PRODUCTION AND EVALUATION OF PROTEINACEOUS EARTHWORM MEAL

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VELLANIKKARA, THRISSUR - 680 656

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THESIS

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2012

DECLARATION

I hereby declare that the thesis entitled **“Production and evaluation of proteinaceous earthworm meal”** is a bonafide record of the research work done by me during the course of research and the thesis has not previously formed the basis for the award to me any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that the thesis entitled **"Production and evaluation of proteinaceous earthworm meal"** is a record of research work done independently by **Miss. Fasila E. K.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Miss. Fasila E. K. (2010-11-127)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Soil Science and Agricultural Chemistry**, agree that the thesis entitled **"Production and evaluation of proteinaceous earthworm meal"** may be submitted by **Miss. Fasila E. K.**, in partial fulfilment of the requirement for the degree.

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Fasila E. K.

Dedicated to my Mother

ABBREVIATIONS

Atmos.	Atmosphere
Ca	Calcium
cm	Centimeter
C/N	Carbon Nitrogen ratio
Cu	Copper
Fe	Iron
FYM	Farm yard manure
Fig	Figure
g	Gram
HCl	Hydrochloric Acid
K	Potassium
KAU	Kerala Agricultural University
kg	Kilogram
l	Litre
m	Metre
m^2	Square metre
m^3	Cubic metre
mg	Milligram
Mg	Magnesium
Mn	Manganese
N	Nitrogen
NH_4^+	Ammonium
OC	Organic Carbon
^0C	Degree Celsius
P	Phosphorus
pH	Hydrogen ion activity
%	Percentage
Std	Standard
S	Sulphur
V	Volume
Zn	Zinc

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Introduction

1. INTRODUCTION

Earthworms are found in all, but the driest and coldest land areas of the world. They include about 3000 species of the suborders Alluroidina, Moniligaotrina and Lumbricina of the subclass Oligochaeta (Annelida: Clitellata) as defined by Jamieson (1978). Most earthworms are inhabitants of soils, including litter layers and above-ground habitats such as animal dung, rubbish heaps, rotting logs on the ground surface, moss and fern covered tree trunks, under the bark of standing trees and in organic material accumulated at the base of epiphytes or in leaf bases of some sub canopy forest trees.

The abrasiveness of coarse textured soils and their susceptibility to drought, as a result of free drainage, influence the species composition and abundance of earthworm population. The clay content of soil is also significant, and in regions of high rainfall or poor drainage soils with high clay content may have no earthworms, because of their susceptibility to become anaerobic when soil water content is high.

Availability of adequate nitrogen appears to be one of the most important factor connecting earthworm population and their distribution, especially in tropical regions, when the nitrogen content of soil is low compared to that of soils of temperate regions. The C/N ratio is a measure of the quality of soil organic matter as an energy source, which is also an important determinant of humus types and is related to soil moisture, pH and many other soil properties.

Vermicomposting is an easy and effective way to recycle agricultural waste, city garbage and kitchen waste, by converting these organic waste materials into nutritious compost by the activities of earthworms. Worm composting yields two economically valuable products, worm cast and worm meal. About 5 kg of fresh earthworms can produce about 1 kg of worm meal. Worm meal is a protein rich meal made from ground earthworm meat. Its protein content is about 46% with 10% fat. It can be used as an ingredient of animal as well as fish feeds.

The potential of earthworms as a food source, and their nutrient and protein content have been reviewed by Sabine (1983). The favoured species for protein production must be *Eisenia foetida* because of its short generating time, extraordinary high productivity and the performance for life in organic matter rich habitats such as compost and dung heaps. The protein and amino acid content of protein meal made from earthworms closely resemble those of meat meal and fish meal which are commonly used as protein sources in feeds prepared for the intensive rearing of livestock. Weight gains of livestock fed by earthworm meal are comparable to those when other generally accepted protein sources are used.

Yaqub (1997) conducted studies on the weight increment in catfish and the best growth performance was recovered by maggot meal followed by earthworm and then only fishmeal. Based on the food conversion ratio, maggot meal was followed by earthworm and then only fishmeal.

Ornamental fish culturing in India has increased at a higher rate, but ornamental fish feed is very costly. Recently with rising cost of imported fish meal, worm meal has been recognized as a potential substrate. Unlike the production of fishmeal which relies on the exploitation of wild fisheries, production of worm meal depends on the recycling of farm waste. It is therefore a more environment friendly product and economically viable production of protein from earthworms is not that much difficult also.

Under these circumstances, the present study on the "*production and evaluation of protienaceous earthworm meal"* was undertaken with the following well defined objectives.

1. To compare the efficiency of different substrates for the mass multiplication of exotic earthworms.
2. To formulate viable techniques for the collection and multiplication of native earthworms.
3. To develop a protocol for the preparation of worm meal using exotic or native earthworms and
4. To evaluate the nutritive content of different worm meal preparations.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Earthworms, the major soil macrofauna

Earthworms are one of the major macrofauna in soil and have been considered as the farmer's friend. They formed a major component of soil system and they had been efficiently ploughing the land for millions of years and assisting the recycling of organic nutrients for efficient growth of plants (Gupta, 2005). Earthworms played a major role in the proper functioning of the soil ecosystem. It acted as a scavenger and helps in recycling of dead and decayed plant materials by feeding on them. Earthworms were the most significant soil macroorganism in humid temperate and tropical systems, especially in relation to their effect on the physical and chemical conditions of soils. Compared to the soil itself, the worm casts were found higher in bacteria, organic matter and available plant nutrients. Vleeschauwer and Lal (1981) compared the physical and chemical properties of the surface (0-10cm) soils along a toposequence developed on basement complex rocks in Southwestern Nigeria and the findings were summarised as follows;

Table 2.1 Comparative characteristics of earthworm casts and soils

Characteristic	**Earthworm casts**	**Soils** (on an average)
Silt and clay (%)	38.80	22.20
Bulk density (Mg m^{-3})	1.11	1.28
Structural stability (number of raindrops required to destroy structural aggregate)	849.00	65.00
Cation exchange capacity ($cmol(p^{+})\ kg^{-1}$)	13.80	3.50
Exchangeable Ca ($cmol(p^{+})\ kg^{-1}$)	8.90	2.00
Exchangeable K ($cmol(p^{+})\ kg^{-1}$)	0.60	0.20
Soluble P (mg kg^{-1})	17.80	6.10
Total N (%)	0.33	0.12

Three types of earthworms were described below; (Bouche, 1977; Lavelle, 1981; Lee, 1985).

Anecic (Greek for "out of the earth") – these were burrowing worms that come to the surface at night to drag food down into their permanent burrows deep within the mineral layers of the soil. Example: Canadian Night crawler.

Endogeic (Greek for "within the earth") – these were also burrowing worms but their burrows are typically more shallow and they feed on the organic matter already in the soil, so they come to the surface only rarely.

Epigeic (Greek for "upon the earth") – these worms lived in the surface litter and feed on decaying organic matter. They were phytophagous and generally have no effect on the soil structure, as they cannot dig into the soil. These "decomposers" are the type of worms used in vermicomposting. Examples: *Eudrilus euginiae*, *Eisenia foetida*, *Perionyx excavatus*.

2.1.1 Taxonomy

Kingdom	Animalia
Phylum	Annelida
Class	Clitellata
Subclass	Oligochaeta
Order	Haplotaxida

There were about 3000 species of earthworms, grouped into five families, Lumbricidae, Moniligastridae, Megascolecidae, Glossoscolecidae and Eudrilidae are found to be distributed all over the world (Edward and Lofty, 1972).

2.1.2 Biology

Earthworm resembled a cylindrical tube with average length of about 25cm. Its body is divided into various segments in which vital organs are present in particular segments. The skin is covered by a moist mucous layer that served the main purpose of respiration through exchange of air. Earthworm did not have any locomotion organ, as it moved by means of muscle contraction and relaxation and they have well-developed nervous, circulatory, digestive, excretory, muscular, and reproductive systems. Earthworm is a hermaphrodite, both male and female sex organs being present in the same body. However reproduction takes place via cross fertilization. Eggs are enclosed in egg case or cocoon, juvenile earthworm resembles an adult worm, except the lack of sex organs. It attains sexual maturity within 2-3 months after hatching (Sherman, 2003).

Table 2.2 The different systems of earthworms with their peculiarities (Lee, 1985).

Earthworm system	**Peculiarities**
Muscular – skeletal	No limbs and they have two sets of muscles; one makes them long and thin and the other one makes them fat.
Digestion	It eats dirt, digesting the plant and animal matters in the dirt and then eliminating the rest. It has an oesophagus for the food to go down, a crop to store the food in, a gizzard that grinds the food down, intestines for the food to pass through and take out nutrients and an anus for the food to come out.
Nervous	A nervous system with a simple brain and nerve cord
Circulation	A blood and blood vessels with multiple(5) hearts
Respiration	No respiratory organ. It takes in oxygen directly through its skin and gives off carbon dioxide. Its skin is always moist
Reproduction	Both sperm and eggs within its body and reproduces sexually. The eggs must be fertilized by the sperm of another worm. It lays a batch of eggs at one time. They do not spend time raising their young, once they are hatched
Excretion	It gets rid of its wastes through tubes called nephridia that lead to pores that allow the wastes out
Symmetry	Bilateral symmetry
Colouration	Earthworm generally has earthtones such as brown, tan, etc. it can be up to eight feet in length

Comparative studies were performed to evaluate composting potential, biomass growth and biology of different worms used for composting. The details were outlined as follows.

Table 2.3 Biology of different earthworm species suitable for composting (Kale, 1998)

Particulars	***Eisenia foetida***	***Eudrilus euginiae***	***Perionyx excavates***
Duration of life cycle (days)	+/- (70)	+/- (60)	+/- (46)
Growth rate (mg/worm/day)	7	12	3.5
Maximum body mass (mg)	1500	4294	600
Maturation attained at age (days)	+/- (50)	+/- (40)	+/- (21)
Start of cocoon production (days)	+/- (55)	+/- (46)	+/- (24)
Cocoon production (per worm/day)	0.35	1.3	1.1
Incubation period (days)	+/- (23)	+/- (16.6)	+/- (18.7)
Mean number of hatchings (per cocoon)	2.7	2.7	1.1
Number of hatchings from one cocoon	1-9	1-5	1-3

2.1.3 Life activities

Earthworms can tolerate a temperature range of 25-35°C. They generally have short regeneration time. They are omnivorous but they mostly derived nutrition from dead organic matter. The quantity of food taken by a worm varied from 100-300 mg/g body weight per day. Earthworms consume soil organic matter and convert it into humus within a short period of time and thereby increase the soil fertility. They induce pores in soil by their burrowing and casting activity (Edwards and Bohlen, 1996).

2.1.4 Food habits

The kind and amount of food materials available influenced the size of earthworm population, species diversity, growth rate and cocoon production. Activated sludge, manure and nitrogen rich diets helped in rapid growth and more cocoon production in earthworms.

Table 2.4 Consumption rate of different species of compost worms (Sushama *et al.*, 2009)

Earthworm species	**Consumption rate (mg substrate/g fresh weight of worms/day)**	**Substrate**
Eisenia foetida	10 – 5000	Activated sludge
Eudrilus euginiae	2000 – 5000	Activated sludge with dead leaves
Perionyx excavatus	700 – 2800	Nitrogen rich materials

2.2 Native Vs Compost worms

The percentage of organic carbon of the culture bedding material declined up to 105 days with *Eisenia foetida* and 120 with *Lumbricus mauritii*. The percentage of nitrogen, phosphorous and potassium increased as a function of the vermicomposting period and cocoon production was higher for *Eisenia foetida* than *Lumbricus mauritii*. The net reproductive rate was 9 per month in the case of *Eisenia foetida* and 1 per month for *Lumbricus mauritii*. (Tripathi and Bhardwaj, 2004). Exotic earthworms (*Eudrilus eugeniae*) were found to degrade the coffee pulp faster (112 days) as compared to the native worms (*Perionyx ceylanesis*) (165 days) and the vermicomposting efficiency (77.9%) and vermicompost yield (389 kg) were found to be significantly higher with native worms. The multiplication rate of earthworms

(280%) and worm yield (3.78 kg) were significantly higher with the exotic earthworms (Raphael and Velmourougan, 2010).

Table 2.5 Niche diversification in different species of earthworms (Sushama *et al*., 2009)

Earthworm species	**Weight of adult worm (g)**	**Temperature tolerance (^{o}C)**	**Moisture tolerance (%)**	**Feeding niche and vertical distribution**	**Active phase**	**Casting activity**
E. euginiae	1.5 – 2.5	18 – 35	20 – 40	Epigeic, surface living in organic matter	Throughout the year, No diapauses	Non-burrower, surface casting, loose, granular
E. foetida	0.3 – 0.7	15 – 30	20 – 40	Epigeic, living in organic matter	Throughout the year, No diapauses	Non-burrower, surface casting, loose, granular
P. excavatus	0.8 – 1.2	8 – 30	30 – 50	Epigeic, living in organic matter	Throughout the year, No diapauses	Non-burrower, surface casting, loose, granular

2.3 Collection and preservation of earthworms

Hand sorting : Soil samples of prescribed dimensions were collected either by spade or plastic or metal core and earthworms and cocoons were sorted out from it by hand.

Washing and sieving : Soil washing and sieving might be conducted with or without previous hand sorting of samples. Simplest methods involved washing soil through sieves using jets of water from a hose. Selection of suitable sieve sizes ensured collection of small earthworms and cocoons.

Use of chemical repellents: The application of solutions of irritant chemicals to the soil induced earthworms to leave. The chemicals included mercuric chloride, potassium permanganate and formaldehyde.

Electrical methods: Some earthworms would come into soil surface if an electrode was pushed into the soil and an alternative current was discharged. The volume of soil affected by current flow was dependent on moisture, electrolyte content of soil water and temperature.

Flotation: This method involved initial hand sorting or washing and sieving of soil samples and then stirring them in solutions with suitable specific gravities such that plant materials and earthworms get floated in the sieves. Earthworms could be handpicked from sieves or separated from plant material by further differential floatation.

Heat extraction: Extracting earthworms from soil samples could be based on repulsion of animals by a heat source. Soil samples were partly immersed in water beneath a bank of light bulbs. Earthworms would leave the heated soil and are collected by hand from water.

Several narcotizing solutions are effective for preservation of earthworms and 5-10% ethyl alcohol or 1% propylene phenoxetol are amongst most convenient. The alcohol should be changed periodically, as the specimens dilute the solution with water from their bodies, and otherwise become macerated and unidentifiable (Lee, 1985)

2.4 Rearing and mass multiplication of earthworms

Earthworms were cultured on animal dung, poultry dropping and vegetable wastes. Culturing can be done in cement tanks, wooden boxes or plastic tubes of 1 x 1 x 1m size. Culture bed or worm bed can be prepared by placing either saw dust or husk or coir waste or sugar cane trash in the bottom of the container. A layer of fine sand should be spread over the culture bed followed by a layer of garden soil. All layers should be moistened with water. Dung of domestic animals such as cattle, sheep, horse, pig or poultry droppings mixed with kitchen wastes form an ideal feed. Best results are obtained if dung and wheat or rice bran + gram bran + vegetable waste or leaf moulds are mixed in 10:1:1 proportion (Sushama *et al*., 2009).

By using *Azolla pinnata* as a substrate for vermicomposting by *Eisenia foetida*, the conversion rate was 17.91±0.30%, 43.46±0.67%, 83.15±0.53% and 100% for first, second, third and fourth fortnight respectively. The worm cast showed increased level of organic nitrogen, total phosphorus, calcium, magnesium, sodium and potassium (Ishtiyaq and Khan, 2010).

2.5 Bioconversion of important substrates used for composting

2.5.1 Animal manures

Chemical composition of animal waste was influenced by feed of animal, bedding material used and the way waste was collected, stored and handled before utilization. Cattle manure was the most suitable of all animal wastes for earthworm biomass increase and was a suitable substrate for vermicomposting (Kemppainnen, 1989). The total carbon of different organic enrichment materials was in the range of 23.01% in rabbit manure to 32.5% in cowdung. The content of nitrogen was highest in goat manure (1.02%) and lowest in rabbit manure (0.27%). The pig manure registered highest phosphorus content (3.30%) and lowest in cowdung (0.43%). Rabbit manure was highest in potassium content (1.60%) and pig manure (0.40%) the lowest (Bindhu, 2010).

2.5.2 Silkworm waste

Silk worm pupae and moth residues were used for bioconversion to make compost. Pupae was one of the potential nutrient organic residue, which on recycling yield valuable and nutrient rich product known as compost. The pupal waste was found to be high in N (8.3%) and P (1.03%). It also contained 0.95% K, 0.58% Ca, 0.21% Mg and rich in micronutrients mainly Zn and Mn (Heenkende, 2008). The effect of silkworm pupae in growth performance, egg production performance and profitability almost linearly increased up to 6% dietary levels of Rhode Island Red and it is demonstrated that the cheaper silkworm pupae was an excellent substitute for costly protein concentrate leading to increase the profitability (Khatun *et al*., 2005).

2.5.3 Azolla

The experimental results revealed that the combination of *Sesbania rostrata* intercropping and *Azolla microphylla* as dual cropping in rice alongwith 50% inorganic nitrogen application both for kharif and rabi seasons followed by summer residual greengram increased the productivity, soil fertility and profitability of wet seeded rice-rice-greengram cropping system (Rajarathinam *et al*., 2007). The yield attributes like number and weight of panicles, number of spikelets, number of filled grains produced were increased in *Azolla*-treated rice plants as compared to the plants received with the recommended dose (RD) of chemical fertilizers and grain protein was increased by 5 to 7% and 29% in *Azolla* + 50/N + full P and K as compared to that of RD of chemical nutrients and control (only FYM) plants respectively (Tripathy *et al*., 2009). Combination of *Azolla* (1000 kg/ha) and 90 kg N/ha exhibited highest grain yield of 58 q/ha with an increase of 85.30% over the uninoculated control (31.3 q/ha) and consecutive application of *Azolla* for two years improved organic carbon (%) and available N (kg/ha) of soil (Ara *et al*., 2008). According to Paudel (2012) mixing of cow dung and azolla in a proportion of 1:0.5 produced highest volume of gas (0.29 m^3

kg^{-1} TS) in a biogas plant within 20 days of Hydraulic Retention Time (HRT) and the proportion of 1:1 favored in terms of N (3.44 per cent) content in biogas slurry followed by 3.23 per cent in 1:0.75 ratio. Addition of azolla increased pH from 7.1 to 7.8 but decreased the total solids of slurry from 5.40 per cent in cow dung alone to 2.68 per cent in the ratio of 1:1.

Azolla meal contained (%DM) 21.4 crude protein, 12.7 crude fibre, 2.7 ether extract, 16.2 ash and 47.0 carbohydrate and a gross energy value of 2039 kcal kg^{-1} was also obtained. The concentrations of calcium, phosphorus, potassium and magnesium were 1.16, 1.29, 1.25 and 0.25%, respectively, while those of sodium, manganese, iron, copper and zinc were 23.79, 174.42, 755.73, 16.74 and 87.59 mg kg^{-1}, respectively. The chemical score index showed the potential of azolla meal as a good source of protein. Leucine, lysine, arginine and valine were the predominant essential amino acids while tryptophan and the sulphur-containing amino acids were deficient (Alaladi and Lyayi, 2006).

2.5.4 Banana pseudostem

Banana pseudostems and leaves were cut into pieces and mixed with cattle dung at different proportions to raise earthworms(*Eisenia foetida*) for maximum production of organic fertilizer and high protein animal feed. The result of this study indicated that the mixture with 80% banana pseudostems and leaves produced higher number of the earthworms and that the organic fertilizer contained the highest level of nitrogen and the mixture with 90% of banana pseudostems and leaves produced a slightly fewer number of earthworms than the 80% mixture, but with no remarkable difference in the worm's weight, and consumed more banana pseudostems and leaves,and the resultant organic fertilizer contained higher organic matter (Shaoming and Qiquan, 2008).

