EFFECT OF FEEDING MATERIALS ON YIELD, QUALITY OF VERMICOMPOST, MULTIPLICATION AND REPRODUCTION OF EISENIA FOETIDA

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ABSTRACT
The most promising earthworm species used for vermicomposting is Eisenia foetida for their tolerance in wide range of moisture levels and temperatures. The earthworms (E. foetida) were grown in cow dung wastes and cow dung wastes supplemented with plant materials of banana pseudo-stem, leaf litter and sawdust respectively. The earthworm population, cocoon number, vermicompost amount, macronutrient contents (N, P, and K), microbial population, pH and electrical conductivity were examined to assess the effect of animal waste (cow dung) and plant material mix on multiplication, reproduction, yield and vermicompost qualities of E. foetida. The multiplication and reproduction were achieved highest in the mixture of cow dung and sawdust vermicompost and lowest in the mixture of cow dung and banana pseudo-stem vermicompost. But the amount of vermicompost was found to be highest in cow dung as sole source of feeding material as compared to the cow dung mixed with plant materials. High quality vermicompost containing nitrogen, phosphorus, potassium and greater microbial population was produced from the mixture of cow dung and banana pseudostem vermicompost as compared to other mixtures. All these vermicomposts showed acceptable level of pH and electrical conductivity values.

Keywords: Vermicompost, Cocoon, Mineralization, Gut, Microbial

INTRODUCTION
Introduction of green revolution in agriculture in South Asia persuaded Nepal to use agrochemicals. Although, agricultural productivity has increased in Nepal, indiscriminate use of agrochemicals has also resulted in air, water and soil pollutions, and losses in agricultural productivity and contributed to health hazards [2, 4, 23, 24]. Since, Nepal does not produce any chemical fertilizers, demand are being met through formal and informal import [27]. The price of chemical fertilizer is also increasing yearly [3]. The scientific community is desperately looking for an economically viable, socially safe & environmentally sustainable alternative to the agrochemicals for crop production. In recent years, there is a greater interest in the utilization of organic amendments such as vermicompost for crop production [12]. A revolution is unfolding in vermiculture studies for vermicomposting of diverse organic wastes
(plant and animal origin) by waste eater earthworms (epigeic in nature) into a nutritive “organic fertilizer” i.e. vermicompost, rich in humus, macronutrients (nitrogen, phosphorus and potassium), micronutrients, beneficial soil microflora, actinomycetes, and plant growth regulators, and using them as an alternative to agrochemicals [1].

In Southeast Asia, *E. foetida* (Savigny 1826) is mostly used because these species being epigeic (Bouché 1977) display characteristics like high rates of processing of organic wastes, high reproductive rates and tolerant to wide range of environmental factors [9, 13]. The multiplication, reproduction, vermicompost yield and quality have been suggested to be stimulated by quality and intake of feed by earthworms [5]. Also mixture of cow manure with plant residues like saw dust, banana pseudostem and leaf litter respectively as feeding materials for earthworms showed different responses regarding multiplication and reproduction of *E. foetida* [8, 18, 19, 20, 30]. Moreover yield and qualities of produced vermicomposts were also differed [9, 15].

In this part of research we have focused to investigate the effect of cow dung mixed with plant materials like banana pseudostem, leaf litter and saw dust respectively on yield, quality of vermicompost, multiplication (earthworm number) and reproduction (cocoon number) of *E. foetida*.

**MATERIALS AND METHODOLOGY**

**Collection of earthworm samples**
*Eisenia foetida* were brought from Praramva Biotech Pvt. Ltd., Nepal and were raised in plastic worm bin until the required amounts of healthy earthworms were achieved for the experiment.

**Preparation of different feeding materials**

1. **Cow dung (CD) processing:**
   Dry cow dung was collected from nearby cow farm and ground to powdery form and was then packed in sack.

2. **Banana pseudostem (BP) processing:**
   Banana pseudostem was collected from one of the farmers nearby Kathmandu University (KU), sun-dried for 3 days and was cut into small pieces of size 1 inch.

3. **Leaf litter (LL) processing:**
   Leaf litter was collected from KU premises and was cut into small pieces of size 1 inch.
4. Saw dust (SD) processing:
Saw dust was collected from furniture nearby KU.

Experimental Setup
Plastic bins of internal diameter 26cm and height 9cm were used for vermicomposting. Cardboard paper soaked with water was used as bedding (B) material for earthworms. 10g of bedding material was placed at the bottom of all the bins. Four setups (treatments) containing different feeding materials (Cow dung (CD) alone and CD supplemented with BP, LL and SD respectively) with three replications of each were prepared according to following ratios.

