Influence of wastes of taro leaf, sugar beet and saw dust on physiochemical parameters of produced vermicompost

Abstract:

Climatic changes and its negative impacts may is represented one of big global challenges and eliminate soil and water availability time by time. Thereby, adding organic fertilizers (i.e vermicompost) as soil amelioration may consider one of effective approaches in order to recover soil degradation and enhance water retention in soil. Through vermicomposting, agro-wastes are converted into vermicompost that rich in humus, growth promoters (i.e. amino acids, growth hormones) and nutrients.

Current work was carried out during 2019-2020 to investigate impact of different agro-wastes types i.e. Taro leaf (TF), Saw Dust (SD) and Sugar Beet (SB) on vermicompost quality. Obtained results indicated that these agro-wastes resulted in varying of physiochemical parameters and vermicompost content of amino acids and growth promoters. Whereas, adding SD to Cow dung during vermicomposting resulted in raising Organic matter and N content. Meanwhile, adding SD to FS resulted in increment in C:N ratio and P content in vermicompost. Besides, adding TL to cow dung during vermicomposting led to increment in amino acids. In addition, adding SB to cow dung during vermicomposting resulted in raising ABA and GA3 content. Moreover, these different agro-wastes resulted in varying microbial activity and the highest activities produced when TL adding to FS during vermicomposting. Finally, these different agro-wastes led to differing in antimicrobial activity in produced vermicompost. From these resulted, research team concluded that there is a great potential to produce vermicompost with specific qualitythat may play a crucial role in combat climatic changes particularly reinforce tolerant plant to drought stress.

Keywords : Vermicompost, Cow dung, Fish Sludge, Microbial activity, Antimicrobial, Nutrient contents, Growth promoters and Amino acids content

Introduction:

Converting agro-wastes into vermicompost is known globally as recycling organic wastes. Through this process, earthworm have a crucial role in degrading these agro-wastes through fragmentation and ingestion of organic matter (agro-wastes) and produce an efficient organic fertilizer (vermicompost)that rich in humus and nutrients. Moreover, various micro-organisms including bacteria, fungi, and actinomycetes help earthworm in its crucial role and vermicompost processing agro-wastes. Vermicomposting has been arising as an innovative eco-technology for the conversion of various types of wastes into vermicompost (Khan and Tripathi, 2020).

This vermicompost may be an effective substitute for chemical fertilizers. Whereas, Ceritoglu *et al.*, 2018 report that the vermicompost products are good sources for plant nutrient elements, various hormones, enzymes, humic substances and especially organic matter when added the soil.

Also, Rekha *et al.*, 2018 emphasized that through their study, vermicompost contains a combination of macro- and micro-nutrients and the uptake of the nutrients has a positive effect on plant nutrition, growth, photosynthesis and chlorophyll content of the leaves.

Application vermicompost may play an effective role in encourage plant growth through direct or indirect way. Vermicompost as any organic fertilizer is rich material in terms of nutrition, antioxidants, vitamins, humic and phenolic substances and various hormones (Joseph, 2019). All these substances play a crucial role in promoting plant growth.

Besides , vermicompost may improve plant growth performance though its positive effect on leaf chlorophyll content, and photochemical efficiency, yield, and electron transport rate (ETR) of mature leaves, as well as increased leaf succulence, and carotenoid, protein, and amino acid content (Chenping and Mou, 2016).

In respect for indirect way, vermicompost application may improve soil conditions or works as soil amendment. Whereas several studies indicated that applying vermicompost resulted in enhancing soil properties particularly porosity, water holding capacity (WHC), cation exchange capacity (CEC) and occurrence of macronutrients (Vasanth *et al.*, 2020 Ceritogluand Erma, 2020).Also, vermicompost as any organic matter resulted in promoting microorganisms activity in soil and that will reflect on facilitate nutrients in root zone and improving soil condition in root zone (Pathma and Sakthivel, 2012) .