2.5.5 Other wastes

Vermicomposts prepared from different sources of green leaf materials were differed in their nutrient status (Raghavendra, 1998). Substrate combination of banana pseudostem: glyricidia leaves: coconut leaves : cowdung in ratio 2:2:2:1 registered the least C:N ratio of 12.25 which attained the compost maturity within 47 days and was identified as the best substrate controlled environment for vermicompost production. Banana pseudostem favoured the flourishment of microbial and higher worm population (Thankamony, 2005). The physicochemical analysis of the vermicompost produced by native earthworm (*Lampito mauritti*) from the leaf litter wastes of three different plants namely mango, guava and custard apple resulted in a substantial increase in the nutrient composition of the compost produced (Sumathi and Isaiarasu, 2008). In vegetable-market solid waste vermicomposting caused a decrease in organic C (12.7–28%) and C:N ratio (42.4–57.8), while increase in total N (50.6–75.8%), available P (42.5–110.4%), and exchangeable K (36.0–78.4%) contents. This concluded that vermicomposting could be an efficient technology to convert negligible vegetable-market solid wastes into nutrient-rich biofertilizer if mixed with bulking materials in appropriate ratios. (Suthar, S., 2008). Rate of reproduction in rubber leaf litter diet for both *Eudrilus eugeniae* (1.4 young worm^{-1} week^{-1}) and *Eisenia fetida* (1.3 young worm^{-1} week^{-1}) was significantly higher than that in *Perionyx excavatus* (0.2 young worm^{-1} week^{-1}) over a period of 62 days and it appeared that the suitability of rubber leaf litters as a vermiculture substrate for the species was in the descending order: *Eudrilus eugeniae* > *Eisenia foetida* > *Perionyx excavatus*. (Chaudhuri *et al*., 2003).

As per the studies of Preetha (2003) the analysis of alkaloid rich oushadi wastes with aromatic odour revealed that it contains the nutrients N, P and K as 2.03, 0.33 and 0.85%, respectively. Biochemical composition of the same included crude protein (12.67%), crude fibre (33.4%), crude lipid (6.3%), cellulose (35.7%) and lignin (37.9%).

2.6 Vermicomposting of agro wastes

Earthworm action was shown to enhance natural biodegradation and decomposition of wastes (60–80%) under optimum conditions, thus significantly reducing the composting time by several weeks. Within 5 to 6 weeks, 95–100 per cent degradation of all cellulosic materials was achieved. Even hard fruit and egg shells and bones could be degraded, although these might take longer time (Sinha *et al*., 2002). Vermicomposting is an ecofriendly technology and has a tremendous scope in the recycling of sericultural residue. Proper utilization of sericulture waste as a raw material for vermicomposting served as an organic manure which helps in improvement of soil health and nutrient availability to plants with improved crop quality (Venugopal *et al*., 2010). All worm cast and compost amendments significantly increased wheat P and K uptake compared to either the non-amended control or the mineral fertilizer treatment. The results showed that worm casts were an efficient source of plant nutrients and they were less likely to produce salinity stress in container as compared to compost and synthetic fertilizers (Chaoui *et al*., 2003). Humic materials and other plant growth-influencing substances, such as plant growth hormones, produced by microorganisms during vermicomposting, produced after increased microbial biomass, activity in soils, might have been responsible for the increased pepper growth and yields, independent of nutrient availability (Arancon and Edwards, 2005). Vermicomposting of partially composted waste (2 weeks), for a further 6 weeks, reduced volatile solids content significantly more than for composting fresh waste for 8 weeks and it was concluded that *E. andrei* was capable of attaining good rates of growth and reproduction in fresh green waste and that vermicomposting would result in a more stable material (with lower volatile solids content) as compared to composting (Frederickson *et al*., 1997).

Table 2.6 Nutrient status of vermicompost on using different organic wastes (Kale, 1998)

Nutrient	**Content**
Organic carbon	9.15 - 17.98%
Total nitrogen	0.5 – 1.15%
Available phosphorus	0.1 – 0.3%
Available sodium	0.06 – 0.3%
Calcium and Magnesium	22.67 – 70 meq/100g
Copper	2 – 9.5 mg kg^{-1}
Iron	2 – 9.3 mg kg^{-1}
Zinc	5.7 – 11.5 mg kg^{-1}
Available sulphur	128 – 548 mg kg^{-1}

2.6.1 Physicochemical properties of compost

Vermicomposting of spent mushroom compost using epigeic earthworms *Eisenia foetida* and *Eisenia andrei* caused significant reduction in pH (8), total organic carbon (35%), C/N ratio (56), K (68%), Na (10%) and increased in available macro and micronutrients such as P by 3-fold,N 1.37-fold, B 1.29-fold, S 1.59-fold, Fe 2.1-fold, Cu 1.89-fold, Zn 1.68-fold, Mn 1.2-fold, Ca 1.92-fold and Mg 1.72-fold as compared to those of the initial substrate (Tajbakhsh, *et al*., 2008). Vermicomposting of paper waste resulted in net reduction in ash content and total organic carbon (42.5–56.8%) but increment in total Kjeldhal nitrogen (2.0–2.4-fold), total potassium (2.0-fold), and total phosphorous (1.4–1.8-fold) was achieved after 91 days of worms' activity (Gupta and Garg, 2008). Vermicomposts showed decrease in pH and organic C, but increase in EC, total Kjeldhal N, total available P and total K contents and the C/N ratio of final vermicomposts also reduced to 10.7–12.7 from 22.8 to 56 in different waste combinations (Yadav and Garg, 2011). The addition of vemicompost had significant positive effects on the soil physical, chemical properties and plant growth parameters (Tharmaraj *et al*., 2011). Organic amendments like vermicompost increased the organic matter content necessary for the maintenance of soil properties, which was beneficial for long-term sustainability and crop productivity and it was proposed that large-scale production of vermicompost through vermitech to recycle organic waste could effectively help in managing solid waste, and farmers for crop productivity could apply vermicompost thus produced and this could lead to a suitable environment-friendly effort towards a balanced ecosystem (Ansari, 2011). A comparative study of garden compost and vermicompost were done, in which the garden compost was made by heaping the organic wastes for decomposition and the vermicomposting was done by the earthworms.

Table 2.7 Chemical characteristics of garden compost and vermicompost (Gupta, 2005).

Parameter	**Garden compost**	**Vermicompost**
pH	7.80	6.80
Electrical conductivity (dS m^{-1})	3.60	11.70
Total nitrogen (%)	0.80	1.94
Phosphorus (%)	0.35	0.47
Potassium (%)	0.48	0.70
Calcium (%)	2.27	4.40
Sodium (%)	<0.01	0.02
Magnesium (%)	0.57	0.46
Iron (mg kg^{-1})	11690.00	7563.00
Zinc (mg kg^{-1})	128.00	278.00
Manganese (mg kg^{-1})	414.00	475.00
Copper (mg kg^{-1})	17.00	27.00
Boron (mg kg^{-1})	25.00	34.00
Aluminium (mg kg^{-1})	7380.00	7012.00

2.6.2 Accessory products of compost

The study by Suthar (2007) indicated that vermicomposting of crop residues and cattle shed wastes could not only produced a value added product (vermicomposting) but at the same time acted as best culture medium for large-scale production of earthworms. Higher decline in organic carbon and higher content of nitrogen and phosphorous along with lower electrical conductivity and higher pH of the products of vermicomposting indicated that *E. foetida* helped in fast conversion of toxic paper mill sludge into a soil conditioner in 100 days (Kaur *et al*., 2010). Vermiwash prepared using tea organic wastes showed good nutritive values which comprised of major and secondary nutrients like N, P, K, Ca, Mg, Zn and Fe in varying proportions and the per cent available nutrients varied depending on the raw materials used and their composition (Radhakrishnan, 2009). Aqueous extracts from vermicomposts (compost tea) have suppressed plant pathogens such as *Plectosporium*, *Verticillium*, and *Rhizoctonia* significantly in laboratory and greenhouse experiments (Arancon and Edwards, 2002).

2.8 Earthworm meal

2.8.1 Importance and composition

The protein efficiency ratio values were higher in the fish based earthworm diet as compared to plant based diets. The earthworm *Eisenia foetida* powder has antifungal properties which would be effective in treating fungal infections, such as candidosis (Ansari and Sitaram, 2011). The earthworm powder fed group of avian broilers had the best humoral immunities, its total serum protein increased by 20.11% and 17.13%, serum albumin increased by 13.76% and 11.67%, serum globulin increased by 28.79% and 24.49% and albumin or globulin decreased by 11.76% and 10.45% as compared to the control and fishmeal fed group (Yujing, *et al*.,2010). Earthworm meal (*Eisenia* *foetida*) inclusion in the diet for 42 days could represent a proteic complement to improve the dressing percentage and nutritional profile of quail meat (Feunmayor *et al*., 2008). According to (Prayogi, 2011) 0.2% earthworm meal supplementation in 55 weeks old laying hens diet improved the laying performance and ratio of egg yolk fatty acid contents. Supplementing 0.3% earthworm meal in 55 weeks old laying hens diet, improved the laying performance and egg quality. Supplementation of 10% earthworm meal in diet gave a good growth performance of the quail because it had low feed conversion and high body weight gain.

2.8.2 Amino acid composition of earthworm meal

The value of essential amino acid index obtained from earthworm meal was higher (58.67%) than those from earthworm (21.33%) and it showed that powdering method of earthworm by using formic acid addition had higher amino acid balance than earthworm and the essential amino acid of earthworm was dominated by histidine (0.63% of dry matter basis), meanwhile the earthworm meal was dominated by isoleucine (1.98% of dry matter basis). The nonessential amino acid of earthworm and earthworm meal was dominated by glutamic acid (1.52% and 3.60% of dry matter basis respectively). The value of essential amino acid index obtained from earthworm meal was higher (58.67%) than those from earthworm (21.23%). (Istiqomah, *et al*., 2009).

2.8.3 Possibility of use as a protein feed

Worm meal produced from cultured *Perionyx excavatus* and *Eudrilus eugeniae* had been found to be an efficient and cost effective replacement for fishmeal in the diets of the cage cultured fresh water fish (*Orcochromis niloticus*) and poultry (chicken and quail) (Guerrero, 2009). The lowest feed conversion ratio of 1.51, highest protein efficiency ratio and productive protein value of 1.52 and 52.48, respectively were also recorded in fish fed 25% earthworm meal inclusion diet. So the 25% replacement of fishmeal by earthworm meal is recommended in the diet of *Heterobranchus longifilis* finger lings for profitable and sustainable aquaculture practices (Sogbesan and Madu, 2008).

Table 2.8 Protein requirements of ornamental fish species (Sales and Janssens, 2003)

Common name	**Species name**	**Initial size (g)**	**Energy**	**Protein source**	**Parameters**	**Dietary requirement of protein (%)**
Guppy	*Poecilia reticulate*	0.10	13.10 kJ/g ME	Fish meal, casein	Weight gain, feed conversion, gonadal development	30-40
Goldfish	*Carassius auratus*	0.20	11.72 kJ/g DE	Fish meal, casein	Weight gain, feed conversion, protein efficiency ratio	29
		0.008	20.3 kJ/g GE	Fish meal, casein	Specific growth rate, feed efficiency, nutrient retention	53
Tin foil barb	*Barbodes altus*	0.81	20.38 kJ/g GE	Casein	Weight gain	41.7
Discus	*Synphisodon aequifasciata*	4.45-4.65	21.65 kJ/g GE	Fish meal, casein	Specific growth rate	44.9-50.1
Redhead cichlid	*Cichlasoma synspilum*	0.28	1.55 kJ/g DE	Fish meal	Specific growth rate	40.81

GE: Gross Energy, ME: Metabolisable Energy, DE: Digestible Energy

2.8.4 Comparative efficiency of earthworm meal

The utilization of cellulose substrate was recommended for the culture of earthworm and the inclusion of earthworm meal was guaranteed as a reliable and nutritional dependable fish meal supplement (Sogbesan *et al*., 2007). Stafford and Tacon (2008) found that the dried earthworm meal derived from the species *Eisenia foetida* was nutritionally evaluated as a replacement for herring meal in production diets of rainbow trout, *Salmo gairdneri* and it was found that there was significant increase in the whole carcass lipid content of fish fed with diets containing dried *Eisenia foetida* meal. Nandeesha *et al*. (2003) conducted studies on three new fish feeds, formulated using dried earthworm meal obtained from *Eudrilus eugeniae* as a replacement for fish meal and were evaluated in a culture trial with common carp. The diet which was enriched with sardine oil was found to be the best.

Materials and Methods

3. MATERIALS AND METHODS

The study on production and evaluation of protienaceous earthworm meal was conducted at College of Horticulture, Vellanikkara during May 2011 to May 2012. The entire study consisted of three parts.

1. Comparative evaluation of different substrates for the mass multiplication of exotic earthworm *Eisenia foetida*
2. Viable techniques for culturing native earthworms.
3. Preparation of earthworm meal and comparative evaluation of nutritive content of different earthworm meals.

MATERIALS USED FOR THE STUDY

Silkworm waste, azolla, banana pseudostem and cow dung were used for the mass multiplication of exotic and native earthworms. Silkworm waste for the culturing of worms was procured from Almighty silk industry, Thiruvalluvar nagar, Coimbatore. Sericulture wastes including double cocoon, urinated cocoon, diseased and dead larvae cocoon and waterjelly were used as silkworm waste for multiplication of earthworms.

Azolla used for the earthworm multiplication were reared in specially designed water tanks at Vermiculture unit, College of Horticulture, Vellanikkara. About 20 shallow cemented tanks of 2.5 m x 2 m x 15 cm size, available at the vermiculture unit of College of Horticulture were used for mass multiplication of azolla. About 25 kg of fertile sieved soil, 10 kg cow dung and 60 g Rajphose were added and mixed in each tank. Water level was maintained at 10 cm depth. To this, azolla was placed at the rate of 500 g m^{-2}.

Banana pseudostems of Nendran variety were collected from the plot maintained by Vermiculture unit at College of Horticulture. The banana pseudostem

a) Silkworm waste

b) Banana pseudostem

c) Azolla

Plate 1. Substrates used for the study

a) Waste generation in silkworm reeling unit

b) Sericulture wastes including double cocoon, urinated cocoon, diseased and dead larvae cocoon

Plate 2. Silkworm waste

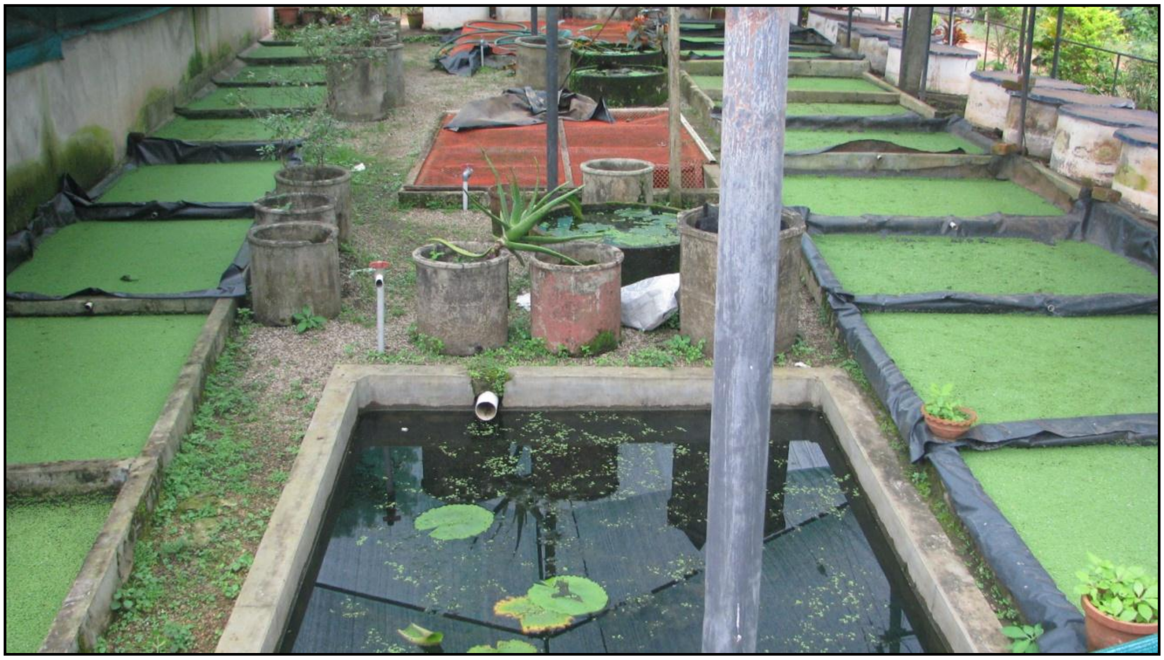


Plate 3. Mass multiplication of azolla at Vermicomposting unit

Plate 4. Mass multiplication of exotic/native earthworms

a) Eisenia foetida

b) Perionyx excavatus

Plate 5. Earthworm species used for the study

used for the multiplication of worms were chopped into small pieces and used for worm rearing.

3.1 COMPARATIVE EVALUATION OF DIFFERENT SUBSTRATES FOR THE MASS MULTIPLICATION OF EXOTIC EARTHWORM *Eisenia foetida*

The exotic earthworms were procured from Vermiculture unit maintained by the Department of Soil Science and Agricultural Chemistry, College of Horticulture, Vellanikkara.

Exotic earthworms: *Eisenia foetida* (Family: Lumbricidae) Worms appear coloured (red, brown or purple or even darker) and have dorsally coloured bands. On maturity, clitellum spreads over 7-9 segments in length and ridges will develop on maturity.

The mass multiplication of *Eisenia foetida* was done in plastic buckets lined with jute bags and their lids provided holes for aeration. The study was mainly focused on the identification of best substrate for the multiplication of exotic worms. For this, the three different substrates silkworm waste, banana pseudostem and azolla were used.

3.1.1 Methodology

The design followed was CRD with five replications for each treatment. The different treatments were tried at 1:1 ratios at volume basis.

Treatments

T_1 – cow dung: silkworm waste (1:1 by volume)

In each plastic bucket lined with jute bag, silkworm waste and cow dung were added in equal volume in the ratio 1:1. Into these buckets 100 adult exotic worms (*Eisenia foetida*) were released.

T_2 – cow dung: banana pseudostem (1:1 by volume)

In each plastic bucket lined with jute bag, banana pseudostem of Nendran variety, chopped into smaller sizes and cow dung were added in equal volumes in the ratio 1:1. To all these buckets 100 adult exotic worms (*Eisenia foetida*) were released.

T_3 – cow dung: azolla (1:1 by volume)

In each plastic bucket lined with jute bag, freshly harvested azolla and cow dung were added in equal volume in the ratio 1:1. To all these buckets 100 adult exotic worms (*Eisenia foetida*) were released.

Observation

Crude fibre, crude protein, crude fat and minerals such as calcium, magnesium, iron, chlorine, nitrogen, phosphorus and potassium of different agro wastes were analyzed before the initiation of the experiment, as indicated in table 3.1.

The number of adult worms was counted at weekly intervals till the compost maturity.

Major nutrients such as carbon, nitrogen, phosphorus, potassium and heavy metals of the worm casts were analyzed at the mature stage of compost.

