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Nitrogen Recovery and Reproductive Performance of *Eisenia fetida* through Composting of Biowastes under Himalayan Ecosystem

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A B S T R A C T

This study examines the potential of indigenous *Eisenia fetida* in the conversion of pre-composted substrate mixtures of cow dung, chinar leaves (*Platanus orientalis*), paper and kitchen waste and quantification of nitrogen recovery from the waste combinations through economical methodology. The experiment was conducted at Mountain Livestock Research Institute, Manasbal, Kashmir. The treatments consisted of four pre-decomposed biowaste substrate combinations of cow dung, chinar leaves, paper waste and kitchen with different ratios. Locally found *Eisenia fetida* were inoculated with 35-days-oldworms. Kjeldahl method was applied in determination of total N percentage after digestion of biowastes samples. Vermicastings produced from T_5 (cow dung, kitchen waste and chinar leaves 2:1:1), T_1 (cow dung and chinar leaves in the ratio of 3:1), and T_2 (cow dung, paper waste 3:1) recorded an increase in nitrogen by 40.86 %, 35.77 % and 28.57 % respectively at 90 days after stocking. Maximum gain in earthworm number and highest growth rate was significantly higher in T_5 (93.80%), followed by T_1 (85.20) at 90 days after stocking. The waste-to-nutrients when set up with such consideration, can effectively bridge the gap between waste recycling, composting and landfilling, for tackling the increasing problems of waste disposal in the urban areas, in an environmentally friendly manner, besides augmenting recycling of nitrogen for agricultural use.

Introduction

Inhabitations of human beings and animals generate huge load of organic wastes and their open decomposition affects the quality of soil, air and water. Most of the biosolid wastes are highly infectious as they contain an array of pathogenic microorganisms. Their disposal into the environment without prior disinfection causes health and environmental risks. World waste industry has been registering a growth rate of 2.8% per annum. Vermicomposting helps to convert organic wastes (agro-wastes, animal manure and domestic refuse) into highly nutrient fertilizers for plant and soil (Yvonne *et al.*, 2019). One of the main concerns regarding the environmental impact of agriculture is nutrient management and maintenance of soil health. This is especially important as agricultural sector is predicted to increase further due to estimated global population growth of about 35% by 2050 (UN, 2013). All plants utilize nitrogen in the form of NO_3^- and NH_4^+. It is most imperative element for proper growth and development of plants which significantly increases and enhances the yield and its quality by playing a vital role in biochemical and physiological functions of plant (Leghari *et al.,* 2018). In cereals, it affects the proportion of lysine and threonine amino acid. Kernel quality and strength increases with enhanced nitrogen content which in turn positively affects the milling properties. In oil seed, protein content and pod formation significantly increases with nitrogen nutrition (Arshad *et al*., 2018). In fruit crops, nitrogen positively affects several quality aspects such as size, shape, color, texture, flavor, and composition (Carranca *et al.*, 2017). The largest fraction of nitrogen and phosphorus deriving from agriculture is found in organic waste fractions of agriculture farms, households and the food industry (Wivstad *et al*., 2009).

Earthworms are one of the most important organisms among soil invertebrates owing to their beneficial effects on soil physical properties and impact on decomposition of soil organic matter (Kangmin *et al.*, 2010). Earthworms play an important role in decomposition of organic matter and soil metabolism through feeding, fragmentation, aeration, turnover and dispersion (Shuster *et al*., 2000). Earthworms are important drivers of waste management process by conditioning the substrate and altering the biological activity (Aira *et al*., 2007; Amruta *et al.*, 2018). Applied use of earthworms in breakdown of a wide range of organic residues, including sewage sludge, animal wastes, crop residues, kitchen waste and industrial refuse to produce vermicompost, has been recommended by Dominguez and Edwards(2004). Earthworms play an important role in determining the balance of greenhouse gases from soils, and their impact is expected to increase in the coming decades (Lubbers *et al.,* 2013). The worms actually enhance microbial activity and diversity and lead to rapid degradation of waste and recovery of nutrients. The organic rich diet is an ideal medium where biomass of earthworms and their enzymatic activity are high due to variety of microbes (Sumathi and Thaddeus, 2013). Microflora present in the intestine of worms and gut enzymes, as in waste, is involved in decomposition. Suriyanayanam *et al., (*2010) carried out a study and reported that kitchen waste can be used as a good bulking agent or source of carbon in composting. 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 rate of cocoon production and their further development by influencing the nutritional need of protein for earthworms. In view of the above, the present study aimed to investigate the effect of different bio-wastes (cow dung, kitchen waste, chinar leaves and paper waste) on the reproductive performance and cocoon production *of E. foetida*. It was also hypothesized that combination of different biowastes would influence the availability of nitrogen due to difference in their physico-chemical characteristics.