In addition, Ilker and Tavali (2014) mentioned that vermicompost appeared to more significantly increase bacterial number in soil and it has a potential to be used as an alternative to farmyard manure to improve and maintain soil biological activity. Besides, Singh *et al*., (2011) showed that applying vermicompost resulted in increasing amount of humus in soil which resulted in favourable changes in physical, chemical and biological properties of soil, and in enhancing the water-holding capacity.

Generally, several studies reported that use of vermicompost is effective for improving soil aggregation, structure, aeration and fertility; contains most of the nutrients in plant-available form such as nitrates, phosphates, exchangeable calcium and soluble potassium; increases beneficial microbial population diversity and activity; improves soil moisture-holding capacity; contains vitamins, enzymes and hormones; and accelerates the population and activity of earthworms (Aggelides & Londra 1999, Mascolo *et al*. 1999, Albiach *et al*. 2000, Marinari *et al*. 2000, Sailajakumari & Ushakumari 2002, Arancon *et al*. 2006, Prabha *et al*. 2007, Azarmi *et al*. 2008).

Moreover, Karmakar *et al*., (2015) mentioned that the application of vermicompost showed better result in comparison to chemical fertilizers in terms of soil physical and chemical properties as well as productivity of soil.

Few studies indicate effect of type agro-wastes (whether crops wastes or animal wastes) on quality of vermicompost. Ceritoglu *et al*., 2021 indicated that quality of vermicompost is affected by input organic matter). Also, Moustafa *et al*., (2021) studied potential of utilizing different agro-wastes (banana leaves, Rice straw and sugarcane) in feeding earthworm and effect of these different agro-wastes on quality of produced vermicompost. Their results showed that type of agro-wastes have different impact on quality of vermicompost. They suggested that there is a great potential to produce vermicompost with specific quality as growers needs or requirements of plant stage via using different agro wastes.

In this context, Blouin *et. al*. (2019) showed that the best original material to be used for vermicompost production was cattle manure. Also, the maximum positive effect occurred when vermicompost represented 30 to 50% of the soil volume.

Current work investigates impact of different agro-wastes (i.s. taro leaf, sawdust and sugar beet) on vermicompost quality. Specially, these wastes differ among them self in its nutrient, amino acids contents and other composites.

MATERIALS and METHODS

This work was carried out during 2019-2020 through cooperation both of National Research Center, the Central Laboratory for Aquaculture Research (CLAR) and Faculty of Science, Tanta University. Whereas vermicompost types were produced at the Central Laboratory for Aquaculture Research (CLAR) during (2019-2020) and all needed analysis were done at the National Research Centre (NRC), except microbiological analysis was done at the Faculty of Science, Tanta University.

Specimen collection:

Mixing three species of earthworm (*Eiseniafetida, lumbricusrubellus*and*Perionyxexcavatus*) were raised on cow dung and fish sludge until utilized in the experiment

Preparation of different feeding materials

1. **Cow dung** (CD) **processing**: Fresh cow dung was obtained from a cow farmer adjacent to the central lab for aquaculture research (CLAR) and applied directly to the treatments, assuming the moisture is about 50% of the wet weight.

2- Fish sludge collection and preparation: Fishsludge (FS) was collected from the concrete ponds of Nile tilapia *Oreochromisniloticus*brood stock and fry, at the Nile tilapia hatchery belonging to CLAR; during fry harvesting from the brood stock ponds as well as from fry rearing ponds. The produced FS, with a moisture content of 96.5% and dry solid content of 3.5%, was collected in barrels and then spread out in a thin layer on a cement floor for drying over fourteen days, so it can be stored safely until being used.

3.**Taro Leaves (TL) processing**: Taro leaves were collected from CLAR nearby farm, then sun-dried for 3 days and werecrushed into small pieces.

4. **Saw dust (SD)**: saw dust was bought from CLAR nearby carpentry shop. Saw dust soaked in water for three days before utilized in vermicompost producing.