Table 3.1 Analytical methods used for the determination of biochemical and mineral composition of agro wastes and worm cast

Property	Method	References
Crude protein	Microkjeldahl digestion and distillation for nitrogen and multiplication of nitrogen content by factor 6.25	Thimmaiah, 1989
Crude fat	Extraction with petroleum ether using soxhlet apparatus and estimation by gravimetry	
Crude fibre	Acid alkali treatment for extraction and estimation by gravimetry	
Carbon	Ashing (550°C for 5 hr)	Jackson, 1958
Nitrogen	Microkjeldahl digestion and distillation	
Phosphorus	Diacid extract – spectrophotometry	
Potassium	Diacid extract –flame photometry	
Calcium	Diacid extract –atomic absorption spectrophotometry	Perkin-Elmer, 1982
Magnesium	Diacid extract – atomic absorption spectrophotometry	
Micronutrients	Diacid extract – atomic absorption spectrophotometry	
Heavy metals	Diacid extract – atomic absorption spectrophotometry	

3.2 VIABLE TECHNIQUES FOR CULTURING NATIVE EARTHWORMS

3.2.1 Collection of native earthworms

For the multiplication of native earthworms, the collection was done from different localities which were marked. For the collection, a handful of cow dung was scattered over the marked one square meter area. Then leaf litter was added as organic matter source and later covered with an old jute bag. Each area was kept moistened by regular watering (not flooded with water). In about a fortnight time worms were observed in surface and subsurface areas. For the collection of worms, digging, hand sorting and wet sieving; with the help of suitable digging tool, cores or quadrates of soil of exact dimension are taken for accurate population estimate. Usually 16 sample units of $25m^2$ with 20cm depth provide adequate estimation of medium sized worms. Other methods like chemical extraction method, electrical extraction or heat extraction methods were also tried and it was found that the effective method for worm collection was digging, hand sorting and wet sieving. These worms from different localities were collected and identical worms were used for culturing. For the identification of native earthworms, the monographs by Gates (1972) and Stephenson (1930). were referred and the descriptions agreeing with that of collected worms were taken as *Perionyx excavatus*.

Native species: *Perionyx excavatus* (Family: Megascolecidae) Body colouration on dorsal surface (upper part) deep purple to reddish brown and lower side pale. Total length 23-120mm; diameter, 2.5mm; clitellum ring shaped, covering five or less segments. Male pores were seen in a depression and spermathecal pores in 7/8 and 8/9 segment, nearly at same distance as male pores.

3.2.2 Rearing of native earthworms

The mass multiplication of *Perionyx excavatus* was done in plastic buckets lined with jute bags and their lids were provided holes for aeration. For the multiplication of native earthworms three different substrates silkworm waste, azolla and banana pseudostem were used. The soil used for the study was laterite of the order Oxisols belonging to Vellanikkara series.

The design followed was CRD with five replications for each treatment. The different treatments were tried at 1:1:1 ratios of substrate: cow dung: soil in equal volumes.

Treatments

T_1 – cow dung: silkworm waste: soil (1:1:1 by volume)

In each plastic bucket lined with jute bag, the silkworm waste, cow dung and soil were added in equal volumes in the ratio 1:1:1. To all these buckets 100 adult native worms (*Perionyx excavatus*) were introduced.

T_2 – cow dung: banana pseudostem: soil (1:1:1 by volume)

In each plastic bucket lined with jute bag, banana pseudostem, cow dung and soil were added in equal volumes in the ratio 1:1:1. To all these buckets 100 adult native worms (*Perionyx excavatus*) were introduced.

T_3 – cow dung: azolla: soil (1:1:1 by volume)

In each plastic bucket lined with jute bag, azolla, cow dung and soil were added in equal volumes in the ratio 1:1:1. To all these buckets 100 adult native worms (*Perionyx excavatus*) were introduced.

Observations

Worm count at weekly interval was noted till the compost maturity.

Physicochemical properties of the soil such as soil texture, pH, organic carbon, nitrogen, phosphorus and potassium were analyzed.

Manurial value such as carbon, nitrogen, phosphorus and potassium of worm cast under each treatment were analyzed along with heavy metals.

Table 3.2 Analytical methods followed for the determination of physicochemical properties of soil

Property	**Method**	**Reference**
Soil texture	International pipette method	Robinson, 1922
pH	1:2.5 suspension	Jackson, 1958
Organic carbon	Walkley and Black method	Walkley and Black, 1934
Available Nitrogen	Alkaline permanganate method	Subbiah and Asija, 1956
Available Phosphorus	Bray's extractant (0.03 *N* NH_4F in 0.025 *N*HCL)	Bray and Kurtz, 1945
Available Potassium	Neutral normal ammonium acetate method using flame photometer	Jackson, 1958
Exchangeable Calcium	Versenate titration method	
Exchangeable Magnesium	Versenate titration method	

3.3 PREPARATION OF EARTHWORM MEAL AND COMPARATIVE EVALUATION OF NUTRITIVE CONTENT OF DIFFERENT EARTHWORM MEALS

3.3.1 Preparation of earthworm meal

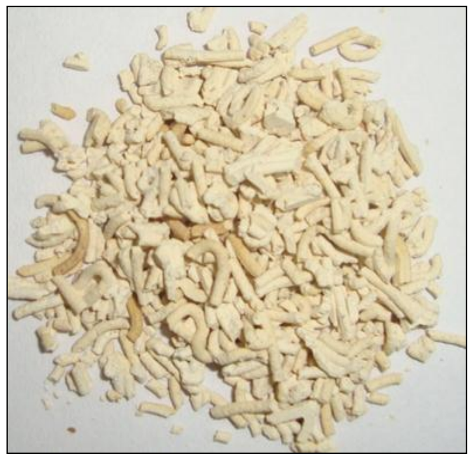
After the maturity of compost, the adult worms were collected and washed thoroughly to remove the soil particles sticking on the body. Then they were left in clean containers containing wet cellulose material such as waste paper. The containers are covered tightly to prevent the worms from crawling out. When the worm start excreting the paper alone indicating the gut clearance (of soil and organic materials),

Plate 6. Different stages in the preparation of earthworm meal

Exotic worm meal

Native worm meal

Readymade fish feed (Tubifex)



Locally prepared fish feed

Protein substituted with exotic worm meal

Protein substituted with native worm meal

Plate 7. Different fish feed preparations

they were removed and dried under shade. The air dried worms were powdered and used as worm meal.

3.3.2 Comparative evaluation of nutritive content of different earthworm meals

Under this experiment, six different fish feeds were analyzed for the nutritive content and for the identification of the best feed for ornamental fishes. The general composition of fish feed as recommended by FAO (1990) is given below;

Carbohydrate : 40%

Protein : 40%

Fat and oil : 15 - 20%

Minerals and vitamins: 1%

The different feeds were prepared to obtain the above mentioned contents. The experiment was done in CRD with 6 treatments and 3 replications.

Treatments

1. Exotic earthworm meal – as described under section 3.3.1
2. Native earthworm meal – as described under section 3.3.1
3. Readymade fish meal (Tubifex) obtained from the market

The readymade fish meal, Tubifex was usually prepared from the live tubifex worms by freeze dried process and marketed by Taiyo pet products Pvt Ltd.

4. Locally prepared fishmeal with crude protein content 40-50%

Locally prepared fishmeal was prepared by using prawn powder as a source for protein, tapioca flour as carbohydrate source, groundnut powder for oil and small quantities of mineral and vitamin powders as detailed in section 3.3.2.

5. Crude protein of (4) substituted by exotic earthworm meal

In locally prepared fishmeal the protein source was substituted by exotic earthworm meal.

6. Crude protein of (4) substituted by native earthworm meal

In locally prepared fishmeal the protein source was substituted by native earthworm meal.

Observations

The nutritive value such as content of crude fibre, crude fat, crude protein and minerals such as calcium, magnesium, iron, phosphorus and potassium were analyzed as per the procedures outlined in table 3.1.

The total carbohydrate and total protein in different fish feeds were determined. Also qualitative determinations of amino acids were undertaken.

The heavy metal contents in fish feeds were analyzed as detailed in section 3.1.1.

Table 3.3 Analytical methods used for the determination of primary metabolites in feed materials

Property	**Method**	**Reference**
Total carbohydrate	Phenol sulphuric acid method	Sadasivam and Manickam, 1991
Total protein	Lowry's method	
Amino acids	Paper chromatography	

3.3.3 Suitability of earthworm meal for aquaculture

Each fish feed was evaluated by conducting feeding trials in six aquariums with the ornamental fish, Red Oscar. The aquarium water was well aerated continuously with the help of air pumps so as to maintain the required dissolved oxygen during the period of investigation. Fishes were fed with the feeds once in a day and the measurement of pH and electrical conductivity of the aquarium water were observed at weekly intervals.

Red Oscar – Ornamental fish used for the study

Plate 8. Suitability of earthworm meal for aquaculture – a pilot study

3.3.4 Comparative economics

Comparative economics of the six fish feeds were also calculated in terms of protein, carbohydrate and fat equivalents.

3.4.STATISTICAL ANALYSIS

The data obtained was statistically analyzed by the method of analysis of variance (ANOVA) (Panse and Sukhatme, 1985) and using DMRT by M STATC programme.

Results

4. RESULTS

4.1 COMPARATIVE EVALUATION OF DIFFERENT SUBSTRATES FOR THE MASS MULTIPLICATION OF EXOTIC EARTHWORM *Eisenia foetida*

The experiment was done to identify the best substrate for the mass multiplication/vermicomposting using exotic earthworm, *Eisenia foetida*. The influence of nutrient status of the substrates used for vermicomposting on the earthworm count, heavy metal contents and the manurial value of the worm casts were studied as a part of this experiment.

4.1.1 Biochemical properties of substrates

The substrates used for worm multiplication were cow dung, silkworm waste, banana pseudostem and azolla (*Azolla pinnata*).

Table 4.1 Biochemical composition of substrates used for vermicomposting (% on fresh weight basis)

Substrate	**Moisture**	**Crude protein**	**Crude fibre**	**Crude fat**	**Other components**
Cow dung	34.37	3.20	7.55	ND	54.88
Silkworm waste	16.70	19.83	2.71	9.66	51.10
Banana pseudostem	58.20	1.38	8.99	0.42	31.01
Azolla	90.78	1.91	1.52	0.26	5.53

ND – Not Detected

The biochemical composition of the substrates used for vermicomposting is presented in table 4.1. The moisture content of cow dung, silkworm waste, banana pseudostem and azolla were 34.37, 16.70, 58.20 and 90.78 per cent, respectively. The highest crude protein was recorded in silkworm waste (19.83%) and the lowest was recorded in banana pseudostem (1.38%). The crude protein content of cow dung and azolla were 3.20 and 1.91 per cent, respectively. The crude fibre content of cow dung, silkworm waste, banana pseudostem and azolla were 7.55, 2.71, 8.99 and 1.52 per cent, respectively. Among the substrates the crude fat content was found to be maximum in silkworm waste (9.66%) and minimum in azolla (0.26%). The crude fat of banana pseudostem was recorded as 0.42 per cent and there was no detectable amount of crude fat in cow dung.

4.1.2 Physicochemical properties of substrates

Table 4.2 pH and nutrient contents of different substrates used for vermicomposting (% on oven dry basis)

pH and nutrient content	**Substrates**			
	Cow dung	**Silkworm waste**	**Banana pseudostem**	**Azolla**
pH (1:1 extract)	6.10	6.70	7.50	7.90
Carbon (%)	32.50	87.02	38.60	51.54
Nitrogen (%)	0.78	3.81	0.53	3.32
Phosphorus (%)	0.55	0.31	0.07	0.52
Potassium (%)	0.37	0.67	1.34	3.15
Calcium (%)	1.01	0.04	0.42	0.22
Magnesium (%)	0.89	0.47	0.67	0.60

Table 4.2 illustrates the pH and nutrient contents of different substrates used for vermicomposting on oven dry basis. The pH of cow dung, silkworm waste, banana

pseudostem and azolla recorded were 6.1, 6.7, 7.5 and 7.9. The organic carbon content of cow dung, silkworm waste, banana pseudostem and azolla were 32.50, 87.02, 38.60 and 51.54 per cent, respectively. The N, P and K contents of cow dung were 0.78, 0.55 and 0.37 per cent, respectively. The total N, P and K contents of silkworm waste were 3.81, 0.31 and 0.67 per cent, respectively. The banana pseudostem recorded 0.53, 0.06 and 1.34 per cent and that for azolla, the values were found to be as 3.32, 0.52 and 3.15 per cent N, P and K, respectively. Among the substrates, cow dung recorded the highest content of calcium (1.01%) and magnesium (0.89%). The lowest calcium and magnesium content was recorded in silkworm waste as 0.04 and 0.47 per cent, respectively. For banana pseudostem the calcium content was 0.42 per cent and magnesium was 0.67 per cent and for azolla 0.22 per cent calcium and 0.60 per cent magnesium were also recorded.

Table 4.3 Micronutrient and heavy metal contents in different substrates used for vermicomposting

Micronutrients & heavy metals (mg kg^{-1})	**Substrates**			
	Cow dung	**Silkworm waste**	**Banana pseudostem**	**Azolla**
Iron	2527.40	2.80	1.30	1135.10
Manganese	884.60	9.10	275.70	853.10
Zinc	71.80	94.00	37.40	9.00
Copper	32.10	2.10	6.50	16.30
Cadmium	15.30	ND	11.80	22.20
Lead	10.07	ND	12.92	8.12
Chromium	32.00	23.60	16.82	8.06
Nickel	3.89	4.03	2.76	8.47
Cobalt	ND	ND	ND	1.50

ND- Not Detected

Micronutrient and heavy metal contents of different substrates used for composting are furnished in table 4.3. The highest iron content (2527.4 mg kg^{-1}) was recorded in cow dung and the lowest (1.3 mg kg^{-1}) was found in banana pseudostem. For silkworm waste and azolla the iron content was found to be 2.8 and 1135.1 mg kg^{-1} , respectively. Manganese content of substrates also showed a wide variation. The manganese content of cow dung, silkworm waste, banana pseudostem and azolla was found to be 884.6, 9.1, 275.7 and 853.1 mg kg^{-1}, respectively. The zinc and copper content of substrates were 71.8 and 32.1 mg kg^{-1} for cow dung, 94 and 2.1 mg kg^{-1} for silkworm waste, 37.4 and 6.5 mg kg^{-1} for banana pseudostem and 9 and 16.3 mg kg^{-1} for azolla. Cadmium and lead content of cow dung, banana pseudostem and azolla were 15.3 and 10.07 mg kg^{-1}, 11.8 and 12.92 mg kg^{-1} and 22.2 and 8.12 mg kg^{-1}, respectively. There was no detectable amount of cadmium and lead in silkworm waste. The chromium and nickel content of cow dung was 32 and 3.89 mg kg^{-1}, silkworm waste 23.6 and 4.03 mg kg^{-1}, banana pseudostem 16.82 and 2.76 mg kg^{-1} and azolla 8.06 and 8.47 mg kg^{-1}, respectively. The cobalt content of azolla was 1.5 mg kg^{-1} and cobalt was absent in cow dung, silkworm waste and banana pseudostem.

4.1.3 Influence of substrates on earthworm count

The data pertaining to the rate of earthworm multiplication during composting were furnished at weekly intervals in table 4.4. Earthworms were introduced @ 100 numbers/10 kg substrate. The earthworms multiplied up to compost maturity which was found to be varied for different substrates. The maximum multiplication was noticed in T_3 cow dung: azolla (1:1 proportion) and the lowest multiplication noticed in T_2 cow dung: banana pseudostem (1:1 proportion) within a period of 6 weeks. For the silkworm waste, the vermicompost matured only within 12 weeks and the multiplication was found to be less than the other substrates at 6 weeks interval. But the same treatment recorded the highest earthworm count at its compost maturity period of 12 weeks.

Table 4.4 Earthworm count as influenced by different treatments used for vermicomposting with Eisenia foetida

Weeks after the introduction of worms	**Treatments**		
	Silkworm waste + cow dung (T_1)	Banana pseudostem + cow dung (T_2)	Azolla + cow dung (T_3)
1	125	164	342
2	214	386	1196
3	584	1010	2720
4	1034	1376	3240
5	1520	1970	4000
6	2275	2600	5100
7	2340	-	-
8	2480	-	-
9	3340	-	-
10	4160	-	-
11	4660	-	-
12	4800	-	-

4.1.4 Manurial value of worm casts as influenced by different treatments

Table 4.5. Effect of different treatments on pH and nutrient contents (% on oven dry basis) of worm casts at compost maturity

Content	Silkworm waste + cow dung (T_1)	Banana pseudostem + cow dung (T_2)	Azolla + cow dung (T_3)
pH	7.02^a	7.40^b	7.80^c
Carbon	27.05^a	21.35^b	9.56^c
Nitrogen	1.43^a	1.34^a	1.40^a
Phosphorus	0.81^a	0.73^b	0.91^c
Potassium	1.09^a	0.91^a	1.29^b
Calcium	0.76^a	0.76^a	0.79^a
Magnesium	0.83^a	0.79^a	0.81^a

Treatment values in a row followed by the same superscript do not differ significantly.

pH

Different substrate combinations of each treatment influenced the pH of worm casts significantly. The highest pH (7.80) was recorded in T_3 (cow dung: azolla in 1:1 proportion) and the lowest pH (7.02) was recorded in T_1 (cow dung: silkworm waste in 1:1 proportion). A pH value of 7.4 was recorded in T_2 (cow dung: banana pseudostem in 1:1 proportion).

Carbon

The highest carbon content was recorded in T_1 (27.05%), followed by T_2 (21.35%) and the lowest carbon content was recorded in T_3 (9.56%). It was found that there was significant difference among the treatments with respect to carbon content and the highest value was recorded in treatment combination cow dung : silkworm waste (1:1 proportion) and the lowest value was recorded in treatment combination cow dung : azolla (1:1 proportion).

Nitrogen

No significant difference in nitrogen content due to different treatment combinations was observed. The highest nitrogen content (1.43%) was recorded in T_1 and the lowest nitrogen content (1.34%) was observed in T_2. However the T_3 registered a nitrogen content of 1.40 per cent.

Phosphorus

The phosphorus content of worm casts significantly varied from each other with 0.73 per cent in T_2, 0.81 per cent in T_1 and 0.91 per cent in T_3. On considering the substrate influence, the treatment with cow dung and azolla as substrates had the highest phosphorus content and the treatment with cow dung: silkworm waste had recorded the next highest value and the treatment with cow dung and banana pseudostem had the lowest. The phosphorus content of all the three treatments differed significantly.

Potassium

The maximum potassium content was recorded in T_3 with 1.29 per cent and the minimum value recorded in T_2 with 0.91 per cent. The T_1 recorded a potassium content of 1.09 per cent. The T_1 and T_2 were on par with respect to the potassium content but the T_3 differed significantly with T_1 and T_2.

Calcium

There was no significant difference between different substrate combinations on calcium status. The highest calcium status (0.79%) was recorded in T_3 (cow dung: azolla in 1:1 proportion). For T_1 (cow dung: silkworm waste) and T_2 (cow dung: banana pseudostem) the calcium status recorded was 0.76 per cent.

Magnesium

The maximum magnesium content (0.83%) was recorded in T_1 (cow dung: silkworm waste in 1:1 proportion) and the minimum magnesium content (0.79%) was recorded in T_2 (cow dung: banana pseudostem in 1:1 proportion). In T_3 (cow dung:

azolla in 1:1 proportion) the magnesium content was found as 0.81 per cent. There was no significant difference between different substrate combinations on magnesium status.

The micronutrient and heavy metal contents in worm casts of different treatment combinations are given in table 4.6. FCO specifications for vermicompost as detailed in schedule IV Part-A of Fertilizer Control Order, 1985 were provided in appendix I for comparing the results obtained.

Table 4.6 Micronutrient and heavy metal contents in worm casts (mg kg^{-1} on dry weight basis)

Micronutrients and heavy metals (mg kg^{-1})	**Silkworm waste + cow dung (T_1)**	**Banana pseudostem + cow dung (T_2)**	**Azolla + cow dung (T_3)**
Iron	7451.00[a]	5199.00[b]	8401.00[a]
Manganese	687.00[a]	794.00[a]	727.00[a]
Zinc	89.00[a]	56.00[b]	140.00[c]
Copper	34.00[a]	31.00[a]	40.00[a]
Cadmium	1.00[a]	4.00[a]	3.00[a]
Lead	88.00[a]	10.00[b]	23.00[b]
Chromium	33.70[ab]	39.10[a]	21.70[b]
Nickel	6.30[a]	3.30[b]	5.30[ab]
Cobalt	ND	ND	1.00[a]

ND- Not Detected

Treatment values in a row followed by the same superscript do not differ significantly.

