



## Study of Some Physiological And Biochemical of Earthworm Eiseniafetida during the Vermicomposting of Organic Plants

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### Abstract

Earthworms *Eiseniafetida*, are treated in Sawdust + Tomato (STM), Sawdust + Tobacco (STB), Sawdust +Sugar beet (SSB), Sawdust +Tomato + Tobacco (STMTB), Sawdust +Sugar beet Tobacco (SSBTB), Sawdust Tomato + Sugar beet (STMSB) and Sawdust Tomato +Sugar beet + Tobacco (STMSBTB) substrates with four replications. The effects of treatment, time and interaction of treatment\*time are assessed with respect to variability of physicochemical parameters of substrates and the growth and reproduction of *E.fetida* for a period of 45 days with intervals of 15 days sampling. The two-way ANOVA indicates the statistical significances effect of (P<0.01). The parameters: EC, pH, C: N, moisture%, weight, number of earthworm, number of cocoon and number of hatchlings' mean involved in treatments above indicate scientific differences with respect to physicochemical parameters of substrates and the growth and reproduction of *E.fetida*. Regarding time effect the findings on treatment effect hold true in its own sense.

## 1. Introduction

The Food and Agriculture Organization of the United Nations [1] reported that the annual total amounts of global food waste (FW) generation are approximately 1.3 billion tones. The FW related issues in developing countries is currently considered to be a major threatening factor regarding sustainable development in addition to this management systems [2]. Gustavsson et al. [3] separated the FW generation in five sources: agricultural, postharvest handling and storage, processing, distribution and consumption, moreover Thi et al. [4] reported that the per capita FW in developed countries and developing countries are 107 kg/year and 56 kg/year, respectively. Bureau Population [5] reported that the population constitutes developed countries is 1.2 billion, and 6 billion in less developed countries. Although today, there is a wide recognition of the major environmental implications of food production [6], studies on agri-food processing wastes so far have not shown any impacts of resources wastage from a global environmental perspective [7]. The valorization strategies of the wastes from agri-food processes are intertwined with clean technological approaches and eco-industrial management [8]. Biological waste treatment technologies such as composting and vermicomposting are widely regarded as a clean and sustainable method in organic waste management [9]. Vermicomposting, a novel technique of converting decomposable organic waste into valuable vermicompost through earthworm activity is a faster and better process when compared with the conventional methods of composting [10]. Vermicomposting is adopted for the management of organic waste in industry [11]. It is well established that organic wastes can be ingested by earthworms and egested as peat like material termed as vermicompost [12]. The quality and amount of the available food influences the size of earthworm populations [13]. The growth, maturation, cocoon production and reproductive potential which is strongly affected by the quality and availability of food [14, 15]. The pH and the moisture contents of the substrate is reported by [16,17]. The consumption of mixed material in feeding the epigeic composting species [18-20] leads to an improvement in the C: N ratio by supplying C and, at the same time, preventing N losses by ammonia volatilization [18]. The C: N ratio is a factor related to decomposition of plant residues [21] and is recognized as a factor correlated with earthworm density a negative manner [22]. Several epigeic earthworms, e.g., *E. fetida*, *P. excavatus*, *P. sansibaricus*, *E. eugeniae*, and *E. andrei* are

identified as detritus feeders and as a potential in minimizing the anthropogenic wastes from different sources [23-25]. The *E. fetida* was, and still is, the favored earthworm species for laboratory trail experiments on vermin composting due to its tolerance against environmental variables (pH, moisture content, temperature, etc.). For this study *E. fetida* is used as the vermin composting agent. The objective of this study is to assess the effect of treatment, time and treatment \* time interaction on the variability with respect to: (i) the EC, pH, moisture content and C: N parameters and (ii) growth, production of cocoon and number of hatchlings successfully emerged from cocoons.

