Online First Article

Solid Waste Management: Impact of Organic Waste on Growth and Reproduction of Earthworm *Eisenia fetida* via Vermicomposting

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**ABSTRACT**

The objective of this study was to investigate physicochemical parameter's impact on the growth and reproduction of earthworm *Eisenia fetida* during vermi-composting. Our study revealed that maximum weight/worm (0.35±0.00 mg/worms), length/worm (7.1±0.07 cm), the maximum number of cocoons (186), and juveniles (360) were recorded during 12 weeks of composting due to the high metabolic rate of earthworms. During the process of composting, pH, temperature, electrical conductivity, C: N ratio and moisture decreased till the end of the experiment and the final vermi-compost had pH (7.8), temperature (25 °C), electrical conductivity (2.82 mS/cm), C: N ratio (11:1), and moisture (25%). The maximum NPK content and various trace metals were also recorded in vermi-compost compared to cow dung which indicated its efficiency to promote the growth of plants. It was concluded that *E. fetida* could be used as a potential animal source for the solid waste management via vermi-composting.

**INTRODUCTION**

The inadequate waste management practices cause alteration in the ecosystems including air, water, and soil pollution, thus it represents a real threat to human health due to the increase in greenhouse gas emissions (Weigand *et al.*, 2003). For preventing any serious environmental health hazard wastes management is strongly required (Hussein and Mansour, 2018). Studies have also been directed to the novel technologies decomposition of organic waste. Organic solid waste (OSW) management solutions must be financially sustainable, technically feasible, socially, legally acceptable and friendly (Alqadar and Hamad, 2012). However, if properly recycled, rich sources of organic wastes such as food, agricultural, household, sugar, cotton and animal wastes can be successfully converted in to organic fertilizers through composting, nutrient enrichment and blending with specific plant growth regulators (*Ahmad* *et al.*, 2008). Although most of the studies have been focused on the impacts of some widely-known organic amendments such as animal manures, green manures and compost, but the recent interest in other alternative organic amendments such as vermi-compost is gaining importance (*Ramnarain* *et al.*, 2019).

Vermi-composting is a biotechnological method of composting by using different species of earthworms to boost organic waste conversion mechanism and achieve better product (*Adhikary*, 2012). Vermi-composting is a joint action of microorganisms and earthworms (vermi-culture) for degradation/breakdown of organic materials and is best for organic farming (*Soni and Sharma*, 2016). Previous literature indicated that tiger worm (*E. fetida*), red tiger worm (*E. andrei*), Indian blue worm (*Perionyx excavatus*), African night crawler (*Eudrilus eugeniae*), and red worm (*Lumbricus rubellus*) are best suited for vermi-composting.
Earthworms promote the growth of ‘beneficial decomposer aerobic bacteria’ in waste biomass and also act as an aerator, grinder, crusher, chemical degrader, biological stimulator, and so called soil ecosystem engineers (Dash, 1978; Binet et al., 1998; Khan, 2014). Earthworms host millions of microbes in their gut (Singleton et al., 2003) act as decomposer (bio-degrader). Edward and Fletcher (1988) showed that the number of bacteria and actinomyces contained in the ingested material increased up to 1000 fold while passing through the gut. Under favorable conditions, earthworms and microorganisms act symbiotically and synergistically to accelerate and enhance the decomposition of the organic matter in the waste.

Vermi-compost improves soil aeration and structure via reducing soil compaction, promotes better root growth and nutrient absorption, and improves nutrient status of soil (both macro-nutrients and micro-nutrients) (Lazcano and Dominguez, 2010). Thus, the objective of the present study was to use *Eisenia fetida* for decomposition of organic waste into vermi-compost, to investigate the physicochemical parameters of vermi-compost so that it can be used as effective fertilizer in agriculture, and to evaluate the impact of organic waste on growth and reproduction of *E. fetida*. This study is aimed at technology development and modifications for the production of good quality vermi-compost from locally available organic waste materials using composting earthworm.