5. **Sugar beet (SB)**: Sugar Beet wasteswere collected from sugar beet factory and sun-dried for 3 days then minced into small pieces by machine.

Earthworm inoculation and Vermicompost production:

Both of fresh cow dung and dried fish sludge were mixed individually with agro-wastes (SD, SB or TL) at a ratio of 2:3respectively and moistened to 60-70% in Styrofoam boxes with dimensions of 60× 40× 30 cm. After 24 h, three species of earthworm (*Esienafetida*; *Perionyxexcavatus* and *Lumbricusrubellus*) were added to the media at a rate of 50 g worm per 1000g media. For eight weeks the boxes were checked weekly and re-moistened and mixed until the vermicompost matured. All boxes were kept indoors and the temperature maintained between 18-25°C during the vermicompost maturation. At harvest time, vermicompost was checked manually on white plastic surface and the adult as well as pre-adult earthworms were collected then the vermicompost was returned to the boxes again for one more month. Later, the vermicompost was re-checked again and all hatched earthworms were collected. The harvested vermicompost was packed in plastic bags and delivered to laboratories to be analyzed.

Experimental treatments: eight setup (treatments) containing different feeding materials (Cow dung (CD) alone, Fish sludge alone (FS) and CD or FS supplemented with TL, SD and SB respectively) with three replicates of each were prepared as following

CD, FS, CD+ TL, CD+SD, CD+ SB, FS+ TL, FS+SD and FS+ SB. Samples of all these treatments delivered to different laboratories in order to be analyzed.

<u>Analysis of chemical and microbial parameters</u>

- Dry weight (g)
- Organic matter (%)
- Humidity (%)
- Ash
- C/N ratio
- Nitrogen % (Kjeldalh method)
- Available Phosphorus (Modified Olsen's method, 1958)
- Available Potassium (Ammonium acetate method)
- Amino acids content (mg/100g dry weight)
- Growth promoters content (g/100gsample)
- Bacterial and Fungal population (Serial dilution and plate count method)

Physiochemical analysis for vermicompost:

Vermicompost samples were dried in a ventilated oven at 70 ^{o}C to constant weight for determined dry weight and chemical analysis.

Macronutrients were extracted using the dry ashing digestion method according to Chapman and Pratt[23]. Nitrogen was determined by using the Kjeldahl method, the ash was dissolved in HCl (2N) and phosphorus was photometrical determined in the digested solution using vanado-molybdate color reaction according to the method described by Jackson [24]. Potassium was measured in the digested suspension using the Flamephotometer, (Eppendorof, DR Lang). Organic matter content was determined according to Walkely and Black[25].

Free amino acids and growth promoter's analysis:

To determine total free amino acids, the modified of ninhydrinecolorimetric method that described by **(**Rosein1957 & Selim *et al.*, 1978) was used for this purpose.

Besides, growth promoters in samples of vermicompost were estimated according to method described by (Dobrev *et al*., 2005)

Microbiological analysis of vermicompost samples:

Sampling and sample preparation:

Five grams from vermicompost samples were placed in sterile Stomacher bags and treated by a Stomacher 400 Circulator for 60 s at middle speed after adding 45 ml sterile 0.85% NaCl. The Stomacher blending step was repeated three times and the microbial suspension was obtained.

Estimation the counts of total viable bacteria count:

Tenfold serial dilution of the microbial suspensions obtained with the protocol described above made with sterile 0.85% NaCl were plated onto plate count agar medium for the estimation of total viable counts, counts of colony forming units (CFU) were estimated after 3 days of incubation at 28°C and were calculated per gram vermicompost. The total resistant bacteria were estimated by planting the same dilution onto plate count agar medium sublimated with (20mg/l) for (penicillin, ampicillin, erythromycin and tetracycline respectively).

