Full Length Research Paper

Growth and reproductive performance of *Eisenia fetida* in three varieties of flower (rose, carnation and hypericum) leftovers

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Understanding the growth and reproductive efficiency of vermicomposting worms in various substrates is highly essential for effective utilization of earthworms in sustainable waste management system. The growth and reproductive performance of *Eisenia fetida* in rose, hypericum and carnation flower wastes was evaluated in this microcosm study. Determination of cocoon production rate, hatching success, number of hatchling, biomass and growth in all the three flower waste type were conducted in cylindrical plastic containers. The experiment were designed under three treatment: T1 (rose leftover + cow dung), T2 (hypericum leftover + cow dung) and T3 (carnation leftover + cow dung). Cocoon production started early (within 2 weeks of clitellated worm introduction) in T1 and T2, while it took 4 weeks in T3. The highest total number of cocoon was attained in T2 (178.3 ± 2.8), whereas the minimum cocoon number (109 ± 2.6) was recorded in T3. Significant difference was recorded between the cocoon production performance of *E. fetida* in all the three varieties of flower leftovers (P< 0.05). The highest total number of hatchlings (52 ± 0.88) was recorded in T1, while the least (30.33 ± 0.6) was observed in T3. Maximum weight increase (0.86 ± 0.006 g/worm) of *E. fetida* was observed in T1. The overall result of this study showed that better hatching success of cocoons and number of hatchling/cocoon were obtained by experimental species incubated in rose leftover.

Key words: Carnation, *Eisenia fetida,* Hypericum, Rose, vermicompost.

INTRODUCTION

Earthworms are hermaphrodites, both male and female reproductive organs are present in every single earthworm but self-fertilization does not generally occur. Earthworms have beneficial physical, chemical and biological effects on soil and many researchers have documented that these effects can increase the plant growth and productivity (Sinha et al., 2002; Amador et al, 2013).Since earthworms represent the domina group in

the soil habitat, currently they are becoming the best candidates for soil fertility tests as well as suitable bio indicators of chemical contamination of the soil (Sorour and Larink, 2001; Bustos-Obreg and Goicochea, 2002). Certain species of earthworms have also a great advantage for biological transformation of a wide range of organic wastes into valuable biological fertilizer or vermicomposting.

Understanding the growth and reproductive efficiency of vermicomposting worms in various substrates is highly essential for effective utilization of earthworms in sustainable waste management system (Appelhof et al., 1996; Jesikha and Lekeshmanaswamy, 2013). As huge amount of wastes can be managed through more population of earthworms (Garg and Kaushik, 2005), reproductive and growth performance of various species of earthworms in a range of substrates can act as useful biomarkers to measure the efficiency of an earthworm species in vermicomposting or earthworm based biotechnology (Suthar, 2007; Jesikha and Lekeshmanaswamy, 2013). Neuhauser et al. (1980) have reported that the weight gain by *Eisenia fetida* is positively correlated with food type. Similarly Nath and Chaudhuri (2014) and Nath et al. (2009) have also described substrates that provide earthworms with sufficient amount of easily metabolizable organic matter, facilitate growth and reproduction.

Because of the direct correlation between food availability and quality with the growth and reproduction of earthworms (Dabral et al., 2013), different researchers investigated the growth and reproductive potential of different species of earthworms on various wastes generated from both industrial and household sources. The reproductive and growth performance of *Hyperiodrius africanus* was evaluated in tropical weed (*Chromolaena odorata)* and coffee residues by Tandon (1998). Bhat et al. (2015) measured the growth and reproduction of *E. fetida* in bagasse feed, while Parthasarathi and Ranganathan (1999) studied the growth and reproductive potential of *Perionix excvatus* and *E. fetida* on pressmud. Govindarajan et al. (2008) made growth and reproductive assessment of *E. fetida* on *Leucaena glauca* leaf litter, Nath et al. (2009) studied the effect of different combinations of animal dung and agro/kitchen wastes on growth and development of *E. fetida*. In addition to the food quality of the substrate, other factors such as temperature, pH and moisture of substrate have effect on growth and survival of the worm (Jicong et al., 2005).

