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Growth and Reproductive Dynamics of *Eisenia fetida* during Vermicomposting of Mango Pulp Along with Fish Waste and Biofertilizers (*Azospirillum*, *Phosphobacterium* and *Rhizobium*)

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Abstract

Vermicomposting is a green technique used to produce organic compost from organic waste with the aid of specific earthworm species. The resulting compost is rich in nutrients that can improve plant health and fertility. This study was conducted to produce organic compost using a developed vermicomposting technique and to enhance the exchangeable nutritional content in the soil for utilization in sustainable agriculture. The experiment was carried out with *Eisenia fetida* worms in a treatment technique using fish waste, mango pulp and biofertilizer. Six feeding compositions – different combination of fish waste (FW) + Mango pulp (MP) waste (C1, C2 and C3) alone; C1/C2/C3 + Earthworms (E. fetida) EW (C1E1/ C2E1/ C3E1); C1/C2/C3 + EW + *Azospirillum- AZ* (C1E2/ C2E2/ C3E2); C1/C2/C3 + EW+ Phosphobacteria - Pb (C1E3/ C2E3/ C3E3); C1/C2/C3 + EW+ Rhizobium-Rb (C1E4/ C2E4/ C3E4); C1/C2/C3 + EW + AZ+Pb+Rb (C1E5/ C2E5/ C3E5). The physiochemical parameters, namely pH, electrical conductivity and seven different nutrients, were assessed in different compost samples and the growth and reproductive performance of epigeic earthworm *Eisenia fetida* in biofertilizers enriched FW + MP vermicomposting was studied. The vermicomposting co-efficient of physicochemical parameters shows an increased trend in EC, OC, N, P, K, S, Zn and Fe, except pH. Maximum number of earthworms was recorded in C1E3 and the highest average body weight and body length were observed in C3E5. The microbial populations and availability of food materials from biofertilizers enriched fish waste with fruit waste increases the biomass, average body weight, average body length and also increases the reproductive

Key words: Vermicompost, Biofertilizer, Earthworm, Mano Pulp, Fish Waste, Nutrient

Fishing generates large quantities of waste daily in fish markets and fish processing industries (Canneries, fresh and frozen fish processing plants, etc.). Fish waste becomes the organic fertilizer. Fish waste composting is an old method but unfortunately in inland fisheries sector, this technique is not used so far. India is No. 2 in Fresh water aquaculture next to China. Composting initiatives using fish waste have been carried out in various parts of the world in search of alternative and viable techniques for transforming fish waste into useful agricultural products [1-2]. Composting fish wastes is a relatively new, simple and environment friendly alternative to disposing of fish wastes [3]. Composting made from fish wastes could provide an effective source of nutrient-rich fertilizer. Conversion of fish waste into compost is cost effective for large fish processing plants. Because of high nutrient content in fish waste, they can be used for local gardening and agricultural crop production [4].

Mango is a fruit which has many varieties and is grown in many places all over the world. Mango processing is a traditional activity; production began to produce juice from the mango without intending to satisfy the demands of the international fresh fruit market. The mango processing industry, only 50% of the mango becomes juice and the rest is seed, skin, or fruit pulp which is removed during the process [5]. Usage of fruits produces two types of waste a solid waste of peel/skin, seeds, stones etc. a liquid waste of juice and wash water [6]. This kind of waste is not only a problem for tropical production, but also an issue of waste management for the society. The waste decomposes rapidly, and gives rise to the hatching of many fly larvae [5]. To avoid this, waste may be reused or submitted to physical, chemical, biological, thermal or mixed treatments, among which we can find feeding animals, crushing, composting, anaerobic digestion and vermicomposting. Of the treatments mentioned, vermicomposting is presented as a viable alternative to degradation.