Iron

The iron content of $T_{1,}$ T_2 and T_3 were recorded as 7451, 5199 and 8401 mg kg^{-1} , respectively. The T_1 with substrate combination cow dung: silkworm waste was on par with T_3 with a substrate combination of cow dung: azolla. The lowest value for iron was recorded in T_2 with substrate cow dung: banana pseudostem.

Manganese

The manganese content was recorded as 687, 794 and 727 mg kg^{-1} for $T_{1,}$ T_2 and T_3, respectively. There were no significant difference in manganese content of each treatment and all the three treatments were on par.

Zinc

The highest value (140 mg kg^{-1}) of zinc was recorded in T_3 (cow dung: azolla in 1:1 proportion). The next high value was recorded in T_1 cow dung: silkworm waste in 1:1 proportion (89 mg kg^{-1}) and then for $T_{2,}$ cow dung: banana pseudostem (56 mg kg^{-1}). There was significant difference between the treatments in case of zinc content.

Copper

There were no significant difference in case of copper contents among the three treatments and all treatments were on par. The value for copper content T_1, T_2 and T_3 were 34, 31 and 40 mg kg^{-1}, respectively.

Cadmium

The value for cadmium content for T_1, T_2 and T_3 were 1, 4 and 3 mg kg^{-1}, respectively. There was no significant difference in cadmium content of worm cast with respect to different treatments applied.

Lead

The maximum value for lead was recorded in T_1 (88 mg kg^{-1}). The next high value was recorded in T_3 (23 mg kg^{-1}) and the lowest value recorded for T_2 (10 mg kg^{-1}) which is on par with T_3.

Chromium

The highest value was recorded in T_2 (39.1 mg kg^{-1}), the next high value in T_1 (33.7 mg kg^{-1}) and the lowest in T_3 (21.7 mg kg^{-1}). T_1 was on par with T_2 and T_3.

Nickel

The nickel content in T_1 was 6.3, in T_2 3.3 and in T_3 5.3 mg kg^{-1}. All the treatments were on par. However the highest value was recorded in T_1 and the lowest in T_2.

Cobalt

There were no significant difference in cobalt content of worm casts for the different treatments and there was only negligible amount of cobalt in T_3 (1 mg kg^{-1}). T_1 and T_2 contained no detectable amounts of cobalt.

4.2 VIABLE TECHNIQUES FOR CULTURING NATIVE EARTHWORMS

This experiment was done to develop suitable technique for collection of native earthworms and to identify suitable substrates for the mass multiplication of native earthworm (*Perionyx excavatus*). The physicochemical properties of soil, earthworm multiplication at weekly intervals, mineral nutrients and heavy metal contents of worm cast were investigated in detail.

4.2.1 Physicochemical properties of soil

For the mass multiplication of native earthworm, the substrates used were cow dung, silkworm waste, banana pseudostem and azolla along with soil. The different physico-chemical properties of laterite soil of the order Oxisol belonging to Vellanikkara series, used for native worm (*Perionyx excavatus*) multiplication were presented in table 4.7.

Table 4.7 Physicochemical properties of laterite soil (Order Oxisol-Vellanikkara series)

S No.	Properties	Value
1	Soil texture	Sandy clay loam
2	pH	6.30
3	Organic carbon (%)	0.86
4	Available N (kg ha^{-1})	232.08
5	Available P (kg ha^{-1})	6.80
6	Available K (kg ha^{-1})	72.04
7	Exchangeable Ca $(cmol(p^+)\ kg^{-1})$	2.20
8	Exchangeable Mg $(cmol(p^+)\ kg^{-1})$	0.23

The basic properties of soils were studied before the conduct of the experiment. Soil samples were collected for the mass multiplication of earthworms, and were analyzed for soil texture, pH, organic carbon, available N, P and K content employing standard procedures. The soil was acidic in reaction with a pH of 6.3. The organic carbon content of soil was 0.86 per cent. Among the major nutrients, the content of available N, P and K were 232.08, 6.80 and 72.04 kg ha^{-1}, respectively. The soil was low in fertility status for nitrogen, phosphorus and potassium contents. The exchangeable calcium and magnesium content of soil was found to be 2.20 and 0.23 $cmol(p^+)\ kg^{-1}$, respectively.

4.2.2 Influence of substrates on earthworm count

The rates of earthworm multiplication at weekly intervals during composting were furnished in table 4.8. Native earthworms were introduced to each bucket @ 100 worms/10 kg material. Initial increase in the worm count and gradual decrease was observed in each treatment during the period of composting.

Table 4.8 Earthworm count as influenced by different treatments

Weeks after introduction of worms	**Treatments**		
	Silkworm waste + cow dung + soil (T_1)	Banana pseudostem + cow dung + soil (T_2)	Azolla + cow dung + soil (T_3)
1	109	102	109
2	133	107	125
3	148	110	137
4	170	86	133
5	184	49	132
6	203	30	130
7	246	-	-
8	229	-	-
9	208	-	-
10	197	-	-
11	196	-	-
12	185	-	-

4.2.3 Manurial value of worm casts as influenced by different treatments

The effect of different treatments on the pH and nutrient contents at compost maturity illustrated in table 4.9.

Table 4.9. Effect of different treatments on the pH and nutrient content (% on oven dry basis) at compost maturity

Content	Silkworm waste + cow dung + soil (T_1)	Banana pseudostem +cow dung + soil (T_2)	Azolla +cow dung +soil (T_3)
pH	7.03^{a}	7.40^{b}	7.80^{c}
Carbon (%)	2.07^{a}	2.09^{a}	2.90^{b}
Nitrogen (%)	0.50^{a}	0.56^{ab}	0.78^{b}
Phosphorus (%)	0.23^{a}	0.26^{b}	0.39^{c}
Potassium (%)	0.29^{a}	0.17^{a}	0.59^{b}
Calcium (%)	0.03^{a}	0.04^{a}	0.17^{b}
Magnesium (%)	0.43^{a}	0.43^{a}	0.47^{b}

Treatment values in a row followed by the same superscript do not differ significantly.

pH

The pH of T_1, T_2 and T_3 were recorded 7.03, 7.4 and 7.8, respectively. The pH values of different treatments varied significantly. The highest pH value was found in the third treatment with a substrate combination of cow dung: azolla: soil in 1:1:1 proportion and the lowest value was recorded in T_1 with substrate combination of cow dung: silkworm waste: soil in 1:1:1 proportion.

Carbon

The highest carbon content was recorded in treatment T_3 (2.90%). The next treatment recording the highest carbon content was T_2 (2.09%) which was on par with T_1 (2.07%). There was no significant difference between the treatments T_2 and T_1.

Nitrogen

The nitrogen content of $T_{1,}$ T_2 and T_3 were 0.50, 0.56 and 0.78 per cent, respectively. Among all the treatments T_2 was not showing any significant difference with T_1 and T_3 in nitrogen content but the T_1 and T_3 differed significantly from each other.

Phosphorus

The highest phosphorus content (0.39%) was recorded in T_3, the next highest phosphorus content (0.26%) in T_2 and the lowest phosphorus content (0.23%) was recorded in T_1. While considering the different substrate combinations there was significant difference for the phosphorus content between treatments.

Potassium

The maximum potassium content was recorded in T_3 (0.59%) the next highest value in T_1 (0.29%) and the lowest value in T_2 (0.17%). The treatments T_1 and T_2 did not differ significantly and the treatment T_3 differed significantly from T_1 and $T_{2.}$

Calcium

The highest calcium content was recorded in treatment T_3 (0.17%). The lowest calcium content was in T_1 (0.03%) which was on par with treatment T_2 (0.04%). The treatment T_3 was significantly different from treatments T_1 and T_2.

Magnesium

The highest magnesium content was recorded in treatment T_3 (0.47%). The lowest magnesium content was recorded in treatment T_1 (0.42%) which was on par with the treatment T_2 (0.43%). The treatment T_3 was significantly different from treatments T_1 and T_2.

The micronutrient and heavy metal contents in worm casts for different treatments at compost maturity are presented in table 4.10.

Table 4.10 Micronutrient and heavy metal contents in worm casts (mg kg^{-1} on oven dry basis).

Micronutrients and heavy metals (mg kg^{-1})	**Silkworm waste + cow dung + soil (T_1)**	**Banana pseudostem +cow dung + soil (T_2)**	**Azolla +cow dung + soil (T_3)**
Iron	11533.00[a]	11513.00[a]	11358.00[a]
Manganese	1027.00[a]	875.00[b]	549.00[c]
Zinc	9.00[a]	2.00[a]	54.00[b]
Copper	61.00[a]	43.00[b]	12.00[c]
Cadmium	1.00[a]	1.00[a]	2.00[a]
Lead	68.00[a]	2.00[b]	68.00[a]
Chromium	28.00[a]	21.70[a]	28.80[a]
Nickel	7.40[a]	12.20[b]	5.80[a]
Cobalt	2.00[a]	1.00[ab]	1.00[b]

Treatment values in a row followed by the same superscript do not differ significantly.

Iron

The iron content of worm casts were 11533, 11513 and 11358 mg kg^{-1} for T_1, T_2 and T_3, respectively. There was no significant difference in iron content among the treatments.

Manganese

There was significant difference observed in manganese content of different worm casts. The highest value was recorded in T_1 (1027 mg kg^{-1}). The next high value was recorded in T_2 (875 mg kg^{-1}) and the lowest value in T_3 (549 mg kg^{-1}).

Zinc

The zinc content of T_1, T_2 and T_3 were 9, 2 and 54 mg kg^{-1}, respectively. The treatment, T_3 was significantly different from T_1 and T_2. The treatments T_1 and T_2 were on par.

Copper

Significant differences were observed in the copper content of all the three treatments. The value ranged from 12 mg kg^{-1} to 61 mg kg^{-1}. The highest value was recorded in T_1 (61 mg kg^{-1}), the next highest value was in T_2 (43 mg kg^{-1}) and the lowest value in T_3 (12 mg kg^{-1}).

Cadmium

There was no significant difference in cadmium content of worm casts in different treatments. The cadmium content of T_1 and T_2 was 1 mg kg^{-1} and that for T_3 was 2 mg kg^{-1}.

Lead

The lowest lead content was recorded in T_2 (2 mg kg^{-1}). For T_1 and T_3 the value for lead was recorded as 68 mg kg^{-1} and the treatments (T_1 and T_3) were on par.

Chromium

There was no significant difference between the three treatments. The chromium content of three treatments was 28.0, 21.7 and 28.8 mg kg^{-1} for T_1, T_2 and T_3, respectively.

Nickel

The nickel content of worm casts ranged from 5.8 mg kg^{-1} to 12.2 mg kg^{-1}. The high value was recorded in T_2 (12.2 mg kg^{-1}). The lowest value was recorded in T_3 (5.8 mg kg^{-1}) which was on par with T_1 (7.4 mg kg^{-1}).

Cobalt

The cobalt content of T_1 was 2 mg kg^{-1}, T_2 was 1 mg kg^{-1} and that for T_3 was 1 mg kg^{-1}. The treatment, T_2 was on par with T_1 and T_3, but significant difference was observed between T_1 and T_3.

4.3 PREPARATION OF EARTHWORM MEAL AND COMPARATIVE EVALUATION OF NUTRITIVE CONTENT OF DIFFERENT EARTHWORM MEALS

As described in section 3.3.1 the worm meals were prepared and both the exotic as well as native worm meals were compared to locally prepared and readymade fish feeds for the nutritive content and biochemical composition in order to check the suitability of worm meal as a fish feed.

4.3.1 Biochemical composition of different worm meals as compared to locally prepared and readymade fish feeds

The biochemical composition of different worm meals as compared to locally prepared and readymade fish feeds are given in table 4.11. The biochemical constituents of worm meal studied were crude protein, crude fibre and crude fat along with moisture content.

Table 4.11 Biochemical composition of different feed materials (% on fresh weight basis)

Feed material	Moisture	Crude protein	Crude fibre	Crude fat
Exotic worm meal (T_1)	11.90[a]	46.37[a]	1.00[a]	10.33[a]
Native worm meal (T_2)	10.60[b]	44.63[b]	0.75[a]	10.33[a]
Readymade fish feed (Tubifex) (T_3)	8.00[c]	48.13[c]	1.00[a]	6.15[b]
Locally prepared fishmeal (T_4)	12.50[a]	42.00[d]	3.00[b]	17.33[c]
Protein substituted with exotic worm meal (T_5)	14.10[d]	43.36[e]	0.76[a]	17.83[c]
Protein substituted with native worm meal (T_6)	13.30[e]	41.75[d]	0.75[a]	18.66[d]

Treatment values in a column followed by the same superscript do not differ significantly.

Moisture

Moisture content of all the six feed samples varied widely from 8 to 14.1 per cent. The highest moisture content (14.1%) was recorded in T_5 and lowest moisture content (8%) in T_3. The moisture content of the treatments T_1, T_2, T_4 and T_6 were 11.90, 10.60, 12.50 and 13.30 per cent, respectively.

Crude protein

The crude protein content of all the six fish feeds showed significant difference and the lowest value of crude protein (41.75%) was recorded in protein substituted fish feed, T_6 and highest value (48.13%) in readymade fish feed, Tubifex. The crude protein content of exotic worm meal, native worm meal, readymade fish feed, locally prepared fish feed, protein of locally prepared feed substituted with exotic worm meal and protein substituted with native fish feed were 46.37, 44.63, 48.13, 42.00, 43.36 and 41.75 per cent, respectively.

Crude fibre

The crude fibre content of locally prepared fish feed differed significantly with all other fish feeds and the highest value recorded was 3.00 per cent for locally prepared fish feed. The crude fibre content of exotic worm meal, native worm meal, readymade fish feed, protein of locally prepared feed substituted with exotic worm meal and protein substituted with native fish feed were 1.00, 0.75, 1.00, 0.76 and 0.75 per cent, respectively.

Crude fat

The crude fat content of exotic earthworm meal and native earthworm meal was found to be on par with the value of 10.33 per cent. The lowest value was recorded for readymade fish feed (6.15%). The crude fat content of locally prepared feed, feed in which the protein source substituted by exotic worm meal and native worm meal was 17.33, 17.83 and 18.66 per cent, respectively. The treatments T_1 and T_2 were on par, the treatments T_4 and T_5 were on par where as the treatments T_3 and T_6 were differed significantly from all other treatments.

4.3.2. Primary metabolites of feed materials

Primary metabolites of feed materials were given in the table 4.12. The primary metabolites of feed materials studied were total carbohydrate, total protein and ash content.

Table 4.12 Primary metabolites of different feed materials (% on fresh weight basis)

Feed material	Total carbohydrate	Total protein	Ash
Exotic worm meal (T_1)	15.03[a]	43.45[a]	7.83[a]
Native worm meal (T_2)	19.60[b]	41.61[b]	27.91[b]
Readymade fish feed (Tubifex) (T_3)	21.56[c]	39.73[c]	5.97[c]
Locally prepared fishmeal (T_4)	41.13[d]	40.85[bc]	5.55[cd]
Protein substituted with exotic worm meal (T_5)	43.78[e]	41.31[bc]	4.67[d]
Protein substituted with native worm meal (T_6)	45.04[f]	40.06[bc]	10.38[e]

Treatment values in a column followed by the same superscript do not differ significantly.

Total carbohydrate

The total carbohydrate was found to be significantly high (45.04%) in T_6 (protein substituted with native worm meal) followed by T_5 (43.78%) with protein substituted with exotic worm meal. The lowest carbohydrate (15.03%) was recorded in T_1 (exotic worm meal) followed by T_2 (19.60%) in native worm meal. Readymade fish feed recorded a value of 21.56% and locally prepared fish feed showed 41.13%. All the six feed materials showed significant variation.

Total protein

The total protein was significantly high (43.45%) in T_1 followed by T_2 (41.61%). Readymade fish feed registered the lowest value of 39.73% for total protein. The total protein content of locally prepared fish feed, protein substituted fish feed by exotic worm meal and native worm meal was 40.85, 41.31 and 40.06 per cent, respectively. The treatment T_1 differed significantly from other treatments and all other treatments were on par with respect to the protein content.

Amino acids

The qualitative determination of 20 amino acids was done in all the six feed samples and the results showed that about 16 amino acids were present in all the feed samples. The amino acids present in the feed samples were phenyl alanine, isoleucine, arginine, glycine, methionine, DL-alanine, L-leucine, L-tyrosine, glutamic acid, DL-serine, aspartic acid, nor-leucine, L-ornithine monohydrate, lysine monohydrate, histidine and amino butyric acid (Appendix II).

Ash

A high level (27.91%) of ash was observed in native worm meal, T_2 and the lowest level (4.67%) was observed in T_5 (protein substituted feed by exotic worm meal). Ash content in exotic worm meal, T_1 was 7.83%, in readymade fish feed, T_3 it was 5.97%, locally prepared fish feed, T_4 5.55% and protein substituted with native worm meal, T_6 10.38%. The treatment, T_4 was found to be on par with T_3 and T_5 and all other treatments differed significantly.

4.3.3. Mineral content of feed materials

The mineral contents of different feed materials are furnished in table 4.13. The mineral contents analyzed were calcium, magnesium, iron, phosphorus and potassium.

Table 4.13 Mineral contents of feed materials (% on dry weight basis)

Feed material	Calcium	Magnesium	Iron	Phosphorus	Potassium
Exotic worm meal (T_1)	0.09^a	0.45^a	0.13^a	0.41^a	3.51^a
Native worm meal (T_2)	0.06^b	0.52^a	0.35^b	0.36^b	3.94^b
Readymade fish feed (Tubifex) (T_3)	0.05^c	0.24^b	0.09^{ac}	0.41^a	0.35^c
Locally prepared fishmeal (T_4)	0.98^d	0.59^{ac}	0.03^c	0.32^c	2.95^d
Protein substituted with exotic worm meal (T_5)	0.10^e	0.69^c	0.10^a	0.46^d	6.46^e
Protein substituted with native worm meal (T_6)	0.08^a	0.86^d	0.66^d	0.40^a	7.32^f

Treatment values in a column followed by the same superscript do not differ significantly.

Calcium

Calcium content of the six fish feeds ranged from 0.05% to 0.98%. The highest calcium content was recorded in T_4 and the lowest calcium content was recorded in T_3. The calcium content of treatments T_1, T_2, T_5 and T_6 were 0.09, 0.06, 0.10 and 0.08 per cent, respectively. The treatments T_1 and T_6 were on par, and the treatments, T_1, T_2, T_3, T_4 and T_5 differed significantly.

Magnesium

The highest magnesium content was observed in T_6 (0.86%) and the lowest in T_3 (0.24%). The magnesium content of T_1 was 0.45% which was on par with T_2 (0.52%). The magnesium content of T_4 was 0.59% and that for T_5 was 0.69%. All other treatments differed significantly with respect to the magnesium content.

Iron

The iron content of different fish feeds were 0.13, 0.35, 0.09, 0.03, 0.10 and 0.66 per cent for T_1, T_2, T_3, T_4, T_5 and T_6, respectively. The treatment T_1 was on par with

T_3 and T_5. The treatment T_2 and T_6 were significantly different from all other treatments.

Phosphorus

The phosphorus content of fish feeds ranged from 0.32 to 0.46 per cent. The highest value for phosphorus was in protein substituted fish feed, T_5 and lowest value in locally prepared feed, T_4. The phosphorus content of exotic worm meal, native worm meal, readymade fish feed and protein substituted with native worm meal were 0.41, 0.36, 0.41 and 0.40 per cent, respectively. The treatments, T_1, T_3 and T_6 were on par and the treatments, T_2, T_4 and T_5 differed significantly from each other.

