate + Earthworms + Rhizobium.

Biofertilizers were mixed at the rate of 1 g per kg of substrate [24]. Triplicates were maintained for all experiment and control. Five weeks old 25 clitellate *E. fetida* were stocked in each experimental container containing 5 kg of substrate material. The moisture level was maintained around 60-80% and throughout the study period by periodic sprinkling of adequate quantity of tape water. To prevent the moisture loss, the experimental containers were covered with jute bags, containers were kept in dark humid place with a temperature of 27 – 30ºC. The samples were drawn at a regular interval of 20 days for chemical analysis i.e., Organic carbon, Total nitrogen, phosphorus, potassium and C: N ratio up to 80days.

The physicochemical parameters of bedding material and vermicompost produced during the experiment were analyzed by using standard methods. The pH was determined using a double distilled water suspension of vermicompost in the ratio of 1:10 (W/V) that was agitated mechanically for 30 min and filtered through whatman no.1 filter paper [25]. Moisture content was estimated after oven drying at 105ºC to a constant weight. Total organic carbon (TOC) of the vermicompost was estimated by the method of [26]; Total Kjeldahl nitrogen (TN) by [27]; Available phosphorus by [28], potassium by [29]. C: N ratio was calculated by dividing the percent of carbon with percent of nitrogen.

Vermicomposting Co-efficient (VC) was calculated by using the following formula [30]).

$$VC = \frac{Total\ increase/decrease\ during\ vermicomposting\ (Experiment)}{Total\ increase/decrease\ during\ composting\ (Control)}$$

The growth and reproductive performance of earthworms in different experimental containers were measured at the same interval. The earthworm population was counted by the hand-sorting method as suggested by [20] at 20, 40, 60 and 80 days after the start of the experiment and the material was returned to the same containers.

Growth rate determination, $R = (N_2 - N_1) / T$

Where R = Growth rate; N_1= Initial earthworm biomass (mg); N_2= Final earthworm biomass (mg); T= Time period of the experimental day.

Reproductive performance:

This was determined as the Net Reproductive Rate (Nrr) and was computed following [31] as stated below:

$$Nrr = \frac{Total\ Number\ of\ earthworms\ harvested}{Total\ number\ of\ earthworm\ stocked\ x\ Experimental\ period\ (weeks)}$$

All the Data were analyzed using SPSS statistical package. Differences among different experimental parameters were calculated by using one way ANOVA ($p<0.05$) and Duncan's multiple range test (DMRT).

3. RESULTS AND DISCUSSION

The vermicomposting co-efficient of physicochemical parameters shows an increased trend in Moisture, Nitrogen, Phosphorus and Potassium, decreases in pH, Temperature, Total Organic Carbon and C: N ratio (Table 1). Maximum pH reduction was recorded in A and minimum in B and C. When compare to temperature a higher reduction in A and least in C and Co, where as highest reduction of TOC observed in E and D, and lowest in Co. A high C: N reduction noted in E and low in A and Co. The increase in earthworms' growth may also be attributed to a low C: N ratio of the pre-decomposed substrate and positive role of bio-inoculants used in the present study [32].

Increased moisture content was recorded in A (82.02%) and B (76.6%) where as reduced level in C (69.58%). The moisture content of the substrate can influence the worm's growth directly or indirectly. The rate of mineralization and decomposition becomes faster with the optimum moisture content [33]. The direct influence concerns the activity (feeding activity) of the worms. According to [34] the feeding activity of certain earthworm species depends on the moisture content of the substrate. *E. fetida* only attains sexual maturity between moisture levels of 65 and 80% [35]. Edwards and Bater [36] reported that optimum moisture content for growth of earthworm's *E. fetida*, *E. eugeniae* and *P. excavatus* was 85% in organic waste management. Hartenstein et al. [12] and Kaplan et al. [17] indicate the greatest biomass and maximum weight gain of earthworms in domestic dung or activated sludge to be achieved at temperatures of 20ºC -29ºC and at moisture levels of 70%-85%.The major nutrients N (B and E), P (A) and K (B) were increased and decreased in Co. Several studies have shown that when soil N content increases, the abundance, biomass and reproduction of earthworms also increase [37, 38]. The presence of large number of microflora in the gut of earthworm might play an important role in increasing P and K content in the process of vermicomposting. The P content also a direct action of earthworm gut enzymes and indirectly by stimulation of the microflora [39] due to bacterial and faecal phosphatase activity of earthworms that probably lead towards mineralization and mobilization of phosphorus [40].

Table 1. Vermicomposting coefficient (VC) of different chemical parameters in control and experimental bins

Parameters	Co	A	B	C	D	E
pH	0.9250	0.9189	0.9730	0.9730	0.9459	0.9594
Moisture (%)	1.0000	1.1014	1.0274	0.9337	0.9889	0.9889
Temperature (℃)	1.0000	0.8333	0.875	0.9167	0.8958	0.875
TOC (%)	0.851	0.836	0.826	0.840	0.789	0.776
TN (%)	1.5	1.692	1.709	1.692	1.618	1.709
Phosphorus (%)	2.263	2.765	2.722	2.684	2.594	2.390
Potassium (%)	1.632	1.721	2.969	2.676	2.067	2.232
C:N ratio	1.0000	0.9122	0.8513	0.8466	0.8311	0.7770