In spite of various studies on reproductive and growth potential of popular vermicomposting species of earthworms, mainly on *E. fetida*, on a range of wastes from various origins, no study have been carried out on the life cycle pattern of this worm on commonly dumped and littered wastes in flower farms of Ethiopia. In view of this gap in knowledge, this study was particularly carried out to evaluate the suitability of carnation, hypericum and rose wastes, which are hardy herbaceous plant (except hypericum) (Tah and Mamgain, 2013) for survival of the vermicomposting species, *E. fetida* in terms of growth and reproductive parameters.

MATERIALS AND METHODS

Life cycle experiments were carried out in a laboratory scale with average room temperature of 27°C and 60 to 70% moisture of the culture media. For moisture determination, the sample substrates were taken regularly (at 3 days interval) weighed, oven dried at 105°C and cooled in desiccators for 1 h and reweighed. The difference between moist and dried samples were taken and then the moisture content of beddings was adjusted to 60 to 70% and the temperature was kept within the range of 23-27°C throughout the study period (Chauhan and Joshi, 2010). Each experimental waste in this experiment was designated as follows. T1 = rose leftover + cow dung (3:1), T2 = hypericum leftover + cow dung (3:1), T3 = carnation leftover + cow dung (3:1).

Experimental design for reproductive potential (cocoon production rate) determination

Since worms could not survive in fresh cattle and vegetable wastes (Gunadi and Edwards, 2003), all the waste materials and the cow dung used for the experiment were air dried for 48 h and chopped into small pieces and sieved with 2 mm mesh and left over for 48 h to stabilize before laying them in to experimental containers. The experiments were conducted in cylindrical plastic containers. Each container was filled with each experimental waste material based on the feeding rate explanation of Ndegwa et al. (2000). Three replicates were prepared for each substrate and worm combination. 5 freshly clitellated *E. fetida,* in good health condition, were collected from the stock culture and rinsed with distilled water to remove any adhering material, dried briefly on paper towel, weighed with electronic balance and finally introduced in each container containing one of the experimental treatments. The substrates in each treatment container were examined daily in order to determine the onset of cocoon production. Once the cocoons appeared, they were separated by hand sorting, washed lightly in distilled water and counted so as to determine total number of cocoon and the fecundity or reproductive rate (cocoon/worm/day).

Experimental design to study cocoon incubation period, hatching success and number of hatchlings

To determine the incubation period (time lapse from cocoon formation until the first hatchling emerged in days), hatching success (total number of hatched cocoons), and number of hatchlings per cocoons, fifteen freshly laid cocoons from each treatment were taken from the above containers and placed in containers which contained the same material in which their parents were reared. Three replicates were prepared for each treatment. The beddings were observed daily for the emergence of hatchlings in order to determine the incubation period. As soon as the appearance of hatchling started, they were removed daily using a fine painting brush and counted by hand sorting in order to determine the total number of hatchlings that emerged from a single cocoon. The number of unhatched cocoons was also counted in order to determine the hatching success of cocoons.

Table 1. Cocoon production by *E. fetida* after introduction into experimental treatments.

Treatment	T1	T2	T3
Developmental time to start cocoon production	2nd week	2nd week	3rd week
Mean number of cocoon produced	173±0.88	178.3±2.8	109.4±2.6
Cocoon production/worm	32.8	29	21.88
Mean cocoon production/week(in batch)	18.2	16.1	12.15
Mean cocoon production/worm/week	3.6	3.2	2.43
Mean cocoon production/worm/day	0.52	0.46	0.35
Time to cease cocoon production	11th week	11th week	11th week

T1= rose leftover +cow dung (3:1), T2= hypericum leftover + cow dung (3:1), T3 = carnation leftover + cow dung (3:1).

Experimental design for biomass, maturation and cocoon production date

For biomass determination, exactly the same kind of experimental treatments and containers were used as for the above reproductive potential determination. Each container was filled with each experimental treatment based on the feeding rate determination of Ndegwa et al. (2000). Five hatchlings (approximately 2 weeks old) of *E. fetida* in good health condition, were taken from the above containers for reproductive potential determination. The hatchlings were rinsed with distilled water to remove any adhering material, dried briefly on paper towel, weighed on electronic balance and finally introduced in each respective experimental container with each type of experimental treatments. The weight of the hatchling in the initial day was in the range of 0.2 to 0.2g. Three replicates for each treatment were established.