Potassium

All the six treatments differed significantly in potassium content. The highest value (7.32%) for potassium was recorded in treatment T_6 (protein replaced with native worm meal feed) and the lowest value (0.35%) was in readymade fish feed, T_3. The potassium content of exotic worm meal (T_1), native worm meal (T_2), locally prepared fish feed (T_4), protein substituted with exotic worm meal in locally prepared fish feed (T_5) were found to be 3.51, 3.94, 2.95 and 6.46 per cent, respectively.

4.3.4. Heavy metal contents of feed materials.

The heavy metal contents of different feed materials were presented in table 4.14. Only negligible amounts of heavy metals were observed in different feed materials.

Table 4.14 Heavy metal contents of feed materials (mg kg^{-1} on dry weight basis).

Feed material	Cadmium	Lead	Chromium	Nickel	Cobalt
Exotic worm meal (T_1)	ND	5.2^{a}	3.88^{a}	3.3^{a}	1^{a}
Native worm meal (T_2)	ND	4.9^{a}	0.18^{b}	7.5^{ab}	ND
Readymade fish feed (Tubifex) (T_3)	1.1^{a}	5.2^{a}	6.08^{c}	4.7^{ab}	ND
Locally prepared fishmeal (T_4)	1.0^{a}	ND	3.40^{a}	12.5^{b}	4^{b}
Protein substituted with exotic worm meal (T_5)	ND	5.2^{a}	1.47^{b}	7.3^{ab}	1^{a}
Protein substituted with native worm meal (T_6)	ND	2.8^{c}	4.45^{ac}	10.3^{ab}	ND

ND- Not Detected

Treatment values in a column followed by the same superscript do not differ significantly.

Cadmium

There were no detectable amounts of cadmium in $T_{1,}$ $T_{2,}$ T_5 and $T_{6.}$ Only a negligible amount of cadmium was recorded in T_3 and T_4 as 1.1 and 1.0 mg kg^{-1}, respectively. There was no significant difference in cadmium content of T_3 and $T_{4.}$

Lead

The lowest lead content was recorded in T_6 (2.8 mg kg^{-1}). The treatments $T_{1,}$ $T_{2,}$ T_3 and T_5 were on par and the value were 5.2, 4.9, 5.2 and 5.2 mg kg^{-1}, respectively. No detectable amount of lead was reported in T_4.

Chromium

The lowest value of chromium was recorded in T_2 (0.18 mg kg^{-1}) and the highest chromium content recorded in T_3 (6.08 mg kg^{-1}). The chromium content for treatment $T_{1,}$ $T_{4,}$ T_5 and T_6 were 3.88, 3.40, 1.47 and 4.45 mg kg^{-1}, respectively. The treatment T_1 was on par with T_4 and $T_{6.}$ Treatment T_2 was on par with $T_{5.}$

Nickel

The nickel content of different fish feeds were 3.3, 7.5, 4.7, 12.5, 7.3 and 10.3 mg kg^{-1} for $T_{1,}$ $T_{2,}$ $T_{3,}$ $T_{4,}$ T_5 and T_6, respectively. The treatment T_1 was found to be on par with $T_{2,}$ $T_{3,}$ T_5 and $T_{6.}$ Treatment T_4 was on par with $T_{2,}$ $T_{3,}$ T_5 and $T_{6.}$

Cobalt

The cobalt content of treatments T_1 and T_5 were recorded as 1 mg kg^{-1}. The highest cobalt content was recorded in T_4 (4 mg kg^{-1}) and no detectable amount of cobalt were reported in T_2, T_3 and $T_{6.}$ The treatment T_4 was significantly different from all other treatments.

4.3.5. Suitability of earthworm meal for aquaculture

Each fish feed was evaluated by feeding trials in six aquariums with ornamental fish, Red Oscar. Fishes were fed with the feeds once in a day and the changes in pH and electrical conductivity of the aquarium water were observed at weekly intervals and the data are given below in table 4.15 and 4.16;

Table 4.15 pH of aquarium water observed at weekly intervals

Week	**Treatments**					
	T_1	T_2	T_3	T_4	T_5	T_6
1	7.04	7.04	7.04	7.04	7.04	7.04
2	7.26	7.31	7.98	7.89	7.70	7.81
3	7.81	7.90	8.49	8.61	7.87	7.89
4	8.02	8.10	8.97	9.00	8.04	8.07
5	8.55	8.52	9.44	9.48	8.37	8.36
6	8.72	8.74	9.44	9.48	8.40	8.39
7	8.81	8.79	9.46	9.48	8.43	8.44
8	8.92	8.94	9.46	9.49	8.49	8.51
9	9.00	9.01	9.48	9.49	8.53	8.55
10	9.02	9.04	9.51	9.54	8.68	8.75
11	9.04	9.04	9.58	9.58	8.79	8.88
12	9.04	9.05	9.58	9.59	8.88	8.90

The original pH of aquarium water was 7.04 in all the fish tanks. Gradual increase can be seen in all the fish tanks up to 9 to 10 weeks. The increases in pH of all the fish tanks were in a comparable range. Almost constant pH values were recorded for all the treatments within 12 weeks after initiation of the experiment.

Table 4.16 Electrical conductivity (dS m^{-1}) of aquarium water observed at weekly intervals

Week	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
1	0.071	0.071	0.071	0.071	0.071	0.071
2	0.109	0.110	0.137	0.132	0.078	0.079
3	0.223	0.232	0.201	0.198	0.081	0.081
4	0.298	0.330	0.289	0.291	0.089	0.090
5	0.338	0.338	0.333	0.333	0.105	0.105
6	0.339	0.339	0.338	0.337	0.108	0.107
7	0.341	0.343	0.339	0.340	0.110	0.111
8	0.347	0.348	0.342	0.343	0.114	0.114
9	0.348	0.348	0.343	0.343	0.115	0.115
10	0.348	0.348	0.343	0.343	0.115	0.115
11	0.349	0.348	0.343	0.343	0.115	0.115
12	0.349	0.348	0.343	0.343	0.115	0.115

The original electrical conductivity (EC) of all the fish tanks was 0.071 dS m^{-1} in initial days. A gradual increase of EC was registered in all the six fish tanks. The EC value registered were high in treatments, T_1, T_2, T_3 and T_4. Low values were recorded in treatments, T_5 and T_6.

Comparative economics

The economics of six feed materials were calculated in terms of protein, carbohydrate and fat equivalents. The values are presented in table 4.17

Table 4.17 Comparative economics of six feed materials

Feed material	Cost of production for 1 kg feed material	Protein equivalent	Carbohydrate equivalent	Fat equivalent	Composite value
	Rs/kg				
Exotic worm meal (T_1)	5700.00	131.18	379.24	551.79	354.07
Native worm meal (T_2)	3850.00	92.53	196.43	372.70	220.55
Readymade fish feed (Tubifex) (T_3)	6666.66	167.78	309.18	1083.90	520.28
Locally prepared fishmeal (T_4)	830.00	20.32	20.18	47.89	29.47
Protein substituted with exotic worm meal (T_5)	4200.00	101.67	95.93	235.55	144.38
Protein substituted with native worm meal (T_6)	4854.00	121.16	101.04	260.13	160.77

Among the six feed materials, locally prepared fish feed (29.47 Rs/kg) was found to be the cheapest followed by protein substituted with exotic worm meal (144.38 Rs/kg) and native worm meal (160.77 Rs/kg) in terms of protein, carbohydrate and fat equivalents. The readymade fish feed, Tubifex was very costly (520.28 Rs/kg). The composite values for exotic worm meal and native worm meal were 354.07 and 220.55 Rs/kg, respectively.

Discussion

5. DISCUSSION

The results of various experiments of the project on "production and evaluation of proteinaceous earthworm meal" presented in chapter 4 are discussed below:

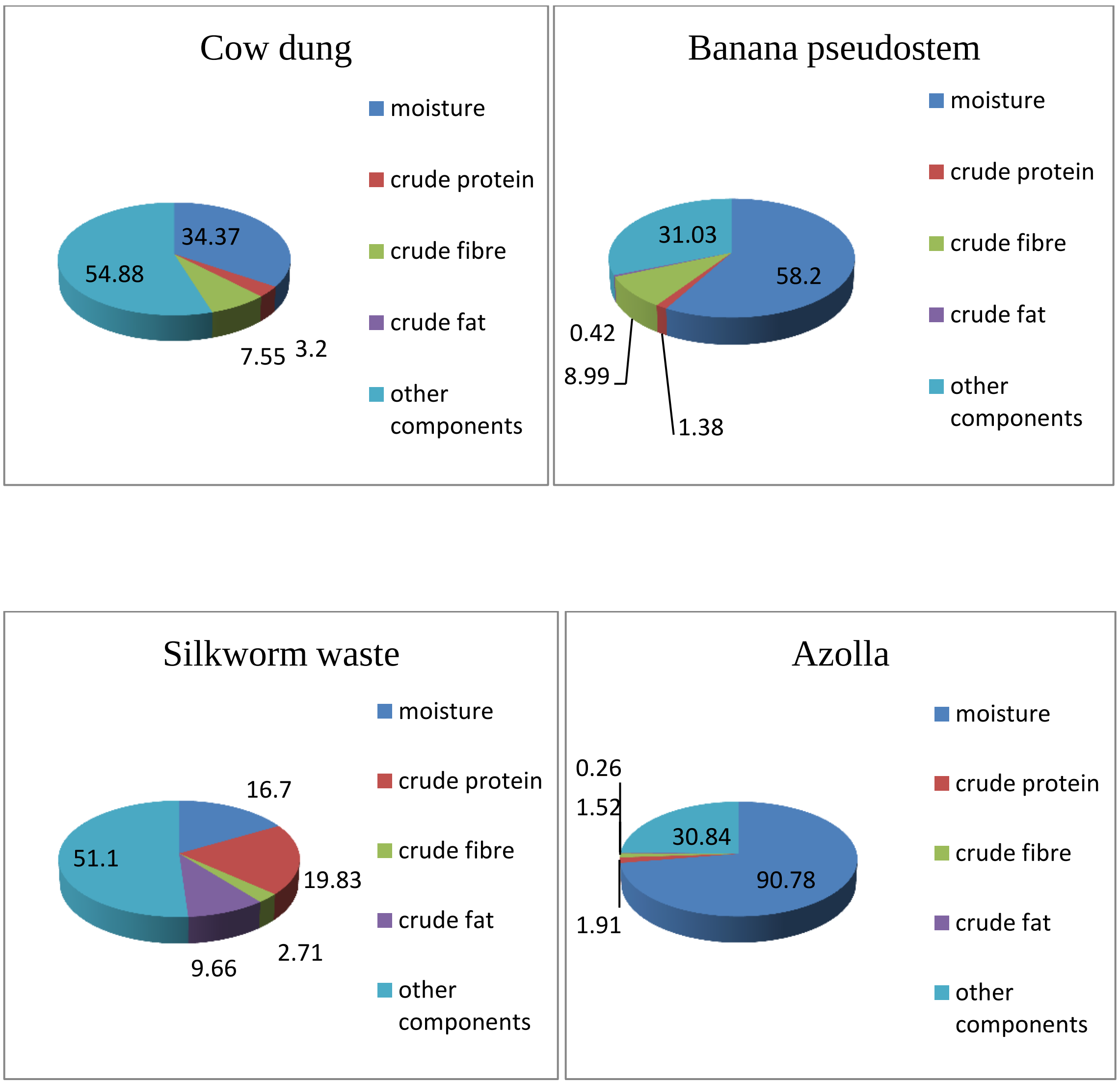
5.1 COMPARATIVE EVALUATION OF DIFFERENT SUBSTRATES FOR THE MASS MULTIPLICATION OF EXOTIC EARTHWORM, *Eisenia foetida*

5.1.1 Biochemical properties of different substrates used for vermicomposting

As detailed in section 4.1.1., the biochemical properties of all the three substrates showed wide variations in their important biochemical components along with their respective moisture contents. The data are depicted in fig. 5.1. The highest moisture content was found in water fern azolla (90.78%) followed by fibrous banana pseudostem (58.20%) and cow dung (34.37%). The lowest moisture content (16.70%) was recorded in silkworm wastes, including double cocoon, urinated cocoon, diseased and dead larvae cocoon and waterjelly. Cow dung was the ideal substrate for vermicomposting with its optimum moisture content which favoured high proliferation of micro fauna and flora. Banana pseudostem was not ideal since the moisture content of the substrate may result in water logged conditions in the compost bins. A cost effective vermicomposting technique is recommended in the Kerala Agricultural University Package of Practices of Crops (KAU, 2007) using banana pseudostem and cow dung in the ratio 8:1. In order to increase the microbial load, the different substrates are to be mixed with cow dung in vermicomposting process.

Crude protein is the main source of nitrogen which is required for the body buildup of earthworms. The highest content (19.83%) was observed in silkworm waste which comprised of the cocoon waste and pupae of silkworms as the source of protein. This is followed by azolla which fixes the atmospheric nitrogen with the help of blue green algae *Anabena azolla* in the heterocysts and so the host tissues of the fern, azolla

Figure 5.1. Biochemical composition of substrates used for vermicomposting



is rich in crude protein content (1.91%). The crude protein content of cow dung was 3.20% and banana pseudo stem was 1.38%.

In contrast to the contents of crude protein, the highest crude fibre content (8.99%) was recorded for banana pseudostem, followed by cow dung (7.55%) and silkworm waste (2.71%). The lowest crude fibre content (1.52%) was estimated for azolla.

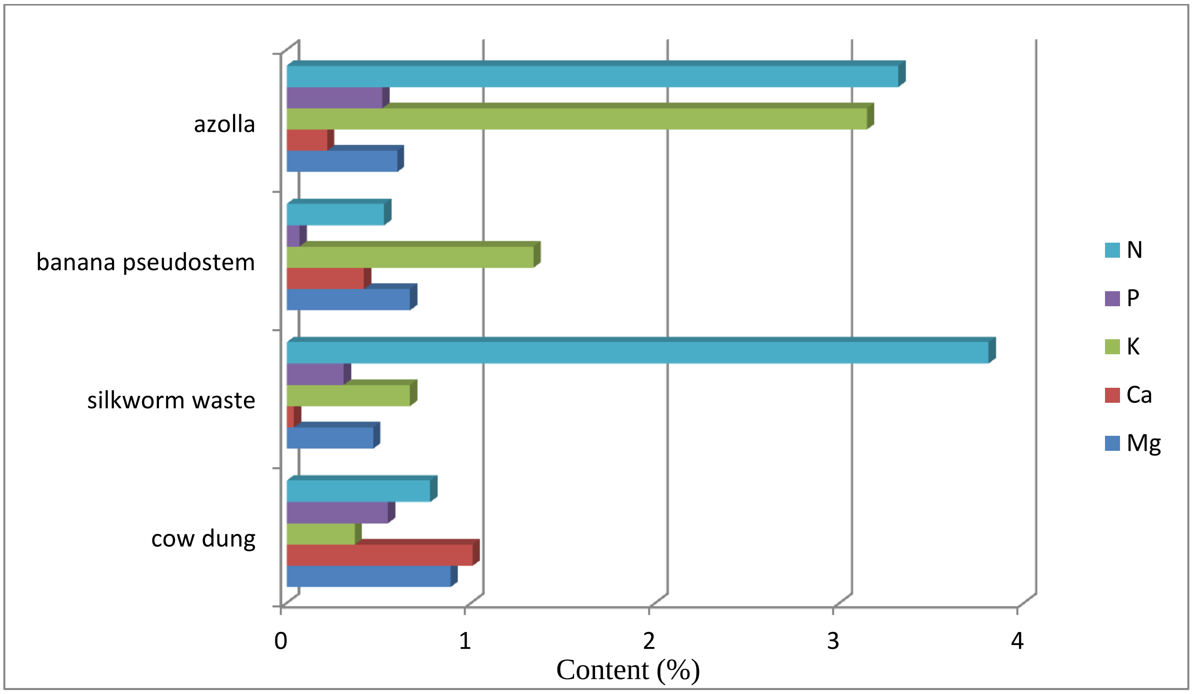
Along with crude protein, the silkworm waste is found to be rich in crude fat (9.66%). Both azolla and banana pseudostem registered low values for crude fat and there is no crude fat content detected in cow dung. As earthworms are saprophagous animals, their diet comprised mainly of organic detritus in various stages of decay. In order to fasten the decomposition of organic matter, cow dung with living micro organisms, microfauna and mesofauna is necessary. Datar *et al*. (1997) reported that cow dung was the most suitable of all the animal wastes for earthworm rearing.

5.1.2. Physicochemical properties of the substrates

The substrates pH, carbon, nitrogen, phosphorous, potassium, calcium and magnesium are presented in table 4.2. and are graphically represented in fig. 5.2. An optimum pH of 6.7 was registered in silkworm waste and in cow dung it was 6.1 and all other substrates recorded an alkaline pH range. In the case of banana pseudostem it was 7.5 and azolla it was 7.9. A wide range of carbon content was reported in substrates and due to the wide variations in carbon content, the decomposition of the substrates may vary. The carbon content also influenced the proliferation of earthworms depending upon its palatability and easy availability. A high value of carbon (87.02%) was registered in silkworm waste. The lowest value (32.50%) was recorded in cow dung.

The nitrogen content was also high in silkworm waste (3.81%) followed by azolla (3.32%) and the lowest nitrogen content (0.53%) was reported in banana pseudostem. The variation in the nitrogen content of different substrates may affect the

Figure 5.2. Nutrient contents of different substrates used for vermicomposting



worm multiplication since nitrogen acted as the major body building component of earthworms.

The C/N ratio of the different substrates and the worm casts resulted are depicted in fig. 5.3. and 5.4. This ratio affected the mineralization efficiency of the substrate material. The loss of carbon as carbon dioxide through microbial respiration and simultaneous addition of nitrogen by microflora and fauna are very important in any composting process. The ideal C/N ratio for vermicomposting ranged from 20 to 25 (Ndegwa and Thompson, 2000). So the silkworm waste with C/N ratio 22.89 was found to be the most ideal one. This is being compared with banana pseudostem with wide C/N ratio (72.83) and narrow C/N ratio (15.52) of azolla. However, cow dung with C/N ratio 41.66 is already proven as an excellent growth medium for earthworms with its readily available carbon and low concentration of growth retarding substances. Moreover, the vermi-beds with cow dung may supply easily metabolisable organic matter and non assimilated carbohydrates. Moreover the crop residues such as banana pseudostem or silkworm waste have different palatability, protein and crude fibre contents. Even some plant metabolites that are present in the azolla may restrict the growth of microbes along with the narrow C/N ratio. However it is suggested that cow dung is to be mixed with appropriate quantities of other carbonaceous substances so as to favour microbial enrichment which ensures the composting process, since vermicomposting is a non thermophilic biodegradation of organic materials through the interaction between the earthworms and microorganisms resulting the production of vermicompost (Edward and Burrows, 1988).

In the case of phosphorous content, a high value was reported in cow dung (0.55%) followed by azolla (0.52%) and silkworm waste (0.31%). A low value of 0.07% was recorded in banana pseudostem. The maximum value of potassium was reported in azolla (3.15%) followed by banana pseudostem (1.34%). The minimum values were recorded in cow dung (0.37%) and silkworm waste (0.67%). Micronutrients and heavy metals were also seen in all the substrates. The variation in heavy metal contents are attributed to their contents in feed stock given to the animals.