- Treatment 1 (CD): 500g of Cow dung only
- Treatment 2 (CD+BP): 250g of CD + 250g of BP
- Treatment 3 (CD+LL): 250g of CD + 250g of LL
- Treatment 4 (CD+SD): 250g of CD + 250g of SD

All these setups were left for pre-composting for 21 days to avoid the mortality of worms as some volatile gases are toxic to worms. Also, partial decomposition of feeding materials occurs in this stage by microbial action. Hence, partially decomposed feeding materials are suitable for earthworms. 84 earthworms each weighing 1g (according to the stocking density of 1600g-worms/m²) were added to each treatment after 21 days of pre-composting. All these bins were covered with black muslin cloth from the top and were kept in closed house at 24-32°C. The moisture content was maintained to 65-70% with periodic sprinkling of an adequate quantity of water. No additional feed was added at any stage during the study periods. After 120 days of vermicomposting, Earthworm and cocoon number in the finished vermicomposts were counted by hand sorting and vermicompost yield was calculated using weighing balance. The produced vermicomposts from different feeding materials were then packed in polythene bags with loosely tightened and stored at room temperature for 45 days. After 45 days of storage, the chemical and microbial parameters of these vermicomposts were analyzed as the quality indicators.

Analysis of chemical and microbial parameters

- pH (pH probe method)
- Electrical conductivity [EC] (Conductivity method)
- Nitrogen (Kjeldahl method)
- Available Phosphorus (Modified Olsen’s method)
- Available Potassium (Ammonium acetate method)
- Bacterial and Fungal population (Serial dilution and plate count method)
Data analysis

Vermicompost samples were analyzed using the standard procedures in the laboratory at Kathmandu University. All data are the means of triplicates. The data obtained were analyzed and processed using one-way ANOVA (Analysis of Variance) with the significance level of 0.05 in MS-Excel Office Package 2010.

RESULTS AND DISCUSSIONS

Earthworm multiplication, reproduction and vermicompost yield

ANOVA test showed the significant difference (P<0.05) among the different treatments that influences these parameters (Table 1). The earthworm number (915±62) and cocoon number (823±39) were found to be highest in the vermicompost prepared from CD+SD as feeding material. Saw dust is an ideal bedding material for growth and reproduction of epigeic earthworms [30]. In a similar study using epigeic earthworm *Eudrilus eugeniae*, high number of worms and cocoon were observed in cow dung and saw dust mixture (1:1 and 1:1.5) than the pure cow dung [8]. Also, higher numbers of earthworms were observed in the feeding mixture of cow dung and leaf litter as compared to the cow dung as a sole feeding material. This might be due to the presence of leaf litter in the mixture which favored earthworm multiplication. In the similar study, maximum increase in population of *E. foetida* was observed in tree leaves as a sole feeding material than cow dung only [20]. The number of worm population is related to cocoon production and vice versa [19]. The lowest number of worm population (159±26) were recorded in CD+BP vermicompost as the cocoon number was less as compared to cocoon produced in cow dung only. This result is in agreement with the other findings [18].

Yield was significantly higher in CD (432.33±7.50 g) as compared to other treatments (Table 1). Cow dung as bedding material might contain easily metabolizable organic matter and low concentration of growth retarding substances that favored the worm’s growth in the substrate leading to more yield of compost [15]. Low yield (170±10g) was obtained in BP+CD vermicompost. This might be due to high moisture content in banana pseudostem [21].
Table 1. Mean ± SD Earthworm number, cocoon number and vermicompost yield (in gm.) in different feeding materials

| Type of feeding materials | Total Earthworm number | Total Cocoon Number | Yield (in gm.) |
|---------------------------|------------------------|---------------------|---------------|
| T1: Cow dung (CD)         | 246±33                 | 476±33              | 432.33±7.50   |
| T2: Cow dung + Banana pseudostem (CD+BP) | 159±26               | 225±21              | 170±10        |
| T3: Cow dung + Leaf litter (CD+LL) | 714±15               | 777±47              | 290±8.66      |
| T4: Cow dung + Saw dust (CD+SD) | 915±62               | 823±39              | 325.66±10.06  |

*All data are mean of triplicates.

Quality of Vermicompost

1. pH and Electrical Conductivity (EC)

A significance difference (P<0.05) was observed among different treatments such pH and electrical conductivity (Table 2). In all the treatments, pH was around neutrality value (pH=7), the highest pH was recorded in CD+BP (8.11±0.26). The pH value of vermicomposts around neutral might have been attributed by the secretion of NH$_4^+$ ions during the vermicomposting process that reduce the pool of H$^+$ ions and the catalytic fixation of CO$_2$ as CaCO$_3$ by carbonic anhydrase in the earthworms’ gut [22]. Highly acidic soil is harmful for growth and development of most of the plants [14]. These near-neutral and slightly alkaline pH values of these vermicomposts show great potential to use as soil amendment for crop production [25].