## 2. Material and Methods

### 2.1. Collection of *E. fetida* earthworm, cow dung and food processing waste

*E. fetida* earthworm species, cultivated at a research institute of vermin compost in the Islamic Azad University of Isfahan (Khorasgan branch), Iran, is used for inoculating it in the substrates of vermin composting. Cow dung is collected from Research Institute of Agriculture of native cattle breeding, Isfahan. Sawdust is prepared from the Research Institute of Wood and Paper Science and Technology, Gilan, Iran. Rotted tomatoes are prepared from fruit and vegetable market, and processed of sugar beet waste is prepared from the sugar factory of Isfahan. Tobacco is consumed as breeding substrates of earthworm, prepared from Research Tobacco Center at the Ministry of Agriculture Research Farm, Iran. These prepared materials are dried in the laboratory at ambient temperature and then pre-composted in plastic bags for about 21 days. The cow dung is used as bulky agent for preparation of Vermibeds/feed materials for *E. fetida*.

### 2.2. Experimental design

The experiments are run in seven treatments with four replicates in a completely randomized block design. The produced waste is mixed with cow dung in different proportions (Table 1). Total mashed plastic bins of 45×30×30 cm are used as the worm bins. The walls, with the bottom and the top of the bin are corroding with a thin layer of newspaper for adsorption and prevention of earthworm escape. Each treatment consists of four replicates (400 g of feed materials in each replicate). The cow dung weight used in all treatments is 280g. The remained 120 g is proportional in mixtures to desired ratios for seven treatments, Table (1). One cm thick farm soil is spread on the bottom of the bin, next the mixture which is pre-composted for 21 days is poured on it and covered with one layer by 1 cm farm soil. This is to prevent the released bad odor and gathering of insects produced among the mixture. Cow dung (CD) is used only as a supplement and bedding material for earthworms. After substrate preparation, 8 g of the matured earthworms (*E. fetida*) with approximately same size are inoculated in each treatment bin, The process takes approximately days 45 since sexual maturity period for this species is 4–6 weeks under favorable conditions. Moisture content of the treatment is maintained at about 60–80% by spraying the surface with water every two days using a wash bottle (50–100 ml per plot). The bins are kept in the laboratory at room temperature.

**Table 1.** Preparation of substrates for inoculation *E. fetida* earthworm

Name of treatment	Type of organic waste	Ratio of cow dung (CD)/ Organic waste
T1	Sawdust + Tomato (STM)	2:1:1
T2	Sawdust + Tobacco (STB)	2,1:1
T3	Sawdust + Sugar beet (SSB)	2,1:1
T4	Sawdust + Tomato + Tobacco (STMTB)	2:1:1:1
T5	Sawdust + Sugar beet+ Tobacco (SSBTB)	2,1:1:1
T6	Sawdust + Tomato + Sugar beet (STMSB)	2,1:1:1
T7	Sawdust+ Tomato +Sugar beet + Tobacco (STMSBTB)	2,1:1:1:1

Source: the authors

### 2.3. Chemical analysis

In order to measure the variability of physico-chemical characteristics of this waste mixture during vermicomposting, the homogenized samples are drawn from each experimental container at days 0, 15, 30 and 45 after inoculation. The samples are oven dried for 48 h at 60 °C, and stored in sterilized plastic airtight containers for further physico-chemical analysis. To determine the electrical conductivity (EC) and acidity (pH) the dried samples are mixed with distilled water in the volume ratio of 1: 5, the mixture was placed in the shaker

for an hour. The amounts of two parameters are measured by the EC meter (model: Metrohm, 712) and pH meter (model: Metrohm, 788). The organic carbon percentage was determined by Walkley-Black method. The organic carbon was oxidized by consuming potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) in an acidic environment full of H<sub>2</sub>SO<sub>4</sub>. Total nitrogen is determined by Kjeldahl method (wet oxidation). The C: N ratio is determined by the measured values of total nitrogen and total organic carbon. The earthworms and cocoons and hatchlings emerged from cocoons were separated from the feed manually, next these are counted and weighed washed and dried with paper towels.

## 2.4. Statistical analysis

The data of the physicochemical characteristics are analyzed through Statistix 8.0. Two way analysis of variance (ANOVA) is carried out and in order to statistical significant, p value is obtained in 0.1 -0.05 level. Next, the characteristics of physicochemical parameters of treatment, time and interaction of treatment \* time are compared based on all-Pairwise means throaty LSD test, at p<0.05

## 3. Results and discussion

### 3.1. Two-way ANOVA

Two-way ANOVA revealed the significant effect at (p<0.01) on all parameters of treatment and time. The same effect is overdone from interaction between treatment \* time regarding the characteristics of pH, C: N, number of cocoon and hatchlings. The significant effect (p<0.05) is observed on the weight with respect to treatment \* time interaction (Table2).