Antimicrobial activity assay:

This method was done by agar well diffusion test according to (Schillinger and Luck, 1989). To determine the antimicrobial activity of the vermicompost samples against the selected identified pathogenic bacteria e.g. *Citrobacterfreundii, Enterobacter cloacae, Pseudomonas aeruginosa*, and*Klebsiellapneumonia.* Theprepared nutrient agar plates were overlayed with 100 µl of overnight culture of tested pathogens (in nutrient broth), then spread well with L- shaped glass rod. After 15 min, wells of 5 mm diameter were made with a sterile cork borer. Samples of vermicomposts extract were placed into wells. Plates were then incubated at 30°C for 12 hrs. The inhibition zones were measured to assay the antimicrobial activity of vermicompost samples.

Data analysis: Vermicompost samples were analyzed using the standard procedures in the laboratory at National research Centre and Faculty of Science, Zoology department, Tanta University. All data are the means of triplicates. Statistical analysis of data, analysis of variance (ANOVA) and mean separation were carried out using Duncan's multiple range test and significance were determined at the ($P \leq 0.01$) level (Duncan, 1955). Data analysis was performed using ASSISTAT version 7.7 beta (2015).

Results and Discussion:

Table1 showed that utilizing Saw Dust (SD) in producing vermicompost was resulting in higher dry weight and organic matter comparing with other types of vermicompost types. In respect for ash percentage, data was indicated that the highest ash percentage achieved with (FS) alone or (FS+TL). However low ash (%) was achieved when (CD) used alone to produce vermicompost. In respect to nutrients content utilizing SD in vermicompost production resulted in increasing N % content with CD and P% content with FS. However, higher K % content was obtained when (FS+SB) utilized in vermicompost producing.

In addition table (2) represented data concern amino acids and growth promoters influenced with different carbon sources. Higher amino acids content was obtained when using TL wastes with CD in producing vermicompost. Meanwhile utilizing SB wastes with CD in vermicompost lead to increasing ABA (1.62g/100g) and GA_3 (2.23) comparing with two other carbon sources either (SD) or (TL) wastes. Besides, using wastes of SB with FS was resulted in producing the highest content of IAA (0.19g/100g).

There was many variation were observed in physio-chemical results, the vermicompost that produced from Cow Dung organic matter represented high results comparison with Fish Sludge organic matter in all treatments (Table 1). The organic matter results was 46.6, 50.15, 33.00 and 37.33 with Cow Dung vermicompost (Cow dung, Saw Dust, Sugar beet and Taro Leaves respectively), while, the results with Fish Sludge was 21.06, 32.14, 26.48 and 19.73 (Fish sludge, Saw Dust, Sugar beet and Taro Leaves respectively).

Similar to the aforementioned results in organic matter, Cow Dung vermicomposttypes represented the highest results comparing with Fish Sludgetypes in the case of total nitrogen.

The opposite result occurred on C:N, i.e. the most of Fish Sludge results increase comparison to Cow Dung vermicompost except with Cow Dung alone. Hui *et al.* (2007) reported that compost made from the mixture spent mushroom waste with livestock manure, and soybean cake had a C/N ratio of 30. The compost was produced through composting alone (Hui *et al.,* 2007). Based on the results of statistical analysis, composition of vermicompost materials affected the total organic C, total N and C/N ratio of the vermicompost. It showed that vermicomposting can lead to mineralization process which reduced C by N ratio of vermicompost. Our results were in line with previous report according to the research of Hui *et al.* (2007).

