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Evaluating the Changes in Quantities and Types of Substrates Containing Organic Wastes on the Growth and Reproduction of the Earthworm (*Eisenia fetida*)

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ABSTRACT

Background: The feasibility of converting organic wastes into vermicompost and the effect of adding such wastes into the substrate on the growth and reproduction of the earthworm (*Eisenia fetida*) were investigated in the present study through a two-factor factorial experiment with a completely randomized design in triplicate.

Methods: The first factor with nine different treatment combinations included control; potato (P); carrot pulp (C); vegetables (V); sawdust (S); potato, vegetables, and carrot pulp (PVC); sawdust, potato, and carrot pulp (SPC); vegetables, sawdust, and potato (VSP); and carrot pulp, vegetables, potato, sawdust (CVPS). The second factor was related to two different amounts of 100 and 150 g. This research was carried out using the analysis of basket trials in Tonekabon County, Mazandaran Province, Iran, in 2021. The base substrate of earthworms was animal manure positioned in boxes and washed with urban tap water to reach a pH value between 6.5 to 7. After ripening the vermicompost, the boxes were emptied, and the worms were counted.

Results: The analysis of variance showed a significant difference in the number of cocoons between the studied treatments ($P<0.1$), but there was no significant difference in the number of worms between groups. The maximum number of cocoons (mean=605) corresponded to the sawdust treatment combination (S), and the minimum number of cocoons (mean=185) was found in the vegetable treatment combination (V).

Conclusion: The treatment of manure bed with sawdust wastes provided the optimal conditions for the growth and reproduction of *E. fetida*.

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1. Introduction

The vermicompost production from animal manure and food waste is now a well-described and understandable process in agriculture and horticulture because the resulting product is applied as a useful fertilizer. The use of earthworms in producing high-quality compost from various organic wastes has greatly expanded in recent years [1, 2]. Vermicomposting can be exploited as a method of biological management and a process on organic waste to prepare animal feed with high nutritional value [3, 4].

One of the fundamental criteria for selecting superior worm species is its high reproduction rate. *Eisenia fetida* has drawn further attention than the other earthworm species because of its rapid growth and reproduction rate and significant potential to consume various organic wastes [5]. The increased rate of this earthworm biomass depends on the density of the population and the type of nutrients consumed. The increase in net earthworm biomass varies per feed unit [6]. The results of Ramnarain et al. [3] study on vermicomposting of different organic materials revealed that a combination of rice straw and grass had the maximum vermicomposting efficiency (105 kg/m^2), followed by grass (102.5 kg/m^2) and rice straw (87 kg/m^2) after 120 days. Therefore, the growth and reproduction of earthworms are significantly affected by nutrition [6].

Edwards and Bohlen [7] investigated the reproduction rate of earthworms on cattle dung substrates and digested sewage sludge. Their results revealed an impressive relationship between the efficiency of cocoons production and the quality of consumed organic wastes and residues (different substrates) as determining factors for starting reproduction and reaching sexual maturity.

Omara et al. [8] evaluated the weight gain and some other growth indices of earthworms in vermicompost from horse and sheep dungs in combination with wheat straw and alfalfa wastes in different volume ratios. Their results indicated that the highest weight of adult worms was observed in the treatment of sheep dung plus alfalfa waste at a volume ratio of 50 to 50, and the highest weight of young worms was obtained in the treatment of wheat straw plus sheep dung at a ratio of 25-75. In addition, the highest weight of baby worms and final weight of worms were found in the treatment of wheat straw plus sheep dung at a volume ratio of 1:1. In general, their results demonstrated that the treatment of sheep dung in combination with wheat straw and alfalfa wastes led to the highest weight gain of earthworms.

Chauhan and Singh [9] studied the effects of various agricultural wastes, including wheat straw, banana peel, and bran (barley and rice), in combination with cattle and goat dungs at a ratio of 1: 1: 1 on the growth and reproduction of *E. fetida*. Their findings revealed a significant difference in the number of cocoons produced in different combination treatments ($P<0.05$). Overall, it is essential to understand how the earthworms grow and reproduce in different substrates to choose the right conditions and suitable substrates [10, 11]. Moreover, the chemical composition of organic residues is affected by various factors, such as climatic conditions, type of used substrate, collection, storage, and maintenance; each can significantly affect the growth and reproduction of earthworms [12-14]. Large quantities of summer fruits, vegetables, carrot pulp, and sawdust are produced annually, whose disposal causes the production of large volumes of leachate in the landfill and the penetration into the environment resulting in soil and climate pollution [15-17]. Accordingly, the present study aimed to convert potato peel waste, carrot pulp, vegetable waste, and sawdust into valuable compost fertilizer and to determine the most suitable substrate for the reproduction of *E. fetida*.

