Journal of Crop and Weed, 17(2): 72-79 (2021)
ISSN- O : 2349 9400 ; P : 0974 6315

http://cwssbckv.org
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Assessment of the suitability of agricultural waste-based substrate for vermicompost production

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Received : 29.05.2021 ; Revised : 11.06.2021 ; Accepted : 12.06.2021

DOI: https://doi.org/10.22271/09746315.2021.v17.i2.1454

ABSTRACT

Current study was conducted to assess the suitability of different agricultural and fruit waste substrates (slurry based) with respect to vermicompost and earthworm production for 90 days in high density polyethylene vermi bags (2.78 m^2). The area was divided into two sections (1.39 m^2) to make the duplicates of the four different treatments of four agro-wastes viz. without any slurry (T_1), ripen pumpkin (T_2), bottle gourd (T_3) and papaya (T_4) and earthworm was stocked @ 550 numbers (220±1.5g)in 1.39m^{2}areas.Among all the substrates, papaya-based substrate significantly enhanced the nutrients level in vermicompost and the substrate enhanced the earthworm's number, biomass and nutritional quality within the short period of duration. So, the above findings revealed that slurry of rotten papaya could act as best substrate for production of vermicompost and good quality earthworm to act as excellent live food source in aquaculture.

Keywords: Agri and fruit waste**,** substrate, earthworm, microbial population, nutrients, vermicompost

Globally, food security is considered as burning issue due to the over increasing population, over utilization of natural resources, exhaustion of resources and impact of climate change on environment which lead to major challenge to the agricultural and allied industries to reduce the overdependence on non-renewable resources (Godfray *et al.*, 2010). Utilization of waste can be the great opportunity to the farmers and industries for utilization of renewable resource and to enhance per unit crop production. So, organic waste which consists of sewage sludge, animal residues, agricultural residues and food processing wastes serve as excellent raw materials for composting to use as renewable resource (Misselbrook *et al.*, 2012). Huge application of inorganic fertilizers provokes high cost of agricultural production and caused nutrients deficiency. Aulakh and Bahl (2001) has reported that in Punjab, the nutrient removal trends are very high for production of wheat and rice as both the crops are very efficient to exploit huge amount of nitrogen (83%), phosphorus (83%), potassium (83%), sulfur (80%) and zinc (77%). Production of organic manure reduces the volume and weight of the agro-residues to about 50 % and results in stable product that can be used to enhance the fertility of the soil (Kumar and Goh, 2000).

Utilization of agri-waste for vermicompost production is one of the best possible ways for recycling of waste and organic manure and also production of good quality earthworm. In India, agriculture wastes are becoming huge loss from significant portion of farm produce due to a weak cold chain infrastructure as every year, near about 16% of fruits and vegetables are being wasted without any kind of management by government. (Sharma, 2019). The *in-situ* physical, chemical and microbial transformations of organic matter and ingestion biomass by earthworm help to produce vermicompost successfully. The vegetable and fruit waste can act as good substrates for vermicompost through substituting the nutrients in plants and maintaining the good condition of soil health which are the main strategies of sustainable organic farming (Chatterjee *et al.*, 2014). Vermicompost is considered as one of the best organic animal manure which might be due to presence of high available nutrients (Edwards and Burrows, 1988), plant growth promoting substances such as auxins, gibberellins, and cytokinins (Krishnamoorthy and Vajrabhiah, 1986), nitrogen fixing and phosphate solubilizing bacteria, enzymes and vitamins (Ismail, 1997). In vermicomposting process, the degradation rate of organic matter by earthworm is very fast which enhances the faster mineralization, humification of organic matter and also, increases microbial population in vermicompost (Atiyeh *et al.*, 2002).

Globally, the over increasing demand on food resources led to facing difficulties to supply animal protein sources. In livestock industry, protein component in animal feed is considered as the major concern for environmental and economic sustainability issue (Khan *et al.*, 2016). Nowadays, the market price of conventional feeds like fish meal, soybean meal is increasing in domestic and international markets. It has

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become major problems for farmers in livestock and aquaculture industries which lead to require production and supply of alternative protein sources in sustainable way (Veldkamp and Bosch, 2015). Earthworm can be used as an alternative protein source for feeding fish and poultry (Pereira and Gomes, 1995; Parolini *et al*., 2020). Earthworm meal contains good percentage of dry matter (90%), protein (58-71%), fat (5-7%) and lysine (4%) (Bahadori *et al*., 2015; Khan *et al*., 2016) Production of earthworm through vermicompost technology can be considered as an eco-friendly technology for cleaning the environment as well as mitigation of the issue like food security globally.