**MATERIALS AND METHODS**

**Earthworms, cow dung, fruits and vegetable waste collection**

Locally available earthworms *E. fetida* were collected from Mahajar camp, Muzaffarabad, Azad Jammu and Kashmir (AJK), Pakistan, using hand sorting method (Zicsi, 1962) and storing in sealing bags or plastic bottles. Animal waste (cow dung) was collected from local residential houses of Muzaffarabad, AJK, Pakistan. The cow dung was air-dried for 10 to 15 days to remove the various associated organisms (beetles, spiders, snails, etc.), noxious gases, and chopped into small pieces (Bhattacharyya and Palta, 1996). The organic waste materials such as grass clippings, raw vegetables and fruits (their peels as well), maize straw, coconut peel, rice straw, eggshells, raw papers, and dry leaves were also collected from the residential houses and purchased as well from the local market of Muzaffarabad. Raw waste products such as vegetables and fruits were pre-digested before use (Chauhan and Singh, 2013). Fresh spinach or green vegetables were used for vermi-culturing. Waste papers were dipped in water before bedding. All material and earthworms were brought in Vermi-tech Unit, Zoology Department, University of AJK.

**Vermi-bed preparation and vermi-composting**

For mass culturing of earthworms and to allow earthworms to move horizontally and vertically for compost formation, vermi-bed was prepared in cemented pits (10 x 5 feet) (Supplementary Fig. 1). Worm bed was first prepared by using coconut peel/moisten papers/shredded twigs/grass clippings/crushed eggshells, varying according to what is available locally. A small amount of dried cow dung was mixed with pre-digested vegetables and fruits waste in a 3:1 ratio and spread on the shredded papers and coconut peel. At the beginning of vermi-composting, mature clitellate worms (800 to 1000) were acclimatized by releasing onto the vermi-bed for 2 h and feed matter like wheat straw, green and brown leaves, crushed eggshells, fresh green vegetable waste, and tea bags were given to earthworms. To protect the earthworm population from predators such as birds, rats, mice, cockroaches, and ants the pre-compost material was covered by either banana leaves, wire mesh, or cloth cover. The vermi-composting process was checked once in a week and thorough mixing of all substrate material was done to ensure the availability of all feeding materials that required for vermi-culturing. The moisture content was adjusted to 70–80% during the study period by spraying a sufficient quantity of distilled water. Temperature, moisture, and pH of the vermi-bed during vermi-composting were recorded using hygrometer and pH meter. After 3 months, earthy smell and blackish-brown color were observed which indicated the formation of vermi-compost (VC). Finally, composting material was sieved through 2 mm sieve and stored for further process.

**Physicochemical analysis of vermi-compost and cow dung**

All physicochemical parameters were measured at the facility available at the Soil Testing Laboratory of Soil Research Institute, Gojra, AJK, Muzaffarabad, Pakistan. Physical parameters of vermi-compost and cow dung such as texture, appearance, color, electrical conductivity, temperature, moisture, pH, and the smell was recorded. The total organic matter (%), total carbon (%), nitrogen (%) were measured using the Walkley-Black method whereas potassium (%) and phosphorus (%) levels in the vermi-compost and cow dung were measured by ammonium acetate method and modified Olsen method, respectively (Watanabe and Olsen, 1965). Besides, the concentration of trace metals like manganese, calcium, sulfates, copper, magnesium, iron, and zinc were measured via atomic absorption spectrometry (Naouni et al., 2011; Shahnamsouri et al., 2005).

During vermi-composting, variations in
physicochemical parameters were also recorded every week for up to three months regularly via above mentioned methods.

**Earthworm growth rate and cocoon production during vermi-composting**

Worm biomass, worm length, the total number of adult, juvenile worms, and production of cocoons were recorded weekly for 12 weeks during the vermi-composting. For biomass and length measurement three worms were randomly picked from three different pits. Earthworms, juveniles, and cocoons were separated from the feed by hand sorting method, washed with water and dried by paper towels, then they were counted.

**Statistical analysis**

Each experiment was repeated in triplicates and Mean±Standard Deviation from absolute data was calculated: (http://easycalculation.com/statistics/standard-deviation.php).

