

Studies on the biological parameters of compost and vermicompost made from different livestock dung with Guava leaf wastes using indigenous earthworms

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Abstract

The guava (*Psidium guajava* L.) is a widely consumed fruit known as the “apple of the tropics.” Lignocellulosic wastes of guava leaves and three different livestock dungs (cow dung, sheep dung, and horse dung) introduced with two Indigenous earthworms, *Lampito mauritti* and *Perionyx excavates*, were taken for the study. Experimental set up of four different combinations of each animal dung (cow dung, sheep dung, and horse dung) = (dung 1000g), (dung 750g + guava leaves 250g), (dung 500g + Guava leaves 500g), and (dung 250g + Guava leaves 750g). Compost was prepared by mixing guava leaves with four combinations of three different species of animals studied in the present investigation. After 60 days of observation, both vermicompost and non-vermicompost were studied. The biological composition of compost and vermicompost was examined at the end of the study. The earthworm biomass, cocoon production, hatching number, and vermicompost recovery activities of two types of earthworms were also studied. The SAS (Statistical Analysis System) version 9.4 is used for analysis. Using different biological parameters, the total number of microbial populations, dehydrogenase, lignin, cellulose, hemicellulose, phenol, and humic acid was estimated. Therefore, the present investigation shows vermicompost was the best of the two types of compost studied, and its nutrient content was slightly higher than compost. Consequently, applying vermicomposting increases the soil’s nutrient content and fertility over time. Also, vermicomposting enhances plant growth and crop yield.

Keywords: Guava leaves, cattle dung's, vermicompost, *Lampito mauritti*, *Perionyx excavatus*, SAS (Statistical Analysis System)

Introduction

One of the most significant commercial fruits in India is guava (*Psidium guajava*). The guava (*Psidium guajava*), a small tropical fruit tree belonging to the *Myrtaceae* family, is sometimes referred to as the “poor man's apple” (Das, 2020) ^[5]. It has been under cultivation in India since the early 17th century. It has developed into a significant fruit crop in India, accounting for 4% of the country's total fruit production, with 1.75 million metric tons estimated to be produced from 0.25 million ha. Among the main Indian states that produce guavas are Uttar Pradesh, Bihar, West Bengal, Maharashtra, Chhattisgarh, Tamil Nadu, Karnataka, Madhya Pradesh, Gujarat, and Andhra Pradesh. Guava is typically grown primarily using a traditional method, which makes it challenging to reach the necessary level of production due to the size of the trees (Pilania *et al.*, 2010) ^[23]. In India, guava production increased by 55% from 11 lakh metric tons to 17 lakh metric tons, while the area under cultivation increased by 64% from 94 thousand hectares in 1991-1992 to 155 thousand hectares in 2015-16 (Palanna, 2015) ^[17]. While there has been methodical guava manuring since 1960 in India, region-specific manurial schedules incorporating both organic and inorganic manures were advised (Naik and Hari, 2005) ^[16].

Disposal of animal dung materials is a serious problem. Excreta from livestock, bedding, rain or other water, soil, hair, feathers, and other debris typically found in animal waste handling procedures are all considered “livestock waste” (Parihar *et al.*, 2019) ^[18]. According to the 19th livestock census conducted in India, there were approximately 299.9 million cows, 65.06 million sheep, 135.17 million goats, 10.29 million pigs, and other livestock and poultry, totalling about 512.05 million (Gupta., 2019)

^[10]. If leaf litter undergoes a natural and biological conversion to organic matter, it has the potential to be a valuable source of energy. An example of an organic waste product is leaf litter, which can be turned into nutrient-rich vermicompost by earthworm processing and then applied to crops as a biofertilizer (Joseph and Kathireswari, 2020) ^[11]. An essential link in the global food chain that reduces poverty, upholds food and nutrition security and fosters economic expansion is the livestock sector.

The global production of lignocellulosic biomass is estimated to exceed 200 million tons annually. A widely accessible and thoroughly tested feedstock for the production of biofuels, bioenergy, and various value-added products is lignocellulosic biomass (Banu *et al.*, 2021) ^[2]. Lignocellulosic leaf wastes are found abundantly on the earth, but they are rarely used. Lignocellulose is a type of organic waste that is primarily made up of a blend of cellulose (40%) and hemicellulose (20-30%) as well as lignin (20-30%) (Sjostrom, 2013) ^[26]. On the other hand, improper management and/or use of the 25-30 kg of leaf litter that plants produce annually as lignocellulosic waste results in environmental pollution issues (Parthasarathi *et al.*, 2016) ^[20]. A further unethical method of cleaning up plant waste is burning dry leaves, which not only pollutes the air but also depletes the soil of vital nutrients (Kumar *et al.*, 2015 and Muthuraman and Ramaswamy, 2019) ^[12, 15]. Thus, the goals of this study were to choose a lignocellulosic waste material, such as guava leaves, and combine it with the dung of three different animal species (cow, sheep, and horse), then compost it using native earthworm species, specifically *Lampito mauritti* and *Perionyx excavatus*. The biological composition of vermicompost (microbial population, dehydrogenase, lignin,

cellulose, hemicellulose, phenol, and humic acid), as well as earthworm biomass, cocoon production, hatching number, and vermicompost recovery of the prepared compost, were also examined after the study.