Earthworm and other beneficial microbes are very efficient for conversion of plant biomass into quality organic manure to be used in both the agriculture and aquaculture sectors (Rajkhowa *et al*., 2019). The efficiency of decomposition may be changed with respect to different kinds of plant biomass used in vermicompost preparation (Mahanta *et al*., 2012). Earthworm is having good content of favorable amino acids (Istiqomah *et al*., 2009), ω-3 fatty acids, varying levels of protein (50-70%) and lipid (5-10%) contents (Dynes, 2003). Being a non-conventional protein source and bio-remediator, earthworm plays an important role in vermicompost production through utilization and recycling of agriculture wastes like vegetable and fruit which acted as good substrate. The importance of earthworm in vermicomposting process and its efficient utilization in vermicompost technology can be good approach to the farmers. Recycling of agri-waste through the vermicompost production technology can reduce the wastage of agri-waste residues. Considering the above benefits, vermicompost technology has the great potential to minimize the application of inorganic fertilizers in agriculture. So, the present study was aimed to evaluate the suitability and utility of different vegetables and fruits waste for production of quality vermicompost as well as healthy nutritious earthworm in short duration of production period.

MATERIALS AND METHODS

The study was conducted for the period of 90 days (March – May, 2019) in HDPE (high density polyethylene) vermi bags. The total area of one vermi bag was 2.78 m^2 which was divided into two sections (1.39 m^2 each) by using synthetic net to make the duplicates of the four different treatments *viz*., T_1: control without any slurry; T_2:slurry of ripen pumpkin; T_3: slurry of bottle gourd and T_4: slurry of papaya at College of Fisheries, GADVASU, Ludhiana (Punjab), India (30.54^{0}N latitude, 75.48^{0}E longitude). The filling materials used for vermi-bed preparation were as follows: green leaves and grass (bottom layer), agriculture farm soil (column layer) and semi-decomposed cow dung (gas free like methane, CO_2) was finally filled (upper layer). Vermi-bed was covered by perforated jute bags to prevent direct penetration of sun light as earthworm loves to inhabit in dark condition and bed was kept moist by sprinkling of water regularly. Mature earthworm (*Eisenia foetida*) which was ready to reproduction, used for vermicompost preparation. Initially, mature earthworm was stocked @ 550 numbers (220±1.5g) in each treatment (1.39 m^2) on the basis of previous traits conducted at college. The previous trials showed that optimum stocking density of mature can be within 350-400 nos. m^{-2}. All the vegetables and fruit were kept separately in plastic bag in separate small earthen pits for 10-15 days for aerobic decomposition to make slurry which was acted as substrate for red wigglers. Cow dung was racked up and down for proper mixing at 3-5 days intervals. Addition of water, feeding and racking was continued during the entire period of the experiment. At the end of the experiment, the harvesting of vermicompost and earthworm biomass, cocoons and juvenile production rate, adult earthworm number were recorded.Worms were measured and recorded for the growth and productivity indices at 15 days intervals by taking one kg vermicompost sample. Sample of vermicompost and earthworm from each treatment was taken at every 30 days interval for macro and micronutrient analysis in vermicompost and nutrient quality of red earthworm at the beginning and end of the experiment. Quantification of earthworm was done by using quadrat (0.9 m^2) by digging 15 cm substrate base. The nutrient qualities of earthworm were determined according to the standard methods (AOAC, 2000).