Materials and Methods

The experiment was conducted at Mountain Livestock Research Institute, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Manasbal during 2013 and 2014. The location represents temperate climate with annual mean minimum and maximum air temperature of 13.5 and 29.7, respectively. The institute supports a mixed farming system and is an ideal model of Integrated Farming System (IFS) in this region. The treatments consisted of six pre-decomposed combinations of different biowaste substrates as designated in Table 1. These treatments were laid out as Randomized Block Design (RBD) with three replications. The carbon: nitrogen (C:N) ratio of biowastes was determined by taking the substrate samples to the laboratory for carbon and total nitrogen analysis using Walkley (1934) and Kjedahl method (Subbiah and Asija, 1956), respectively. The C/N ratio was obtained by dividing carbon content with nitrogen content of organic material (Table 2).

As per requirement, different biomass was collected from premises of the institution. The experimental units of dimension 4.0 ft x 7.0 ft with 2.0 ft depth were filled up with pre-decomposed biowaste material as per the treatment details, using indigenous *Eisenia fetida*. Each combination of biowaste substrates under experimentation were inoculated with 35–day-old worms, fifty in number. All the treatments were given similar management practices atdifferent time intervals. To study the effect on growth and reproduction of *Eisenia fetida*, 50 adults of this species were inoculated in substrate (in triplicate) and monitored for mortality, sexual maturity and cocoon production. Cocoon production was recorded weekly; cocoons were separated from the localized area by hand sorting. These cocoons were further used for studying different life stages of *Eisenia fetida*. Few freshly laid cocoons were kept under separate tray (1.0´ x 0.8´ x 0.3´ deep) with substrate in all the six ratios. Cocoons were observed after every second day to observe hatching. The feed in the container was turned out, earthworms were separated by hand sorting, after which they were examined for clitellum development. The total nitrogen in organic material before and after experimentation was estimated by following the principle of Pellett and Young (1980).

Statistical analysis

Data were analyzed for ANOVA subjected to Duncan's multiple-ranged tests to differentiate the statistical difference between results of earthworm growth, cocoon production rate, number of total earthworm population in different feeding material. Pearson's correlation coefficient was also calculated between nitrogen recovery parameter from different substrates. The data was analyzed by using SPS-software.

Results and Discussion

Mixtures of cow dung, kitchen waste with chinar leaves were placed in heap for 45 days to overcome higher temperatures by hypothermal process, which led to release of gases like ammonia, methane and leaching out of uric acid from the cow dung, heaping also caused the better structure of waste substrates. Cecilia (2003) reported that there is heat production in aerobic decomposition of biowastes which is a highly exothermic process. A loss of 25 % in total fresh mass was also recorded in the biowaste mixture of cow dung, kitchen with chinar chips after 6weeks of pre-composting. Higher relative weight loss was also found with alone raw kitchen waste (44 % on average) compared to pre-composted cowdung (32 % on average). Addition of chinar leaves into waste mixture of cowdung and kitchen caused faster and substantial loss of weight during vermicomposting.