**Table2.** Analysis of variance based on the physico-chemical characteristics

Source variable	df	MS							
		EC ds/m	pH	C:N Ratio	Moisture%	Weight(g)	Number of earthworm	Number of cocoon	Number of hatchlings
treatment	6	18.66**	1.76**	0.02**	106.05**	27.71**	50.82**	856.97**	2498.70**
time	3	2.21**	8.43**	0.01**	123.14**	113.16**	128.40**	2553.56**	9537.85**
Treatment*Time	18	0.13	0.33**	0.00	32.16**	5.81*	10.19	224.83**	853.14**
Error	84	0.11	0.05	0.16	13.81	3.06	9.56	87.34	206.71

Note: \*\*, \* are significant at 1% and 5% probability level, respectively

EC: Electrical Conductivity, pH: Acidity

Source: the authors

### 3.2 The Effect of treatment, time and treatment\* time on EC

Comparison the means Tables (3, 4 and 5) reveal that the EC varies significantly between treatments, yet there significant difference is not for EC in treatments of SSBTB and STMSB. The highest and lowest obtained EC means related to the STMTB is 4.53 and to SSB is 1.54. The effect of time indicates the EC increased significantly after inoculation compared to day 0, and continued up to days 30 and 45 in relation to day 15.

**Table 3.** Mean comparison of the physic-chemical characteristics the treatments

Treatment	characteristic							
	EC(ds/m)	PH	C:N	Moisture%	Weight(g)	Number of earthworm	Number of cocoon	Number of hatchlings
T1	3.26 <sup>c</sup>	7.88 <sup>c</sup>	0.12 <sup>a</sup>	23.61 <sup>d</sup>	6.54 <sup>ab</sup>	10.43 <sup>a</sup>	12.00 <sup>bc</sup>	20.81 <sup>b</sup>
T2	1.79 <sup>e</sup>	8.49 <sup>b</sup>	0.15 <sup>a</sup>	29.23 <sup>ab</sup>	6.01 <sup>ab</sup>	10.25 <sup>a</sup>	15.31 <sup>b</sup>	33.62 <sup>a</sup>
T3	1.54 <sup>f</sup>	8.36 <sup>b</sup>	0.13 <sup>a</sup>	24.95 <sup>cd</sup>	7.14 <sup>a</sup>	10.18 <sup>a</sup>	13.37 <sup>bc</sup>	27.93 <sup>ab</sup>
T4	4.53 <sup>a</sup>	7.93 <sup>c</sup>	0.12 <sup>a</sup>	27.39 <sup>bc</sup>	3.28 <sup>c</sup>	5.68 <sup>c</sup>	1.87 <sup>d</sup>	3.12 <sup>c</sup>
T5	3.77 <sup>b</sup>	8.46 <sup>b</sup>	0.06 <sup>b</sup>	25.96 <sup>cd</sup>	5.36 <sup>b</sup>	8.43 <sup>ab</sup>	5.25 <sup>d</sup>	2.43 <sup>c</sup>
T6	3.65 <sup>b</sup>	7.95 <sup>c</sup>	0.07 <sup>b</sup>	31.22 <sup>a</sup>	5.46 <sup>b</sup>	7.50 <sup>bc</sup>	7.18 <sup>cd</sup>	7.06 <sup>c</sup>
T7	2.94 <sup>d</sup>	8.72 <sup>a</sup>	0.04 <sup>b</sup>	26.86 <sup>bc</sup>	6.95 <sup>a</sup>	9.75 <sup>a</sup>	23.87 <sup>a</sup>	21.87 <sup>b</sup>

Means followed by the same letter within each column are not significantly different at p < 0.05 according to LSD test