### RESULTS

**E. fetida**

The earthworms were collected from the local soil of Muzaffarabad city of Azad Jammu and Kashmir, Pakistan, and identified by the experts in Indonesia and Spain. *E. fetida* is an epigeic species associated with high organic matter content (Elvira et al., 1996). *E. fetida* lives on the surface of the soil as epigeic worms. Some external features were recorded based on color, number of segments, prostomium shape, clitellium shape, and segments. *E. fetida* is (35-130 mm) in length, 3-5 mm in diameter, clitellum over segments either 24, 25, 26-32, 80-120 segments, first dorsal pore between 4/5 or 5/6 and spermatheca, two pairs in 9/10 and 10/1. Earthworms come in various colors. The dorsal side of the worm is darker whereas the ventral side appears paler in color. The colors range from brownish-black to purple with the most common color being reddish-brown due to the presence of hemoglobin in the blood. *E. fetida* may also be known as Tiger worm, Red worm, Manure worm, and Red Wiggler. The optimum growing temperature for *E. fetida* is (20° to 25°C) but they can tolerate a range between 4° to 27°C. On the other hand, *E. fetida* becomes overheated at 85°F and can quickly die at above 90°F (Domínguez, 2004). Iron appears as the trace element with the highest concentration in dried *Eisenia fetida* (280 mg/l) while magnesium exists as the lowest concentration at 0.046 mg/l. The trace element concentrations ranked from highest to lowest are iron, zinc, manganese, copper, nitrogen, phosphorous, potassium, calcium, and magnesium, respectively. The concentration of elements obtained from dried *E. fetida* contained a composition of vermi-compost elements as reported previously (Dickerson, 1994; Mihara et al., 1999).

### Vermi-compost production

Vermi-compost has been produced on large scale (approx. 700kg to 1000 kg) at Vermi-tech Unit, Department of Zoology, University of Azad Jammu, and Kashmir (UAJ and K), Muzaffarabad using various organic waste materials and *E. fetida* earthworm species. Approximately, after 3 months vermi-compost contents looked like black rather than bedding that used at the beginning of the experimental setup. All the waste material completely converted into humus like material (vermi-compost) by *E. fetida*.

### Physical parameters variations during vermi-composting

Physical properties such as color, smell, appearance, temperature, pH, electrical conductivity, and moisture contents were recorded at the beginning and end of the experimental work. Results showed that during vermi-composting, significant physical variations were observed due to the biodegradation of organic matter and earthworms and microbial interaction. Variations in physical parameters were observed during three months of the experiment (Table 1). Results showed that initially pH was increased from 7.86±0.047 to 8.53±0.047 during the composting period. From 3rd week to 5th week pH was declined to 8.4±0.00 to 7.9±0.00 and finally further decreased to 7.8±0.00 from 6th week to the end of the experiment (Table 1). The EC values decreased gradually over initial values throughout the composting process. The observed highest EC value in 1st week and 2nd week was 3.39±0.008 mS/cm and 3.33±0.009 mS/cm respectively. From 3rd week EC values decreased gradually from 3.21±0.026 mS/cm to 2.83±0.04 mS/cm. At the 12th week of experiment minimum value of EC (2.83±0.04 mS/cm) was recorded (Table I).

The results showed the significant variations in temperature during various stages of vermi-composting from 1st to 12th week. During the initial stages of vermi-composting (1st to 4th week), the temperature recorded between 22±0.00 to 25.3±0.047 °C during the experiment, followed by 5th week (28.1±0.23 °C) and 6th week (30.1±0.23 °C) while it gradually increased during the middle stages 7th week (35.4±0.09 °C) and 8th week (36.8±0.047 °C) and again decline to 30.1±0.23 °C in 9th week, 28.1±0.23 °C in 10th week and finally decline to 25.0±0.00 °C during last two weeks as given in Table I.

Moisture content varied widely and gradually decreased throughout the vermi-composting. At the
beginning of experiment from 1st to 4th-week moisture content was between 59.1±0.00 to 60.0±0.00 %, from 5th to 6th week (40.0±0.00 to 41.23±0.87 %), from 7th to 8th week (35.5±0.00 to 35.3±0.23 %), from 9th to 10th week (25.0±0.00 to 25.6±0.12 %), and from week 11th to 12th (20.16±0.23 to 25.0±0.00 %) were recorded (Table I). Physical properties of finally produced vermi-compost were recorded as blackish-brown in color, fine grain and porous in appearance with an earthy smell, 25.0±0.0 °C temperature, 7.8±0.0 pH, 2.83±0.04 mS/cm EC, and 25.0±0.0 % moisture contents, respectively (Table II).

Table I. Physical parameters (Mean±SD) variations during vermi-composting.