Materials and methods

Collection of earthworms and inoculation

Native species of *perionyx excavatus* (epigeic) and *lampito mauritti* (anecic) earthworms were collected from Vermibotechnology lab, Department of Zoology, Annamalai University, to conduct this study.

Collection of cattle dungs

The cow dung (CD) was gathered from a cow farm at the Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, horse dung (HD) was collected from Ilamainayakkarkulam, Chidambaram, and sheep dung (SD) was collected from Parangipettai, Chidambaram.

Collection of guava leaves

The lignocellulosic wastes of guava leaf litter (GLL) were collected from Siththarasur guava garden, Cuddalore district, Tamil Nadu. The biological composition of guava leaf wastes used for the experiment is given in Table 1.

Table 1: Biological composition of Guava leaf wastes

Parameters	Cow dung	Horse dung	Sheep dung	Guava leaves
Total microbial population CFU × 10 ⁶ g ⁻¹	262	246	229	89
Dehydrogenase*	4.35	3.91	3.85	1.43
Lignin (mg/g)	22.1	19.4	18.7	195
Cellulose (mg/g)	86.1	79.2	74.2	478
Hemicellulose (mg/g)	14.1	12.3	10.6	51
Phenol (mg/100g)	29.1	24.1	21.8	71
Humic acid (mg/5g)	6.07	5.84	5.77	0.68

*-µl H/ 5g substrate

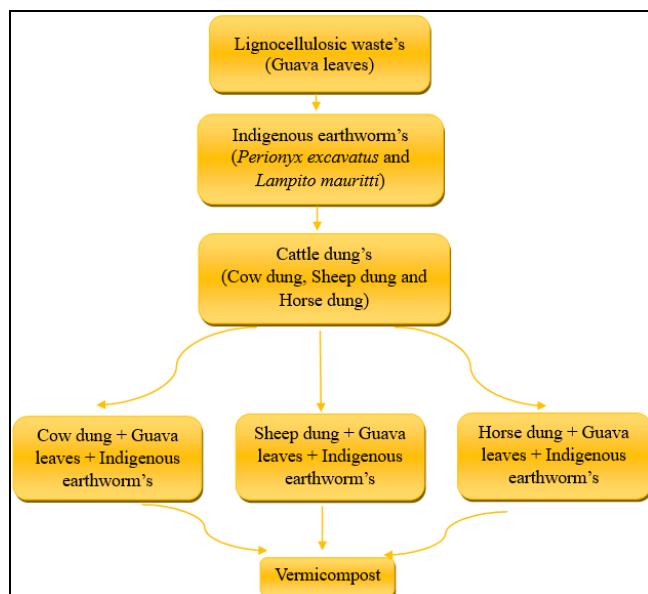


Fig 1: Work design

Experimental preparation

Four different combinations were prepared with each animal's dung and guava leaf. They are cow dung (4:0) (1000g), cow dung + guava leaves (3:1) (750g + 250g), cow

dung+ guava leaves (2:2) (500g + 500g), cow dung + guava leaves (1:3) (250g +750g). The same another two setups of experiments only change the animal dung instead of cow dung. They used sheep and horse dung with the same proportion. The above-mentioned powdered cattle dung and chopped guava leaves (2-5 cm) were weighed (dry weight) and placed into 36 plastic containers with a diameter of 14 cm and a depth of 12 cm. They were thoroughly mixed. Thirty-six plastic containers were kept in the lab at a temperature of 25±1 °C, for 60 days. Experiment setups were maintained at 65-70% moisture sprinkling water. A hole is placed at the bottom of each plastic container to avoid stagnant water after pre- decomposition 24 experiment plastic containers were inoculated with 25g of sexually immature preclitellate *Perionyx excavatus* (34-36 number of earthworms, 15-18 days old) and *Lampito mauritii* (22-25 number of earthworms, 30-32 days old). Another 12-plastic container for compost (without worms). All the plastic containers were maintained with thick mosquito netting because earthworms were protected from rats, moles, frogs, birds, flatworms, red ants, centipedes, and other insects. At the end of the experiment on the sixtieth day the compost and vermicompost were gathered from all experiments, then weighted and shade-dried to measure biological parameters.

Earthworm activity in experiments (vermibeds)

Using a manual sorting method, the reproductive parameters, such as the number of cocoons produced, earthworm biomass, recovery of vermicompost, and hatchlings, were counted on the sixtieth day.