EC: Electrical Conductivity, pH: Acidity

Source: the authors

Obtained details from the analysis of the interaction between treatment\* time confirmed that the EC of SSBTB

and STMSB have no significant difference, moreover, the EC of STB and SSB changes not significantly. The effect of all the treatments on EC is ranked in the following order STMTB> SSBTB> STMSB> STM> STMSBTB> STB> SSB. Interaction analysis do not approve the significant difference for EC of STM compared to SSBTB at day 30, STMSBTB at day 15 and STMSB at days 30 and 45. A significant difference in EC of STMTB in relation to SSBTB and STMSB is observed after inoculation. A significant difference in EC of SSBTB and STMSB compared to STMSBTB related to the time after inoculation. Analysis of the interaction specified that the time effect caused a significant difference the EC in between days 0 and 45 through STB, SSBTB and STMSBTB, it is worth mention that an increase in EC might be due to loss of organic matter and release of different mineral salts as phosphate, ammonium, potassium, etc. [26-28]. Garg et al. [29] reported a gradual increase in EC all the reactors with respect to a increase in decomposition time, which, in this work the same phenomena is observed with respect to the parameters of treatment and time.

**Table 4.** Mean comparison of the physic-chemical characteristics for the time

Time	characteristic							
	EC(ds/m)	PH	C:N Ratio	Moisture%	Weight(g)	Number of earthworm	Number of cocoon	Number of hatchlings
0	2.71 <sup>c</sup>	9.02 <sup>a</sup>	0.08 <sup>b</sup>	30.13 <sup>a</sup>	8.00 <sup>a</sup>	11.39 <sup>a</sup>	0.00 <sup>c</sup>	0.00 <sup>b</sup>
15	2.99 <sup>b</sup>	8.23 <sup>b</sup>	0.08 <sup>b</sup>	25.96 <sup>b</sup>	7.03 <sup>b</sup>	9.92 <sup>a</sup>	23.00 <sup>a</sup>	1.64 <sup>b</sup>
30	3.24 <sup>a</sup>	8.03 <sup>c</sup>	0.10 <sup>b</sup>	25.58 <sup>b</sup>	4.45 <sup>c</sup>	7.42 <sup>b</sup>	13.14 <sup>b</sup>	30.07 <sup>a</sup>
45	3.34 <sup>a</sup>	7.74 <sup>d</sup>	0.13 <sup>a</sup>	26.45 <sup>b</sup>	3.81 <sup>c</sup>	6.82 <sup>b</sup>	8.92 <sup>b</sup>	35.07 <sup>a</sup>

Means followed by the same letter within each column are not significantly different at  $p < 0.05$  according to LSD test

