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Effect of neem leaves and stock density of earthworm (Eisenia fetida) on quality of rice straw vermicompost

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Abstract. The sustainable management of rice straw is essential for protection of human health and environment. This study assesses the impact of stock density of earthworm (*Eisenia fetida*) and Neem leaves (*Azadirachta indica*) on the quality of the final vermicompost. The vermicompost is produced using different combinations of rice straw, Neem leaves, and cow dung (bulking agent) by varying stock density of earthworms. The vermicomposting experiments are performed in plastic containers (32 cm × 28 cm × 28 cm) in open for 90 days under laboratory conditions. The stock density of the earthworm is found to be an important factor to influence nutritional quality of the final vermicompost. There is observed significant improvement in the total nitrogen (91.8%), phosphate (73.4%), potassium (38.8%), and calcium (59.05%) content of the vermicompost produced with the highest stock density of the earthworms. All the treatments showed decrease in TOC and C:N content after 90 days of vermicomposting. The treatment with Neem leaves showed maximum growth of earthworms (2.65 fold). Neem leaves brought positive changes in the quality of final vermicompost by enhancing the growth and reproduction of the earthworms. The calcium content increased by 39% in the final vermicompost with the addition of Neem leaves at the same stock density of the earthworms. The stock density of the earthworms and Neem leaves are found to significantly improve quality of the final vermicompost as compared with the compost (control). The surface morphology in SEM images showed high degree of fragmentation in the vermicompost as compared with the compost. The combined action of microbes and earthworms resulted in high degree of disintegration in the vermicompost.

Keywords: C/N ratio; *Eisenia fetida*; neem leaves; rice straw; vermicomposting

1. Introduction

India produces large quantity of food grains to fulfill domestic and global food demand. It stands second in terms of production of rice and wheat globally. The production of rice and wheat has increased to 122.27 and 109.52 million tonnes, respectively, in 2020-21 (The Economic Times 2021). A bumper crop leaves huge amount of residue particularly after the mechanized harvesting.

The rice and wheat represent about 70% of the total crop residue (500 Million tons) produced

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annually in India (Bhuvaneshwari *et al.* 2019, MNRE 2019). The rice straw represent around 43% of the same (Jain *et al.* 2014). Generally, farmers burn the extra straw to clear the field in time for another crop. The burning is considered an easy and cost effective way to manage excess straw as compared to tilling in the field (Hung *et al.* 2019, Mendoza and Mendoza 2016). About 8 - 80% rice straw is annually burnt across all states in India (Jain *et al.* 2014). The burning is one of the potential sources of air pollution. The burning converts straw carbon, sulphur, and nitrogen into harmful gases which pose threat to human health and the environment. It also produces huge amount of black soot which results in poor visibility and increased incidences of accident. The crop residue burning also emits greenhouse gases (GHGs) which are responsible for climate change. It also causes severe pollution of water and land resources at local and regional scale. The crop residue burning adversely affects the nutrient budget and composition of the soil to affect plants health. Hence, there is an urgent need to provide an economical and sustainable alternative for in-field crop residue management.

The rice straw is a ligno-cellulosic biomass which contains about 12% lignin, 38% cellulose, and 25% hemicellulose. It is a potential resource which can be effectively utilized for composting to recycle nutrients in the soil. The composting is an economical and natural process for effective management of crop residue. It involves aerobic decomposition of organic wastes by microorganisms under preferable conditions of moisture, pH, and temperature (40-65°C) (Mendoza and Mendoza 2016). Whereas, the vermicomposting involves combined action of earthworms and microorganisms for accelerated decomposition of organic wastes. The earthworms play a crucial role to stimulate and enhance biological activity by ingestion and fragmentation of organic material which increases surface area for microbial action. The microbial enzymes cause biochemical decomposition of the organic wastes. The vermicomposting has been effectively utilized for the decomposition of a wide variety of organic wastes, i.e. industrial and sewage sludge (Garg and Kaushik 2005, Huang *et al.* 2022, Nogales *et al.* 2005), crop residues (Yadav and Garg 2010), cotton waste (Albanell *et al.* 1988), hospital waste (Singh *et al.* 2010), and food waste etc. (Ameen and Homaidan 2020).