To monitor the growth and maturation progress weekly, for 17 weeks, the biomass was measured in batch of earthworm in each container and three phases of the life cycle were observed, Preclitelleate (incipient development of the clitellum, identified by appearance of tuberculapubertasis), clitelleate (well-developed clitellum) and regression (loss of the clitellum). At the same time, the first cocoon production date for each worm-waste combination was also determined. The worms were weighed without voiding their gut content. No corrections were applied for gut content to any data in this study. No additional feed was added at any stage during the study period. On the basis of the obtained data on the biomass of the worms other parameters of earthworm such as growth rate or biomass increase rate (g/earthworm/day), maximum weight achieved, net weight gain, relative growth rate and specific growth rate were calculated. To calculate the relative growth rate (the percentage ratio of the maximum weight gained to the initial body weight) and specific growth rate (the percentage weight gained with time), the following formula were applied (Sogbesan and Ugwumba, 2006):

$$RGR \text{ (Relative growth rate)} = \frac{Wm \times 100}{Wi}$$

Wm = maximum weight attained; Wi = initial weight:

$$SGR \text{ (specific growth rate)} = \frac{Log\ Wm - Log\ Wi}{t}$$

Log Wm = logarithm of maximum weight attained; Log Wi = logarithm of initial weight; t = Experimental period in days. Weight gain was calculated as the difference between the initial biomass of worms inoculated and the maximum biomass of worms produced.

Statistical analysis

All results are given as the average value of a single measurement on each of the three replicates. Data were subjected for one way analysis of variance (ANOVA) followed by Duncan's multiple-ranged tests at significant difference $P < 0.05$ using SPSS 15.0 software package to differentiate the statistical difference of the growth, cocoon production rate, hatchling success and number of hatchlings per cocoon among the three experimental wastes. All results are given as the average value of a single measurement on each of the three replicates.

RESULT

Cocoon production rate

Influence of feeding substrate was observed on total cocoon production rate. After the introduction of clitellate worms into the experimental containers, *E. fetida* cultured in T1 and T2 started to release cocoon on the 2nd week and those cultured in T3 started to release cocoon on the 4th week. Once cocoon production started, it continued for nine successive weeks in all experimental substrates. Within nine weeks, the highest total number of cocoon was attained in T2 (hypericum leftover + cow dung) (Table 1). (178.3 ± 2.8 cocoons at a reproduction rate of 0.5 cocoon/worm/day), whereas the minimum cocoon number (109 ± 2.6 cocoons at cocoon production rate of 0.39 cocoon/worm/day) was recorded in T3 (carnation leftover + cow dung).

After cocoon production started, progressive rise in cocoon production was seen for five successive weeks in T1 and T2, whereas in T3, this successive increment in cocoon production continued for 6 weeks. The total cocoon number of *E. fetida* in T2 was 8 and 39% higher than the total cocoon number in T1 and T3, respectively.

Significant difference was recorded between the cocoon production performance of *E. fetida* in T1 and T3 at the same time significant difference was observed between T2 and T3 ($P < 0.05$). Cocoon produced in T1 and T2 were greater than cocoon produced in T3 by 37 and 39%, respectively.

Table 2. Incubation period and hatching performance of cocoons of *E. fetida.*

	T1	T2	T3
Incubation period(in days)	22-25	21-24	32-42
Total number of hatched cocoons	13±0.05	12.3±0.03	11 ±0.03
Hatching success	86.6%	80%	73%
Total number of hatchlings emerged	52±0.88	48±1.15	30±0.33
Hatchlings/cocoon	3.5	3.2	2

T1= Rose leftover +cow dung (3:1), T2= hypericum leftover + cow dung (3:1), T3 = carnation leftover + cow dung (3:1).