Analysis of vermicompost samples (on dry weight basis) was carried out at 30 days interval from each treatment. The physical properties were determined like temperature, absorbency, bulk potential, bulk density and moisture. Temperature level of vermicompost was checked by using digital soil thermometer MAXTECH DT-9, Absorbency of vermicompost was regularly checked by observing the bedding material and moisture level. Bulking potential was determined through overall porosity of the bedding like the texture, and the strength and rigidity of its structure. Bulk density (kg. m^{-3}) was determined in vermicompost using the core method (Vomocil, 1965). Moisture content was estimated according to Kaiîtis and Imants Nulle (2017) as follows: 30g samples were taken for estimation of initial and final moisture content. Samples were weighed before and after the experiment. The samples were fully dried at 105 ºC temperature and weighed again. Initial and final moisture were calculated using equation: moisture content (%) = [1- sample mass after drying (g) / sample mass before drying (g)]x100.

The chemical properties were determined like pH, electrical conductivity (EC), organic carbon (OC). pH was measured by using digital pH meter (model HI 98107). pH was measured by dissolving and stirring 10 g of a sample in distilled water and using the digital pH meter after waiting for one hour. Electrical conductivity (EC) was measured by using conductivity meter (model HI98331). Organic carbon (OC) was determined using Walkley and Black acid digestion method (Walkley and Black, 1934; Pattnaik and Reddy, 2010) as following process: addition of 5.0 ml of aqueous potassium dichromate ($K_2Cr_2O_7$) and 7.5 ml of sulfuric acid(H_2SO_4) to 0.5 g of the prepared sample in a block digester tube which was then placed in a pre-heated block at 145-155ÚC for 30 minutes and left for cooling at room temperature. Then the digestate sample was titrated with ferrous ammonium sulfate (FAS) solution until a color change from green to brown was attained. The amount of $K_2Cr_2O_7$ consumed during the chemical reaction indicated the total organic carbon content of the feedstock.

The macro and micronutrients were analysed like total Kjeldahl nitrogen (TKN), total phosphorus (TP), total potassium (TK) and total micronutrient cations. Total Kjeldahl nitrogen (TKN) content was determined by micro-Kjeldahl method (Jackson, 1973). Total phosphorus (TP) content was determined in triple-acid (HNO_3: H_2SO_4: $HClO_4$; 10:3:1) digests using the ammonium molybdate method for phosphorus (Nelson and Sommers, 1996). Total potassium (TK) content was determined by flame photometry method after digesting an oven-dried sample for 2 hours at 360^0C (Westerman, 1990). Total micronutrient cations concentrations were determined in diacid mixture (HNO_3: $HClO_4$; 4:1) digests by using the method as described by Page *et al.* (1982). After appropriate dilution with double distilled water, the micronutrient content in digested materials was estimated by using an atomic absorption spectrophotometer (AAS). C/N ratio was obtained by dividing the organic carbon composition of the samples by their nitrogen content. C/P ratio was obtained by dividing the total organic carbon composition of the samples by their phosphorus content.

Microbial populations in vermicompost were enumerated at every thirty days intervals. The enumeration of total aerobic heterotrophic bacteria (AHB), nitrogen fixing bacteria (NFB), phosphate solubilizing bacteria (PSB), cellulose decomposing bacteria (CDB) were carried out by spread plate method (Sanders, 2012). Population of aerobic heterotrophic bacteria was grown in nutrient agar medium supplemented with 1.0% w/v NaCl (Eaton *et al.*, 1998). The composition of the medium for nitrogen fixing bacteria was used as per Alexander (1978). PSB are capable of utilizing tri-calcium phosphate. The production of clear zone around the colonies of the organisms was an indication of the presence of PSB. The composition of modified Pikovskaya's agar medium was used as per the medium used by Rao (2002). The composition of the medium for CDB was used as per the medium used by Sarkhel (2002).

One-way analysis of variance (Montgomery 2001) was performedusing SPSS (Statistics v. 20.0) to test the significance in difference among the treatments. Statistical tests were considered significant at $p < 0.05$. Duncan's multiple range test (DMRT) was performed to find out the homogeneity among the mean of every possible pair of treatment combinations and to test the level of significance of difference between the treatments.