Perionyx excavates, Lampito mauritii, and *Eisenia fetida* are the commonly used local species of earthworms for vermicomposting in India (Ray 2007). *Eisenia fetida* has been widely utilized for vermicomposting in many previous studies because of rapid growth rate and tolerance to wide range of temperature and moisture conditions. Boruah *et al.* (2019) investigated potential of citronella bagasse and paper mill sludge for vermicomposting by employing *Eisenia fetida*. Recently, Gusain and Suthar (2020) reported vermicomposting of duckweed (*Spirodela polyrhiza*) by using *Eisenia fetida*. The use of compost as organic fertilizer improves soil quality, structure, and biodiversity. It buffers soil from chemical imbalances, and also provide biological control for certain pests. The present study aims to investigate impact of addition of Neem leaves (*Azadirachta indica*) and stock density of earthworms (*Eisenia fetida)* on the physico-chemical quality parameters of the final rice straw vermicompost.

2. Materials and methods

2.1 The feedstock and earthworms

The rice straw was collected from the agricultural fields situated near to Gurugram, Haryana, India. The rice straw was air dried and cut into small fragments (1-2 cm) for use in the vermicomposting experiments. The earthworms (*Eisenia fetida*) were directly procured from vermicomposting plant of Krishi Vigyan Kendra, Shikhohpur, Gurugram, Haryana, India. The earthworm stock culture was maintained under laboratory conditions for use in the experiments. The Neem leaves were collected from roadside Neem plants (*Azadirachta indica*) at Gurugram, Haryana, India. The cow dung was directly collected from a nearby cow-shed at Gurugram, Haryana, India.

2.2 Experimental set up

The vermicomposting experiments were performed using four plastic containers of 2 L capacity (A, B, C, and D) and size 32 cm × 28 cm × 28 cm. The vermibeds were made by keeping decomposed cow dung (350 g) and soil (175 g) in 2:1 proportion at bottom of each container. There was added 700 g of rice straw in each container. There were also added 300 g of Neem leaves in the container B. The feedstock was moistened regularly by sprinkling distilled water to hold about 80% of moisture (Yadav and Garg 2009) and pre-decomposed for 30 days. The pre-decomposition helps in stabilization of feedstock in terms of pH, moisture content, and further mass reduction to ensure suitable environment for the growth and development of the earthworms (Kaur 2019). The feedstock was manually mixed to ensure sufficient aeration and prevent development of anaerobic conditions which lead to generation of foul odour. The feedstock samples were drawn from each container after 30 days of pre-decomposition phase and analyzed to measure initial nutritional quality parameters (Table 1). The mature earthworms (*Eisenia fetida*) were introduced into the containers on 31st day. The stock density of the earthworms was varied as 20, 20, and 40 earthworms in containers A, B, and C, respectively, while container D (compost) was used as control with no earthworms. The vermicomposting of the feedstock was conducted for 90 days under the shade at ambient conditions. All the experiments were performed in triplicate for statistical comparison. The final vermicompost was harvested after development of dark black granules on the top surface of the containers and analyzed for nutritional quality parameters.

2.3 Earthworm growth

All the earthworms (adult and hatchlings) were hand sorted from the vermicompost of each container after 90 days and counted. The reproductive growth of earthworms was calculated on the basis of worm number for different containers.

2.4 Feedstock, vermicompost, and compost analysis

The air dried samples of feedstock, vermicompost, and compost (control) were analyzed in terms of various physico-chemical quality parameters, i.e., moisture content, bulk density, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (TN), total potassium (K), phosphate (P), calcium (Ca), C:N ratio, and heavy metals to evaluate physico-chemical changes using standard methods (Table 1) (BOFFO 1985). The analysis of samples was performed in triplicate and average values were used for comparison. TOC was determined by the loss on ignition of dried samples at 650-700 ^{o}C for 6-7 hours in a muffle furnace. TN was estimated using Kjeldhal method. C:N ratio was computed using values of TOC and TN. The total phosphorus in the samples was determined using gravimetric quinoline molybdate method. Ca and K content were analyzed using flame photometer after dry ashing at 650 - 700^{o}C and digestion with

Table 1 The physico-chemical characteristics of the feedstock after pre-decomposition