EC: Electrical Conductivity, pH: Acidity

Source: the author

**Table 5.** Mean comparison of the interaction effect of the treatment \* time on the physic-chemical characteristics

Treatment	Time	characteristic							
		EC(ds/m)	PH	C:N Ratio	Moisture%	Weight(g)	Number of earthworm	Number of cocoon	Number of hatchlings
T1	0	3.10 <sup>efg</sup>	8.85 <sup>cd</sup>	0.10 <sup>cdefghi</sup>	25.03 <sup>e-i</sup>	8.00 <sup>a-d</sup>	11.25 <sup>abcd</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T1	15	3.08 <sup>efg</sup>	7.78 <sup>kl</sup>	0.09 <sup>defghij</sup>	22.46 <sup>ghi</sup>	7.49 <sup>a-e</sup>	10.25 <sup>a-d</sup>	27.00 <sup>ab</sup>	0.75 <sup>e</sup>
T1	30	3.40 <sup>cde</sup>	7.57 <sup>lm</sup>	0.121 <sup>b-h</sup>	22.74 <sup>ghi</sup>	5.49 <sup>e-h</sup>	9.75 <sup>a-e</sup>	11.75 <sup>cde</sup>	41.50 <sup>c</sup>
T1	45	3.48 <sup>cde</sup>	7.32 <sup>m</sup>	0.17 <sup>bc</sup>	24.20 <sup>e-i</sup>	5.19 <sup>e-h</sup>	10.50 <sup>abcd</sup>	9.25 <sup>de</sup>	41.00 <sup>c</sup>
T2	0	1.34 <sup>j</sup>	9.19 <sup>ab</sup>	0.10 <sup>d-i</sup>	36.05 <sup>a</sup>	8.00 <sup>a-d</sup>	11.50 <sup>abcd</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T2	15	1.70 <sup>hij</sup>	8.49 <sup>efgh</sup>	0.10 <sup>e-j</sup>	27.25 <sup>c-h</sup>	8.77 <sup>ab</sup>	11.50 <sup>abcd</sup>	38.75 <sup>a</sup>	4.00 <sup>e</sup>
T2	30	2.01 <sup>hi</sup>	8.30 <sup>ghi</sup>	0.16 <sup>bcd</sup>	27.05 <sup>d-h</sup>	3.30 <sup>h-l</sup>	8.00 <sup>cdef</sup>	13.75 <sup>cd</sup>	58.00 <sup>abc</sup>
T2	45	2.12 <sup>h</sup>	7.97 <sup>jk</sup>	0.25 <sup>a</sup>	26.57 <sup>e-h</sup>	3.96 <sup>g-k</sup>	10.00 <sup>abcd</sup>	8.75 <sup>de</sup>	72.50 <sup>a</sup>
T3	0	1.31 <sup>j</sup>	8.72 <sup>cdef</sup>	0.10 <sup>e-j</sup>	20.89 <sup>i</sup>	8.00 <sup>a-d</sup>	13.00 <sup>a</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T3	15	1.45 <sup>j</sup>	8.50 <sup>efg</sup>	0.11 <sup>e-i</sup>	24.93 <sup>e-i</sup>	9.26 <sup>a</sup>	12.50 <sup>ab</sup>	34.50 <sup>ab</sup>	0.00 <sup>e</sup>
T3	30	1.63 <sup>ij</sup>	8.20 <sup>ghij</sup>	0.14 <sup>b-f</sup>	25.75 <sup>e-i</sup>	5.86 <sup>c-g</sup>	7.75 <sup>c-g</sup>	9.50 <sup>de</sup>	49.75 <sup>bc</sup>
T3	45	1.77 <sup>hij</sup>	8.02 <sup>ijk</sup>	0.18 <sup>b</sup>	28.22 <sup>c-f</sup>	5.45 <sup>e-h</sup>	7.50 <sup>d-g</sup>	9.50 <sup>de</sup>	62.00 <sup>ab</sup>
T4	0	3.60 <sup>bcd</sup>	9.00 <sup>bc</sup>	0.11 <sup>cdefghi</sup>	29.18 <sup>b-e</sup>	8.00 <sup>a-d</sup>	11.50 <sup>abcd</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T4	15	4.65 <sup>a</sup>	7.76 <sup>kl</sup>	0.11 <sup>b-i</sup>	27.49 <sup>c-g</sup>	2.14 <sup>ijkl</sup>	5.00 <sup>fgh</sup>	2.25 <sup>cd</sup>	0.00 <sup>e</sup>
T4	30	4.93 <sup>a</sup>	7.53 <sup>lm</sup>	0.13 <sup>b-g</sup>	27.11 <sup>d-h</sup>	1.70 <sup>kl</sup>	3.50 <sup>gh</sup>	1.75 <sup>de</sup>	6.00 <sup>e</sup>
T4	45	4.97 <sup>a</sup>	7.42 <sup>m</sup>	0.15 <sup>bcde</sup>	25.77 <sup>e-i</sup>	1.27 <sup>l</sup>	2.75 <sup>h</sup>	3.50 <sup>de</sup>	6.50 <sup>e</sup>
T5	0	3.54 <sup>cde</sup>	8.74 <sup>cdef</sup>	.05 <sup>hij</sup>	33.66 <sup>ab</sup>	8.00 <sup>a-d</sup>	12.00 <sup>abc</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T5	15	3.66 <sup>bcd</sup>	8.68 <sup>cdef</sup>	0.05 <sup>hij</sup>	22.03 <sup>b-i</sup>	7.49 <sup>a-e</sup>	11.50 <sup>abcd</sup>	13.50 <sup>cd</sup>	1.00 <sup>e</sup>
T5	30	3.85 <sup>bc</sup>	8.44 <sup>fgh</sup>	0.06 <sup>ghij</sup>	23.54 <sup>f-i</sup>	3.62 <sup>g-l</sup>	4.75 <sup>fgh</sup>	6.25 <sup>cd</sup>	3.50 <sup>e</sup>
T5	45	4.03 <sup>b</sup>	7.99 <sup>ijk</sup>	0.08 <sup>e-j</sup>	24.62 <sup>e-i</sup>	2.35 <sup>i-l</sup>	5.50 <sup>efgh</sup>	3.75 <sup>de</sup>	5.25 <sup>e</sup>
T6	0	3.41 <sup>cde</sup>	9.40 <sup>a</sup>	0.09 <sup>d-j</sup>	33.96 <sup>ab</sup>	8.00 <sup>a-d</sup>	9.50 <sup>a-e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T6	15	3.60 <sup>bcd</sup>	7.61 <sup>lm</sup>	0.05 <sup>hij</sup>	32.44 <sup>abc</sup>	5.73 <sup>d-h</sup>	7.75 <sup>c-g</sup>	13.50 <sup>cd</sup>	3.25 <sup>e</sup>
T6	30	3.77 <sup>bc</sup>	7.48 <sup>lm</sup>	0.05 <sup>hij</sup>	29.36 <sup>b-e</sup>	4.41 <sup>f-j</sup>	5.50 <sup>efgh</sup>	10.50 <sup>cde</sup>	5.50 <sup>e</sup>
T6	45	3.82 <sup>bc</sup>	7.32 <sup>m</sup>	0.07 <sup>f-j</sup>	29.13 <sup>b-e</sup>	3.71 <sup>g-l</sup>	7.25 <sup>d-g</sup>	4.75 <sup>de</sup>	19.50 <sup>de</sup>
T7	0	3.21 <sup>def</sup>	9.26 <sup>ab</sup>	0.03 <sup>j</sup>	32.16 <sup>a-d</sup>	8.00 <sup>a-d</sup>	11.00 <sup>abcd</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
T7	15	3.102 <sup>efg</sup>	8.78 <sup>cde</sup>	0.03 <sup>j</sup>	25.11 <sup>e-i</sup>	8.32 <sup>abc</sup>	11.00 <sup>a-d</sup>	34.00 <sup>ab</sup>	2.50 <sup>e</sup>
T7	30	2.76 <sup>fg</sup>	8.66 <sup>def</sup>	0.04 <sup>ij</sup>	24.87 <sup>e-i</sup>	6.76 <sup>b-f</sup>	8.50 <sup>b-f</sup>	38.50 <sup>a</sup>	46.25 <sup>bc</sup>
T7	45	2.70 <sup>g</sup>	8.17 <sup>hij</sup>	0.05 <sup>ij</sup>	25.30 <sup>e-i</sup>	4.74 <sup>f-i</sup>	8.50 <sup>b-f</sup>	23.00 <sup>bc</sup>	38.75 <sup>cd</sup>