Figure 5.3. A comparison between the C/N ratio of cow dung, substrates and their worm casts

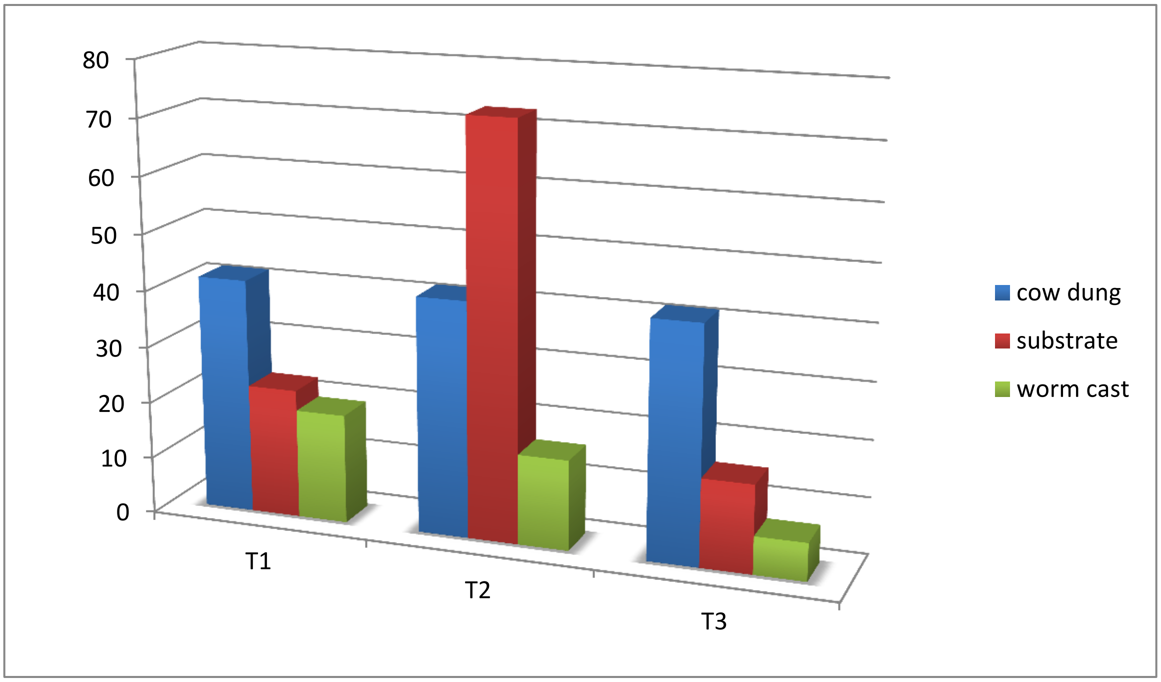
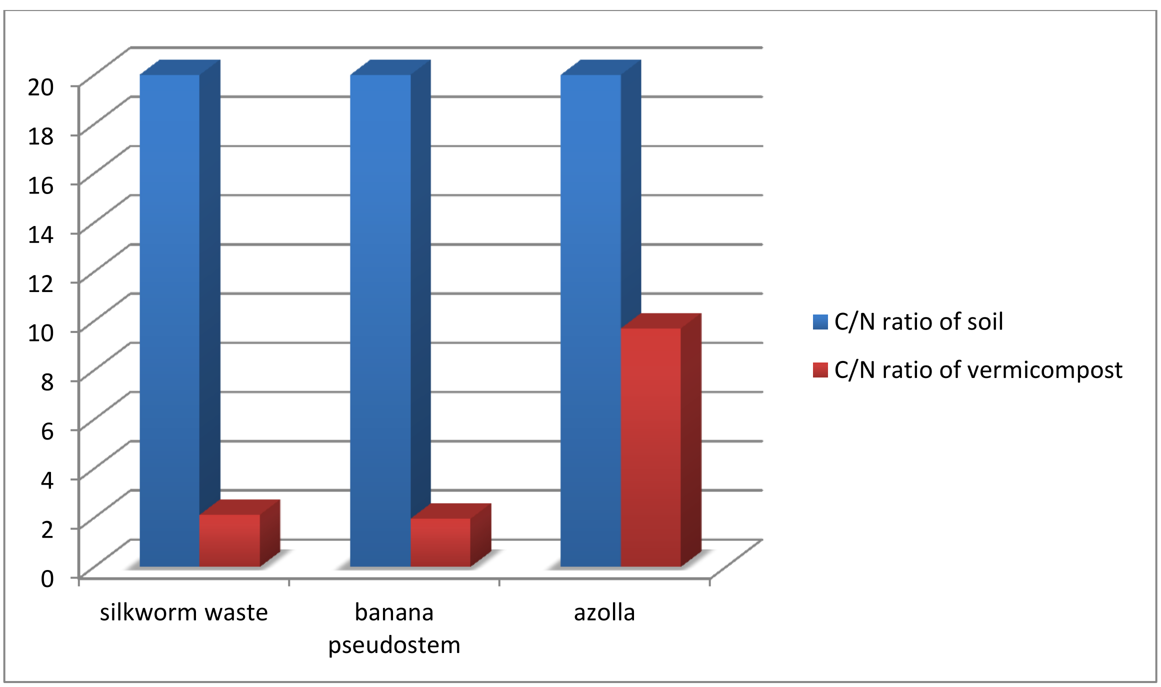


Figure 5.4. A comparison between the C/N ratio of laterite soil and vermicompost including soil



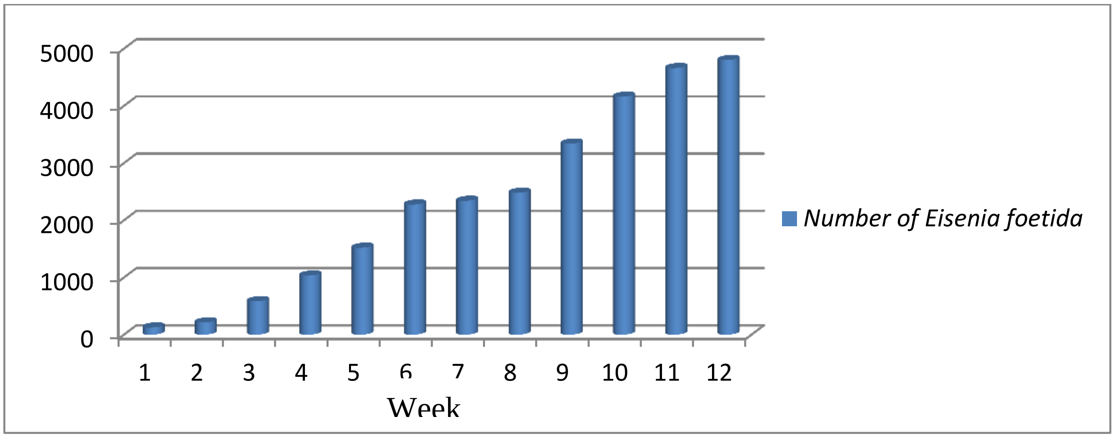
Since the cow being a milch animal, may be fed with concentrates rich in minerals, with traces of heavy metals. Among the substrates azolla recorded a high amount of heavy metals and this may be due to the application of rajphos during azolla multiplication. It was recorded that rajphos, a rock phosphate source contributed substantial amount of cadmium (35mg kg^{-1}) and lead (91mg kg^{-1}). This is in conformity with the findings of Vanishri (2004).

5.1.3. Influence of substrates on earthworm count

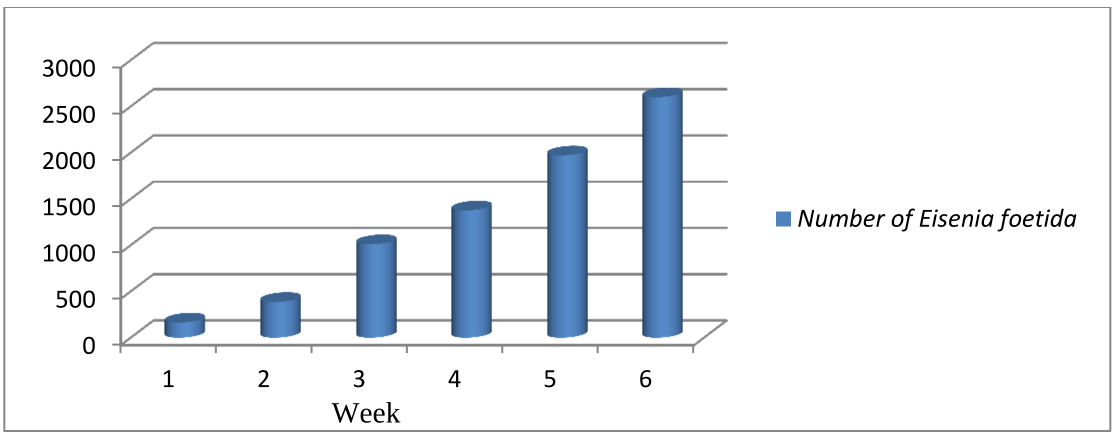
Earthworms are able to consume a wide range of organic wastes such as sewage sludge, animal dung, crop residues and industrial refuse. The factors relating to growth of earthworm may be considered in terms of physicochemical and nutrient characteristics of waste feed stock (Suthar, 2007). The earthworm counts (*Eisenia foetida*) at different stages of vermicomposting as influenced by different substrates are depicted in fig. 5.5. The maximum number of worm multiplication was found in T_3 (cow dung: azolla) followed by T_1 (cow dung: silkworm waste). The high rate of worm multiplication in T_1 and T_3 may be due to the presence of high nitrogen content. The earthworm multiplication was found to be minimum in T_2 with cow dung and banana pseudostem as substrates. The low multiplication of worms in T_2 may be due to the high moisture content in banana pseudostem as discussed in section 4.1.1. Even though the worm multiplication in T_2 was at low rate, the compost matured within 6 week time along with T_3. The T_1 takes 12 weeks time for attaining the compost maturity. This is because the decomposition of silkworm waste was very slow compared to the banana pseudostem and azolla due to the high organic carbon content as indicated in section 4.1.1. The earthworms are getting more organic matter to flourish in T_3 which may not be available in T_1 and T_2. In the latter cases, the substrate carbon is getting reduced due to the rapid mineralization process. Because of the slow decomposition of substrate material it takes more time for compost maturity and a high rate of worm multiplication was obtained at the compost maturity period.

Figure 5.5. The earthworm count (*Eisenia foetida*) at different stages of vermicomposting as influenced by different substrates

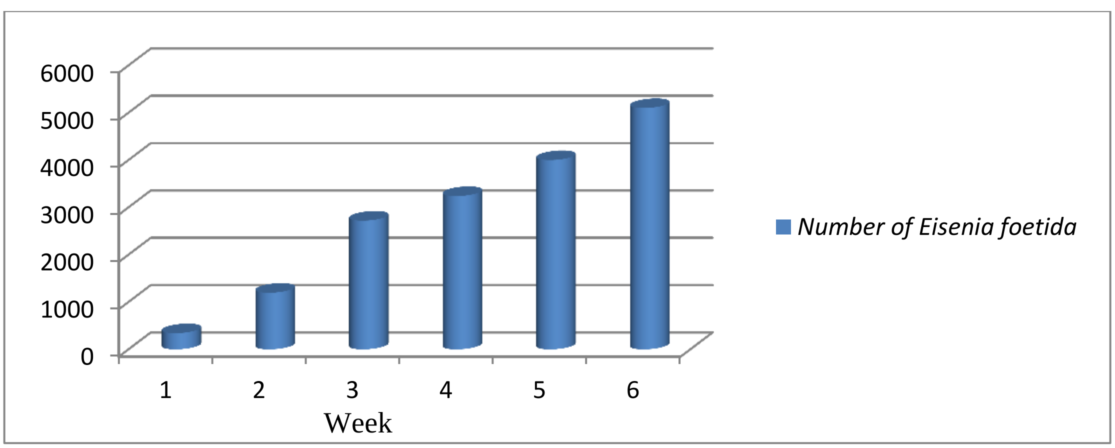
a) Silkworm waste as the main substrate



b) Banana pseudostem as the main substrate



c) Azolla as the main substrate



5.1.4. Manurial value of worm cast as influenced by different treatments

The manurial value of worm casts as influenced by different treatments are presented in table 4.5 and depicted in fig. 5.6. Worm castings are the earthworm excreta together with their cocoons and undigested feed. Earthworms eat a wide variant of organic matter. These feed materials undergo complex biochemical changes in the intestine of worms and are excreted in the form of granular worm casts.

pH

Worm cast is in general neutral in pH and a rich source of calcium as the earthworms possess calciferous glands which contain calcigenous cells which secrete amorphous calcium carbonate which crystallizes in the lumen of the oesophageal pouch to form spherolite of calcite, which are excreted through their casts. This also increases the pH of worm cast. pH of different substrates and their influence on worm casts were presented in fig. 5.7.

Carbon

The carbon content of T_1 was found to be 27.05%, of T_2 21.35% and T_3, 9.56%. The earthworm promotes microbial proliferation in compost bins that increases the loss of organic carbon from substrates through microbial respiration. A part of the carbon in the decomposing substrate evolved as carbon dioxide and a part was assimilated by microbial biomass. It was reported that there will be 58.4% reduction in the organic carbon during vermicomposting of cow dung for 90 days (Garg *et al*., 2005). Reduced concentration of carbon and enhanced nitrogen level as a consequence of microbial activity during humification process resulted in reduction of C/N ratio ranging from 10-12 which is usually considered to be an indication of stable and decomposed organic matter (Jimenez and Garcia, 1992).

Nitrogen

The nitrogen content of worm cast is the resultant of initial nitrogen content of the substrate and the degree of decomposition of organic matter. So the highest

Figure 5.6.Mineral composition (N, P, K, Ca and Mg) of the exotic earthworm cast

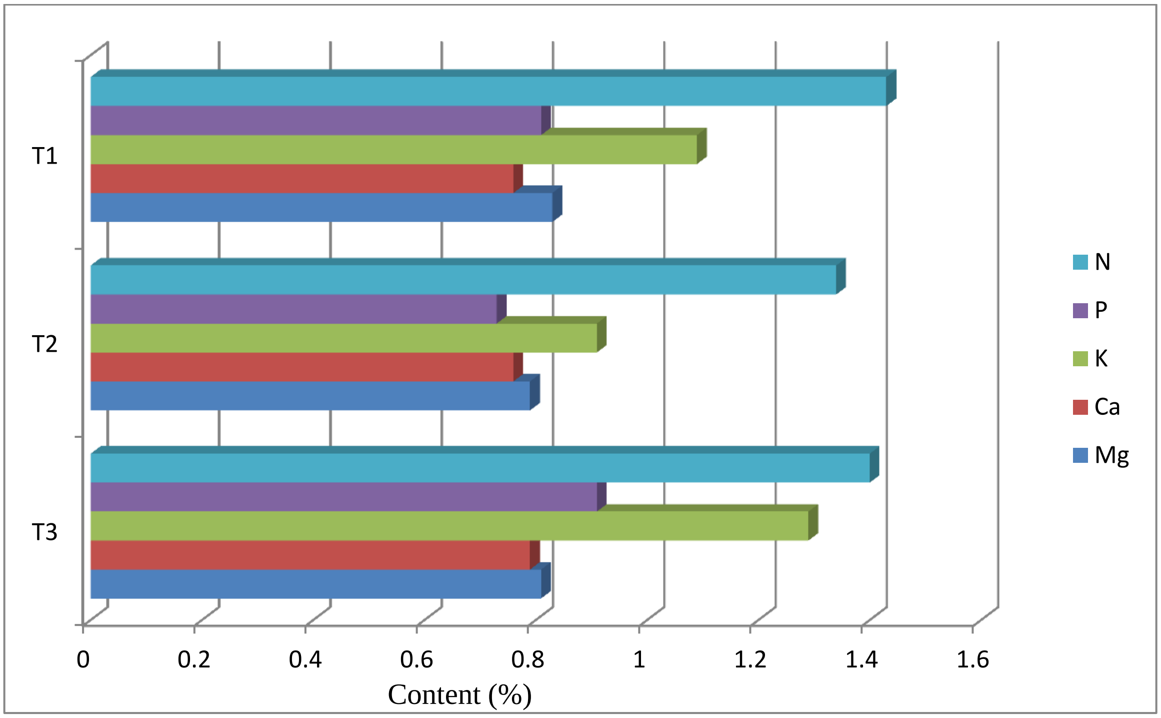
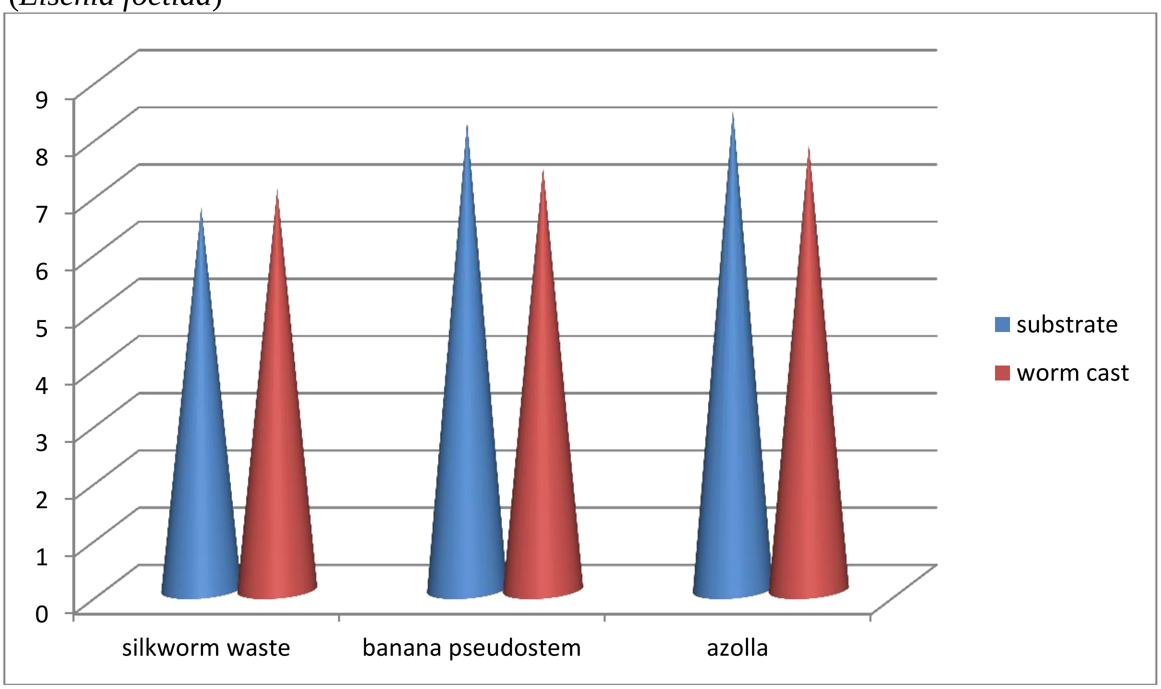


Figure 5.7 pH of different substrates and their influence on pH of worm casts (*Eisenia foetida*)



nitrogen content in worm cast was recorded in T_1 (1.43%) with cow dung: silkworm followed by T_3 (1.40%) with cow dung: azolla and the lowest value (1.34%) was registered in T_2 with cow dung: banana pseudostem. Even though the different substrates used for worm multiplication varied widely in nitrogen content, the worm cast showed no significant difference in nitrogen content. But the increase and decrease in the nitrogen content of substrates may be due to the mineralization of organic matter which included not only the substrates but also the cow dung with high nitrogen content. At this point it is also evident that the worm cast contained nitrogen in the form of nitrogenous excretory substances, growth hormones and enzymes.

Phosphorous

There is an increase in phosphorus content of worm casts compared with the substrates due to vermicomposting. Due to the direct action of worm gut enzymes and stimulation of microflora the phosphorus content increased. The increase in phosphorus content may be due to the bacterial and faecal phosphate activity of earthworms (Garg *et al*., 2006).

Potassium

The highest potassium content was recorded in T_3 (cow dung: azolla in 1:1 proportion) with 3.58% and the lowest value (0.90%) was recorded in T_2 (cow dung: banana pseudostem in 1:1 proportion). While considering the K content of substrate, an increase in K content was not at all observed in any of the treatments. There was significant reduction in total K content by the end of vermicomposting due to high water solubility and leaching of potassium during composting (Elvira *et al*., 1998). As detailed in section 4.1.2 there was high potassium content in azolla and so T_3 recorded the highest potassium content. Among different substrate combinations with cow dung, the maximum K content was registered with cow dung: azolla in 1:1 proportion and the resulting worm cast also registered high K content.