The sexual development and multiplication rate of earthworms in various feed stocks has been depicted in Figure 2. The initial stocking of 50 young worms were uniform to each feed stuff under same management practices. Table 3 reveals corresponding increase in earthworm stocking as 13.60; 55.80 and 93.80 at 30, 60 and 90 days after stocking inoculation respectively. This is in line with the study of Mahalakshmi *et al.,* (2018) who reported that at the end of 60^{th} day, the numbers of earthworms in each vemicomposting pit increased from 25 to 136, 214 and 432, respectively. Growth rate is a good indicator for comparing the growth of earthworms in different wastes. Lalthanzara *et al.,* (2011) reported that the growth rate in earthworms might have enhanced due to higher food intake. Similar results were also reported by Neuhauser *et al.,* (1980) suggesting that the rate of weight gain by *E. fetida* is dependent on population density and the type of food stuff. Development of individual clitellum in earthworms was found on 26^{th} to 30^{th} day after stocking in different food substrates. The results corroborate the Gunadi and Edwards (2003) who studied growth, reproduction and mortality of *E. fetida* for over a year in solid manure, pig manure and supermarket waste solids. Nath *et al*. (2009) also studied the effect of different combinations of animal dung and agro/kitchen wastes on growth and development of *E. fetida*.

Earthworms also had a great impact on nitrogen transformation in the compost material. Total nitrogen increased at all treatments (Table 4). Vermicompost from bio-waste showed higher total nitrogen content than initial fedstock (Fig. 2). Addition of paper caused decreased total nitrogen content in the initial mixture, however, increased the nitrogen content in the final vermicompost of mineral nitrogen in final product of T_4 could be explained by the initial low N richness in substrate mixtures and also by volatilization of ammonia during pre-composting (stalking) process. However, it is also speculated that increased nitrogen (24.39 %) content in T_4 (kitchen waste + paper waste) treatment at the last phase of vermicomposting could be attributed to nitrogen released from dead earthworms and a low pH values. Suthar and Singh (2008) also reported that enhancement nitrogen content in vermicompost was probably due to mineralization of organic matter containing proteins from dead worms and conversion of ammonium nitrogen into nitrate. The highest percentage of nitrogen was recovered from T_1 (cow dung 75 %+ chinar leaves 25 %) treatment at a tune of 1.62±0.04 at the end of composting period as compared to 1.15±0.03 of same materials before composting, showing an increment of 40.86 % nitrogen. The increase in the nitrogen value in the final compost is result of higher proportion of cow dung, carbon loss and probably due to mineralization of organic matter. These results are in line with the results of Kaushik *et al*., (2003) and Chandra *et al.*, (2018) who reported that the final nitrogen content of compost is also dependent on the initial N present in the waste and the extent of decomposition. Cow dung along with chinar leaves (T_1) also showed higher nitrogen content (40.86 %) on average, compared to initial content of same feedstock mixtures, followed by T_5 (35.77%) at 90 days after earthworm stocking. The results are in line with the reports of Frederickson *et al.*, (2007) and Gupta and Garg (2009) who reported that appropriate feed composition for earthworms can optimize vermicompost. Nitrogen content showed increasing trend during vermicomposting on average in all treatments (Fig. 1). Chinar leaves had a more positive effect on the nitrogen content in the final vermicompost compared to the paper mixture irrespective of pre-composting. The correlation coefficient "R" computed for different treatments T_1, T_2, T_3, T_4, T_5 and T_6 are as follows: 0.81, 0.80, 0.74, 0.71, 0.58 and 0.44, with an increase in nitrogen content of 40.86, 33.33, 31.15, 24.39, 35.77 and 32.00% respectively at 90 days after earthworm inoculation. Earthworms have a great impact on nitrogen transformations, by enhancing nitrogen mineralization, so that mineral nitrogen may be retained in the nitrate form. Atiyeh *et al*., (2000) reported that lower contents of $N\text{-}NO_3$ in pre-composted materials than in final materials can be partially explained due to higher pH levels in the substrate material.

Influence of feeding substrate was also observed on cocoon production rate. After introduction of clitellate worms into the experimental containers, *E. fetida* cultured in T_1 and T_2 started releasing cocoon in 3rdweek and those cultured in T_3 started releasing cocoon in 4thweek after stocking. The cumulative cocoon production by earthworms in different feeds is depicted in Figure 2. Cocoon production rates may be related to biochemical differences between the quality of feed substrates, which are important in determining the time to reach sexual maturity and begin reproduction. The results corroborated with the previous studies of Sifolo (2019), who reported the number of cocoons produced per worm per day in cashew residues (0.28 ± 0.03) was significantly higher than those observed in cocoa residues (0.17 ± 0.02) and in the mixture of the two residues (0.21 ± 0.01).