Hoitink and Boehm, (1999) reported that C/N ratio is one of parameter used for measuring compost maturity. Researchers have suggested various ideal C/N ratios ranging from >12 to <25 (Brewer and Sullivan, 2003). According to Gomez-Brandon*et al.* (2008) the stabilized compost has a C/N ratio from 10 to 15. Majlessi*et al.* (2012) showed that to produce a stable vermicompost of food waste needed 7 weeks of the process duration. The vermicomposting duration in our research was four weeks and then continued by composting for two weeks**.** Domiguez, (2011) reported that the decaying organic in vermicomposting system is a spatially and temporally heterogeneous matrix of organic resources with contrasting qualities that result from the different rates of degradation, occur during decomposition**.** This means that the vermicompost still needs the composting process. To improve the quality of generated vermicompost, the systematic study of composting process and the addition of additives material such as fish meal and egg shell flour was conducted. After the composting process, the quality of vermicompost increased which marked by a lower C/N ratio of vermicompost (average 15.3). The decrease in C/N ratio was caused by the decomposition process of microorganisms during the composting process. During decomposition process, soil microorganisms burn carbon as a source of energy, but not all of the carbon remains in its body; a certain amount is lost as carbon dioxide during respiration (Frankenberger and Abdelmagid, 1985).The decrease in C/N ratio of compost improve the compost quality. Frankenberger and Abdelmagid, (1985) states that the organic matter with a value of C/N ratio lower than 20 include high quality of organic matter and will undergo mineralization in the soil**.** Majlessi*et al.* (2012) stated that the vermicompost with low C/N ratio (14-30) indicate a mature and stable vermicompost**.**

In addition, Igbokwe*et al.* (2015) showed that the mixing sawdust with the other organic waste in composting process can increase N content of the organic fertilizer (Mtambanengwe and Kirchmann, 1995) and increase in N content lower the C/N ratio.

Table 1: effect of different agro-wastes (TL, Sb and SD) as carbon sources on physiochemical parameters of vermicompost

TRT	FW (g)	Dry Weight (g)		O.M (%)		Humidity (%)		Ash		N (%)		C / N ratio		P (%)		K (%)	
CD (Control)	10	6.70	b	46.60	a	33.00	b	20.40	d	1.33	b	20.40	a	0.35	ba	1.08	c
CD + SD	10	7.68	a	50.15	a	23.20	d	26.65	cd	2.51	a	11.48	b	0.12	b	1.65	b
CD + SB	10	6.80	b	33.00	ab	32.00	b	30.00	bcd	1.87	ab	11.58	b	0.18	ba	1.70	b
CD + TL	10	6.01	c	37.33	ab	39.90	a	22.77	d	1.87	ab	11.23	c	0.45	ba	1.58	b
FS (Control)	10	7.27	a	21.06	b	27.33	c	54.94	a	1.09	b	11.58	b	0.40	ba	1.88	a b
FS + SD	10	7.62	a	32.14	ab	23.83	cd	44.03	abc	1.61	b	11.63	b	0.48	a	1.53	b
FS + SB	10	7.45	a	26.48	b	25.50	cd	46.02	ab	1.54	b	11.50	b	0.42	ba	2.13	a
FS + TL	10	7.52	a	19.73	b	24.83	cd	52.10	a	0.99	b	11.58	b	0.36	ba	1.42	b

Means were represented as average of replicates.
Different letters are express for significant differences while the same letters are non-significant at L.S.D. p>0.05.
CD= cow dung, SD= Saw dust, SB= Sugar beet, TL= Tour leaf FS= Fish

Table 2: Effect of different agro-wastes (TL, SB and SD) as carbon sources on amino acids and growth promoters in produced vermicompost

	Amino acids (mg/g DW)	ABA (g/100 g)	GA3 (g/100 g)	IAA (g/100 g)
CD (Control)	0.27 c	0.33 c	1.08 c	0.04 c
CD + SD	0.41 c	0.70 b	0.37 f	0.05 c
CD + SB	1.43 b	1.62 a	2.23 a	0.15 b
CD + TL	1.82 a	0.19 d	0.94 d	0.05 c
FS (Control)	0.44 c	0.01 e	0.16 g	0.03 c
FS + SD	0.29 c	0.01 e	0.92 d	0.02 c
FS + SB	0.46 c	0.17 d	1.59 b	0.19 a
FS + TL	0.34 c	0.67 b	0.78 e	0.13 B