Means followed by the same letter within each column are not significantly different at  $p < 0.05$  according to LSD test

EC: Electrical Conductivity, pH: Acidity

Source: the authors

### 3.3 The Effect of treatment, time and treatment\* time on pH

The effect of treatment indicates that when tobacco added to STM which changed to STMTB and when sugar beet added to STM it changed to STMSB, which lead to a significant change in pH. The highest pH is 8.72 among all treatments and is obtained in STMSBTB and the lowest is 7.93 in STMTB. The effect of the treatments on PH is ranked in the following order STMSBTB > STB > SSBTB > SSB > STMSB > STMTB > STM. The time effect indicates that the PH reduced significantly during vermicomposting, Interaction analysis show a significant difference for pH of STMSB in relation to STM and STMTB at day 0. The pH of STM is significantly lower compared to STB and STMSBTB during vermicomposting; it is significantly lower than SSB and SSBTB after inoculation. STMTB is a treatment with significantly less pH than STB, STB, SSBTB and STMSBTB after inoculation. PH of STB is lower in STMSB at day 0 which increases after inoculation, moreover it is significantly lower than STMSBTB at days 15 and 30.

A significant difference is observed in pH of SSB when merged with tomato changed to STMSB which is low, the pH in SSB is lower in regarding STMSB and STMTB after inoculation. SSBTB and STMSBTB have high pH compared to STMTB, this is related to the time after inoculation. The pH of SSBTB compared to STMSB varies over time, while the difference between the STMSB and STMSBTB is related to after inoculation Tables (3, 4 and 5).