Calcium

The calcium content of the worm casts from different substrates showed no significant difference. There is no influence for the different substrates on the calcium content of resultant worm casts. It may be due to antagonism reaction between alkali and alkaline earth elements, which is present in the final product. Due to composting, the total calcium increased in the worm cast than in the substrates. So the calcium and magnesium contents of the worm cast were dependent on the chemical nature of raw materials. The decrease in calcium and increase in magnesium was due to the antagonism between the two elements.

Magnesium

The magnesium content of the worm casts from different substrates showed no significant difference. It is evident that the magnesium content of different substrates does not influence the magnesium content of worm casts.

Micronutrients and heavy metals in worm casts

The micronutrient and heavy metal contents in worm casts were given in table 4.5. The worm casts are found to be lesser in the contents of cadmium, lead, chromium, nickel and cobalt. The increase in the contents of micronutrients may be due to the increase in the mineralization of organic matter. With the substrate, carbonaceous silkworm waste the increase in contents is found to be prominent. The presence of heavy metals is more common in the case of agro wastes like banana pseudostem, silkworm waste and farm yard manure. This may result in accumulation of heavy metals in worm casts. The reduction in contents of heavy metals in worm casts may be due to the bioaccumulation of the same in the bodies of earthworms. As compared to different substrates, the contents of heavy metals are less in worm casts.

An increase in the iron content of substrates during vermicomposting was observed in all the three treatments. This may be due to the worm and microbial activities during composting. In case of manganese also, there is an increase in worm casts as compared to different substrates due to the worm activity. Zinc content of worm cast is found to be increased due to vermicomposting of substrates along with cow dung. A high content of copper was also observed in worm casts of all treatments than the substrates. Irrespective of the treatments, the micronutrient contents are found to be increased with the mineralization of carbon present in the substrates.

The cadmium content of worm casts was found to be low when compared with the contents in substrates. Considerable decrease in lead content was also observed during vermicomposting. Chromium content was found to be decreased during vermicomposting. Nickel content was also found to be reduced during composting. There was no detectable amount of cobalt in treatments, T_1 and T_2. In T_3 a negligible amount of cobalt was reported. This may be due to the presence of cobalt in azolla as indicated in section 4.1.2.

5.2. VIABLE TECHNIQUES FOR CULTURING NATIVE EARTHWORMS

5.2.1. Physicochemical properties of soil

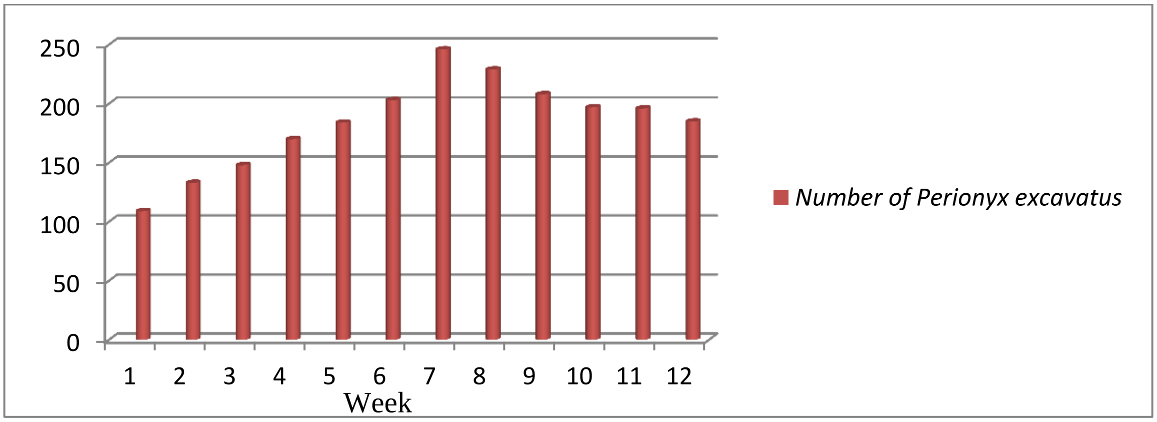
The soil selected for the study was slightly acidic in action with a pH of 6.3 and the organic carbon content was at the medium level (0.86%). The available nutrient contents of soil was 232.08 kg ha^{-1} available N, 6.80 kg ha^{-1} available P, 72.04 kg ha^{-1} available K, 2.20 cmol(p^+) kg^{-1} Ca and 0.23 cmol(p^+) kg^{-1} Mg.

5.2.2. Influence of substrates on earthworm count

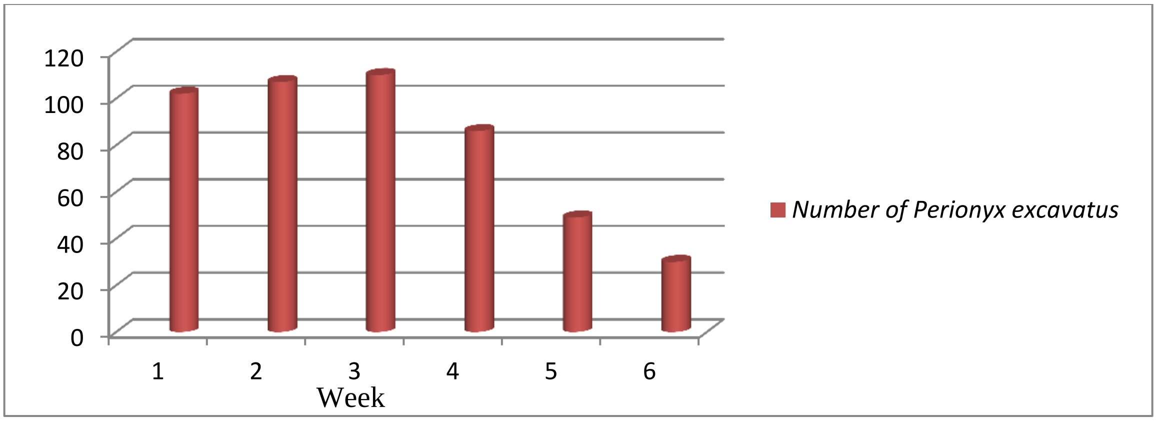
An initial increase in worm count and gradual decrease was observed in all the three treatments during the period of vermicomposting. The earthworm count (*Perionyx excavatus*) at different stages of vermicomposting, influenced by different substrates is presented in fig. 5.8. The increase in number of worms was observed up to three weeks interval in T_2 and T_3 and then a mortality of worms was observed after three weeks. The worm multiplication was recorded up to seven weeks in T_1 and then a mortality of worms was observed afterwards. Eventhough a mortality of worm was

Figure 5.8. The earthworm count (*Perionyx excavatus*) at different stages of vermicomposting as influenced by different substrates

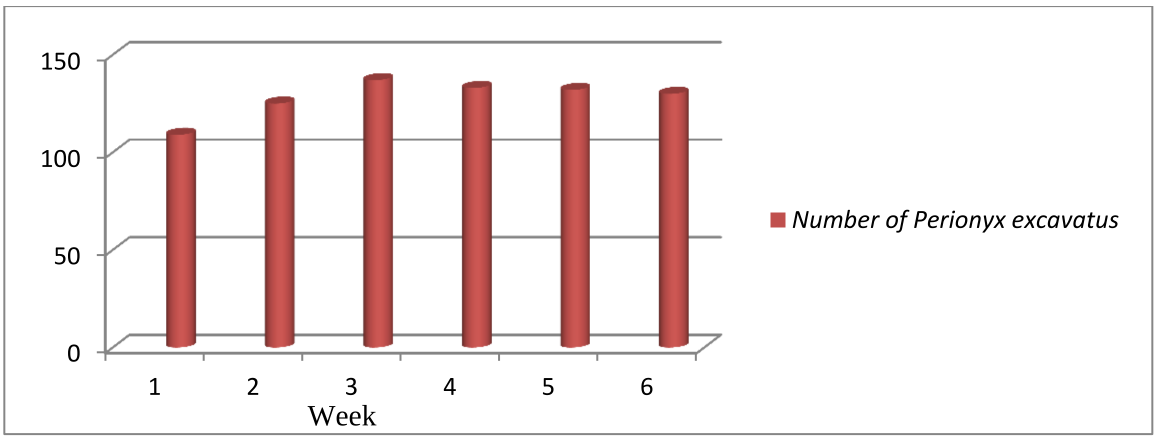
a) Silkworm waste as the main substrate



b) Banana pseudostem as the main substrate



c) Azolla as the main substrate



reported in T_2 the worm multiplication was found to be high in comparison with the T_1 and T_3.

The worm multiplication rate was higher in T_1 (silkworm waste: cow dung: soil) as substrate and this is due to the high nitrogen content of the substrates, silkworm waste together with cow dung and soil. Nitrogen imparted a part of protein and this will help the multiplication of worms. Mortality of worms was observed in all treatments and this may be due to the lack of favourable environmental conditions suitable for the worm multiplication of native worms. The native worms were usually adapted to the natural environmental condition and their reproduction may be affected by the climatic conditions.

5.2.3. Manurial value of worm casts

The manurial value of worm casts of all the three treatments are presented in table 4.9 and depicted in fig. 5.9.

pH

The worm cast is in general neutral in pH irrespective of the pH of substrates. The pH of different substrates and their influence on worm cast are depicted in fig. 5.10.

Carbon

The highest carbon content (2.90%) was recorded in T_3 with cow dung: azolla: soil in 1:1:1 proportion and the lowest value (2.07%) was recorded in T_1 with cow dung: silkworm waste: soil in 1:1:1 proportion.

Nitrogen

The highest N content (0.78%) was recorded where the main substrate used was a nitrogen fixing fern azolla. The low N content (0.50%) was observed in treatment with silkworm waste as the main substrate.

Figure 5.9. Mineral composition (N, P, K, Ca and Mg) of native earthworm cast

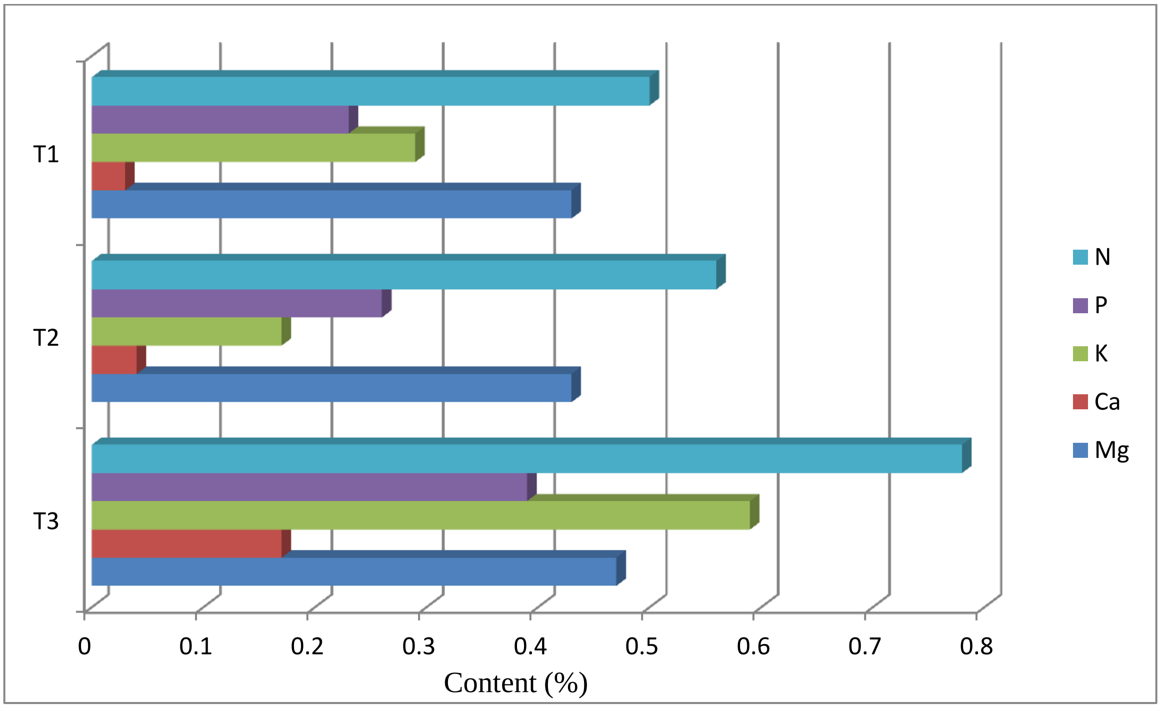
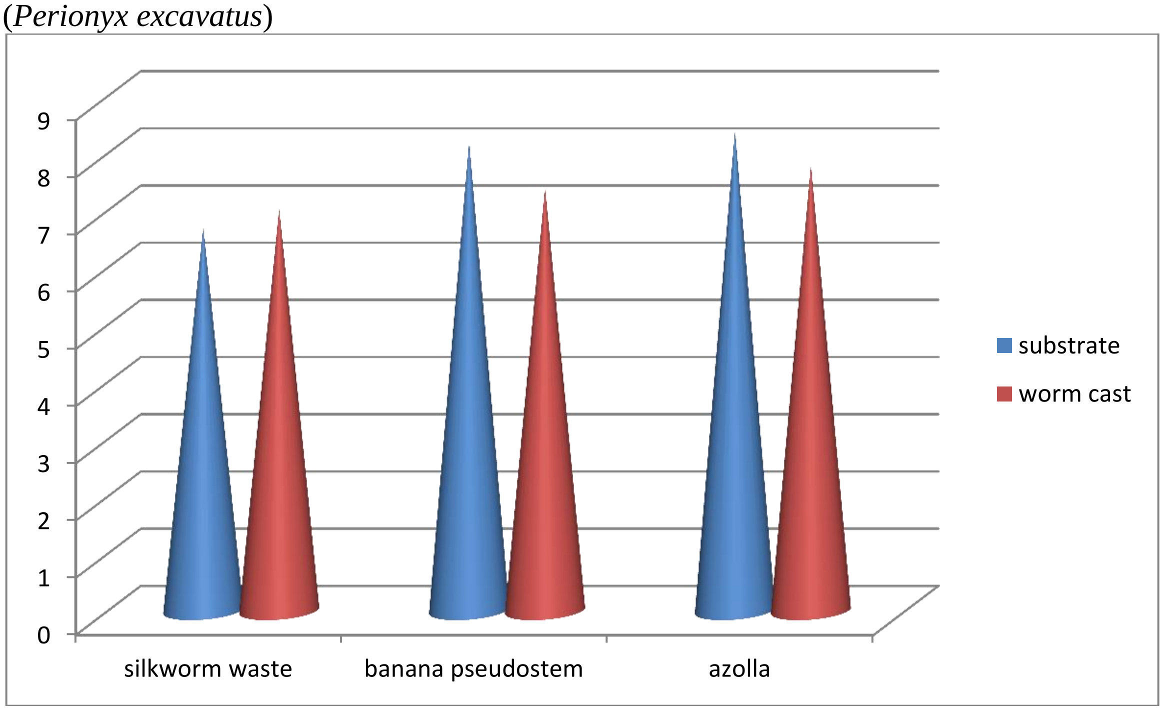


Figure 5.10 pH of different substrates and their influence on pH of worm casts (*Perionyx excavatus*)



Phosphorus

There was an increase in phosphorus content of worm cast as compared to the P content of substrates due to vermicomposting.

Potassium

The potassium content of worm cast was found to be decreased due to the high solubility of potassium, which lead to loss by leaching and due to the adsorption by clay and organic colloids.

Calcium

The highest calcium content was recorded in T_3 followed by T_2 and T_1.

Magnesium

The treatment, T_3 with cow dung: azolla: soil in 1:1:1 proportion showed the highest magnesium content.

Micronutrient and heavy metal contents in worm casts under different treatments

The iron content of worm cast was found to be increased at a high rate during vermicomposting than the substrate's iron content. Manganese content of both the substrates and worm casts varied widely. An increase in manganese content was observed in worm casts. The zinc content was found to be low in T_1 (9 mg kg^{-1}) and T_2 (2 mg kg^{-1}). The zinc content was found to be high in T_3 (54 mg kg^{-1}). The copper content of worm casts was increased when compared to the substrates. All these observations lead to the conclusion that there is rapid mineralization and release of micronutrients during vermicomposting process.

A low level of cadmium was registered in all the three treatments. The lead content of T_1 and T_3 was found to be 68 mg kg^{-1}. A low lead content was recorded in T_2. A decrease in chromium content was observed in worm casts compared to the substrates. Nickel content was found to be decreased. A negligible amount of cobalt was reported in worm casts irrespective of the treatments. Bioaccumulation of heavy metals in the worm bodies are the main reason this phenomenon.

5.3. PREPARATION OF EARTHWORM MEAL AND COMPARATIVE EVALUATION OF NUTRITIVE CONTENT OF DIFFERENT EARTHWORM MEALS

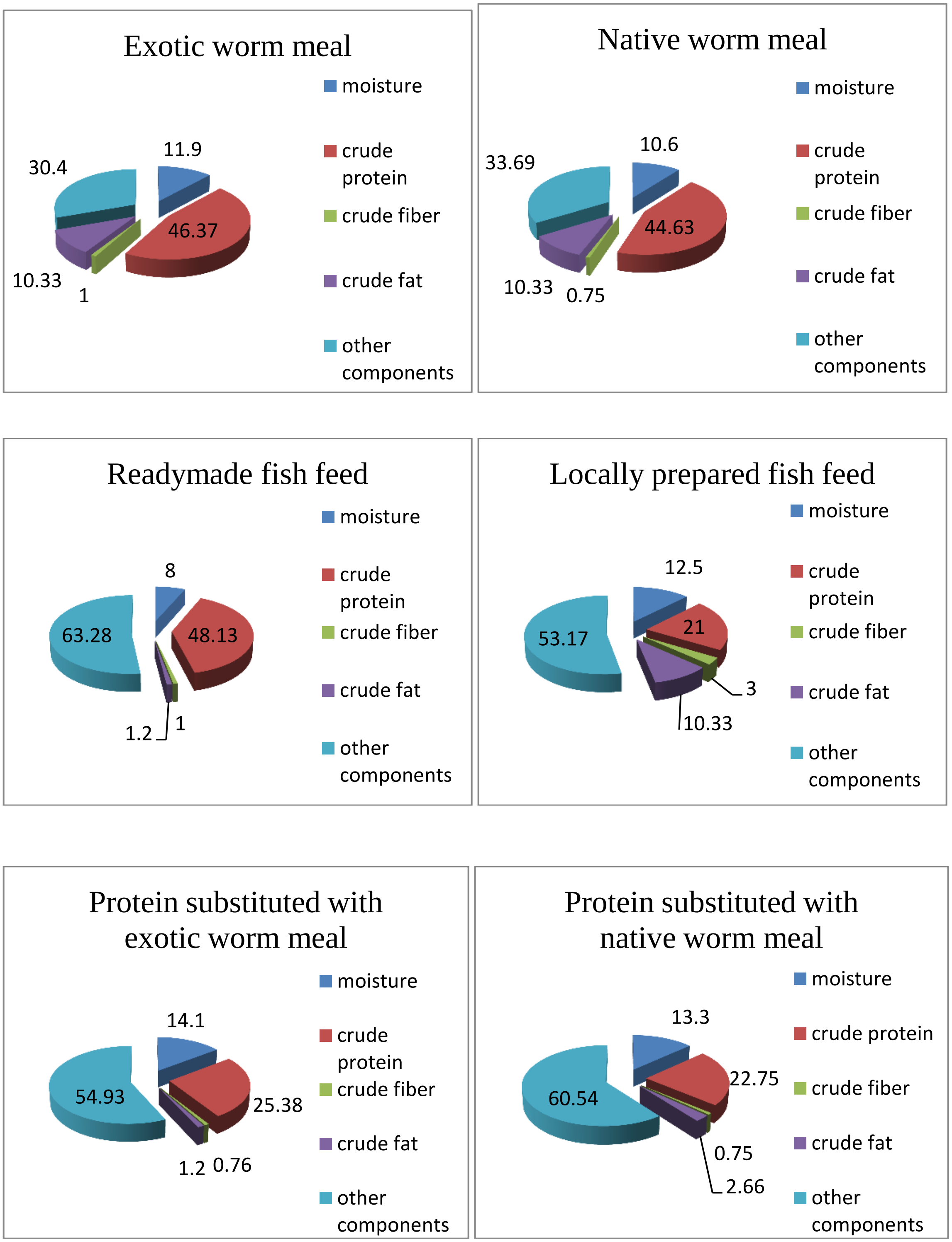
As described in section 3.3.1, different worm meals were prepared and were compared to locally prepared and readymade fish feeds for the biochemical composition and nutritive value in order to check the suitability of using worm meal as a fish feed. Mineral and heavy metal contents of the feeds are evaluated and presented in section 4.3.3.