Parameters	Container A	Container B	Container C	Container D
Bulk density (g/cm^3)	0.817 ± 0.003 a	0.817 ± 0.003 a	0.817 ± 0.003 a	0.827 ± 0.003 a
Moisture content (%)	81.533 ± 0.003 ac	81.540 ± 0.000 a	81.520 ± 0.006 cb	81.510 ± 0.000 b
TOC (%)	18.240 ± 0.000 b	20.250 ± 0.006 a	18.240 ± 0.000 b	18.230 ± 0.000 b
TN (%)	1.100 ± 0.000 b	1.160 ± 0.000 a	1.100 ± 0.000 a	1.100 ± 0.000 c
K (%)	2.253 ± 0.003 c	2.560 ± 0.000 a	2.233 ± 0.007 d	2.277 ± 0.003 b
P (%)	1.237 ± 0.003 b	1.420 ± 0.006 a	1.237 ± 0.003 b	1.237 ± 0.003 b
Ca (%)	2.100 ± 0.000 c	2.227 ± 0.003 a	2.127 ± 0.003 b	2.100 ± 0.000 c
C:N ratio	16.580 ± 0.000 a	17.460 ± 0.000 b	16.580 ± 0.000 c	16.570 ± 0.000 d
EC (mS/cm)	0.837 ± 0.007 a	0.850 ± 0.000 a	0.850 ± 0.000 a	0.847 ± 0.003 a
Fe	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg
Mn	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg
Zn	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg
Cu	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg

Parameter values in each row followed by different letters are significantly different $p < 0.05$ (one way ANOVA followed by post hoc Tukey's all pair wise multiple comparison test)

concentrated HCl (Beckman Model DU). The electrical conductivity was measured with the help of conductivity meter (Chemito 130). The samples were analyzed for heavy metals after acid digestion using atomic absorption spectrophotometer (BOFFO 1985). The morphology of vermicompost and compost (control) samples were studied using scanning electron micrographs. The samples were dried to a constant weight at 70 ± 2℃ and fixed on metal sample holder with the help of an adhesive carbon tape (Boruah *et al.* 2019) which was followed by gold coating using sputter coater for better visibility. The micrographs were recorded using Scanning Electron Microscope (ZEISS, EVO 18) at AIIMS, New Delhi, India.

2.5 Statistical analysis

The statistical analysis of data was done using SPSS software (version 23). Analysis of variance (one way ANOVA) was performed followed by post hoc Tukey's all pair wise multiple comparison test ($p < 0.05$) to compare the means of different physico-chemical quality parameters of feedstock, compost, and vermicompost samples.

3. Results and discussion

3.1 Earthworm growth and reproduction

There is observed sufficient increase in the earthworm population in containers A, B, and C. The container D (compost) is used as control with no addition of earthworms. The results indicate that the rice straw and cow dung provide a good growth media for *Eisenia fetida*. There is

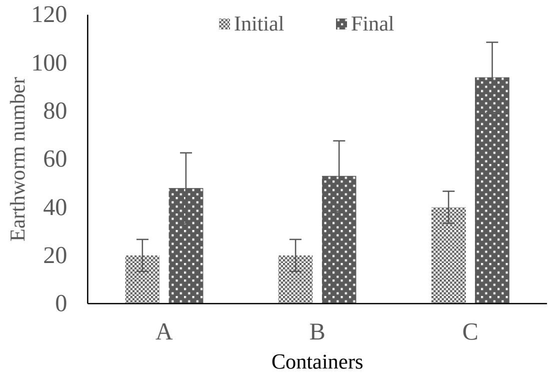

Fig. 1 Growth of earthworms in different containers

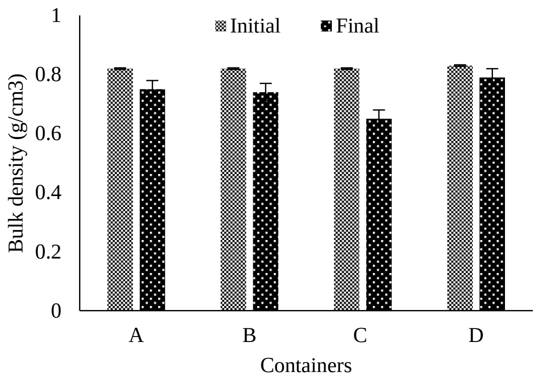

Fig. 2 Comparison of bulk density variation in different containers

observed highest increase in *Eisenia fetida* growth in case of container B (2.65 fold) as compared with container A (2.4 fold) and C (2.35 fold). The addition of Neem leaves (container B) positively impacted the growth and reproduction of *Eisenia fetida* as shown in Fig. 1. There is not observed any mortality in any container. The suitability of a feedstock for earthworm is best compared by evaluating their growth rate (Gupta and Garg 2009). Suthar and co-workers (2008) reported increased weight of earthworms during vermicomposting of wheat straw, slurry, and vegetable waste. The earthworm biomass increases if the feedstock is palatable. The feedstock quality and environmental conditions are important factors to control increase in biomass of the earthworms (Sharma and Garg 2018).