The EC reflects the salinity of any material and is a good indicator of the applicability of vermicompost for crop production as different plants show different responses to EC values. The EC was in the range of 2.96–4.46 millisiemens per centimeter (mS/cm) for different vermicomposts (Table 2). This higher EC in CD+BP vermicompost than CD vermicompost might have been due to release of different mineral ions, such as phosphate, ammonium, potassium etc. in greater amount from the initial feeding material during the vermicomposting process [31].
Table 2. Mean ± SD of pH and Electrical Conductivity (EC) of vermicomposts prepared from different feeding materials

| Type of Vermicomposts | pH      | EC (mS/cm) |
|-----------------------|---------|------------|
| T1: Cow dung (CD)     | 7.40±0.07 | 3.78±0.04  |
| T2: Cow dung + Banana pseudostem (CD+BP) | 8.24±0.08 | 4.46±0.11  |
| T3: Cow dung + Leaf litter (CD+LL) | 6.37±0.10 | 3.54±0.08  |
| T4: Cow dung + Saw dust (CD+SD) | 6.75±0.07 | 2.96±0.15  |

*All data are mean of triplicates

2. Nitrogen, Phosphorus and Potassium

A significant difference in all these vermicomposts was observed for nitrogen (N) and potassium (K). However, phosphorus content did not show significant difference (Table 3). Activities of endosymbiotic microbes and gut enzymes of earthworm aid in transformation of ingested organic matters into vermicompost constituting essential macronutrients such N, P, K in plant available forms [17, 32]. Total nitrogen content in vermicomposts can range quite widely from 0.1% to 4% or more [25]. CD+BP vermicompost showed highest nitrogen (0.43%) content among other vermicomposts. This might be due to the high nitrification rate in which ammonium ions are converted into nitrates [9]. Earthworm processed waste material contains higher concentration of exchangeable K due to enhanced microbial activity during the vermicomposting process, which consequently enhances the rate of mineralization [29]. Highest K content (0.63%) was recorded in CD+BP vermicompost among other vermicompost which might be due to the highest microbial activity that favored mineralization. It was reported that available phosphorus in the vermicomposts to be 0.1%-0.3% [16]. Available phosphorus in all these vermicomposts was found to be 0.3% (Table 3).

Table 3. Mean ± SD Macronutrients (%): Nitrogen, Phosphorus, Potassium in vermicomposts prepared from different feeding materials

| Type of Vermicomposts | Nitrogen (N) | Phosphorus (P) | Potassium (K) |
|-----------------------|--------------|----------------|---------------|
| T1: Cow dung (CD)     | 0.239±0.067  | 0.300±0.005    | 0.492±0.017   |
| T2: Cow dung + Banana pseudostem (CD+BP) | 0.439±0.091 | 0.303±0.003   | 0.638±0.113   |
| T3: Cow dung + Leaf litter (CD+LL) | 0.206±0.018 | 0.302±0.008   | 0.506±0.019   |
| T4: Cow dung + Saw dust (CD+SD) | 0.196±0.018 | 0.305±0.017   | 0.424±0.050   |

*All data are mean of triplicates*
3. Microbial Population
Earthworm enhances microbial diversity and enzymatic activities of ingested microbes that are existent in the initial feed, through gut associated processes [10]. As a result, vermicompost consisted of greater pool of soil friendly microbial population [6, 7]. It was suggested that there is dependence of the size of the microbial population in casts on the quality and types of the feeding materials [11]. The part of study also showed high number of microbial population in all the produced vermicomposts (Table 4). Moreover, the significantly (P<0.05) highest microbial count was observed in CD+BP vermicompost (Figure 1 and 2). The reason might be the excess organic content and ammonia in the vermicompost that lead to more microbial population [26, 28]. However, the fungi count was found to be less than that of the bacterial count. The reason behind this might be the earthworms’ necessities to feed on microbes, particularly fungi for their protein/nitrogen requirement [20].

Table 4. Microbial Population in vermicomposts prepared from different feeding materials

| Type of Vermicomposts | Microbial Population (cfu/ml) |
|-----------------------|-------------------------------|
|                       | Bacterial                     | Fungal                      |
| T1: CD                | 1.03x10^8                     | 6.69x10^4                  |
| T2: CD+BP             | 3.47x10^8                     | 1.93x10^6                  |
| T3: CD+LL             | 4.58x10^7                     | 6.92x10^5                  |
| T4: CD+SD             | 3.63x10^6                     | 3.84x10^4                  |

*All data are mean of triplicates.

**Figure 1.** Bacterial count of CD+BP vermicompost (10^2 dilution) in nutrient agar

**Figure 2.** Fungal count of CD+BP vermicompost (10^2 dilution) in PDA agar
CONCLUSIONS

*E. foetida* showed a great potential of converting the animal waste and plant material mixture into a valuable product, vermicompost. Cow dung mixed with sawdust and cow dung mixed with leaf litter, both, are recommended as suitable feeding material for rearing *E. foetida* and producing vermicompost. However, high yield of vermicompost can be achieved if cow dung is used as a sole feeding material for *E. foetida*. This study revealed that good quality vermicompost containing high amount of nitrogen, phosphorus, potassium and high number of microbial population can be prepared, using *E. foetida*, from the cow dung and banana pseudostem mixture in the equal proportion. Moreover, all these vermicomposts showed acceptable level of pH and electrical conductivity values which indicates the suitability of vermicomposts to use as soil amendment for crop production. This study opens the scope of vermicompost as a good organic fertilizer in organic farming in the countries like Nepal for which a continuous and detailed study must be continued in coming days.

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