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Extensive use of chemical fertilizers and pesticides in agriculture negatively impacts the soil and plants as chemical and pesticide residues are present in nutritional products and they accumulate in the food web and environment. Therefore, developing innovative agricultural practices that employ organic composts and environmentally friendly products will navigate the agricultural sector toward a greener future and production of wholesome and nontoxic food products at affordable prices year-round [7]. Vermicomposting is a green technique that produces vermicompost from different types of organic wastes using specific earthworm species. It helps farmers to reduce their use of chemical fertilizers and the overall production costs [8]. *Eisenia fetida* is one of the earthworm species that works efficiently in breaking down and decaying natural remains and turning these scraps into high-quality organic compost. It is capable of eating as much as half of its weight daily. The behavioral activity of earthworms (feeding, burrowing and casting) enhances the physical, chemical and biological properties of organic matter and soil, thereby augmenting the growth of agricultural crops naturally and safely [9]. Taking all this into account, the main objective of this research is to apply a developed vermicomposting technique to produce vermicompost from organic waste products of mango pulp and fish waste along with biofertizers with the help of *Eisenia fetida* (vermiworms) and enhance the nutrient content of the vermicompost produced for use in sustainable agriculture.

MATERIALS AND METHODS

Samples collection

Fish waste was collected from fish market, Krishnagiri, Tamil Nadu, India and Mango pulp waste collected from Sri Devaraj Agro Industrial, Krishnagiri.

Pre decomposing preparation method of sample

The bulk amount of fish waste and mango pulp waste was taken into the polyethylene bags; Samples of fish waste and mango pulp waste mixed ratio (1:1) pit setup in soil, the samples buried in pit of soil. The pit experiments upper soil weekly two times spared the water and maintains moisture level at 60-70%. After 60th days, finally the pit was opened and collected the pre decomposing samples.

Vermicompost experimental setup

A pilot experiment with six plastic pots (25cm diameter and 40cm height) carries 3.0 kg of pre decomposed matter with a small hole at the bottom to remove the excess water with three replication was carried out in Kandasami Kandars College, Velur, Namakkal, Tamil Nadu, India. Biofertilizers (*Azospirillum*, *Phosphobacterium*, and *Rhizobium*) along with 1.0 g/kg to substrate were added [10] as shown (Table 1). First generation of mature clitellum developed 20 earthworms *Eisenia foetida* where introduced from each experiments. The compost was maintained was 60-70 \% of moisture level throughout the experimental period at 60 days and throughout the study period by periodic sprinkling of adequate of tape water.

Table 1 Decomposed substrates along with Earthworm and biofertilizers

Composition	Mango Pulp Waste + Fish Waste (Ratio)
C1	1 : 1
C2	2 : 1
C3	1 : 2

Experiments			Biofertilizers (1gm/kg)
Control	Control	Control	Compost
C1E1	C2E1	C3E1	Compost + Earthworm
C1E2	C2E2	C3E2	Compost + Earthworm + *Azospirillum*
C1E3	C2E3	C3E3	Compost + Earthworm + *Phosphobacterium*
C1E4	C2E4	C3E4	Compost + Earthworm + *Rhizobium*
C1E5	C2E5	C3E5	Compost + Earthworm + *Azospirillum* + *Phosphobacterium* + *Rhizobium*

C - Composition; E - Experiment

Vermicompost earthworm populations

Initial and 60th days on the total earthworm's population were determined and chemical analysis of the vermicompost was conducted. The total population of earthworms was estimated using a hand sorting method according to Zicsi, [11], samples from the experiments. The earthworm's population consisted of three age group *viz.* Juvenile, Non-Clitellate and Clitellate earthworms.

Physico-chemical analysis

The physicochemical parameters of vermicompost during the experiments were analyzed for standard methods. pH of vermicompost samples was determined using a double DH2O suspension of compost in the ration 1 : 10 (w/v) analyzed in Digital pH meter [12], the total organic carbon content was estimated using the method of [13]. The nitrogen was estimated by Micro Kjeldahl methods [14-15]. Phosphorus was detected by the colorimetric methods [16]. Potassium was determined after digesting the samples in diacid mixture (concentration HNO_3; concentration $HClO_4$, 4:1 v/v), by Flame Photometer [17]. Sulfur, Zinc, Boron and Iron were measured by the diacid digest using an atomic absorption spectrophotometer [12].