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>pH</th>
<th>EC (mS/cm)</th>
<th>Temperature (°C)</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>7.86±0.05</td>
<td>3.39±0.008</td>
<td>22±0.0</td>
<td>59.1±0.0</td>
</tr>
<tr>
<td>2nd</td>
<td>8.53±0.05</td>
<td>3.33±0.009</td>
<td>23.8±0.62</td>
<td>60.0±0.0</td>
</tr>
<tr>
<td>3rd</td>
<td>8.4±0.05</td>
<td>3.21±0.026</td>
<td>3.5±0.40</td>
<td>59.8±0.18</td>
</tr>
<tr>
<td>4th</td>
<td>7.9±0.05</td>
<td>3.21±0.009</td>
<td>25.8±0.047</td>
<td>60.0±0.0</td>
</tr>
<tr>
<td>5th</td>
<td>7.9±0.0</td>
<td>3.14±0.01</td>
<td>28.1±0.23</td>
<td>40.0±0.0</td>
</tr>
<tr>
<td>6th</td>
<td>7.8±0.0</td>
<td>3.8±0.0</td>
<td>30.1±0.23</td>
<td>41.23±0.87</td>
</tr>
<tr>
<td>7th</td>
<td>7.8±0.0</td>
<td>3.02±0.014</td>
<td>35.4±0.09</td>
<td>35.5±0.0</td>
</tr>
<tr>
<td>8th</td>
<td>7.8±0.0</td>
<td>2.96±0.01</td>
<td>36.8±0.04</td>
<td>35.3±0.23</td>
</tr>
<tr>
<td>9th</td>
<td>7.8±0.0</td>
<td>2.93±0.004</td>
<td>30.1±0.23</td>
<td>25.0±0.0</td>
</tr>
<tr>
<td>10th</td>
<td>7.8±0.0</td>
<td>2.91±0.009</td>
<td>28.1±0.23</td>
<td>25.6±0.12</td>
</tr>
<tr>
<td>11th</td>
<td>7.8±0.0</td>
<td>2.86±0.016</td>
<td>25.0±0.0</td>
<td>20.16±0.23</td>
</tr>
<tr>
<td>12th</td>
<td>7.8±0.0</td>
<td>2.83±0.04</td>
<td>25.0±0.0</td>
<td>25.0±0.0</td>
</tr>
</tbody>
</table>

Table II. Comparative physicochemical analysis of vermi-compost and cow dung.

<table>
<thead>
<tr>
<th>Physiochemical parameters</th>
<th>Vermi-compost</th>
<th>Cow dung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Blackish brown</td>
<td>Greenish to dark brown color</td>
</tr>
<tr>
<td>Appearance</td>
<td>Porous and granular</td>
<td>Pasty and pile of shaving cream</td>
</tr>
<tr>
<td>Smell</td>
<td>Earthy</td>
<td>Pungent or rotten egg</td>
</tr>
<tr>
<td>Temperature</td>
<td>25 °C</td>
<td>40-45 °C</td>
</tr>
<tr>
<td>pH</td>
<td>7.8</td>
<td>8.7</td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td>2.82</td>
<td>3.52</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>25.2</td>
<td>50.4</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>43</td>
<td>86</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>2.28</td>
<td>1.86</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.030</td>
<td>0.0098</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>0.033</td>
<td>0.020</td>
</tr>
<tr>
<td>CEC (me/100g)</td>
<td>72.48</td>
<td>62.30</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>11:1</td>
<td>26:1</td>
</tr>
<tr>
<td>Mn (%)</td>
<td>0.2038</td>
<td>0.0414</td>
</tr>
<tr>
<td>Fe (%)</td>
<td>1.3313</td>
<td>1.1690</td>
</tr>
<tr>
<td>Cu (%)</td>
<td>0.0048</td>
<td>0.0017</td>
</tr>
<tr>
<td>Zn (%)</td>
<td>0.110</td>
<td>0.0012</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>1.35</td>
<td>1.0</td>
</tr>
<tr>
<td>SO²⁻ (%)</td>
<td>0.55</td>
<td>0.35</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.78</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Chemical parameters variations during vermi-composting

The current study revealed that vermi-compost possessed a high level of nutrients such as phosphorous, nitrogen, calcium, potassium, and magnesium compared to cow dung manure as well as a reduction in organic carbon and organic matter (Table II). The total organic carbon and organic matter in vermi-compost and values were recorded as 25.2% and 43%, respectively. The percentage of nitrogen (N), phosphorus (P) and potassium (K) was increased in vermi-compost (2.28%, 0.030% and 0.033%, respectively). The recorded value of the cation exchange capacity of vermi-compost was (72.48 me/100g). The level of trace metals in vermi-compost such as sulphates (0.35 %), iron (1.1690 %), zinc (0.0012 %), manganese (0.0414 %), magnesium (0.25 %), Copper (0.0017 %), and calcium (1.0 %) were also measured. From the beginning of the vermi-composting, there has been a reduction in C/N ratio in the worm-worked vermi-composts and final recorded value of carbon to nitrogen (C: N) in mature vermi-compost was 11:1 (Table II).