Table 3. Growth parameters of *Eisenia fetida.*

Treatment	T1	T2	T3
Mean initial weight(g)	0.98 ±0.003	1.01±0.005	1.05±0.008
Mean initial weight/worm(g)	0.2 ±0.003	0.2±0.006	0.21±0.009
Maximum weight achieved	4.3±0.006	3.9±0.005	3.45±0.006
Maximum weight achieved/worm(g)	0.86±0.006	0.78±0.005	0.69±0.006
Maximum weight attained on	7th week	7th week	11th week
Net weight gain	3.32	2.89	2.4
Net weight gain/worm(g)	0.66	0.58	0.48
weight/worm/ day (g)	0.013	0.011	0.006
Worm weight gained/unit weight of substrate(g)	0.007	0.006	0.005
Relative growth rate (%)	338.8	289	240
Specific growth rate (%)	0.65	0.58	0.51

T1= Rose leftover +cow dung (3:1), T2= hypericum leftover + cow dung (3:1), T3 = carnation leftover + cow dung (3:1).

Incubation period, hatching success and number of hatchlings per cocoon

The incubation period of *E. fetida* was completed between 3 and 5 weeks in hypericum (T2) and rose (T1) leftovers, while 5 to 6 weeks in carnation leftovers. The highest total number of hatchlings, 52 ± 0.88 which comprise 3.5 hatchlings/cocoon was recorded in T1, while the least hatchling number 30.33 ± 0.6,which constitute 2 hatchling/cocoon,was observed in T3.(Table 2).

As the worms emerged out of cocoons, they were white with no pigmentation while they progressively developed their characteristic adult pigmentation appeared within few days. The cocoons produced generally demonstrated good hatching performance in T1 and T2 but least performance was recorded in T3.

Growth and maturation

The biomass of *E. fetida* showed progressive raise up to 7th week in T1 and T2, and remained constant up to 9th week. In T3, progressive increment was observed up to 10th week and remained constant up to 12th week. The maximum biomass production per worm per gram of feed substrates for *E. fetida* was observed in T1 (0.007 g), while the minimum was observed in T3 (0.005 g) (Table 3). In T1 where maximum growth attained, *E. fetida* achieved their maximum weight-increase 0.86 ± 0.006 g/worm at a growth rate of 0.013 g/worm/day in the 7th week. The maximum weight achieved by worm in all treatments was significantly varied from initial weight at the beginning of the experiment (P<0.05). Regarding the

sexual maturity (clitellum development), *E. fetida* in T1 and T2 were preclitelated on the second week and mature individual with clitellum totally developed started to emerge on the 3rd week of this experiment. If the additional two weeks before the worms introduced into growth test containers were considered, the total time of maturation from the hatchling emerged out of the cocoon up to complete clitellum development was 5 weeks. Hence, the average time to complete the life cycle (from freshly deposited cocoon, through clitellate worm and the deposition of the next generation of cocoon) was 9 weeks. *E. fetida* cultured in T3 were preclitelated in week 3 and mature individuals of the species start to appear on the 4th week. If the additional two weeks before the worms introduced into growth test containers were considered, the total time of maturation from hatchling emerged out of the cocoon up to clitellum development would be 6 weeks and the full life cycle (which includes the incubation period) was about 12 weeks. Clitellum regression started on some individuals of the species at the final week of the experiment.

DISCUSSION

In order to understand the population dynamics, productivity and energy flow in earthworms, studying their life cycle pattern is highly essential. Particularly, for effective vermiculture, knowledge on life cycle of composting worms on various wastes is crucial (Bhattacharjee and Chaudhuri, 2002). It is well known that food sources influence not only size of an earthworm population but also their growth and reproduction rates (Dominguez, 2004).

Currently, it is known that besides the inherent feature of the worm species, enhanced biomass production and reproductive performance of composting earthworm species could be directly associated with the physico-chemical, palatability, and microbial composition of their feeding substrate (Suthar, 2007; Prasanthrajan and Kannan, 2011). There are ample evidences on the reaction of various species of earthworms to different types of food sources both in the field and in vermicomposting bins. The data obtained from the present experiment has also demonstrated that the quality of the waste material used for vermiculture could influence the reproductive potential and biomass production of composting earthworms; hence the wastes used in this experiment particularly the rose leftover and the hypericum leftover could be a good media for proper growth of composting worms.