RESULTS AND DISCUSSION

All the physico-chemical parameters of vermicompost under the influence of different substrates in different treatments are presented in Table 1 with significant difference ($p<0.05$) as observed during the study period. Initially, temperature of the vermicompost was high and thereafter decreased gradually. The temperature of vermicompost was 24-27^0C. This might be due to the oxidivative action of intensive microbial activity on the organic matter at the initial period (Peigne and Girardin, 2004) followed by the cooling phase during the compost maturation stage later (Zibilske, 1999). Good absorbency was observed in T_4. The results indicated that the bulk density value of vermicompost ranged from 612-636 kg m^{-3} which was within the recommended range of good compost (Mona, 2003). The highest value of bulk density was observed in T_4 and lowest value was observed in T_1. Moisture content in vermicompost was within acceptable range (70.3-76.1%) in all the treatments (Edwards *et al.*, 2011). Significant decrease in pH level in vermicompost irrespective of the treatments was observed during the study period which may be due to production of carbon-di-oxide and organic acid during the microbial metabolism (Ramnarain *et al.*, 2019). The initial electrical conductivity of vermicompost was 220 mhos cm^{-1}. The electrical conductivity in vermicompost increased in all the treatments during the study periodwhich might be related to the reduction in available organic matter as well as production of different mineral salts like phosphate, ammonium, potassium, etc. in the vermicompost (Venkatesh and Eevera, 2008).

Regarding macro and micro-nutrients of vermicompost except organic carbon content, the difference was significant ($p < 0.05$) between all the

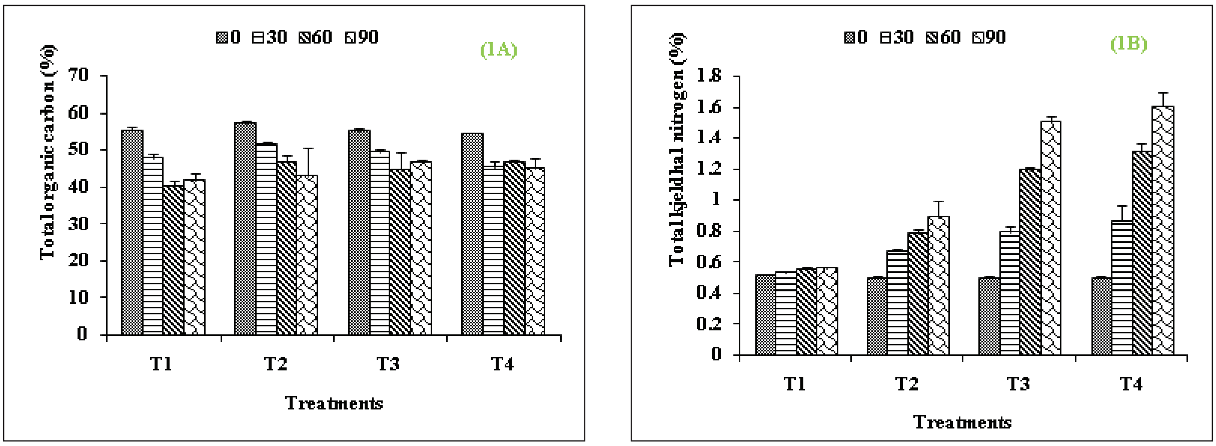

Fig. 1: Changes of organic carbon content (%) (1A) and total Kjeldhal nitrogen content (%) (1B) in vermicompost in different treatments