2. Materials and Methods

The present research was carried out on one of the breeding halls of *E. fetida* and the production of vermicompost fertilizer using a basket method trials in Tonekabon County, Mazandaran Province, in the north of Iran, in 2021 through a two-factor factorial experiment with a completely randomized design (CRD) in triplicate. The first factor with nine different treatment combinations included control; potato (P); carrot pulp (C); vegetables (V); sawdust (S); potato, vegetables, and carrot pulp (PVC); sawdust, potato, and carrot pulp (SPC); vegetables, sawdust, and potato (VSP); and carrot pulp, vegetables, potato, and sawdust (CVPS). The second factor is related to two different amount of 100 and 150 g.

The base substrate for vermicomposting was cattle dung that was first dried at 65°C for 48 h and then crushed and passed through a 0.5-mm sieve. Next, the products were poured into boxes with dimensions of 30×40×60 cm and washed with urban tap water to reach a pH value of 6.5 to 7. Afterward, each box was inoculated three times with 35 adult worms during the vermicomposting period. Throughout the 4-month rearing period, three feeding cycles were performed one month apart in the substrates. In outdoor vermicomposting systems, it is necessary to cover the substrates to retain moisture and avoid direct sunlight [18]. Because of the maximum activity of earthworms at a depth of 15 to 25 cm, adding a thin layer of fresh waste (2 to 3 cm) to the surface of the masses every two or three weeks protects them from extreme heat or cold [19]. Moreover, the frequent addition of wastes could enhance the fertility of worms [20].

The experimental treatments added for the main substrate (animal dung, 100 or 150 g) of earthworm breeding were as follows: 1) control, nothing to add the main substrate; 2) potato (P), 100 g to 100 g animal dung and 150 g to 150 g animal dung; 3) carrot pulp (C), 100 g to 100 g animal dung and 150 g to 150 g animal dung; 4) vegetables (V), 100 g to 100 g animal dung and 150 g to 150 g animal dung; 5) sawdust (S) 100 g to 100 g animal dung and 150 g to 150 g animal dung; 6) potato, vegetables, carrot pulp (PVC); 25 g of each to 100 g animal dung and 37.5 of each to 150 g animal dung; 7) sawdust, potato, carrot pulp (SPC); 33.33 g of each to 100 g animal dung and 50 of each to 150 g animal dung; 8) vegetables, sawdust, potato (VSP); 33.33 g of each to 100 g animal dung and 50 of each to 150 g animal dung; and 9) carrot pulp, vegetables, potato, sawdust (CVPS); 33.33 g of each to 100 g animal dung and 50 of each to 150 g animal dung.

After ripening the vermicompost, the boxes containing vermicompost and cocoons were emptied, and the number of cocoons was counted. Analysis of changes in the number of cocoons and worms in different treatments was performed by the analysis of variance (ANOVA) and the mean comparison using SPSS software v. 22.

3. Results and Discussion

The effects of different combinations of organic wastes (carrot pulp, vegetable waste, potato waste, and sawdust mixed with animal manure) were studied at two different substrate concentrations (of 100 and 150 g) on the changes in the number and reproduction rate of cocoons and earthworms. The qualitative characteristics of vermicompost produced in triplicates for control treatment as the base substrate (animal manure) are presented in Table 1.

The results of ANOVA (Table 2) for the number of cocoons and worms showed a significant difference in the number of cocoons ($P<0.1$) but not in the number of worms between the studied treatments. Therefore, to ensure the evaluation of the studied treatments and complete the information, the mean values were compared by the Duncan test with a 90% confidence interval (90%CI) to examine the changes in the number of cocoons in different treatments.