Statistical analysis

The experimental data was expressed as the mean of three replications. The difference in physico-chemical parameters of fish waste and mango pulp waste vermicompost experiments with biofertilizers with control was statistically computed using One Way Analysis of Variance (ANOVA) was used to define the statistically significant where the Tukey's honestly significant different (HSD) tests at $p < 0.05$ significance level was applied.

RESULTS AND DISCUSSION

Physicochemical characteristics of mango pulp and fish waste with different biofertilizers

Composition 1 with different biofertilizer

The primary physicochemical properties by C1, C1E1, C1E2, C1E3, C1E4 and C1E5 are summarized in (Table 2). The pH varied between 6.32 ± 0.04 and 7.10 ± 0.02. The highest electrical conductivity was in C1E5 (1.75 ± 0.03μS/cm) and the lowest was in C1 (1.26 ± 0.02 μS/cm). The OC ranged from 31.46 ± 1.08% in C1 to 35.01 ± 0.68% in C1E5. The NP were the lowest in C1 and the highest in C1E5, 1.04 ± 0.02% and 1.66 ± 0.03%; 0.57 ± 0.01% and 1.16 ± 0.05, respectively. The potassium content ranged from of 1.35 ± 0.03% in C1 to 1.72 ± 0.04% in C1E3. Sulphur ranged from 1.23 ± 0.02% in C1 to 1.35 ± 0.03% in C1E1. The highest zinc and ferrous content were in C1E5 (1.34 ± 0.02% and 1.11 ± 0.02, respectively) and the lowest was in C1 (1.08 ± 0.02% and 0.52 ± 0.01%, respectively).

Table 2 Physico-chemical properties of decomposing mango pulp along with fish waste with *E. fetida* and biofertilizers at different experiments with composition 1

Days	Control (C1)	C1E1	C1E2	C1E3	C1E4	C1E5
PH	7.10 ± 0.02	6.32 ± 0.04	6.80 ± 0.06	6.68 ± 0.04	7.04 ± 0.02	6.87 ± 0.01
EC	1.26 ± 0.02	1.37 ± 0.05	1.45 ± 0.03	1.66 ± 0.02	1.52 ± 0.04	1.75 ± 0.03
OC	31.46 ± 1.08	32.48 ± 0.97	32.85 ± 0.49	34.42 ± 0.90	31.72 ± 0.64	35.01 ± 0.68
N	1.04 ± 0.02	1.16 ± 0.02	1.29 ± 0.02	1.34 ± 0.02	1.48 ± 0.04	1.66 ± 0.03
P	0.57 ± 0.01	0.85 ± 0.03	0.84 ± 0.02	1.04 ± 0.03	0.82± 0.04	1.16 ± 0.05
K	1.35 ± 0.03	1.45 ± 0.03	1.58± 0.04	1.72 ± 0.04	1.24 ± 0.03	1.47 ± 0.02
S	1.23 ± 0.02	1.35 ± 0.03	1.25 ± 0.02	1.33 ± 0.02	1.25 ± 0.03	1.24 ± 0.02
Zn	1.08 ± 0.02	1.16 ± 0.02	1.33 ± 0.02	1.32 ± 0.02	1.25 ± 0.02	1.34 ± 0.02
Fe	0.52 ± 0.01	0.75 ± 0.03	0.84 ± 0.02	1.04 ± 0.02	0.81 ± 0.03	1.11 ± 0.02