The biomass gain by *E. fetida* in this experiment per gram of feeding substrate was maximum in T1 and minimum in T3. Nath et al. (1999) considered the population density and the nature of feeding waste as a major factor for the difference in biomass gain per gram

of feeding substrate. Despite the need for further studies, the characteristic of the feeding substrate might be a major factor for the differences observed in biomass in relation to per gram of feeding substrates in this study. Since T1 has better nitrogen content (2.45%) and is easily decomposed waste combination, these characteristics might contribute to such kind of result. In the present study, successive weight gain was recorded in the initial weeks of the experiment by the experimental worm in all treatments. But in weeks towards the end of the experiment, progressive worm weight loss was observed in all the tested treatments. In similar pattern, Edwards and Bohlen (1996) and Monroy et al. (2007) also reported that rapid pre-reproductive phase of growth, followed by a phase of progressive biomass and growth reduction once sexual maturity was attained

These losses in worm biomass might be associated with the exhaustion of food. As supported by Neuhauser et al. (1980) who reported that when *E. fetida* received food below a maintenance level, it lost weight. The weight reduction was also occurred because the earthworms attained the matured stage. So, they utilized the energy for reproduction purpose such as laying eggs, mating and cocoon formation (Jesikha and Lekeshmanaswamy, 2013).

Relatively, the highest increase in the worm biomass was observed in T1 and T2, whereas the least rise was observed in T3. This difference could be due to the physical and biochemical quality of the feed substrate, which is an important factor in determining the growth and the time taken to reach sexual maturity (Edwards, 1998). T1 and T2, besides their fast decomposition rates, were rich culture media in the nitrogen content than T3 (1.35%) thus they might provide sufficient protein for fast growth of the vermicomposting worms. Even though this situation needs further studies, the result obtained might indicate that in addition to the nitrogen profile of the substrate, there are other parameters such as the microbial composition, palatability, metabolizable nature of the substrate, low level of growth retarding chemicals, etc, that favor earthworm growth in waste system (Suthar, 2007; Bakar et al., 2014). The maturation period recorded for *E. fetida* in T1, T2, and T3 was different from the findings of Dominguez (2004) who recorded that the average standard time to reach sexual maturity in *E. fetida* was within the range of 21 to 30 days.

The results on maturation time of this study varied from that of Shalabi (2006) who reported that the average standard time taken to reach sexual maturity for *E. fetida* was about 70 days. The result also varied from the result of Venter and Reinecke (1988) who recorded 60 days for *E. fetida* to reach sexual maturity. Other researchers have also reported various maturation times for different species of earthworm in a range of substrates. Garg et al. (2007) reported clietellum development of *E. fetida* achieved in 3rd week in cow, buffalo, horse, donkey,

sheep manure and two weeks in goat as well as 4 weeks in camel wastes. The time needed for sexual maturity varies in direct relationship with nutrient abundance or food quality (Dominguez et al., 2000) and the microbial composition (Suthar, 2007) of the culture material. Therefore, it is possible to hypothesize that the relatively prolonged maturation time of tested worm in T3 was associated with the bio-chemical profile of the substrate, although this needs further experimental confirmation.

It is commonly known that growth is an important step for organisms to attain maturity and enter into reproductive stage; any changes in growth may alter the life cycle of an individual (Lofs-Holmin, 1980). Accordingly, in this study, there was a positive relationship between the weight of the worm and clitellum development. In T1, T2 and T3, the minimum weight for clitellum development in *E. fetida* was within the range of 0.5 and 0.6 g/worm. In line with this finding, Dominguez et al. (2000) established the direct relationship between the weight and clitellum appearance (sexual maturation) of the worm. Similarly, Jager et al. (2006) described that reproduction of various species of earthworms generally starts at a certain minimum body size, which means that any treatment that slows growth automatically leads to a delay on reproduction.

In the present study, better hatching success of cocoons that indicate the cocoon viability (Giraddi et al., 2010), and number of hatchling/cocoon were obtained by experimental species incubated in T1 which has good nitrogen content than other treatments. Since, there are no or few published reports in literature regarding the effect of substrate quality on cocoon hatchling success and number of hatchling that emerge from cocoon, it is difficult to relate totally the direct impact of feeding material on such kind of biological activities. However, Suthar (2007) reported that N-content in substrate might be the primary determinant of cocoon hatching success. The nitrogen content of the culture media affects the cocoon production rate and their further development by influencing the nutritional need of the protein for earthworms. It could be hypothesized that earthworm produce more viable cocoons in nitrogen-rich culture media due to efficient protein supply in their diets.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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