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Study the impacts of bone meal - Cow dung feeding system on *Lampito mauritii* earthworm

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Abstract
In the present study, the bone meal blended cow dung was developed as a feeding material for *Lampito mauritii* earth worm and there impacts were tested based on reproduction cycle. For the studies, initially the bone meal was mixed with cow dung (1:1 ratio) and used for the earth worm feeding. The feeding material impacts were tested up to 150 days based on earth warm biomass, clitellum and cocoon production. The bone meal-cow dung feeding materials showed the maximum biomass value was 1567 ± 0.5 mg and the control showed the biomass value was around 1000 ± 0.7 mg. The Coon production results indicated that the maximum 135 ± 4.1 cocoons were produced in bone meal- cow dung feeding material and the control samples showed the value was around 120 ± 3.4. From these results, the bone meals – cow dung feeding material was highly suitable for *Lampito mauritii* species growth and reproductions. This paper for the first time addresses the bone meal mixed cow dung as a feeding material for *Lampito mauritii* earth worms and it opens up a new possibility for the development of vermi compost production from bone mill wastes.

Keywords: Bone meal, reproduction, growth, cow dung

Introduction
The use of organic fertilizers improved the crop nutrition's, physical, chemical and biological properties of the soil nature, improved the productivity, and yields [1-3]. Past few years, the earth warm based vermicomposting productions and their application play an important role in organic forming [4, 5]. In this technology, the organic materials are biodegraded by earth worms and the product after degradation is known as vermicomposting, and the materials are fine particulate structure with high porous [6, 7]. Previous studies indicated that, the vermicomposting materials showed high-water-holding capability, microbial activity, high nutrition's, it releases the major and minor nutrients very slowly with significant reduction in C/N ratio, and synchronizing with the requirement of plants [8]. For the vermicomposting productions, twenty eight earth warm species are used, and their roles in nature, composting ability, and consumption of feed materials are differing based on species. So it's very important to study the major factors influencing the earth warm growth and their production rates [9]. The bone meal initially used as a feeding material for domestic animals [10] but it was recently banned by European union due to the contamination of animal trans missive spongiform encephalopathies (TSE), and bovine spongiform encephalopathy (BSE)[11]. The elemental composition analysis indicated that the bone meal contained rich amount organic matter, nitrogen, phosphorus, calcium [12] and highly suitable for organic fertilizers. Previously bone meal was utilized as fertilizer along with pig slurry [13], and the soil studies indicated that the available phosphorus level was increased [14]. The green house experiment indicated that, the bone meal is better phosphorus source than commonly used phosphate rock [15].
The purpose of this study was to investigate the growth and reproduction rates of earth worms based on mixed feeding materials up to 150 days. For this study, the *Lampito mauritii* earthworm was used as a model species, the cow dung and bone meal was used as feeding materials. The bone meal was mixed with cow dung at 1:1 ratio, with same moisture conditions. Specially designed concrete pots were used for this study, and this paper for the first time addresses the impacts of bone meal mixed cow dung feeding materials and their effects on vermi composting *Lampito mauritii* earthworm species.

2. Materials and methods

2.1. Preparation of cow dung (CD), bone meal (BM), and earthworm

The bone meal (BM) was collected from a bone crushing industry located at villupuram, tamilnadu, India. The cow dung (CD) was procured from an intensively live stocked farm located at mayiladuthurai, tamil nadu, India. *Lampito mauritii* adult worms were collected from manure pits in and around A.V.C. college of arts and science in mayiladuthurai, and then it was cultured in mass culture tanks containing cow dung medium in the Department of zoology, A.V.C. College of arts and science, mayiladuthurai. For the experiments, the earthworms (adult) were indiscriminately picked from the maintained cultures and each live worm weighed between 0.5 ~ 0.65 g and the study was conducted from 05.03 2015 ~ 05.07.2015.

2.2. Preparation of feeding materials

The feeding materials were prepared with same moisture content (70 −75%), and it was stored separately with suitable identification dataset. The used compositions are (i) control feeding material: – 100% CD (without bone meal) (ii) Test feeding material B: – 50% CD + 50% BM.

2.3. Design of wire mesh attached concrete pots

The concrete pots were constructed with ¾ inch wire mesh, the size of the each pots was 0.25 m and 0.25 m × 0.10 m deep were constructed, the design make multiple holes in the side walls and allow air movements. The reactor design offered low, medium and high stocking density of feeding materials.

2.4. Physico-chemical analysis

Physicochemical analyses of feeding materials were performed based on dry weight. For reproducibility, all the samples were analyzed in triplicate and results were averaged. The pH and electrical conductivity (EC) were determined using a double distilled water suspension of each sample in the ratio of 1:10 (w/v). Total organic carbon was measured by using chromic acid oxidation method. Micro-Kjeldhal method was used for measuring nitrogen [16]. For the estimation of total potassium and total sodium, the samples were initially digested by using diacid mixture and then, the sample was estimated by using flame photometer (Elico, CL 22 D). Spectrophotometric method was used to estimate the available total phosphate, for the estimations, 0.387M ammonium molybdate, 0.25N sulfuric acid, disodium hydrogen phosphate, and sodium sulphide solution were used. And the standard calibration curve, and sample analysis were read at 715 nm. Total heavy metals were determined by atomic absorption spectrophotometer (AAS 6300 Shimadzu, Japan) after digesting the samples with conc. HNO_3 and conc. $HClO_4$ (9:1, v/v).