Composition 2 with different biofertilizer

The primary physicochemical properties by C2, C2E1, C2E2, C2E3, C2E4 and C2E5 are summarized in (Table 3). The pH varied between 6.83 ± 0.02 in C2E2 and 7.44 ± 0.02 in C2. The highest electrical conductivity was in C2E5 (2.36 ± 0.02 µS/cm) and the lowest was in C1 (1.94 ± 0.02 µS/cm). The OC ranged from 28.81 ± 0.47% in C2E1 to 32.45 ± 0.85% in C2. The NPK were the lowest in C2 and the highest in C2E5, 1.17 ± 0.02% and 1.74 ± 0.02%; 0.83 ± 0.01% and 1.44 ± 0.02%; 1.05 ± 0.02% and 1.54 ± 0.02%, respectively. The sulphur content ranged from of 0.94 ± 0.02% in C2E5 in to 1.34 ± 0.02 in C2. The highest zinc and ferrous content were in C2 (1.16 ± 0.02% and 10.74 ± 0.02%, respectively) and the lowest was in C2E5 (0.82 ± 0.02% and 0.30 ± 0.02%, respectively).

Table 3 Physico-chemical properties of decomposing mango pulp along with fish waste with *E. fetida* and biofertilizers at different experiments with composition 2

Days	Control (C1)	C1E1	C1E2	C1E3	C1E4	C1E5
PH	7.44 ± 0.02	6.87 ± 0.02	6.83 ± 0.02	6.93 ± 0.02	7.05 ± 0.02	6.94 ± 0.02
EC	1.94 ± 0.02	2.06 ± 0.02	2.15 ± 0.03	2.32 ± 0.02	2.06 ± 0.01	2.36 ± 0.02
OC	32.45 ± 0.85	28.81 ± 0.47	32.21 ± 0.66	32.40 ± 1.09	29.25 ± 0.54	30.71 ± 0.63
N	1.17 ± 0.02	1.34 ± 0.02	1.44 ± 0.02	1.63 ± 0.02	1.24 ± 0.03	1.74 ± 0.02
P	0.83 ± 0.01	0.94 ± 0.02	1.06 ± 0.02	1.34 ± 0.02	1.04 ± 0.02	1.44 ± 0.02
K	1.05 ± 0.02	1.26 ± 0.02	1.36 ± 0.02	1.44 ± 0.02	1.34 ± 0.02	1.54 ± 0.02
S	1.34 ± 0.02	1.26 ± 0.02	1.15 ± 0.03	1.06 ± 0.01	1.16 ± 0.03	0.94 ± 0.02
Zn	1.16 ± 0.02	1.04 ± 0.02	0.94 ± 0.02	0.84 ± 0.02	0.90 ± 0.02	0.82 ± 0.02
Fe	0.74 ± 0.02	0.64 ± 0.03	0.52 ± 0.02	0.40 ± 0.02	0.44 ± 0.03	0.30 ± 0.02

Composition 3 with different biofertilizer

The primary physicochemical properties by C3, C3E1, C3E2, C3E3, C3E4 and C3E5 are summarized in (Table 4). The pH varied between 6.83 ± 0.02 in C2E2 and 7.44 ± 0.02 in C2. The highest electrical conductivity was in C2E5 (2.36 ± 0.02 µS/cm) and the lowest was in C1 (1.94 ± 0.02 µS/cm). The OC ranged from 28.81 ± 0.47% in C2E1 to 32.45 ± 0.85% in C2. The NPK were the lowest in C2 and the highest in C2E5, 1.17 ± 0.02% and 1.74 ± 0.02%; 0.83 ± 0.01% and 1.44 ± 0.02%; 1.05 ± 0.02% and 1.54 ± 0.02%, respectively. The sulphur content ranged from of 0.94 ± 0.02% in C2E5 in to 1.34 ± 0.02 in C2. The highest zinc and ferrous content were in C2 (1.16 ± 0.02% and 10.74 ± 0.02%, respectively) and the lowest was in C2E5 (0.82 ± 0.02% and 0.30 ± 0.02%, respectively).