Means were represented as average of replicates.
Different letters are express for significant differences while the same letters are non-significant at L.S.D. p>0.05.
CD= cow dung, SD= Saw dust, SB= Sugar beet, TL= Tour leaf FS= Fish

Effect of carbon sources Microbial activity:

Table 3: show Microbial activity in different vermicompost types based different agro-wastes (TL, SB and SD) as carbon source

TRT	CFU
FS + SD	11.33± 2.83
FS + SB	44.00 ± 4.71
FS + TL	56.00 ± 0.00
CD + SD	32.33 ± 4.24
CD + SB	29.17 ± 3.06
CD + TL	28.67 ± 11.79

Values are represented as average of three replicates.
Different letters within the same column express significant differences at L.S.D. p>0.05 .
FS= Fish sludge, CD= cow dung, SD = Saw dust SB= sugar beet, and TL= Taro leaves

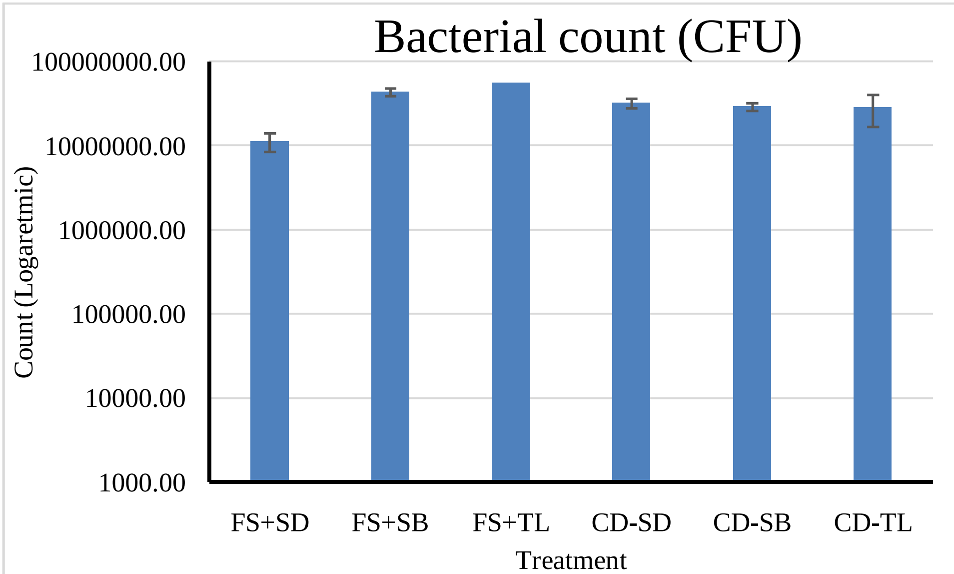

Fig1; microbial activity influenced by utilizing different agro-wastes as carbon source.

Table 3 and Fig 1 represent data concern impact of different carbon source (SD, SB and TL) on microbial activity in produced vermicompost. From first view it was observed that FS vermicompost with whether TL or SB wastes surpassed in microbial activity comparing with CD vermicompost types with the same type of carbon source (TL or SB). Also, incorporation TL wastes with FS during vermicompost processing resulted in considerable increment in microbial activity (56 CFU) comparing with other studied treatments meanwhile adding TL to CD during vermicomposting produced lower value of microbial activity (28.67 ±11.79). The lowest value of microbial activity (11.33± 2.83) was achieved with (FS+ SD).

Also**,** Lores *et al.* (2006) stated that the vermicomposting process involved the activity of earthworms which modify wastes physically and feces excreted by worms can increase the activity of the microorganisms so that the rate of mineralization to be faster**.**

Data in table 4 showed that anti-pathogenic activity of vermicompost was varied with varying added agro-wastes (as carbon source i.e. SD, SB and TL) whereas CD+SD resulted in the highest anti-activity for *Bacillus anthracis* (15mm) comparing with other vermicompost types. Meanwhile this vermicompost has no anti-pathogenic effect for both of *Escherichia coli* and

Pseudomonas aeruginosa. Besides, it can noticed that FS+SD surpassed other types of vermicompost whereas it has higher anti-pathogenic effect for three pathogens *Escherichia coli, Klebsiellapnaumonia*and *Pseudomonas aeruginosa* in addition to *Bacillus anthraci*.