3.2 Change in bulk density

The bulk density is an indicator of soil health and compaction. It influences root penetration, nutrients availability, porosity, water holding capacity, and microorganism activity in the soil. The bulk density of feedstock plays a significant role in the composting process. There is observed decrease in the bulk density in case of all the treatments. The maximum decrease of bulk density is observed for container C (20.73%) which is followed by container B (9.76%), A (8.54%), and D

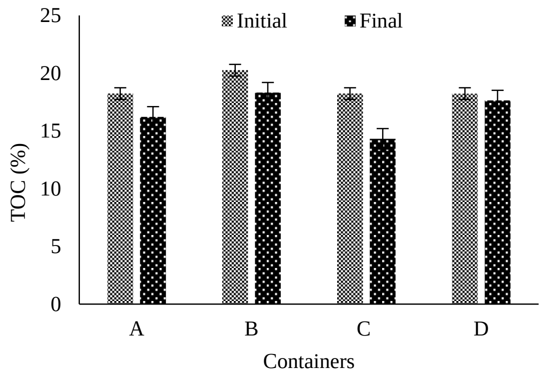

Fig. 3 Comparison of TOC content variation in different containers

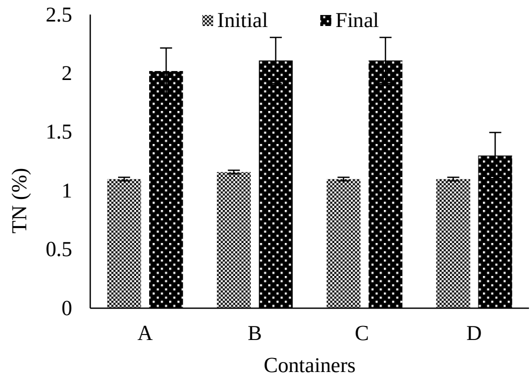

Fig. 4 Comparison of TN content variation in different containers

(4.82%) (Fig. 2). This may be a result of use of higher stock density of the earthworms in case of container C. The Neem leaves addition in container B resulted in more decrease in bulk density (9.42% decrease) as compared with the container A (8.2% decrease) at same stock density of the earthworms. Khater (2015) reported a negative correlation between bulk density and total organic matter of compost. Hence, the container B with higher organic matter (Neem leaves) showed higher reduction in bulk density at same stock density of the earthworms as compared with the container A. It may also be a result of better growth of the earthworms with Neem leaves for the container B. The decrease in bulk density increases soil porosity and water holding capacity which is good for the root penetration and plant growth.

3.3 Change in total organic carbon (TOC)

Total organic carbon (TOC) is the carbon stored in the soil organic matter. The decomposition of the organic matter releases TOC into the soil which is readily available to the plants. There is obtained 14.32 to 18.32% total TOC in the different samples of vermicompost and compost (control) (Table 2). These results are in good agreement with the literature where the optimum value of TOC is reported to be higher than 10% (Batjes 1996, Khater 2015). The maximum TOC reduction is achieved for the container C (21.5%) at maximum stock density of the earthworms which is followed by the container A (11%), B (9.57%), and D (3.24%) (control) (Fig. 3). The results indicate that the stock density of the earthworms has significant influence on TOC reduction of the feedstock.

The earthworms use carbon and change it into their biomass (Sharma and Garg 2017). The similar findings have also been reported by other researchers while working on vermicomposting of municipal waste, tea prunings, rice straw, and vegetable market waste (Hussain *et al.* 2016, Suthar *et al.* 2014). The microbial respiration also results into loss of carbon in the form of carbon dioxide (Nikaeen *et al.* 2015). TOC reduction shows mineralization and conversion of feedstock into the stabilized product. The container B with Neem leaves do not show any significant reduction in TOC of feedstock as compared with the container A at same stock density of the earthworms.