Table 4 Physico-chemical properties of decomposing mango pulp along with fish waste with *E. fetida* and biofertilizers at different experiments with composition 3

Days	Control (C1)	C1E1	C1E2	C1E3	C1E4	C1E5
PH	7.07 ± 0.02	6.97 ± 0.02	6.84 ± 0.01	6.73 ± 0.02	6.84 ± 0.01	6.64 ± 0.02
EC	2.02 ± 0.01	2.13 ± 0.01	2.23 ± 0.02	2.34 ± 0.03	2.14 ± 0.01	2.40 ± 0.02
OC	29.09 ± 0.49	31.43 ± 0.16	32.80 ± 0.55	35.23 ± 0.04	31.73 ± 0.46	36.12 ± 0.23
N	1.15 ± 0.02	1.26 ± 0.02	1.36 ± 0.02	1.66 ± 0.02	1.73 ± 0.02	1.76 ± 0.02
P	0.65 ± 0.03	0.74 ± 0.02	0.86 ± 0.01	0.97 ± 0.01	0.85 ± 0.01	1.13 ± 0.02
K	1.14 ± 0.01	1.26 ± 0.02	1.34 ± 0.02	1.44 ± 0.02	1.36 ± 0.01	1.54 ± 0.02
S	1.96 ± 0.02	2.06 ± 0.02	2.16 ± 0.01	2.30 ± 0.02	2.16 ± 0.02	2.22 ± 0.04
Zn	1.54 ± 0.02	1.53 ± 0.01	1.65 ± 0.03	1.85 ± 0.03	1.74 ± 0.02	1.93 ± 0.03
Fe	1.45 ± 0.10	1.54 ± 0.05	1.54 ± 0.02	1.65 ± 0.03	1.46 ± 0.02	1.67 ± 0.02

Growth of E. fetida in the different composition of compost

Composition 1 with different biofertilizer

No mortality was observed in any compost during the study period. In this experiment, all compost was stirred by hand daily for 15 days to prevent the accumulation of toxic gas. The growth rate of *E. fetida* in C1E1, C1E2, C1E3, C1E4 and C1E5 is given in (Fig 1). The highest number of earthworm was obtained in C1E3 (128.33 ± 3.51) and lowest number was observed in C1E1 (100.67 ± 4.16). Maximum worm weight was obtained in C1E3 (175.33 ± 3.06 mg/earthworm) and minimum in C1E4 (153.67 ± 7.64mg/earthworm. The maximum height was attained by *E. fetida* in C1E3 (20.00 ± 1.00 cm) and minimum was observed in C1E1 (14.87 ± 0.38).

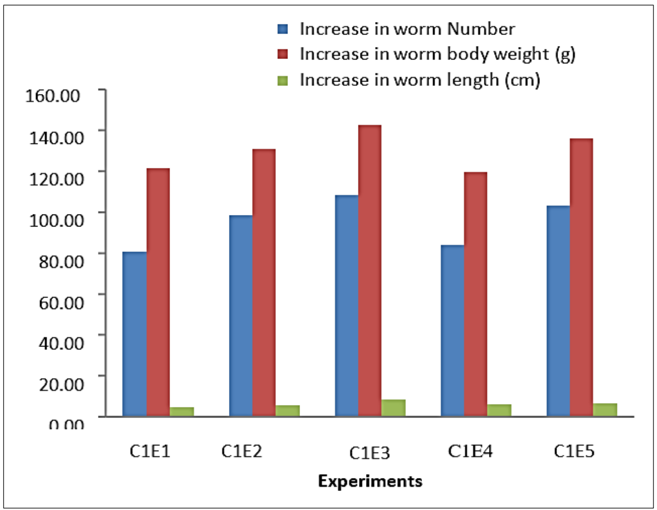

Fig 1 *E. fetida* biomass dynamics during decomposition experiments with composition 1