Physicochemical parameters of cow dung

The total organic carbon and organic matter in cow dung was 50.4% and 86%, respectively. The percentage of nitrogen (N), phosphorus (P), and potassium (K) in cow dung were recorded as 1.86%, 0.0098%, and 0.020%, respectively. The recorded value of the cation exchange capacity of cow dung was 62.30 me/100g. The level of trace metals in cow dung such as sulphates (0.35 %), iron (1.1690 %), zinc (0.0012 %), manganese (0.0414 %), magnesium (0.25 %), Copper (0.0017 %), and calcium (1.0 %) were recorded. C/N ratio recorded in cow dung was (26:1).

Earthworm growth rate and cocoon production during vermi-composting

At the end of 90 days, overall earthworm biomass
had increased, but a number of cocoons varied widely in different stages of vermi-composting (Table III). Maximum worm biomass was attained during the 8th week (0.35±0.0 mg/earthworm) and 7th week (0.27±0.05 mg/earthworm), whereas minimum biomass was recording during the first week of the experiment (0.05±0.0 mg/earthworm). Recorded biomass per earthworm during 2nd, 3rd, 4th, 5th, 6th, 9th, 11th, and 12th week was 0.11±0.01 mg, 0.12±0.05 mg, 0.14±0.05 mg, 0.18±0.05 mg, 0.21±0.01 mg, 0.21±0.01 mg, 0.19±0.0 mg, 0.18±0.0 mg and 0.14±0.0 mg, respectively. Similarly, the maximum length of the worm was also recorded during the 8th week (7.1±0.07 cm/earthworm) and 7th week (6.95±0.02 cm/earthworm), whereas minimum length was recorded during the first week (4.46±0.02 cm/earthworm). Recorded length per earthworm during 2nd, 3rd, 4th, 5th, 6th, 9th, 10th, 11th, and 12th week was 4.86±0.02 cm, 5.54±0.32 cm, 5.59±0.03 cm, 6.06±0.04 cm, 6.55±0.04 cm, 6.46±0.04 cm, 6.22±0.05 cm, 6.32±0.34 cm and 5.85±0.06 cm, respectively.

### Table III. Growth, reproduction, and cocoon production during vermi-composting.

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Weight/earthworm (mg)</th>
<th>Length/worm (cm)</th>
<th>Number of adult worms</th>
<th>Number of juveniles</th>
<th>Number of cocoons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>0.05±0.00</td>
<td>4.46±0.02</td>
<td>996±4.32</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>2nd</td>
<td>0.11±0.01</td>
<td>4.86±0.02</td>
<td>1000±3.68</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>3rd</td>
<td>0.12±0.05</td>
<td>5.54±0.32</td>
<td>1047±3.55</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>4th</td>
<td>0.14±0.05</td>
<td>5.59±0.03</td>
<td>1399±7.36</td>
<td>0.0±0.0</td>
<td>9.0±0.62</td>
</tr>
<tr>
<td>5th</td>
<td>0.18±0.05</td>
<td>6.06±0.04</td>
<td>1694±4.49</td>
<td>64±1.69</td>
<td>15.0±0.47</td>
</tr>
<tr>
<td>6th</td>
<td>0.21±0.01</td>
<td>6.55±0.04</td>
<td>2391±6.48</td>
<td>97±2.05</td>
<td>30.0±0.47</td>
</tr>
<tr>
<td>7th</td>
<td>0.27±0.05</td>
<td>6.95±0.02</td>
<td>3598±2.35</td>
<td>163±3.2</td>
<td>90.0±0.05</td>
</tr>
<tr>
<td>8th</td>
<td>0.35±0.00</td>
<td>7.1±0.07</td>
<td>4103±4.24</td>
<td>240±2.44</td>
<td>100.0±0.01</td>
</tr>
<tr>
<td>9th</td>
<td>0.21±0.01</td>
<td>6.46±0.04</td>
<td>4103±4.24</td>
<td>240±2.44</td>
<td>100.0±0.01</td>
</tr>
<tr>
<td>10th</td>
<td>0.19±0.00</td>
<td>6.22±0.05</td>
<td>4244±6.66</td>
<td>103±0.47</td>
<td>93.0±0.47</td>
</tr>
<tr>
<td>11th</td>
<td>0.18±0.00</td>
<td>6.32±0.34</td>
<td>4506±5.31</td>
<td>94.6±0.47</td>
<td>51.0±0.47</td>
</tr>
<tr>
<td>12th</td>
<td>0.14±0.00</td>
<td>5.85±0.06</td>
<td>4714±10.6</td>
<td>77±2.05</td>
<td>40.0±0.05</td>
</tr>
</tbody>
</table>