Moreover, vermicompost from (FS + TL) showed anti-pathogenic effect for only three of studied pathogenic (*Bacillus anthracis, Escherichia coli*, and *Pseudomonas aeruginosa*).

Obtained results indicated that anti-pathogenic effect differed in vermicompost according for input crops wastes. Also, some crop wastes may be resulted in multi-anti-pathogenic effect than other.

Table 4: antimicrobial activity influenced byutilizing differentagro-wastes (TL, SB and SD) as carbon source

TRT	***Bacillus anthracis***	***Escherichia coli***	***Klebsiella Pneumonia***	***Pseudomonas aeruginosa***
CD+S D	15mm	00	7mm	00
CD + SB	9mm	00	16mm	00
CD + T L	9mm	00	00	25mm
FS + SD	11mm	17mm	23mm	26mm
FS + SB	8mm	00	22mm	13mm
FS + TL	7mm	7mm	00	20mm

Values are represented as average of three replicates.
FS= Fish sludge, CD= cow dung, SD = Saw dust SB= sugar beet, and TL= Taro leaves

Numerous studies (Garczyńska *et al*.,2020) highlight the effectiveness of vermicomposting manure of different origins to reduce pathogenic microorganisms. A reduction in pathogens occurs by passage through the intestines of an earthworm, which obviates the need to raise the temperature. Moreover other studies indicated that earthworm influences microbial community, physical and chemical properties of soil. They breakdown large soil particles and leaf litter and thereby increase the availability of organic matter for microbial degradation and transforms organic wastes into valuable vermicompost by grinding and digesting them with the help of aerobic and anaerobic microbes Maboeta and Van Rensburg (2003). Earthworms' activity is found to enhance the beneficial micro flora and suppress harmful pathogenic microbes. Soil worm casts are rich source of micro and macronutrients, and microbial enzymes Lavelle and Martin (1992).

Other studies indicate that earthworm lives in medium rich in wide range of microbes thereby earthworm strive to cover its bodies with mucus rich in antimicrobial to protect their bodies from pathogenic. These excreted mucus lost in the medium via earthworm movement from a while to while and earthworm excretes new mucus continuously. Thereby vermicast is mixed with mucus secretion of the earthworm's gut wall (Moustafa *et al*., 2021).Whether the antimicrobial produced from gut wall of earthworms or from excreted mucus to protect earthworms bodies against pathogenic microbes, its affected with type of meals (type of agro-wastes) that offer to earthworms as shown in the results of current study.

enerally, these results were supported with findings of (Khwanchai, and Kanokkorn, 2018) who reported that provided new evidence that agricultural waste, especially soybean meal could be used as feeds for the high quality of vermicompost production and earthworm biomass.

Bajal *et al*., 2019 showed that nutrient content of vermicompost was varied significantly among the substrates (Lantana camara, Ageratum conyzoides, banana pseudo stem, garden waste, vegetable waste and cow dung). Also, they reported that Lantana was found most effective with 2.53% N, 1.38% P and 2.28% P.

Ramnarain *et al*., (2019) mentioned that three agro-wastes were used in this study (T1 (Rice straw), T2 (Rice straw + grass) and T3 (Grass)]) and results showed that, the combination of rice straw and grass had the highest rate of vermicompost production of 105 kg/m2 followed by grass and rice straw with 102.5 kg/m2 and 87 kg/m2, respectively, at the end of 120 days. Besides, the harvested vermicompost had an excellent nutrient status, confirmed by the chemical analyses, and contained all the essential macro- and micronutrients.

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