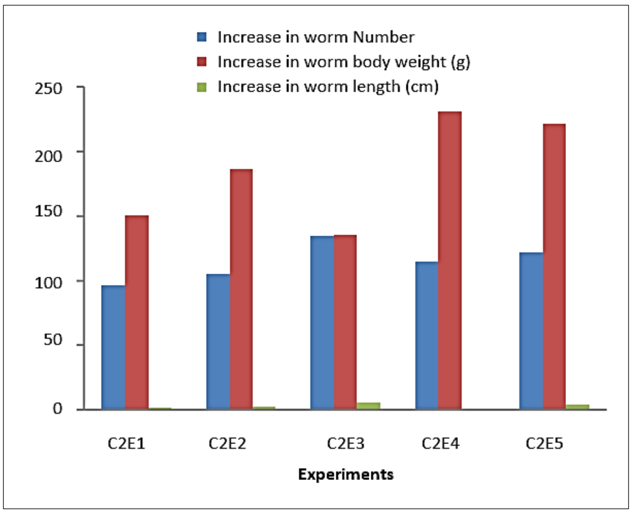

Fig 2 *E. fetida* biomass dynamics during decomposition experiments with composition 2

Composition 2 with different biofertilizer

No mortality was observed in any compost during the study period. In this experiment, all compost was stirred by hand daily for 15 days to prevent the accumulation of toxic gas. The growth rate of *E. fetida* in C2E1, C2E2, C2E3, C2E4 and C2E5 is given in (Fig 2). The maximum number of earthworm was seen in C1E3 (155.00 ± 3.00) and lowest number was observed in C1E1 (116.00 ± 1.00). Maximum worm weight was achieved in C1E4 (263.33 ± 2.52 mg/earthworm) and minimum in C1E3 (171.33 ± 2.52 mg/earthworm. The maximum height was attained by *E. fetida* in C1E3 (20.70 ± 0.61 cm) and minimum was observed in C1E1 (14.87 ± 0.38).

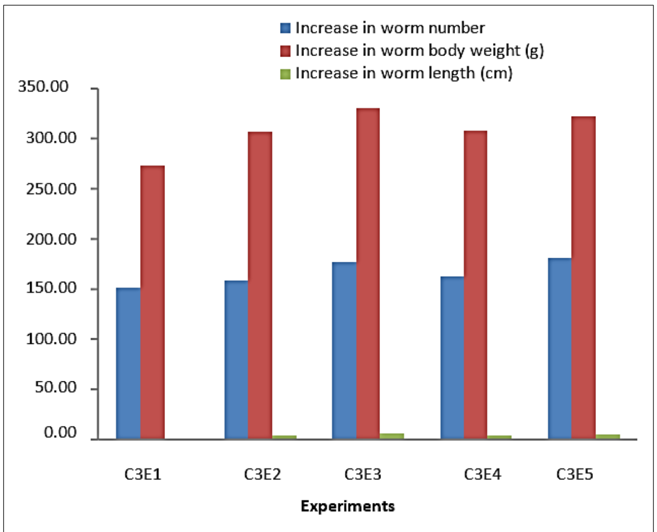

Fig 3 *E. fetida* biomass dynamics during decomposition experiments with composition 3

Composition 3 with different biofertilizer

No mortality was observed in any compost during the study period. In this experiment, all compost was stirred by hand daily for 15 days to prevent the accumulation of toxic gas. The growth rate of *E. fetida* in C3E1, C3E2, C3E3, C3E4 and C3E5 is given in (Fig 1). The highest number of earthworm was obtained in C3E5 (201.33 ± 3.06) and lowest number was observed in C3E1 (171.33 ± 3.06). Maximum worm weight was obtained in C3E3 (363.67 ± 3.79 mg/earthworm) and minimum in C3E1 (309.00 ± 4.00 mg/earthworm. The maximum height was attained by *E. fetida* in C1E3 (20.10 ± 0.36 cm) and minimum was observed in C3E1 (14.67 ± 0.15).