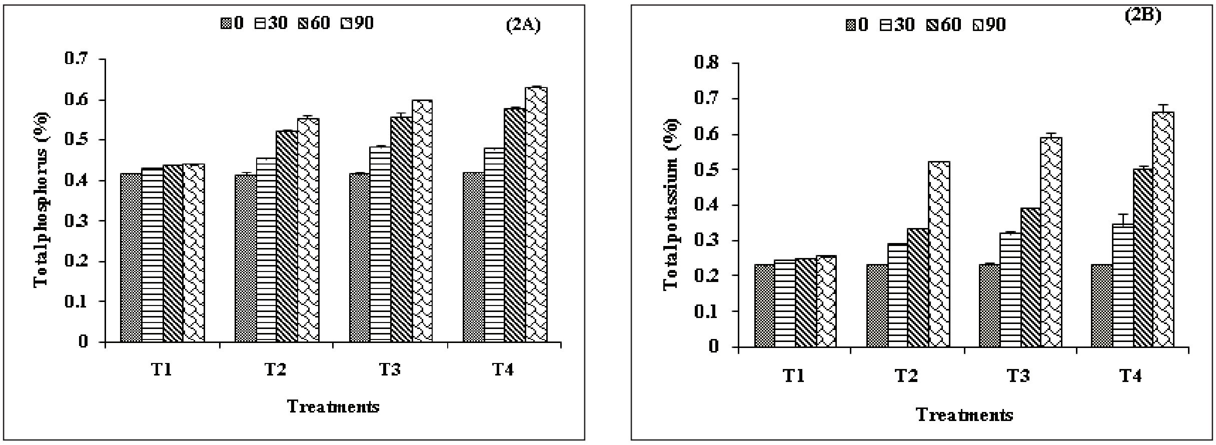

Fig. 2: Changes of total phosphorus (2A) and total potassium (2B) content in vermicompost

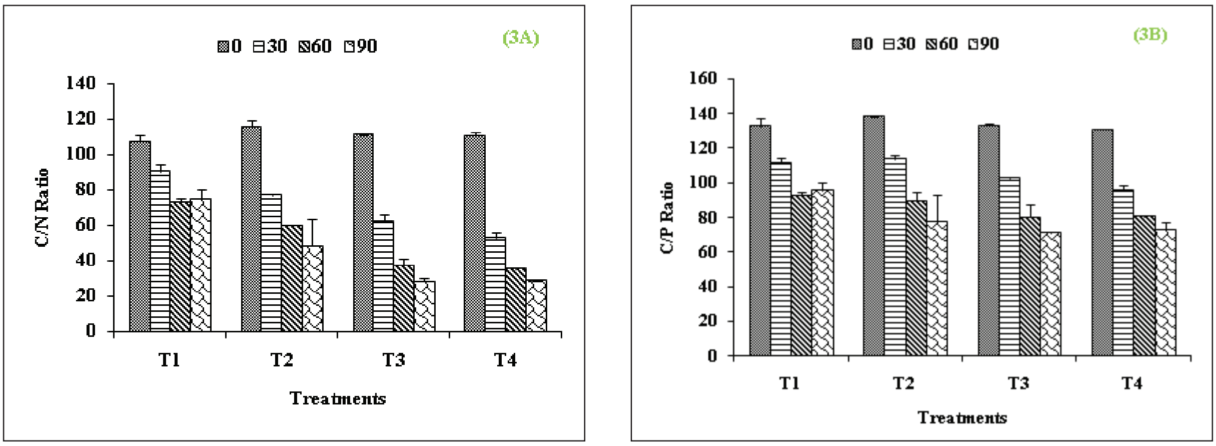

Fig. 3: Changes of C/N ratio (3A) and C/P ratio (3B) in vermicompost in different treatments

Table 1: Different physico-chemical parameters of vermicompost (Mean ± sd; *n* = 3)

Parameters	T_1	T_2	T_3	T_4
Vermicompost weight (kg)	1.00±0.02	1.00±0.01	1.00±0.02	1.00±0.03
Absorbency	Medium	Medium	Good	Medium
Bulking potential	Medium	Medium	Good	Good
Bulk density (kg m^{-3})	612±2.0^{d}	620±2.0^{c}	628±2.0^{b}	636±1.52^{a}
Temperature (^{Ú}C)	25.4±0.01^{c}	27.3±0.02^{a}	26.4±0.03^{b}	24.6±0.02^{d}
Moisture content (%)	70.3±0.03^{c}	70.1±0.01^{c}	75.4±0.05^{b}	76.1±0.02^{a}
pH	7.22±0.04^{c}	7.38±0.01^{b}	7.56±0.01^{a}	7.54±0.03^{a}
EC (mhos cm^{-1})	417.3±0.01^{d}	423.2±0.04^{c}	454.5±0.04^{a}	428.6±0.02^{b}
OC (%)	46.19±6.70	49.5±6.09	48.87±4.46	47.88±4.46
TKN (%)	0.54±0.02^{d}	0.71±0.08^{c}	0.99±0.44^{b}	1.07±0.48^{a}
TP (%)	0.42±0.01^{c}	0.49±0.06^{b}	0.51±0.08^{a}	0.52±0.04^{a}
TK (%)	0.24±0.01^{d}	0.34±0.12^{c}	0.38±0.15^{b}	0.43±0.18^{a}
C/Nratio	86.16±15.75^{a}	74.89±29.03^{b}	59.77±37.01^{c}	56.71±36.98^{c}
C/P ratio	107.92±18.61^{a}	104.67±26.82^{a}	96.74±27.59^{b}	94.92±25.59^{b}
Fe (mg l^{-1})	2252±1.20^{d}	2342±0.88^{c}	2456±0.88^{b}	2486±1.21^{a}
Zn (mg l^{-1})	252±1.21^{d}	268±0.57^{c}	274±0.88^{b}	293±2.18^{a}
Mn (mg l^{-1})	180±0.88^{d}	221±1.15^{c}	236±1.20^{b}	242±0.88^{a}
Cu (mg l^{-1})	24±0.57^{d}	28±0.88^{c}	34±0.88^{b}	38±0.88^{a}