With respect to the physic- chemical amounts a decrease in pH during vermicomposting may be due to CO<sub>2</sub> and organic acids produced by microbial metabolism [30]. Therefore, the effects of earthworms on pH during vermicomposting is probably related to the increases in the mineral nitrogen content of the substrates, changes in the ammonium-nitrate equilibrium and accumulation the organic acids from microbial metabolism or from the production of fulvic and humic acids during decomposition [31]. Ndegwa and Thompson [32] reported that the pH rate change is dynamic and substrate dependent, as well as Khwairakpam and Bhargava [29] developed eight different reactors containing of three monocultures of one earthworm type and four polycultures of *E. fetida*, *E. eugeniae* and *P. excavatus* and one control for the vermicomposting. They reported that pH value in all the reactors varied significantly ( $P < 0.05$ ) on days 30 and 45.

#### **3.4. The effect of treatment, time and treatment\* time on C: N**

The treatment effect on C: N STM in STB, SSB and STMTB is significantly higher compared to the same on SSBTB, STMSB and STMSBTB. The effect of all the treatments on C: N is ranked in the following order STB > SSB > STM and STMTB > STMSB > SSBTB > STMSBTB. The time effect on C: N reveals a significant increase at day 45 in comparison the earlier stages of sampling. Interaction of treatment \* time reveals a significant difference of C: N in STM, STB, SSB and STMTB compared to other treatments at day 45, Tables (3, 4 and 5). The change in C:N ratio reflects the degree of organic waste mineralization and stabilization rate during the process of vermicomposting [33].

#### **3.5. The effect of treatment, time and treatment\* time on moisture%**

Moisture content in STM increased significantly when tobacco added to STM which changes to STMTB. When sugar beet added to STM which changes to STMSB and combinations of these tow makes STMSBTB, with a significant high percent moisture content in STB compared to SSB. The highest moisture content is associated with STMSB, with significant difference with other treatments except STB. The Effect of the treatments on moisture content is ranked in the following order STMSB > STB > STMTB > STMSBTB > SSBTB > SSB > STM. Moisture content from day 0 is significantly higher than after inoculation. Interaction of treatment \* time indicates that moisture content in STM is significantly higher when sugar beet waste added to STM which changed to STMSB at days 0, 15 and 30, this found that the moisture content in STB is higher than SSB at day 0. The interaction between treatment \* time confirmed that the moisture content is reduced after inoculation, however the reduction in moisture content after inoculation in SSB is evident Tables (3, 4 and 5). the moisture content of organic wastes used in vermicomposting is an important parameter influencing the growth of the surface-feeding (epigeic) earthworm species *Eiseniafetida* since the earthworm's body contains about 80% water, according, they were studied the growth and fecundity of the epigeic earthworm *Eiseniafetida* in cattle manure solids and pig manure solids with different moisture contents (70%, 75%, 80%, 85%, 90%) for 30 weeks in the laboratory, the maximum weight of *E. fetida* obtained in the pig manure solids, which had a moisture content of 75%, after 13 weeks and the total number of cocoons and hatchlings produced were lower, compared to those in all the other moisture levels. The total number of cocoons and hatchlings produced was lower, compared to those in all the other moisture levels. Generally, earthworms grew bigger and faster in pig manure solids than in separated cattle manure solids but the mortality at all moisture levels in pig manure solids was high.

### **3.6. The Effect of treatment, time and treatment\* time on weight, number of earthworm, cocoon and hatchlings**

The lowest weight observed in STMTB, it varies significantly compared to other treatments. The weight is significantly higher in SSB and STMSBTB in relation to STMTB and STMSB. The effect of all the treatments on weight is ranked in the following order SSB > STMSBTB > STM > STB > STMSB > SSBTB > STMTB. The earthworm weight is higher in day 0. In all sources becomes lower on days 30 and 45 in relation day15. Interaction analysis confirmed that most weight loss is related to the earthworms inoculated in STMTB. The weight loss is significantly lower in SSB and STMSBTB compared to STMTB. Interaction analysis revealed that significant reduction in weight mainly occurred at days 30 and 45 compared to day 0 Tables (3, 4 and 5).

The number of earthworms cocoons and hatchlings are the lowest in STMTB. The number of earthworm in this treatment varies significantly compared to other treatments, however it do not varies significantly when tobacco is replaced with sugar beet which changes to STMSB, The number of earthworms in STM, STB, SSB and STMSBTB is significantly higher compared with STMSB .