The organic wastes, fish waste and mango pulp waste used in this study were analyzed prior to composting and their Physico-chemical properties are given in (Table 2-4). These data depicted in tables also provide the change in properties after vermicomposting for comparison. There was only a high alteration in some properties. The pH of the fish waste and mango pulp waste increases progressively with the progression of the composting process regardless of types of combinations or proportions. However, the decrease in pH was more pronounced in fish waste, mango pulp waste and mixed biofertilizers (E5) combination where the pH declined from 7.77 to 7.59 during a period of 60^{th} days of vermicomposting. Similar trend of response was observed in fish waste and mango pulp waste with 1:1 ration. The increased pH during the process was probably due to the degradation of short-chained fatty acids and ammonification of organic N [18-19. EC of all vermicomposting experiment was increased initial to final vermicomposting. The EC of fish and mango pulp waste mixtures biofertilizers vermicompost was in the range of 0.33 – 0.43 dSm^{-1} respectively. Statistically, the EC contents in vermicompost were highly significantly in all biofertilizers experiment ($p < 0.05$). This increase in EC may be due to increased level of soluble salts due to mineralization of the waste by worms and microorganisms.

Nitrogen content of the fish waste and mango pulp waste compost initial the process increases the pro decomposition process in all treatments. The enhancement of N in vermicompost was probably due to mineralization of the organic matter containing proteins [17], [20] and conversion of ammonium nitrogen into nitrate [21-22]. The P content nearly minimizes in treatment that has a combination of fish waste and mango pulp waste under 1:1 ration blend during a period of 60^{th} days decomposition in all the treatments. In the present study phosphorus was in the range (1.47 – 1.96%) in all the reactors at initial days. At 60^{th} days the reactor E2 and E5 had the high level of the phosphorus content (1.67±0.15 and 1.60±0.20) respectively. The P content during vermicomposting was through mineralization, release and mobilization of available phosphorus content from organic waste performed partly by earthworm gut phosphates and further release of phosphorus might be due to phosphate solubilizing microorganisms present in worm cast [23].

In the present study, there was a increased potassium content in final feed mixtures ranged from 41.2 to 60% as compared to the initial raw waste mixtures. Likely, Yadav and Garg [24] observed an increase in potassium content of industrial solid wastes and weeds. Sulphur, Zinc and Iron content of vermicompost made out of fish waste and mango pulp waste at 1:1 ratio in all experiments linearly increased to final. Similarly, Ghosh *et al*. [25] reported the results of a study on effect of inclusion of epigeic earthworm *Eisenia foetida* on several organic wastes viz, cow dung, poultry droppings, kitchen wastes, municipal wastes and dry leaves, and observed that the availability of three major nutrient elements viz, N, P and K, and micronutrients viz, Fe, Cu, Mn and Zn was considerably higher in the vermicompost treatments for all the wastes as compared to those without earthworms.

In the present study, the highest worm reproduction in both biomass and number was observed in the treatment Compost 3 + Earthworm + *Azospirillum* + *Phosphobacterium* + *Rhizobium*. The presence of biofertilizer producing organisms of *Azospirillum* + *Phosphobacterium* + *Rhizobium* has increased biomass of earthworms. Similarly, Senthilkumar et al., (26) reported that the maximum number of earthworms was seen in the compost prepared by flower waste + earthworm+ Phosphobacteria. The increased earthworm biomass with phosphobacteria suggests the dual role of bacteria as food material and in enriching the substrate with phosphorus through phosphorus solubilization. This phenomenon has also been reported by [27]. Various studies have shown that earthworm utilize micro-organisms in their substrates as a food source and can digest them selectively [28-29]. The highest average body weight and body length were recorded in C3E3. The growth rate in earthworms mainly depends upon the microbial populations and availability of nutrients in vermibeds [30].

CONCLUSION

In the recent years, vermicomposting is emerging as a simple, easily adoptable for effective recycling wide ranges of organic wastes for agricultural production. On the other hand, vermicomposting can also be taken up at commercial level using municipal and other sources of wastes and the product be marketed as good quality organic manure. In the present studies, organic waste when applied in combination with other bulking material has improved the growth of earthworm’s as well as quality of the final vermicompost. The highly recommended for final vermicompost was higher in available nutrients than traditional compost and has a huge potential for agricultural purposes.

Declaration of competing interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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