The results of the present study demonstrated a significant difference in the number of cocoons counted after the vermicomposting process in different treatments so that the highest number of cocoons (with an average of 605) was found in the sawdust wastes as the best treatment and substrate for the reproduction of worms. The nutrients in this substrate seem to provide better condi-

Table 1. Qualitative characteristics of the final vermicompost produced and the number of cocoons and earthworms in the base substrate (animal manure)

Repeats	Cocoons (No.)	Worms (No.)	N	Ec	pH	C	C/N
1	179	170	1.91	13.4	7.6	29.29	15.33
2	170	183	1.81	13.3	7.5	28	15.47
3	183	177	2.01	13.5	7.7	27.3	13.58

Table 2. ANOVA results for the number of cocoons and worms in different treatments of vermicompost production

Sources of Change	Cocoons (n)			Worms (n)		
	Mean	F	Sig.	Mean	F	Sig.
Treatment	55.728	1.860	0.107	29.580	1.691	0.144
Level	1.359	0.045	0.833	2.895	0.166	0.687
Treatment * level	20.006	0.668	0.697	7.898	0.452	0.862
Error	29.955	-	-	17.489	-	-

Table 3. Comparing the mean number of cocoons and earthworms in the production of vermicompost from various organic wastes

Stations	Cocoons (n)		Worms (n)	
	Code	Number	Code	Number
(Control sample)	c	177.33	ab	176.67
(P)	a-c	396	ab	230.89
(C)	a-c	371.89	ab	195.11
(V)	c	185	b	58.5
(S)	a	605	a	426
(PCVS)	ab	552.5	ab	373.5
(PCV)	bc	240.5	b	61.5
(PSC)	a-c	345	ab	166
(CSV)	a-c	401	ab	222

tions for the growth and maturity of worms [21]. Ebadi et al. [22] reported that the mean final weight of earthworms in the sawdust substrate was at the highest level and acknowledged that the mean daily weight of the earthworms depends on their population density and nutrient type. On the other hand, the number of cocoons in the vegetable treatment (with an average of 185) was at its minimum, so the vegetable could not be a suitable substrate for reproducing worms. Since the vermicompost contains essential micronutrients, the composition of different types of the substrate is directly related to the reproduction rate of cocoons and worms [23]. The difference in the number of cocoons after feeding in different treatments has also been reported in a study done by Gark et al. [24].

The present study revealed no significant difference in the number of worms between the studied treatments. This finding is in line with the findings of Yahyaabadi [5], who compared the biological traits of two earthworm species (*E. fetida* and *Dendrobaena veneta*) on the substrate of waste compost. He found that although the difference in the mean total number of cocoons and the mean number of cocoons per worm per day was significant between the two species ($P<0.05$), these two species had no significant difference in the mean maximum

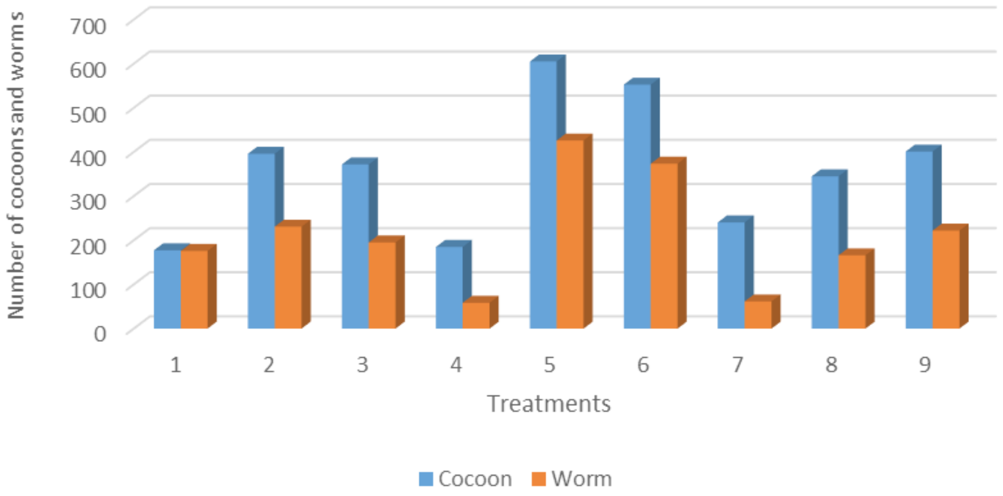

Figure 1. Comparing the number of cocoons and worms generated at the end of the vermicompost production process in 9 different substrate combinations and control sample

number of worms. The difference in the weight of worms under different treatments can be attributed to the presence of various substrates in terms of nutrients. It can be stated that the weight gain of adult, young, and baby worms and that the final weight of worms, as well as the weight of cocoons, depend on the type of treatment fed. This finding agrees with the findings of Nedunchezhiyan et al. [6] and Aquino et al. [25], who found out that the growth and biomass gain of earthworms were dependent on the type of nutrition.