2.5. Vermicomposting experimental design

The experiments were performed in specially designed concrete pot reactors under controlled environmental conditions. For the reproducibility, all the experiments were conducted in triplicates with control, and the pots were placed in dark room at a temperature 27-30 °C (SD = 0.3). The prepared feeding materials were filled in concrete pots (20 cm diameter and 8 cm in depth) with desired number of earth warms, and the moisture level of all feeding materials in vermi beds were maintained around 70–75%, throughout the study period. After 15 days, fresh medium was added to every container in quantities related to the number of worms in the container.

2.6. Data collections

Growth and cocoon productions were measured once every 7 days in each experimental container. For the data collections, the earthworms and total cocoons were separated from the feeding materials by hand sorting, washed with sterilized distilled water to remove adhering material from their earth worm body, wiped with tissue papers and subsequently weighed [live weight basis]. And then all measured earthworms were returned to the concerned container. Separated cocoon were counted and introduced in separate bedding containing the same material in which their parents were reared. Based on the data collections, the growth (weight / worm) and reproduction rates (cocoon/days) were analyzed by the Student's *t* test using MET LAB software (n=6) with respect feeding materials.

3. Results and discussion

In this study, the *Lampito mauritii* earth worm species were used, and it's red in colour, thread-like structure, soft, with a diameter of 1.7-2.0 mm and highly suitable for vermiculture experiments. Previously *Lampito mauritii* earth worms were used to compost biogas slurry waste, cowdung, wheat straw, leaflitter, sawdust and kitchen waste [17]. The quality of the feeding materials directly affect the survival, growth rate and reproduction potential of the earthworms [18] The physico chemical characterizations of the feeding materials were tested, and it was shown in table 1a and b. Based on that, the feeding stocks contained high concentrations of organic carbons, heavy metals, nutrients. Previous studies [19] indicated that, the mixed feeding materials showed more advantages than single waste earth worm feeding system. The bone meal contained rich amount of protein, nitrogen and increase their potential it was mixed with cow dung. The feeding system offered high porosity, uniform particle size and shape, the texture and maintain the moisture level.

Table 1a: Result of physicochemical analysis of cow dung.

Parameters	Results
Moisture content	70.4 ± 5.21
pH	7.98 ±0.02
EC (dSm^{-1})	1.54 ± 0.03
Ash content (g kg^{-1})	270 ±7.4
TOC (gkg^{-1})	410.2 ± 5.23
OM (%)	70 ±0.89
TKN (gkg^{-1})	8.6 ±0.98
TP (gkg^{-1})	7.4 ±0.13
TK (gkg^{-1})	8.2 ±0.22
C:N ratio	49 ±0.01
C:P ratio	54.9 ±1.43
Total -Cu ($mgkg^{-1}$)	87.6 ± 2.3
Total -Fe ($mgkg^{-1}$)	1975 ± 23
Total -Mn ($mgkg^{-1}$)	114 ± 3.8
Total -Zn (mg kg^{-1})	208 ±3.6

Table 1b: Result of physicochemical analysis of bone meal

Parameters	Results
Moisture %	4 ±1.7
Protein %	48.9 ± 1.9
Calcium %	9.94 ± 1.39
Phosphorus %	4.63 ± 0.55
Selenium mg/kg	0.52 ± 0.07
Sulphur %	0.59 ± 0.06
Crude Fat %	12.2 ±2.1
Iron ($mgkg^{-1}$)	572 ± 17
Copper ($mgkg^{-1}$)	14.2 ± 2.4
Manganese ($mgkg^{-1}$)	22
Zinc ($mgkg^{-1}$)	135 ± 16
Magnesium %	0.19 ± 0.02
Potassium %	0.61 ±0.07
Sodium ($mgkg^{-1}$)	7150 ± 65
Molybdenum ($mgkg^{-1}$)	0.4 ±0.01
Cobalt ($mgkg^{-1}$)	0.4 ±0.2

The developed compost showed several advantages such as (i) the mixed feeding material was more acceptable for earthworms (ii) the mixed feeding materials may enhance the nutritive values, microbial concentrations, and enhance the enzyme productions. For the compost preparations the feeding materials were mixed to gather at different ratio and their moisture content was measured. The moisture results indicated that the value was around 70-75%. The temperature plays a vital role and it strongly influences the growth parameters of the earth worms such as activity, metabolism, growth, respiration and reproduction of earthworms. So in this study, the temperature of the environment was fixed as constant around 30 °C. The feeding materials based growth curves of *Lampito mauritii* earth warms were studied using scale and the results were shown in Fig. 1.