Table 2 reveals the earthworm biomass of present study with maximum number in C (439) and E (428) and least in A (207). The increased earthworm biomass with phosphobacteria suggests the dual role of bacteria as food material and in enriching the substrate with phosphorus through phosphorus solubilization. This phenomenon has also been reported by [41]. Various studies have shown that earthworm utilize micro-organisms in their substrates as a food source and can digest them selectively [42, 43]. A highest average body weight was recorded in E (8.8g) and lowest in A (3.3g). Maximum body length was noted in D (1.3cm) and minimum in A and E (0.8cm).The growth rate in earthworms mainly depends upon the microbial populations and availability of nutrients in vermibeds [44]. The presences of fungi during vermicomposting process became additional supplement to the earthworms which contributed to the increased number and weight of the earthworms. Fungi have cell walls composed of chitin that contains high natural protein; amino polysaccharide [45]. In particular, protozoa and fungi are assumed to form a substantial part of their diet [46, 47]. In general, crop residues have different C: N ratios, particle size, proteins and crude fiber content and even some concentration of special plant metabolites i.e., poly-phenols and related substances [48] which may affects the earthworm production rate in the vermibeds.

The earthworms cultured in biofertilizer mixed flower wastes (B,C,D and E) shows high reproductive performance than A in 20th, 40th, 60th and 80th days (Table 3). These results show there was a significant relationship between biofertilizers and earthworm reproductive behaviour. Domínguez et al. [49, 50] found different growth and reproduction rates of *E. andrei* in different diets and they also found that earthworms invested preferentially their energy either to growth or to reproduction depending of the food quality and moreover earthworm's growth is limited by carbon availability [51]. Gunadi et al. [21] found a relationship between increased growth and reproductive rates of *E. fetida* with low C to N ratios of cattle and pig manure, and [32] reported a decrease in growth rates with increased C to N ratio of paper mulch. A better worm growth pattern in *E. fetida* was due to a good supply of easily available metabolizable organic matter, non assimilated carbohydrates and even low concentration of growth retarding substances in vermibeds. The survival of earthworms in waste decomposing sub system mainly depends on physical and initial chemical profile of the feed stuff [52, 53]. The survival, biomass production and reproduction of earthworms are the best indicator to evaluate the vermicomposting process [30]. The earth worm biomass has been increased 7.28 to 16.56 times in the biofertilizers enriched flower waste vermicomposting process of 80 days. Elvira et al. [4] reported earthworm increased 2.2 to 3.9 times total biomass in the combination of paper mill sludge with cattle wastes.

Table 2. Relative growth rate of *Eisenia fetida* in biofertilizers enriched flower waste vermicompost (mean±S.D)

Experiments	**Number of Worms**		**Average Body Weight (g)**		**Average Body Length(cm)**	
	Initial	Final	Initial	Final	Initial	Final
A	25±0.00	206.6±1.140^{d}	8.74±0.051^{a}	12.48±1.134^{b}	6.74±1.920^{a}	6.78±0.083^{c}
B	25±0.00	336.4±2.073^{b}	8.554±0.0357^{a}	15.5±0.20^{a}	5.82±0.148^{a}	7.96±0.712^{a}
C	25±0.00	436.4±1.673^{a}	6.694±0.450^{b}	13.40±2.140^{b}	6.20±0.187^{a}	7.24±0.167^{b}
D	25±0.00	280.2±0.320^{c}	6.14±0.320^{c}	11.7±0.412^{b}	6.42±1.132^{a}	7.38±0.402^{b}
E	25±0.00	431.2±4.868^{a}	6.28±0.148^{c}	16.0±1.581^{a}	6.42±0.506^{a}	7.38±0.192^{b}

Table 3. Reproductive performance of *Eisenia fetida* during vermicomposting (80 days)

Experiments	**Initial stocking**	**20th day**		**40th day**		**60th day**		**80th day**	
		EW	**RP**	**EW**	**RP**	**EW**	**RP**	**EW**	**RP**
A	25.0±0.00	35.6±1.516^{d}	0.47±0.0158^{d}	82.6±1.516^{e}	0.584±0.427^{d}	151.6±2.701^{e}	0.72±0.109^{d}	205.8±1.303^{e}	0.66±0.015^{d}
B	25.0±0.00	74.6±2.966^{b}	1.058±0.019^{b}	152.4±1.673^{c}	1.074±0.015^{b}	183.4±2.073^{c}	0.84±0.01^{c}	334.5±1.581^{c}	0.954±0.036^{b}
C	25.0±0.00	82.8±1.920^{a}	1.19±0.514^{a}	183.8±2.588^{a}	1.28±0.050^{a}	252.8±1.788^{b}	1.16±0.0244^{b}	435.6±2.509^{a}	1.364±0.019^{a}
D	25.0±0.00	54.6±2.073^{c}	0.754±0.078^{c}	131.6±0.894^{d}	0.916±0.078^{c}	175.8±1.303^{d}	0.844±0.033^{c}	278.6±4.929^{d}	0.874±0.011^{c}
E	25.0±0.00	56.0±1.581^{c}	0.758±0.049^{c}	174.0±2.000^{b}	1.226±0.011^{a}	292.6±3.209^{a}	1.356±0.025^{a}	427.6±4.505^{b}	1.388±0.021^{a}

4. CONCLUSION

In the present study highest earthworm biomass were observed in C, E and least in A, biomass has been increased 7.28 to 16.56 times in the biofertilizers enriched flower waste vermicomposting process of 80 days. Based on the present study we conclude biofertilizers enriched flower waste vermicomposting shows better results of earthworm biomass and reproductive performance influence by the biofertilizers added in the substrate material. There are oppurtunities to study the influence of biofertilizers and substrate materials inter relating with environmental factors in the field of vermiculture. Further work is required to establish the exact biofertilizer for improve the production of earthworms for sustainable vermicomposting / vermiculture operations**.**

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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