The maximum number of adult worms were recorded during 12th week (4714±10.6), followed by 11th week (4506±5.31) and 10th week (4244±6.66) while minimum number (996±4.32, 1000±3.68, 1047±3.55) of adult worms were recorded during 1st, 2nd and 3rd week, respectively. Number of adult worms during 4th, 5th, 6th, 7th, 8th and 9th weeks were 1399±7.36, 1694±4.49, 2391±6.48, 3598±2.35, 3887±9.53 and 4103±4.24, respectively. No juvenile worm was seen during the first three weeks of the experiment, however maximum number (360±1.88) of juveniles was recorded during the 8th week of the experiment. The recorded number of juveniles during 4th, 5th, 6th, 7th, 9th, 10th, 11th and 12th week were 30±0.81, 64±1.69, 97±2.05, 163±3.2, 240±2.44, 103±0.47, 94.6±0.47 and 77±2.05 respectively. The number of cocoons increased gradually during the 4th week of the experiment and maximum cocoons were recorded in the middle phase when the metabolic activity of the earthworm was high. In general, the maximum increase in cocoon production was recorded in 8th week (186±0.01), 9th week (100±0.01), 10th week (93±0.47) and 7th week (90±0.05). The number of cocoons declines to 51±0.47 in the 11th week and 40±0.05 in the 12th week. Minimum cocoons were recorded during 4th week (9±0.62), followed by 5th week (15.0±0.47) and 6th week (30.0±0.47). The changes in worm biomass, length, number of cocoons, adult, and juvenile worms over the experimentation period is illustrated in Table III.

### DISCUSSION

Vermi-composting is the conversion of organic waste materials (solid waste) to final humus like product via the mutual act of earthworms and microbes, and is one of the best way to not only enhance the soil fertility but also reduce the soil pollution (Ostos et al., 2008; Deka et al., 2011; Bhat et al., 2016a). Many organic wastes have been used for the vermi-composting such as paper wastes, coconut peel, rice waste, wheat straws, various types of manure (cow, sheep, goat, horse, poultry), leaves, raw vegetable waste, raw fruits wastes, and tea wastes (Acıkbas and Belliturk, 2016). Vermi-compost has been produced using goat and sheep manure, raw vegetables and fruits wastes, tea wastes, paper wastes (Zahmacioglu and Belliturk, 2014), coconut peel, wheat and rice straws (Acıkbas and Belliturk, 2016). In the current research, cow dung manure along with other agro waste was used for vermi-compolyestration and our results agreed with the Acıkbas and Belliturk (2016) and Zahmacioglu and Belliturk (2014).

Several kinds of literature illustrated the utilization of earthworms increase the soil organic matter (Gorres et al., 2011; Bhat et al., 2016). Dominguez et al. (2010) described that earthworms enhance microbial activity especially about soil quality and crop production. In the current research, E. fetida has been used for the vermi-compolyestration. According to Belliturk (2016) and Belliturk et al. (2015a), E. fetida is the best choice for outdoor vermi-compolyestration. No mortality was recorded during the study period. Gunadi and Edwards (2003) illustrated the death of Eisenia fetida after 2 weeks in the fresh cattle, though all other growth parameters were appropriate for the earthworm’s growth. On the other hand,
in the current experiment, wasted material (raw vegetables and fruits) were pre-composed and cow dung was air-dried for 2 weeks, and during this period all the toxic gases might have been eliminated. So, the pre-composting is very essential to avoid the worm’s mortality.

In our study, the maximum weight per earthworm during composting was attained in the 8th week (0.35±0.0 mg) due to high metabolic activities. Initially, worms gained biomass and reached a peak during the middle phase but after few weeks, weight loss by earthworms was observed. This is consistent with the findings of Neuhauser et al. (1980) who reported that loss in worm biomass can be attributed to the reduction of food. In the current research, maximum length per worm (7.1±0.07 cm) was also recorded during the 8th week which can be attributed to extensive metabolic activities and biodegradation of organic wastes during the composting process. It was observed during the composting process that all worms in the feed developed clitellum before day 21 after the start of the experiment which is parallel to the findings of Garg et al. (2005) who suggested in their study that clitellum development in worms started before 21st day of the experiment.