Table.1 Combination of different bio-substrates for conversion through vermicomposting

Treatment Composition	Abbreviations
Pre-composited cowdung waste 75 % + Chinar leaves 25 %	CD+CL
Pre-composited cowdung waste 75 % + Paper waste 25 %	CD+PW
Pre-composited Kitchen waste 75 % + Chinar leaves 25 %	KW+CL
Pre-composited Kitchen waste 75 % + paper waste 25 %	KW+PW
Pre-composited cow dung 50 %+ Kitchen waste 25 % + Chinar leaves 25 %	CD+KW+CL
Pre-composited cow dung 50 %+ Kitchen waste 25 % + Paper waste 25 %	CD+KW+PW

Table.2 Carbon: nitrogen (C:N) ratio of different organic wastes at the start of composting process

Substrate	Carbon (%)	Nitrogen (%)	C:N ratio
Cow dung	45.20	1.32	32:1
Paper waste	68.72	0.50	160:1
Kitchen waste	39.38	2.15	18:1
Chinar leaves	55.45	0.75	56:1

Table.3 Reproductive performance of *Eisenia fetida* during vermicomposting up to 90 days

Substrate	Initial stocking	30th Day		60th Day		90th Day	
	EW	EW	No. of cocoons	EW	No. of cocoons	EW	No. of cocoons
T1	50.0	51.6±1.26	3.21±0.17	74.6±2.66	4.51±0.71	92.6±2.07	6.21±0.31
T2	50.0	55.3±1.10	2.13±0.12	65.6±1.16	3.53±0.41	85.3±1.63	5.53±0.42
T3	50.0	52.8±1.18	2.32±0.16	72.8±1.20	4.66±0.15	89.8±1.49	6.32±0.20
T4	50.0	58.6±1.33	3.12±0.19	78.6±2.73	4.21±0.21	91.6±2.83	6.92±0.89
T5	50.0	61.2±2.09	2.89±0.13	86.2±2.67	6.12±0.39	96.9±2.81	6.89±0.41
T6	50.0	56.8±2.09	2.18±0.09	77.9±1.01	5.03±0.82	82.3±2.67	5.23±0.29

EW= earthworm

Table.4 Nitrogen content (mgkg^{-1}) recovery of different composts in the comparative study to assess the efficiencies of earthworm species for biodegradation of organic wastes at the end of vermin composting

Treatments	Initial (after pre-composting)	Final (after vermicomposting)	Recovery % of nitrogen
T1	1.15 ±0.03	1.62±0.04	40.86
T2	1.05±0.02	1.40±0.04	33.33
T3	0.95±0.02	1.25±0.03	31.15
T4	0.82±0.02	1.02±0.02	24.39
T5	1.09±0.03	1.48±0.04	35.77
T6	1.00±0.02	1.32±0.03	32.00

Fig.1 Correlation coefficient of nitrogen recovery (%) from biowaste food stuffs.