3.4 Change in total nitrogen (TN)

Nitrogen is key component of plant nucleic acid, enzymes, and proteins which facilitate vast array of biochemical reactions. There is observed significant improvement in the total nitrogen (TN) content of all feedstocks after the vermicomposting (Fig. 4). The highest TN content is achieved for container C (91.8%) which is followed by container A (83.6%), B (81.9%), and D (18.1%). The container C showed maximum increase in TN content at the highest stock density of the earthworms whereas minimum increase is seen in case of the container D (control). The results indicate that the stock density of the earthworms plays an important role to increase TN content in feedstock through mineralization of organic matter. Sharma and Garg (2018) reported accumulation of nitrogen in the feedstock with the mineralization of organic waste. Pramanik *et al.* (2016) reported 30.5 – 51.29% increase in the nitrogen content in tea prunings vermicompost. Sudkolai and Nourbakhsh (2017) also reported 1.6 and 3.2 times increase in TN content for cow dung and wheat residue vermicompost, respectively. The increase in TN content after vermicomposting may be due to nitrogen rich secretions of the earthworms like enzymes, hormones, excretory substances, and mucus (Tripathi and Bhardwaj 2004). The addition of Neem leaves is found to have no significant impact on TN content of the feedstock for the container B as compared with the container A at same stock density of the earthworms.

3.5 Change in total phosphorus (P), potassium (K), and calcium (Ca)

The phosphorus (P), potassium (K), and calcium (Ca) are essential nutrients for the plant growth. The concentration of these nutrients improved in all containers after vermicomposting. The highest increase in P content is observed for container C (73.4%), at the highest stock density of the earthworms, which is followed by the container A (50.8%), B (46.15%), and D (3.22%). Arumugam *et al.* (2017) revealed 46.1% increase in P content after vermicomposting of paper cup waste for 90 days. The earthworm gut enzyme phosphatase is responsible for partial mineralization of P. The further phosphorus release is carried out by the microbial communities (Swarnam *et al.* 2016, Vinotha *et al.* 2000). K content increased to the highest extent for the container C (38.8%) which is followed by container A (20.9%), B (19.1%), and D (3.07%). K content increase during the vermicomposting may be due to the production of acids by microorganisms which cause dissolution of K (Kaviraj and Sharma 2003, Suthar 2007). Suthar *et al.* (2014) stated that increase in K content in the vermicompost signifies its use for land application. The highest Ca content is

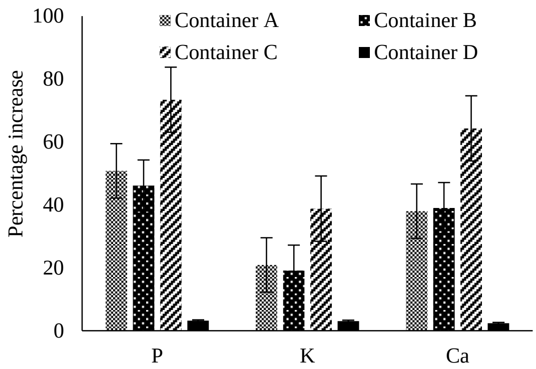

Fig. 5 Comparison of P, K, and Ca content in different containers

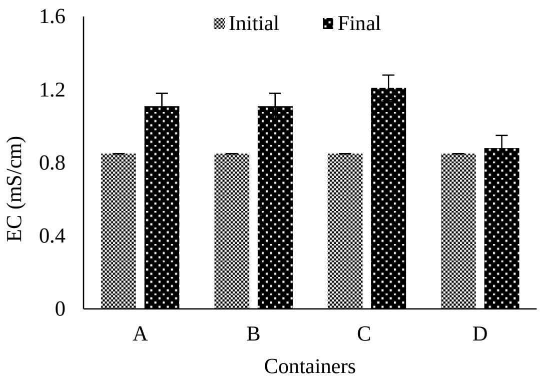

Fig. 6 Comparison of electrical conductivity variation in different containers

observed for the container C (64.6%) which is followed by container B (39%), A (38%), and D (2.38%) (Fig. 5). Ca mineralization starts in the gut of the earthworm and later proceeds through microbial population in the vermicast (Suthar *et al*. 2014). The container B is found to have higher Ca content as compared with the container A.

On the other hand, container A showed higher K and P content as compared with the container B at same stock density of the earthworms. The stock density of the earthworms is found to play an important role to improve nutrient quality of the final vermicompost.