5.3.1. Biochemical composition of different worm meals as compared to locally prepared and readymade fish feeds

The biochemical composition of different worm meals was compared to locally prepared and readymade fish feeds and the results were presented in table 4.11. and depicted in fig. 5.11. From the data it is clear that all the feeds are within the moisture range of 8 to 14% which is within the desirable range. The crude protein content of exotic and native worm meal were found to be 46.37% and 44.63%, respectively. The highest crude protein (48.13%) was recorded in readymade fish feed available in market and the lowest value (41.75%) was recorded in protein substituted with native worm meal. But comparing the locally prepared fish feeds the crude protein content was high in worm meal preparations. Since the protein is essential for the growth and performance of fishes it may suit the fishes as a protein rich feed. The fish feeds mainly contained protein as a major part of their diet about 40% protein is required. The other components are also found higher for the prepared worm meals. It may be concluded that both exotic and native worm meals are as such rich in all components and so may be marketed as a protein rich fish feed.

Even though the crude protein content was low in locally prepared fish feed compared to worm meals, the crude fibre content was found to be high (3.00%) and the

Figure 5.11 Biochemical composition of worm meals



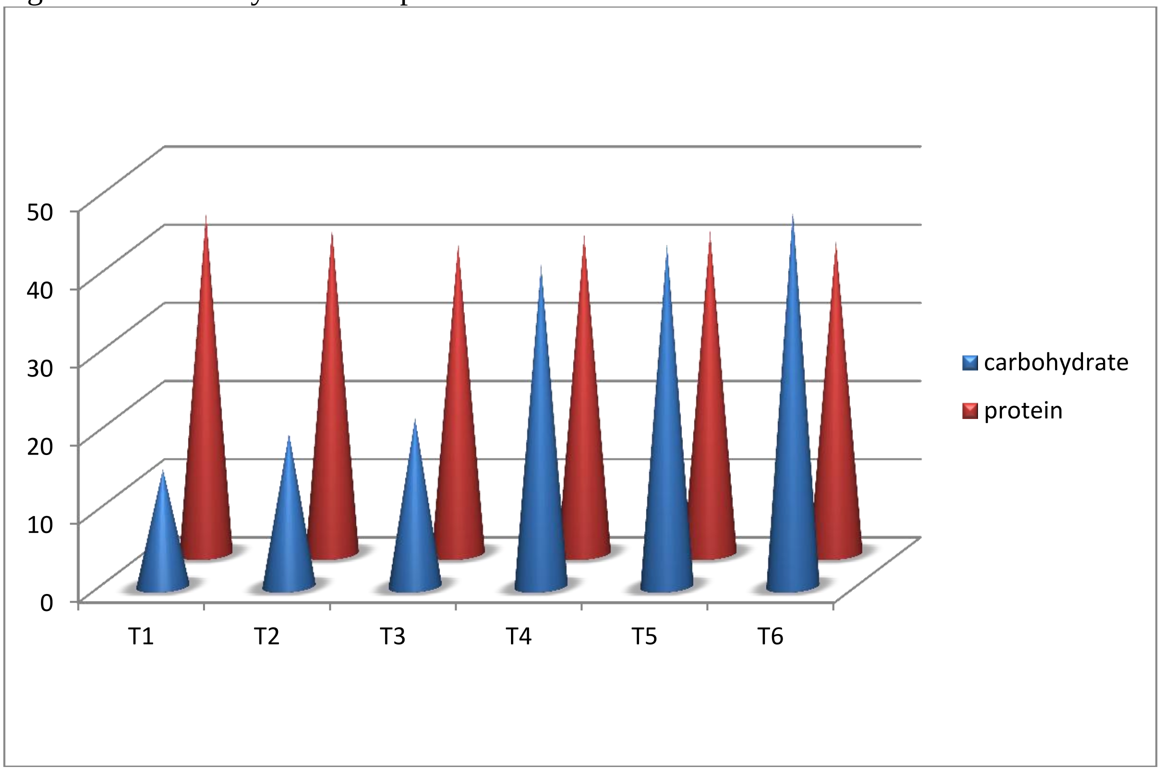
crude fibre content of worm meals were comparatively low (0.75 to 1%). The crude fat content recorded a high value in protein substituted fish feeds by worm meals and the locally prepared feed. There is a low (6.15%) crude fat content reported in readymade fish feed available in market. The per cent moisture content was found to be high in fish feeds prepared by replacing the protein source with exotic and native worm meals.

5.3.2. Primary metabolites of feed materials

The content of primary metabolites in different feed materials was presented in table 4.12 in section 4.3.2. and depicted in fig. 5.12. The highest values for carbohydrates were found in the feed materials which were prepared by substituting the exotic and native worm meals as protein source. This may be due to the addition of carbohydrate rich tapioca flour to the prepared fish feeds. The lower values for carbohydrate were recorded in both exotic and native worm meals and this may be because the worm meals were rich in protein source and not in carbohydrate source. But in the case of locally prepared and readymade fish feeds the carbohydrate content was also at a low level which is significantly higher than the exotic and native worm meals.

The total protein content was found to be high in exotic and native worm meal compared to all other fish feeds. Locally prepared fish feed registered the lowest value for total protein. Even though the readymade fish feed contained high crude protein, the total protein content was more in worm meals. The feeds prepared by using the exotic and native worm meals recorded desired level of total protein because of the addition of protein rich worm meals. However all the fish feeds were found to contain both essential and non essential amino acids except proline, tryptophan, cystine and cystine hydrochloromonohydrate. Though further investigations are necessary the earthworm meals are found to be richer in amino acids.

Figure 5.12 Carbohydrate and protein contents in different fish feeds



The values for total carbohydrate and protein were recorded a comparable values for both exotic and native worm meals, the value recorded for ash content varied widely in exotic and native worm meals.

5.3.3. Mineral contents of feed materials

The mineral contents of different feed materials included calcium, magnesium, iron, phosphorus and potassium. The mineral contents of different feed materials were furnished below in table 4.13.

A high value of calcium is reported in locally prepared fish feed, this may be due to the high calcium content in prawn used for the preparations of this feed. Low values were registered in readymade fish feed, exotic and native worm meals. The magnesium content was also found to be high in protein substituted fish feed by native worm meal followed by substituted feed by exotic worm meal. The magnesium content of worm meals was also high. This may be due to the high magnesium content in the substrates used for the multiplication of worms and the worm casts. Iron content was recorded a high value for protein substituted by native worm meal followed by native worm meal. A low iron content was recorded in locally prepared fish feed. Phosphorus content registered a high value for protein substituted with exotic worm meal (T_5), followed by exotic worm meal (T_1) and readymade fish feed (T_3). The lowest value of phosphorus was recorded in locally prepared fish feed. A high potassium content was registered in prepared fish feed using native worm meal. A comparable amount of potassium was recorded in exotic, native worm meal and also in prepared fish feed with exotic worm meal.

5.3.4. Heavy metal contents of feed materials

Negligible quantities of heavy metals were also seen in feed materials. This may be due to the presence of heavy metals in substrates which are used for the mass multiplication of the exotic and native worm meals.

5.3.5. Suitability of earthworm meal for aquaculture

The pH and EC of aquarium water were observed during the period of feeding of different fish feeds in the different fish tanks. The pH and EC of the aquarium water were found to be increased during the period and attained a high level and then remained almost in a steady state. The increase in the water pH and EC may be due to the presence of excretory materials of fishes and partly due to the different feeding materials.

Comparative economics

While calculating the comparative economics of the six feed materials, locally prepared feed was found to be the cheapest followed by protein substituted with exotic worm meal and native worm meal. Even though the local feed was the cheapest, the quality of protein may vary and the substitution of exotic and native worm meal was also found to be cheapest when compared to the readymade fish feed.

Summary & Conclusion

6. SUMMARY AND CONCLUSION

Study on the "*Production and evaluation of protienaceous earthworm meal*" was conducted at College of Horticulture, Vellanikkara during the period of 2011-2012 with three separate and continuous experiments viz: 1) Comparative evaluation of different substrates for the mass multiplication of exotic earthworm *Eisenia foetida* 2) Viable techniques for culturing native earthworms and 3) Preparation of earthworm meal and comparative evaluation of nutritive content of different earthworm meals.

In order to compare the substrates for the mass multiplication of exotic earthworm, three substrates like silkworm waste, banana pseudostem and azolla were used along with cow dung in 1:1 ratio by volume. For identifying the viable technique for culturing native worms, they were collected from the natural ecosystem and cultured in three different substrates, silkworm waste, banana pseudostem and azolla along with cow dung and soil in 1:1:1 ratio by volume. The nutrient content of six different feed materials, including exotic and native worm meal were analyzed and their suitability as a fish feed was also evaluated. The salient findings are summarized below:

- The crude protein content was higher in silkworm waste (19.83%) as compared to azolla (1.91%) and banana pseudostem (1.38%).
- The carbon and nitrogen content was also higher in silkworm waste where as macro nutrients P, K, Ca and Mg were found to be higher in azolla.
- Multiplication of exotic earthworm (*Eisenia foetida*) was found to be higher in vermicomposting with the use of azolla as the main substrate.
- The compost maturity period was 12 weeks in silkworm waste: cow dung (1:1) where as it was six weeks for both azolla: cow dung (1:1) and banana pseudostem: cow dung (1:1).

- The manurial value of the worm cast prepared by different substrates was found to be better in treatment, azolla: cow dung in 1:1 proportion (1.40% N, 0.91% P and 1.29% K).
- Mortality in native worms (*Perionyx excavatus*) to the extent of 60% was observed within 30 days of vermicomposting with different substrates and soil.
- Irrespective of the substrates and species of earthworms, the worm cast maintained a pH range of 6.5 to 7.8.
- Manurial value of native worm cast influenced by different substrates, also revealed the supremacy of azolla: cow dung: soil in 1:1:1 proportion (0.78% N, 0.39% P and 0.59% K).
- Enrichment of soil with increase in available N, available P, available K, exchangeable Ca and exchangeable Mg on vermicomposting native species of earthworms was recorded.
- Both in case of exotic and native earthworms, there was bioaccumulation of heavy metals such as cadmium, chromium, nickel, cobalt and lead.
- A simple and cost effective method was developed for the preparation of worm meal with the cleaning of earthworm's gut using cellulose.
- The crude protein (46.37%), crude fibre (1.00%) and crude fat (10.33%) were found to be higher in exotic worm meal than the native worm meal with the respective values of 44.63, 0.75 and 10.33 per cent.
- The total protein content was also higher in exotic (43.45%) and native worm meal (41.61%), but the total carbohydrate was low in both cases with the values 15.03 and 19.60 per cent, respectively.
- All the essential and non essential amino acids except proline, tryptophan, cystine and cystine hydrochloromonohydrate were qualitatively detected in all the feeds including worm meals.
- There is no appreciable change in pH and EC of aquatic water with the continuous use of worm meal as a feed for the ornamental fish, Red Oscar.

PRACTICAL UTILITY

- Vermicomposting can be an effective method of bioremediation of urban organic wastes with high heavy metal content.

- Earthworm meal can be used as a protein source for ornamental fishes. It is also found to be compatible with carbohydrate, fat and mineral sources so as to prepare a complete fish feed.

- Since the cost of production of the earthworm meals is comparatively high as compared to locally prepared one, these can be very well utilized for high value ornamental fishes or commercial aquaculture.

- Worm meal preparation can be an additional source of income for farmers who resort vermiculture as an agri-entrepreneurship.

FUTURE LINE OF WORK

- Since the mortality of native worms (*Perionyx excavatus*) was observed during the experimental period, we have to find out better options for the mass multiplication of native worms

- Investigations on the anti-microbial, anti-fungal properties and keeping quality of earthworm meal

- Exploring the possibility of using worm meal as a source of protein for poultry as well as cattle

- Further identification and use of earthworms for decomposition of urban and industrial wastes.

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Appendices

Appendix I

FCO-Specification for Vermicompost

Property	Specification
Moisture %	15-25
Colour	Dark brown to black
Odour	Absence of foul odour
Bulk Density (g cm^{-3})	0.7-0.9
Organic Carbon %	18 (minimum)
Nitrogen %	1 (minimum)
Phosphorus % (as P_2O_5)	0.8
Potassium % (as K_2O)	0.8
Cadmium (mg kg^{-1})	5 (maximum)
Chromium (mg kg^{-1})	50 (maximum)
Nickel (mg kg^{-1})	50 (maximum)
Lead (mg kg^{-1})	100 (maximum)

Appendix II

Amino acids in different fish feed (Rf values)

Sl No.	Amino acids	Rf values					
		Std	**T_1**	**Std**	**T_2**	**Std**	**T_3**
1	Phenyl alanine	0.76	0.73	0.68	0.68	0.63	0.64
2	Isoleucine	0.70	0.71	0.68	0.67	0.63	0.63
3	Arginine	0.34	0.37	0.25	0.25	0.22	0.20
4	Glycine	0.37	0.37	0.26	0.28	0.23	0.27
5	Proline	0.52	ND	0.52	ND	0.39	ND
6	Methionine	0.65	0.65	0.57	0.58	0.50	0.52
7	DL-Alanine	0.44	0.46	0.39	0.37	0.31	0.31
8	L-Leucine	0.77	0.77	0.71	0.70	0.62	0.64
9	Tryptophan	0.55	ND	0.64	ND	0.54	ND
10	L-Tyrosine	0.63	0.62	0.62	0.62	0.48	0.49
11	Cystine	0.34	ND	0.14	ND	0.11	ND
12	Glutamic acid	0.46	0.47	0.42	0.42	0.33	0.34
13	DL-Serine	0.40	0.37	0.31	0.30	0.27	0.25
14	Cystine hydrochloromonohydrate	0.18	ND	0.18	ND	0.11	ND
15	Aspartic acid	0.41	0.40	0.33	0.33	0.27	0.26
16	Nor-leucine	0.70	0.70	0.74	0.74	0.64	0.65
17	L-Ornithine monohydrate	0.29	0.28	0.25	0.23	0.15	0.18
18	Lysine monohydrate	0.34	0.32	0.25	0.25	0.18	0.18
19	Histidine	0.31	0.34	0.25	0.26	0.18	0.18
20	Aminobutyric acid	0.57	0.55	0.47	0.51	0.45	0.44

ND-Not Detected

Appendix continue.......

Sl No.	Amino acids	Rf values					
		Std	T_4	Std	T_5	Std	T_6
1	Phenyl alanine	0.63	0.64	0.66	0.66	0.67	0.66
2	Isoleucine	0.62	0.62	0.66	0.66	0.67	0.67
3	Arginine	0.25	0.25	0.30	0.29	0.32	0.32
4	Glycine	0.26	0.26	0.30	0.30	0.32	0.31
5	Proline	0.35	ND	0.41	ND	0.42	ND
6	Methionine	0.50	0.50	0.56	0.56	0.56	0.56
7	DL-Alanine	0.33	0.33	0.37	0.36	0.37	0.39
8	L-Leucine	0.67	0.66	0.68	0.68	0.68	0.69
9	Tryptophan	0.54	ND	0.60	ND	0.59	ND
10	L-Tyrosine	0.49	0.49	0.57	0.57	0.56	0.56
11	Cystine	0.13	ND	0.18	ND	0.18	ND
12	Glutamic acid	0.32	0.32	0.38	0.39	0.38	0.39
13	DL-Serine	0.24	0.25	0.33	0.34	0.33	0.32
14	Cystine hydrochloromonohydrate	0.13	ND	0.19	ND	0.19	ND
15	Aspartic acid	0.28	0.26	0.36	0.36	0.37	0.36
16	Nor-leucine	0.69	0.67	0.65	0.66	0.65	0.65
17	L-Ornithine monohydrate	0.16	0.16	0.25	0.21	0.25	0.23
18	Lysine monohydrate	0.16	0.15	0.24	0.24	0.26	0.28
19	Histidine	0.16	0.15	0.24	0.23	0.26	0.26
20	Aminobutyric acid	0.39	0.38	0.47	0.55	0.52	0.50

ND-Not Detected

Abstract

PRODUCTION AND EVALUATION OF PROTEINACEOUS EARTHWORM MEAL

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The study on the *"Production and evaluation of proteinaceous earthworm meal"* was conducted at College of Horticulture, Vellanikkara during the period of 2011-2012. The experiment was done to compare the efficiency of different substrates on the mass multiplication of exotic earthworms, to formulate viable techniques for the collection and multiplication of native worms, to develop a protocol for the preparation of worm meal using exotic and native earthworms and to evaluate the nutritive content of different worm meal preparations. In order to attain the objectives three separate experiments were conducted.

The first experiment included the **comparative evaluation of different substrates for the mass multiplication of exotic earthworm *Eisenia foetida***. In order to study the influence of different substrates on the mass multiplication of exotic earthworms, three different substrates like silkworm waste, banana pseudostem and azolla were used along with cow dung in 1:1 ratio by volume. The results revealed that the best substrate for the multiplication of exotic worm was azolla. The contents of crude protein, carbon and nitrogen were higher in silkworm waste as compared to azolla and banana pseudostem. The compost matured within 12 weeks for the treatment with silkworm waste as the main substrate, where as it attained maturity within six weeks with azolla and banana pseudostem. Among the worm casts from different treatments, the worm cast produced from azolla as the main substrate, recorded the contents as 1.43% N, 0.91% P, 1.29% K, 0.79% Ca and 0.83% Mg.

The second experiment mainly included the **viable techniques for culturing native earthworms**. For collecting the native worms, a hand full of cow dung with leaf litter was spread in the surface of soil and covered with wet jute bag. Moisten the bag without flooding. After a fortnight interval worms were found to be at the surface and were collected by digging and hand sorting. The collected worms were identified and cultured for the multiplication with different substrates like silkworm waste, banana pseudostem and azolla along with cow dung and soil in the ratio 1:1:1 by volume. Among the three treatments, the treatment with azolla as main substrate was the most efficient one. However a mortality of native worms

was recorded in all the three treatments within a period of 30 days of vermicomposting. Considering the manurial value of native worm cast, the treatment with azolla as the main substrate was found to be better than other treatments (0.78% N, 0.39% P, 0.59% K, 0.17% Ca and 0.47% Mg). Irrespective of the substrates and types of worms, the worm cast maintained a pH range of 6.5 to 7.8.

The third experiment, **the preparation of earthworm meal and comparative evaluation of nutritive contents of different earthworm meals,** was done to identify the best feed material in terms of protein, along with readymade and locally prepared feeds. A simple and cost effective method was proposed for the preparation of worm meal with the clearing of earthworm's gut using cellulose material. The crude protein (46.37%), crude fibre (1.00%) and crude fat (10.33%) were found to be comparatively rich in exotic worm meal. The total protein content was also higher in exotic (43.45%) than native worm meal (41.61%), but the total carbohydrate was low in both cases with the values 15.03 and 19.06% (as compared to FAO specifications for fish feed) respectively. All the essential and non essential amino acids except proline, tryptophan, cystine and cystine hydrochloromonohydrate were qualitatively detected in all the feeds including worm meals. There is no appreciable change in pH and EC of aquarium water with the continuous use of worm meal as a feed for the ornamental fish, Red Oscar.