^{a,b,c,d}*Values (mean ± sd) with superscripts in same row differ significantly (p <0.05).*

Table 2: Microbial populations in vermicompost under different treatments (Mean ± sd; *n* = 3)

Microbial population	(CFU × 10^{6}cfu g^{-1} vermicompost on dry wt. basis)			
	T_1	T_2	T_3	T_4
AHB	218.4±1.2^{d}	241.3±1.2^{c}	276.2±1.3^{b}	302.4±1.2^{a}
CDB	36.4±1.4^{d}	51.2±1.1^{c}	61.3±1.2^{b}	72.6±1.3^{a}
NFB	29.6±1.1^{d}	40.6±1.3^{c}	52.4±1.1^{b}	59.5±1.1^{a}
PSB	27.6±0.59^{d}	42.3±0.68^{c}	54.2±0.76^{b}	62.4±0.74^{a}

$^{a, b, c, d}$Values (mean ± sd.) with superscripts in same row differed significantly (p< 0.05).

Table 3: Growth parameters of earthworm in different treatments (Mean ± sd; *n* = 3)

Parameters	T_1	T_2	T_3	T_4
Initial avg. biomass (g)	220±1.5	220±1.5	220±1.5	220±1.5
Final avg. biomass (g)	1120±1.2^{d}	1460±1.3^{c}	1725±1.1^{b}	1975±1.1^{a}
Avg. cocoon worm^{-1} day^{-1} (nos.)	0.31±0.002^{d}	0.46±0.001^{c}	0.54±0.001^{b}	0.68±0.001^{a}
Avg. Worm number per cocoon (nos.)	1.8±0.01^{d}	2.2±0.01^{c}	2.4±0.01^{b}	2.6±0.01^{a}
Avg. Cocoon number per 0.9m^{2} (nos.)	156±0.02^{d}	171±0.01^{c}	186±0.01^{b}	198±0.02^{a}
Avg. Adult number per 0.9m^{2} (nos.)	456±0.01^{d}	612±0.01^{c}	668±0.01^{b}	692±0.01^{a}

$^{a, b, c, d}$ *Values (mean ± sd) with superscripts in same row differed significantly (p< 0.05).*

Table 4:Nutrient qualities(% dry matter) of earthworm in different treatments (Mean ± sd; *n* =3)

Parameters	T_1	T_2	T_3	T_4
Moisture (%)	72.25±1.76	72.5±1.41	72.0±1.41	71.75±0.35
Protein (%)	32.26±2.95^{b}	38.38±11.62^{a}	39.35±18.55^{a}	40.15±6.55^{a}
Lipid (%)	10.45± 1.45^{b}	10.89±0.76^{a}	10.93±0.70^{a}	10.87±0.76^{a}
Ash (%)	20.27±1.56^{b}	20.76±0.81^{a}	20.73±0.79^{a}	20.86±0.68^{a}