3.6 Changes in electrical conductivity

There is observed significant increase in the electrical conductivity values of all the vermicompost samples as compared with the compost (control). The highest increase is obtained for container C (42.35%) which is followed by the container A and B (30.59%), and D (3.53%). The order of increase is in accordance with the stock density of the earthworms which shows direct contribution of the earthworm activity to increase electrical conductivity. The addition of Neem leaves is found to have no significant influence on the electrical conductivity of the

Table 2 The physico-chemical characteristics of final vermicompost

Parameters	Container A	Container B	Container C	Container D
Bulk density (g/cm^3)	0.75 ± 0.003 b	0.74 ± 0.003 c	0.65 ± 0.00 d	0.79 ± 0.003 a
Moisture content (%)	80.39 ± 0.137 a	80.52 ± 0.003 a	80.51 ± 0.000 a	80.33 ± 0.177 a
TOC (%)	16.22 ± 0.00 a	18.32 ± 0.003 b	14.32 ± 0.00 c	17.64 ± 0.003 d
TN (%)	2.02 ± 0.003 a	2.11 ± 0.003 b	2.11 ± 0.00 b	1.3 ± 0.00 d
K (%)	2.72 ± 0.009 c	3.05 ± 0.003 b	3.11 ± 0.007 a	2.35 ± 0.006 d
P (%)	1.87 ± 0.00 c	2.09 ± 0.00 b	2.15 ± 0.009 a	1.28 ± 0.007 d
Ca (%)	2.90 ± 0.003 c	3.10 ± 0.003 b	3.50 ± 0.003 a	2.15 ± 0.003 d
C:N ratio	8.03 ± 0.013 c	8.67 ± 0.013 b	6.79 ± 0.00 d	12.60 ± 0.003 a
EC (mS/cm)	1.11 ± 0.003 b	1.11 ± 0.003 b	1.21 ± 0.00 a	0.88 ± 0.007 c
Fe	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg
Mn	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg
Zn	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg
Cu	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg

Parameter values in each row followed by different letters are significantly different $p < 0.05$ (one way ANOVA followed by post hoc Tukey's all pair wise multiple comparison test)

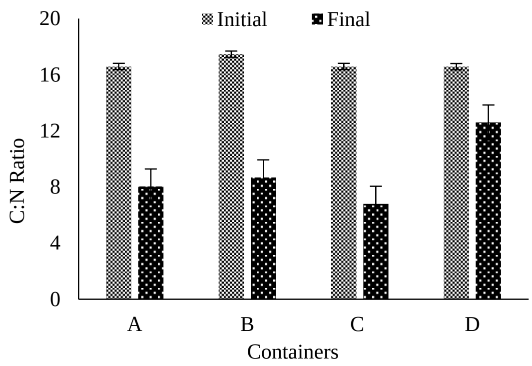

Fig. 7 Comparison of C:N ratio variation in different containers

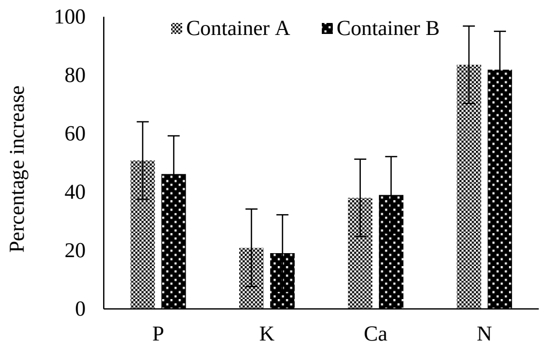

Fig. 8 Comparison of vermicompost nutrient parameters for containers A and B

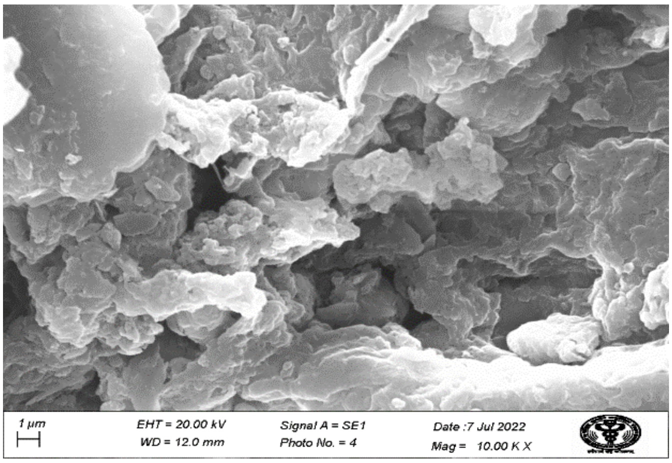

Fig. 9 SEM image of vermicompost

feedstock for the container B as compared with the other treatments (Fig. 6). Gupta and Garg (2009) also reported increase in the electrical conductivity during paper waste and cow dung vermicomposting. The increase in electrical conductivity may be due to weight loss of organic matter and release of soluble mineral salts (i.e. phosphate, potassium, and ammonium) during the decomposition of the feedstock (Kaviraj and Sharma 2003, Yadav and Garg 2011). The better mineralization of the organic matter with higher stock density of the earthworms resulted in higher electrical conductivity for container C.