Comparing the means of the data devoted to the number of cocoons in different treatments during vermicomposting showed that the highest number of cocoons was related to the sawdust treatment (S) with an average of 605, and the lowest was found in the vegetable treatment (V) with an average of 185 (Table 3). It is noteworthy that the number of cocoons was higher in all treatments than in control, indicating the effect of substrate composition diversity due to differences in nutrient compositions on the reproduction of worms during the vermicomposting process. Regarding the comparison of the mean number of worms in different substrates of vermicomposting, although there was no significant difference between the treatments, the results of Duncan test indicated that the highest number of worms was related to the sawdust substrate with an average of 426, and the lowest was found in the vegetable treatment with an average of 58.5. These results showed that the survival of worms in different substrates is different, and the vegetable substrate has the most unfavorable conditions for the transformation of cocoons into worms.

As seen in the present study, sawdust is the most suitable substrate for the growth and reproduction of earthworms. Ebadi et al. [22] studied the growth and reproduction of *E. fetida* on tomato pulp, potato, barley, bagasse, sawdust, and soil in animal manure substrate and observed a significant difference ($P<0.01$) between tested treatments and different substrate concentrations. Maximum yield and weight gain were observed in the sawdust treatment. In the mentioned study, the highest reproduction ratio and the number of worms were seen in the tomato pulp substrate. The lowest yield and reproduction were reported in the barley pulp treatment. Overall, this research exhibited that the substrates of tomato pulp, sawdust, bagasse, animal manure, and soil provided the best conditions for the growth and reproduction of earthworms.

A comparison of changes in the number of cocoons and worms in eight different substrates studied and the control sample is shown in Figure 1. The type of feeding substrates affected the number of cocoons and their transformation into worms. The number of cocoons and worms in the control sample (treatment 1) was almost equal, indicating the desirable conditions of the animal manure substrate for the transformation of cocoons into worms. The lowest rates of cocoons survival and their transformation into worms were related to treatments 7 and 4, respectively; the ratio of cocoons transformation to worms was 240.5 to 61.5 in treatment 7 and 185 to 58.5 in treatment 4.

Omara et al. [8], in determining the best substrate for breeding *E. fetida* using different substrates of horse and sheep dungs in combination with wheat straw and alfalfa residues at different volume ratios, documented significant differences in various substrates between treatments considering the weight gain of adult, young, and baby worms as well as the final weight and growth of worms indices other than the survival rate. One of the reasons for the high number of offspring per producer in a mixture of wheat straw with sheep dung could be attributed to the aeration factor of wheat straw to the substrate, which was in line with the results reported by Edwards and Bohlen [7]. The study by Kale et al. [26] on the effects of nutrition and diet on the worm population shows that the mixture of forage and animal manure generates a much larger population than the other nutrients. Therefore, considering the similarity of physical conditions of wheat straw with sawdust in terms of high porosity in proper aeration of compost mass, the reason for the proper performance of sawdust substrate in a high number of cocoons can be due to the appropriate aeration conditions. Similarly, Edward and Fletcher, and Alayi et al. [27, 28] concluded that a dry food source is beneficial for reproducing worms, and sawdust in the base substrate of animal manure provides the best conditions for the growth and reproduction of *E. fetida*.

4. Conclusion

Different organic wastes provide different conditions for breeding *E. fetida*. The maximum and minimum numbers of cocoons and earthworms were obtained from the sawdust and vegetable treatments after compost processing, respectively. In general, the results indicated the suitability of sawdust substrate and the combination of potato, carrot pulp, vegetable, and sawdust (PCVS) substrate for the reproduction of *E. fetida*. The most changes related to the number of cocoons and worms were observed in these two substrates, indicating the suitability of this substrate for the growth of these earthworms. These worms will have the highest rate of growth and reproduction if the substrate is suitable. The sawdust substrate plus animal manure had the highest reproduction and fertility rates. Furthermore, because of the presence of suitable nutrients in the treatment combination of carrots, vegetables, potatoes, and sawdust, in this substrate, the baby worms grew rapidly and had the highest number of cocoons and earthworms after the vermicomposting period. This result may be related to sawdust treatment because of suitable aeration conditions in this substrate.

According to these results, the reproduction and breeding of earthworms for vermicomposting, in addition to the factor of proper nutritional composition, the appropriate substrate aeration should also be considered by fertilizer producers and earthworm breeders as an essential and effective criterion in the production process. Despite the presence of required nutrients, the worst type of substrate for the growth and reproduction of cocoons and earthworms was the vegetable treatment, which decreased the essential reproduction conditions for cocoons and earthworms because of component adhesion and poor aeration.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors had equal contributions to the writing, review, and final approval of the paper.

Conflict of interest

The authors declared no conflict of interest.

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