Our study thus revealed that cocoon production by earthworms was started by week 4 and then the number of cocoons and juveniles increased gradually from 4th to 8th week, reached a peak during the 8th week (186±0.01 cocoons and 360±1.88 juveniles). Thus, our findings are consistent with the work of previous researchers, that high metabolic and biodegradable phase (7th to 9th week) of composting process favors more cocoon production, thus increasing the number of juveniles, thereby increasing overall sexual reproduction (Edwards, 1988). At the final stage of vermi-composting during the 12th-week maximum number (4714±10.6) of worms were recorded but their biomass was reduced as their activities become slow due to exertion of food (Neuhauser et al., 1980). The current research visibly determines that they significantly increase the macro- and micronutrients level and beneficial microorganisms in vermi-compost are because of an increase level of earthworm reproduction and growth.

The current results indicated that during vermi-composting, significant physical and chemical changes had occurred in the solid waste material due to the biodegradation of organic matter through the synergistic effect of earthworms and microbes. The pH of vermi-compost is very much essential because it affects the availability of nutrients in the soil (Yuvravaj et al., 2019).

In our experiment it was observed that pH was initially increased (8.53±0.047) and later decreased to 7.8±0.0 till the end of the experiment, we might expect that this decrease in pH was due to the generation of CO₂ and organic acids from microbial metabolism along with the decomposition of organic substances. Similar findings were reported by Fu et al. (2015) who suggested that the pH dropped during vermi-composting could be attributed to the production of CO₂ and owing to the metabolic activities of earthworms and microbes, as well as the mineralization of nitrogen and phosphorus. Our results are also agreed with the outcomes of Fu et al. (2014), who reported that inorganic ions and pH increases after 60 days.

Electrical conductivity is a good indicator of the suitability and safety of vermi-compost for agricultural purposes (Lazcano et al., 2008). Current findings showed that electrical conductivity reduced until the formation of mature compost, due to bio-accumulation of minerals in the earthworms’ bodies, and consequently, the reduced amount of soil minerals. According to Shak et al. (2014), the reduction in EC during vermi-composting may be due to the precipitation of soluble salts and organic acid mineralization. The current research showed that pH and EC of the vermi-compost were 7.8 and 2.82 mS/cm, respectively. Similar findings were also reported by (Bhat et al., 2017) who suggested that vermi-composts of pH 6–8.5 and EC value below 4.0 mS/cm are excellent for agricultural soils.

Temperature plays a significant role in earthworm’s growth and development (Yuvravaj et al., 2019). Our results showed significant variations in temperature during various stages of vermi-composting (from 1st to 12th week). At the start of experiments, the temperature was moderate and then gradually increased during mesophilic stage (5th to 8th week) of vermi-composting due to the metabolic activities of microbes and worms, and later the temperature was reduced as vermi-compost was in the final stage of production, which probably due to decreased activity of worms. The recorded temperature of vermi-compost in our study was 25°C, this temperature is in the optimum range for growing media and consistent with the outcomes of Mohee and Mudhoo (2005). Our results are also in agreement with the findings of Peigne and Girardin (2004).

Moisture content during the study period also decreased from 60.0±0.0 to 25.0±0.0 % till the end of the experiment which may be due to higher degradation of wastes by earthworms and maximum microbial population gathered in worm’s gut environment. Edward et al. (2011) reported that the best moisture content (80%) for vermi-composting and Bhat et al. (2017) reported 20-30% moisture contents for maximal microbial activity during vermi-composting. Khater (2015) reported the moisture contents ranged from 23.50 to 32.10% for different compost types, which is in agreement with our results.

Our study revealed that finally prepared compost through activities of Esinea fetida was blackish-brown
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in color, highly porous, well-aerated with an earthy smell which was following the study of Hartenstein and Hartenstein (1981), who reported that vermi-compost has very high aeration, porosity, drainage, and water holding capacity. Our results agreed with the outcomes of Teng et al. (2014) and Kuncoro et al. (2014). They conveyed that healthy soil contains sufficient water, minerals, and air.