3.7 Changes in C:N ratio and heavy metals

The carbon to nitrogen (C:N) ratio is a universal parameter of vermicompost maturity. All the vermicompost and compost (control) samples showed a sharp decrease in C:N ratio as compared with the initial feedstock (Fig. 7). The vermicompost samples showed decrease in C:N ratio to a higher extent as compared with the compost (control). The maximum decrease is obtained for the container C (59.05%), at the highest stock density of the earthworms as compared with the compost (23.96%). Sharma and Garg (2018) reported 19 - 102% decrease in C:N ratio after vermicomposting. The results indicate that the stock density of the earthworms is a significant factor for the decrease in C:N ratio of the feedstock. C:N ratio indicates rate of humification of the organic matter. It may decrease due to addition of nitrogenous components i.e., mucus and excretory products by the earthworms (Lv *et al.* 2018) and the simultaneous loss of carbon as carbon dioxide during microbial and earthworm respiration (Alidadi *et al.* 2016). There is observed no significant change in the heavy metal content of the final vermicompost in all the treatments.

3.8 Effect of addition of neem leaves (Azadirachta Indica)

The Neem (*Azadirachta indica*) is a large and fast growing tree belonging to Meliaceae family. It is likely a native to the Indian subcontinent (Encyclopedia Britannica, Gajalakshmi 2002). The Neem can be used as an environment friendly pesticide because of its insecticidal and pesticidal

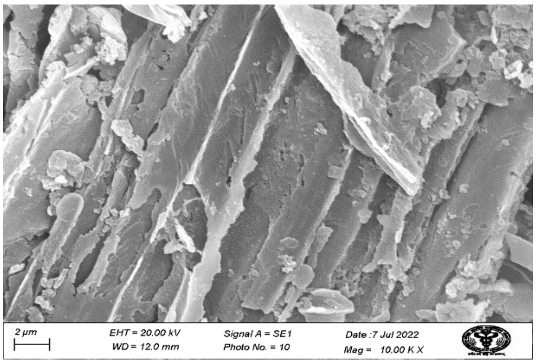

Fig. 10 SEM image of compost (control)

properties (Agarwal 1996). The addition of Neem leaves in container B is found to enhance quality of the final vermicompost as compared with container A (Table 2). Neem leaves have positive influence on the earthworm reproduction due to rich nutrients content. However, significant increase on other quality parameter has not been observed except Ca (Fig. 8).

3.9 SEM analysis

The Scanning Electron Microscope images clearly shows sufficient fragmentation in the vermicompost (Fig. 9). There is observed similar fragmentation of less intensity in the SEM images of compost sample (Fig. 10). The combined action of microbes and earthworms has resulted in higher degree of disintegration in case of the vermicompost sample as compared with the compost sample which is evident from surface morphology in micrographs. The above findings are in accordance with the results reported by previous researchers (Boruah *et al.* 2019).

4. Conclusions

The following conclusions are drawn from the present study:

- Earthworm, *Eisenia fetida*, can be effectively utilized for conversion of rice straw into nutrient rich vermicompost.
- The vermicompost produced with the highest stock density (40) of the earthworms showed the maximum increase in nutritional quality parameters, i.e. TN (91.8%), P (73.4%), K (38.8%), and Ca (64.6%). This showed positive role of earthworms to improve quality of the vermicompost.
- TOC and C:N ratio decreased in final vermicompost and compost (control) samples as compared with the initial feedstock. This shows feedstock mineralization and stabilization in the final product.
- *Eisenia fetida* showed the highest growth (2.65 fold) with Neem leaves (*Azadirachta indica*). The vermicompost produced with Neem leaves also showed higher Ca content with same stock density of the earthworms.
- The vermicompost with improved quality parameters is suitable for use as agricultural fertilizer to boost crop productivity and quality.

Acknowledgments

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