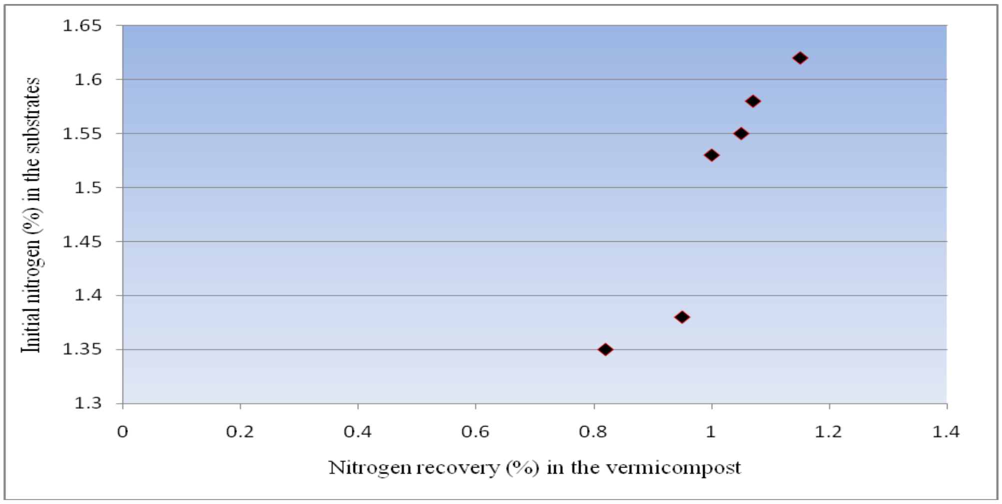
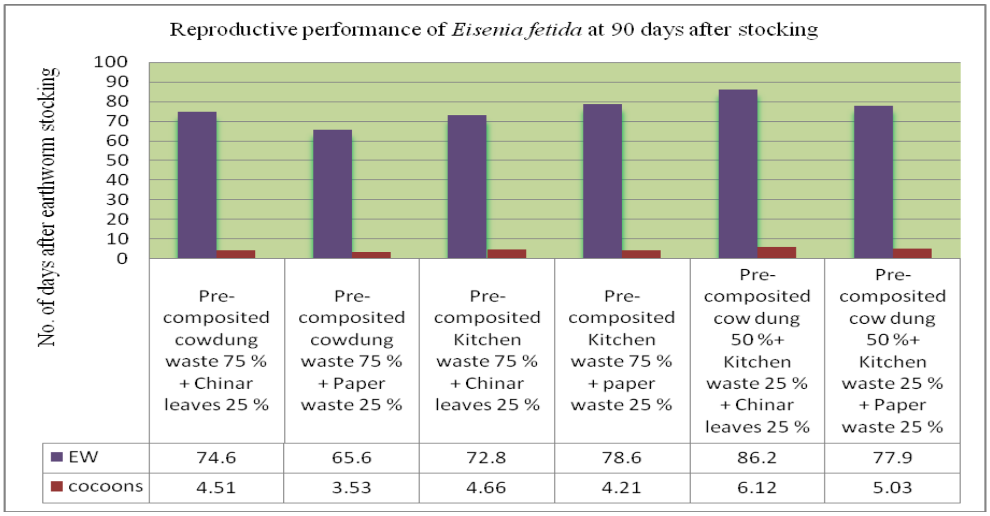


Fig.2 Earthworm multiplication and production of cocoons under different biowastes



There are many other findings about the combined proportions of industrial waste and organic material to optimize earthworm biomass and cocoon production (Yadav and Garg, 2009; Veeresh and Narayan, 2013; Natarajan and Gajendran, 2014). After 12 weeks, maximum cocoons were found in T_4 (6.92) and minimum in T_5(5.23). Cocoon production by earthworms in different substrate mixtures recorded the following pattern from lowest to highest: CD+KW+PW>CD+PW >CD+CL>KW+CL> CD+KW+CL>KW+PW (Table 3). It is suggested that KW+PW followed by CD+KW+CL waste was found highly influential food material for supporting maximum coon production in earthworms (Table 3).

Loh *et al*., (2004) reported higher cocoon production and weight gain by *E. fetida* in cattle waste along with goat waste. However, the maximum number of hatchlings was produced after 12 weeks in CD+KW+CL waste (93.2 %) and the minimum in CD+KW+PW waste (64.6 %). The maximum number of cocoon of *E. fetida* was found in T_4 (6.92) at 90 days after stocking which was higher than all other treatments followed by: T_1 (10.26 %), T_2 (20.08%), T_3(8.67%), T_5(0.43 %) and T_6 (24.42 %). Significant difference was recorded in the cocoon production of *E. fetida* among $T_{4,}T_2$ and $T_{6,}$whereas non-significant difference was observed between T_3 and T_5 ($P< 0.05$) under similar management practices. The cocoon production patterns under all the feeding stuff is illustrated in Figure 2.

In conclusion, utilization of biodegradable wastes blended with cow dung for vermicomposting using earthworm, *Eisenia fetida* can be a feasible eco-friendly technology for the management of emerging wastes menace. The findings of this study will contribute to the knowledge of nitrogen transformations that occur during vermicomposting process. This can help to develop an alternative economic strategy for nutrient recovery from different waste sources and also in maintaining the balance of the ecological environment.

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