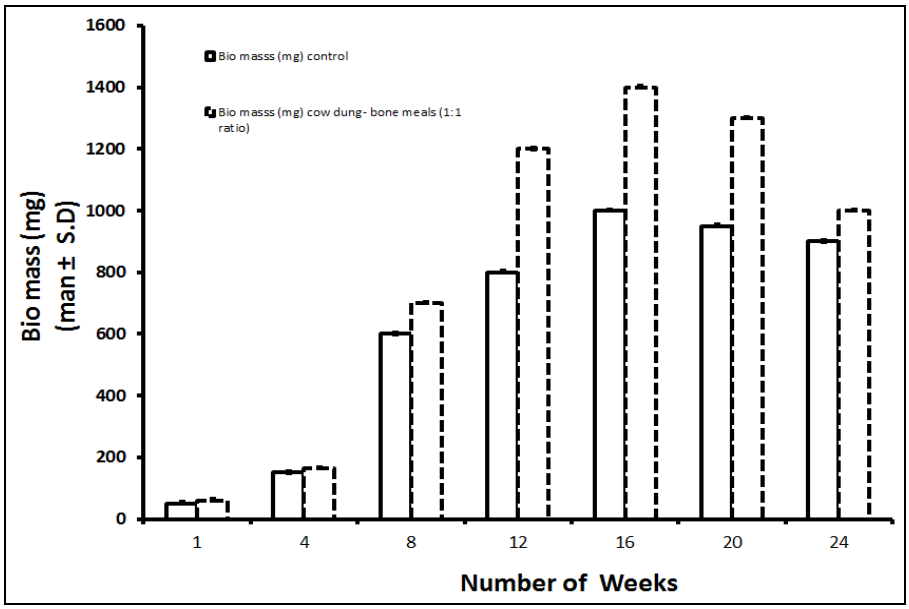

Fig 1: Results of growth rate (mean ± SD; n=6, 150 days) in control and bone meal-cow dung feeding materials.

From the results, the first 21 days the growth rate was almost similar in both feeding materials and there was no significant difference between the samples, and the maximum biomass gain was 81 ± 0.66 mg. After 21 days, the earth warm grew rapidly and the growth rate reached maximum after 16 weeks and the individual biomass was varied based on the feeding materials. In the bone meals mixed feeding materials showed the maximum mean individual biomass was obtained 1567 ± 0.5 mg in 98 days. On the other hand the control sample showed the maximum individual biomass value was 1007 ±0.7 mg and it was recorded in 105 days. From the results, the developed feeding material highly influences the biomass rate

(2 fold) and also produces healthy earth worms. The sexual maturity was measured based on clitellum. Both the feeding materials, the clitellum development was appeared between 25~28 day and the fully developed clitellums was founded between 34~36 day. The time required to reach maturity did not differ much between feeding systems. After 45 days, the cocoon production was started in bone meal-cow dung feeding materials and the control sample. The maximum number of coccons was counted after 150 days, and it was shown in table 2. Based on that, maximum 135 ± 4.1 cocoons were counted in bone meal-cow dung feeding material *Lampito mauritii* showed better reproduction rates in bone meal-cow dung feeding system. The sexual maturity and onset of reproduction are differing between the feeding materials due to the biochemical quality of the feeds. The Bone meal-cow dung mixing combinations provide sufficient amount of easily metabolizable organic matter and non-assimilated carbohydrates, favour growth and reproduction of earthworms. The results also indicated that the nitrogen amount in feeding material play an important role for cocoon production [20-22] and it conclude the feeding system containing appropriate ratio of bulking materials. Previously several reports [23] indicated that the chemical environment, ambient climatic conditions and feedstock directly affect the earthworm population in vermibeds. So the mortality rate in feeding materials were tested, and the results indicated that there is no mortality in the feed mixture, and this may be due to pre-composting of the feeding materials and it may help the worm to handle the waste. Worms do not normally like citrus and acidic waste and, therefore, these wastes are normally excluded from feeding systems [24]. The pre composting process helps to eliminate the toxic gases from the feed stocks.

Table 2: Results of cocoon (mean ± SD; n=6, 150 days) production in control and bone meal-cow dung feeding materials.

Feeding systems	Cocoon production started (days)	Total number of Cocoon after 150 days
Control sample	45	120 ±3.4
Bone meal-cow dung (1:1) feeding material	45	135 ±3.5

4. Conclusion

In conclusion, this report demonstrated that, the mixing of feeding materials directly influences the *Lampito mauritii* earth warm species growth and reproduction rates. The bone meals – cow dung feeding material was more suitable for *Lampito mauritii* earth warm species due to the increased food availability, nutrients and optimum moisture ratio. Based on this study, the bone meal mixed feeding materials opens up a new possibility for the development of vermin compost production from bone mill wastes.

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