The effect of all the treatments on number of earthworm is ranked in the following order STM > STB > SSB > STMSBTB > SSBTB > STMSB > STMTB. Significant reduction in the number of earthworms relates to the last two stages sampling compared to first two stages of sampling. Analysis of interaction in relation to the number of earthworms indicates that STMTB has the lowest number of earthworm compared with other treatments. Interaction of treatment \* time displayed a significant difference obtained by the time between SSB compared with when tobacco is added to SSB which changes to SSBTB Tables (3, 4 and 5).

The number of cocoon is significantly higher in STMSBTB compared to other treatments. A significant reduction on the number of cocoon occurred when tobacco is added to STM, which changes to STMTB, when sugar beet is added to STM, which changes to STMSB, when tomato is added to STB , which changes to STMTB, and sugar beet is added to STB which changes to SSBTB, number of cocoon is significantly higher in STB compared to STMSB, as well as number of cocoon reduced significantly when sugar beet is replaced in SSB with tomato and tobacco which changes to STMTB, moreover when tobacco is added to SSB which changes to SSBTB, the significant decline is observed in number of cocoons. The effect of all the treatments on number of cocoon could be ranked in the following order STMSBTB > STB > SSB > STM > STMSB > SSBTB > STMTB.

The number of cocoons produced by the earthworm have statistical significant on days 30 and 45 compared to day 15. Analysis of interaction revealed that the number of cocoons is higher in STMSBTB compared with STM, STB and SSB at days 30 and 45, similarly, the number of cocoons in STMSBTB is significantly higher than STMTB, SSBTB and STMSB at days 15, 30 and 45. Analysis of interaction revealed that significant changes in number of cocoon among the treatments is relate to day15, likewise interaction of treatment \* time revealed that the number of cocoon is more at day 15 compared with days 30 and 45 in STM, STB and SSB. A difference in number is observes in STMSBTB between days 15 and 45 Tables (3, 4 and 5).

The effect of treatment on number of hatchlings reveals a significant reduction when STM, STB and SSB are replaced with STMTB, SSBTB and STMSB, in the meantime. The number of cocoons is significantly higher in STMSBTB relation to STMTB, SSBTB and STMSB. Number of hatchlings emerged from cocoons in STB is higher significantly compared with when rotter tomato is replaced with tobacco in STB which changed to STM and when rotter tomato and sugar beet is added to STB which is changes to STMSBTB. The Effect of all treatments in the number of hatchlings is ranked in the following order STM> SSB> STMSBTB> STM> STMSB> STMTB> SSBTB. The number of hatchlings emerged from cocoons at days 30 and 45 has statistical significant when compared to days 0 and 15. Interaction analysis confirmed the significant differences obtained through the effect of treatments on the number of hatchlings, this is consistent with STM, STB, SSB and STMSBTB Tables (3, 4 and 5). Domínguez et al. [20] found different growth and reproduction rates of *Eiseniaandrei* in different diets. They also found that the earthworms invested their energy preferentially either on growth or to reproduction depending of the food quality. Gunadi et al. [34] found a correlation between increased growth and reproductive rates of *E. fetida* with low C: N ratios of cattle and pig manure. Ndega et al. [32] reported a decrease in growth rates with a increased C:N ratio of paper mulch. Aira et al. [35] state that after 36 weeks the C:N ratio affected number of earthworms significantly (sevenfold greater in high C: N ratio) and population structure. Thus, at the low C: N ratio treatment the population is consists of mainly mature earthworms (60%), with a higher mean weight than at high C: N ratio treatment. However, in a high C: N ratio treatment, the population is consists of mainly young and hatchlings (70%). in generally the results obtained have support the findings of [13, 27, 36-38] where it is claimed that the availability of food material influences the reproduction and growth of earthworm

## Conclusion

The results of this assessment reveal that STM, STMSBTB, STB and SSB treatments due to the low level of EC have the highest performance with respect to number of earthworms, cocoons hatchlings and weight. The time effect demonstrated a significant increase of EC during vermicomposting, the findings suggest that consumption of tomato, tobacco and sugar beet waste in STM, STB, SSB and STMSBTB have a high potential in the *E.fetida* earthworm's weight gain, and in population growth at 45-day period of vermicomposting. The analysis of interaction revealed more details on the effects of treatments and the time on assessed variables.

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