$^{a, b}$*Values (mean ± sd) with superscripts in same row differed significantly (p< 0.05).*

treatments (Table 1). Organic carbon content (%) decreased in all the treatments with no significant difference ($p>0.05$) among the treatments (Fig. 1A) which might be due to the gradual increasing rate of microbial assimilation of organic matter (Cabrera *et al*. 2005) and increased rate of earthworm digestion efficiency (Pattnaik and Reddy, 2010). Total Kjeldahl nitrogen (Fig. 1B) and phosphorus (Fig. 2A) content (%) was increased in all the treatments during the study period. Total Kjeldahl N and total P level in vermicompost were highest in T_4 and lowest in T_1 (Table 1). Earthworms help in active nitrogen mineralization to increase the available nitrogen content in vermicompost (Mistry *et al*., 2015).The increased level of total phosphorus might be due to the release of available phosphorus content from the organic substrates partially by the earthworm gut phosphatase and the conversion of phosphorus by the phosphorus solubilizing microorganisms present in the worm casts (Goswami *et al*., 2013).

The potassium content was increased (Fig. 2B) in all the treatments which might be due to the alteration in potassium distribution between exchangeable and non-exchangeable form and also, due to the enhanced microbial activity during vermicomposting process which enhanced the mineralization rate. The unavailable potassium transformed to more soluble form when the organic matter passes through the gut of earthworm and the decomposition of organic material by microorganisms increased the available soluble potassium (Joseph, 2019). C/N ratio and C/P ratio were gradually decreased in all the treatments (Fig. 3A, 3B). Higher C/N ratio in T_1, T_2 and T_3 indicated slow degradation of the substrates (Haug, 1993), whereas the lower C/N ratio in T_4 indicated the higher mineralization efficiency by the earthworm (Pattnaik and Reddy, 2010), loss of organic carbon as well as nitrogen assimilation by microorganisms and earthworms (Christry and Ramaligam, 2005). Iron (Fe) content in vermicompost was higher in T_4 compared to other treatments which might be due to the high efficiency of enzymes and co-factors presence in the gut of earthworm increased iron content in the vermicompost (Daman *et al*., 2016). The availability of zinc (Zn) in vermicompost was within the permissible level which can substantially improve plant health and agriculture soil. Copper (Cu) level was higher in T_4which might be due to the presence of copper containing oxidizing enzymes (Daman *et al*., 2016).

The beneficial microbial population numbers like AHB (302.4×10^6cfu g^{-1}), CDB (72.6×10^6cfu g^{-1}), NFB (59.5×10^6cfu g^{-1}) and PSB (62.4×10^6cfu g^{-1}) were encountered highest in T_4 (Table 2) with highly significant differences ($p<0.05$) among the treatments.The substrates can play an important role in vermicomposting process as earthworm increases the microbial conversion efficiency through releasing nitrogen fixing and decomposing microbes along with excreta (Singleton *et al*., 2003, Rajkhowa *et al*., 2019). The average cocoons and juvenile production rate varied across different treatments (Table 3). Body weight of earthworm increased in all the treatments with highly significant difference ($p<0.05$) among the treatment as body weight was highest in T_4 (5.24 g) which was slightly (11.71-45.5%) higher than other treatments (Table 3). Earthworm numbers per kg vermicompost increased in all the treatments and highest population and highest biomass were recorded in T_4 (295.17 nos.) (Table 3) which might be due to the favourable, stable conditions, high growth rate and reproduction rate of earthworms (Domínguez *et al*., 2016).The nutrient qualities of earthworm are presented in Table 4. The protein and lipid content of earthworm were within the acceptable range. Protein and lipid contents of earthworm were varied from 32.26-40.15% and 10.45-10.87%, respectively, which was agreed with the findings of earlier researcher (Sogbesan and Madu, 2008). Ash content decreased in all the treatments at the end of the study. The result was similar to the findings of Hartenstein *et al*. (1980).

Overall, our results indicate that all the vegetables and fruit waste substrates had the potential to act as good substrate for vermicompost production and can be used as organic fertilizer to minimize the cost of application of inorganic fertilizer in agriculture and aquaculture sectors. Papaya based substrate has been proved as best substrate among all the different substrates used with respect to enhance the production performance of earthworm in terms of cocoon, juvenile and adult production rate, nutritional quality of earthworm and vermicompost with the view of future use of this cost-effective technology in both agriculture and aquaculture sectors through improvement of soil quality as well as plant growth.

ACKNOWLEDGMENT

The authors would like to acknowledge the Dean, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India for providing the funding and all the facilities to carry out the research programme.

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