Our study showed a significant increase in NPK level of vermi-compost compared to cow dung. Similarly, increased levels of NPK were reported by Deepa et al. (2008) during prolonged 90 days of vermi-composting of leaf litter using Eudrilus eugenia. Earthworms can lift the level of nitrogen during gut digestion by adding their body fluid, nitrogenous excretory products, enzymes, mucus, and even decaying of dead materials in vermi-composting (Suthar, 2007). The increased nitrogen level stimulates plant growth and vegetative structure. Our findings agreed with Li et al. (2013). The maximum nitrogen contents may be accredited to the release of nitrogenous products of earthworm (Yuvaraj et al., 2019). The enhanced phosphorous contents in our vermi-compost were similar to the findings of Padmavathiamma et al. (2008) who reported that worms during vermi-composting converted the insoluble P into soluble forms with the help of phosphate solubilizing microbes. The release of phosphatases in the earthworm’s gut causing elevates the total phosphorus content. The increase in extractable K in our vermi-compost may be attributed to the earthworm-mediated microbial activity during vermi-composting. On the other hand, decreased levels of organic carbon compared to cow dung were recorded in mature vermi-compost and germination media having vermi-compost, which may be due to the mineralization, effect of earthworm activities and thermophilic phase. Our findings are consistent with the earlier investigations (Garg et al., 2006; Suthar, 2007; Hait and Tare, 2011). The decreased inorganic carbon may be also due to the loss of organic carbon as CO₂ via organic matter mineralization and microbial respiration (Padmavathiamma et al., 2008).

Our analysis of vermi-compost through atomic absorption spectrophotometry indicated the presence of trace metals like manganese, copper, iron, manganese, copper, calcium, sulfates and zinc, which consistent with the findings of Shahmansouri et al. (2005) who reported the presence of trace metals such as manganese, cadmium, copper, chromium, and iron in vermi-compost. The maximum values of Mg and Ca in reported research was also consistent with the findings of Padmavathiamma et al. (2008). Our study revealed that the cation exchange capacity of vermi-compost was maximum (72.48 me/100g) compared to cow dung (62.30 me/100g) and similar to the findings of Xu and Mou (2016). C: N ratio is also a very important parameter for the stability and maturity of vermi-compost. Our results agreed with the previous literature (Suthar, 2008; Singh et al., 2014). The loss of carbon to nitrogen ratio (11:1) is due to the mineralization process and consistent with the findings of Suthar and Singh (2008) and Shak et al. (2014). They reported that C: N less than 12 is preferable for agricultural purposes. The more reduction in the content of the C/N ratio reflected the effective worm activity, accelerated the decomposition and mineralization rate, thereby resulting in high grade, nutrient-rich, and good quality vermi-compost (Yuvaraj et al., 2019).

Suthar and Singh (2008) illustrated the loss of carbon and the addition of nitrogen during the vermi-composting process decreases the C: N ratio in the end product. Current research thus showed the highly porous texture of vermi-compost, rich in nutrients and minerals, which play important role in the enhancement of physical and chemical properties of soil, have a great impact on the movement of nutrients and, water and air, stimulate the plant growth and have a significant effect on the absorption efficiency (Salehi and Maleki, 2012; Chaudhari et al., 2013; Blanchard et al., 2004).

CONCLUSIONS

Our trials demonstrated vermi-composting as an alternate technology for the recycling of different waste materials using an epigeic earthworm Eisenia fetida. Vermi-culture converts farm wastes into organic fertilizer, making it an environment-friendly technology. Earthworms and its metabolic products (vermi-compost) may work as the driving force in sustainable food production while improving soil health and fertility and protecting crop plants from pests and diseases. We recommend that switching over to sustainable agriculture by vermi-culture can truly bring in economic prosperity for the farmers and the nations with environmental security for the earth.

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Supplementary material
REFERENCES


Dickerson, W.D., 1994. Vermicomposting. New Mexico State University, Mexico.


Mohee, R., and Mudhoo, A., 2005. Analysis of the


Solid Waste Management: Impact of Organic Waste on Growth and Reproduction of Earthworm *Eisenia fetida* via Vermicomposting

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Fig. 1. Vermi-bed preparation and vermi-composting process. (A) Pre-digested fruit and vegetable waste, (B-C) Bedding preparation, (D) Introduction of worms, (E) Introduction of fruit and vegetable waste, (F) Mature clitellate worm, (G) Mixing of material, (H) Introduction of spinach as green material, (I-J) Processing of vermi-compost, (K) Worm movements, (L) Watering, (M-N) Sieving of vermicompost.

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