Vermicompost analysis

The biological characteristics of the original (0-day), worm-unworked normal compost, and worm-worked vermicompost substrates were analyzed using standard methods. Total microbiological population (Baron *et al.*, 1994) [3], humic acid (Valdrighi *et al.*, 1996) [29], lignin, cellulose, and hemicellulose (Ververis *et al.*, 2007) [30], and dehydrogenase (Pepper *et al.*, 1995) [22]. The biological parameters studied are given in Table 2.

Statistical analysis

Version 9.4 of the SAS (Statistical Analysis System) Data processing techniques included one-way and two-way ANOVA, which were also used to determine the biological parameters of the prepared vermicompost. The means were separated using a test (CD value, F value).

Result

In comparison to the vermicompost and worm-unworked compost values, the biological parameters of the compost and vermicompost in the vermibeds underwent a significant change after the 60-day experiment Table 2. In comparison to other experiments, the vermicompost of both worms is found to exhibit significantly more declines in lignin, cellulose, hemicellulose, and phenol values in all vermibeds, with the most declines occurring in the 1:1 (500g cow dung + 500g guava leaf), 1:1 (500 g sheep dung + 500 g guava leaf), and 1:1 (500 g horse dung + 500 g guava leaf) combinations. In comparison to the original substrate, regular compost and vermicompost had a substantially larger total microbial population, dehydrogenase activity, and humic acid contents. When comparing these three

experiments, the maximum value of the test is 1:1 (500 g cow dung + 500 g guava leaf), followed by 1:1 (500 g horse dung + 500 g guava leaf), and 1:1 (500 g sheep dung + 500 g guava leaf) compared to other experiments. At the end of the study, a 1:1 (500 g cow dung + 500 g guava leaf) experiment was found to show prolonged and sustainable earthworm activity and quality of vermicompost, followed by earthworm growth, reproduction of earthworms and recovery of vermicompost. An increase in biological parameters total microbial population, dehydrogenase activity, and humic acid and a decrease of lignin, cellulose, hemicellulose, and phenol are found in the other experiments.

Earthworm growth, cocoon production, hatching number, and vermicompost recovery in compost obtained from all experiments on the 60th day after completion of this study are calculated and given in Table 3. The study found that in each experiment, the growth, reproduction, and recovery of both earthworms in vermicompost increased significantly (P<0.05) when guava leaves mixed with different cattle dung were used for vermicomposting. At the end of the study, comparing each experiment, it was found that vermicompost prepared with cow dung and guava leaves had a higher activity of earthworms. Also, comparisons of biological parameters are given in Graph 1.

Table 2: Biological parameters of compost and vermicompost obtained from guava leaves (n=5)

Biological Parameters	Experiments (Vermibeds)												
	4:0 CD	3:1 CD:GL	2:2 CD:GL	1:3 CD:GL	4:0 HD	3:1 HD:GL	2:2 HD:GL	1:3 HD:GL	4:0 SD	3:1 SD:GL	2:2 SD:GL	1:3 SD:GL	
Total microbial population (CFU×10) ⁶ g ⁻¹													
ID	262	283	290	165	246	254	262	129	229	236	243	118	
NC	314	354	388	202	295	342	366	188	266	327	347	174	
VC	A	419	435	465	356	404	417	433	323	380	401	312	
	B	463	486	502	389	428	443	469	354	403	422	333	
Dehydrogenase*													
ID	4.35	4.85	5.11	3.57	3.91	4.46	4.33	3.19	3.85	4.32	4.19	3.07	
NC	5.09	5.65	6.04	4.44	4.72	5.21	5.35	4.07	4.41	4.97	4.77	3.83	
VC	A	6.35	6.88	7.12	5.49	5.65	6.11	6.72	4.63	5.42	5.95	4.41	
	B	6.46	6.95	7.29	6.13	6.18	6.38	7.04	5.77	5.84	6.22	5.42	
Lignin(mg/g)													
ID	22.1	48.1	95.3	126.1	19.4	43.1	86.8	117.7	18.7	41.6	84.4	114.4	
NC	19.7	42.7	92.1	123.5	17.6	39.8	81.7	113.6	16.9	38.5	80.7	108.7	
VC	A	10.4	26.7	71.3	97.4	11.1	29.8	73.3	101.5	12.7	31.1	74.6	
	B	9.51	24.2	66.4	90.1	10.7	23.6	69.1	92.6	11.1	28.3	67.5	
Cellulose (mg/g)													
ID	86.1	171.7	256.4	323.3	79.2	158.7	208.8	309.8	74.2	151.1	182.3	301.8	
NC	78.2	162.3	237.4	296.4	67.1	144.1	184.6	277.3	63.1	138.3	169.5	284.7	
VC	A	64.1	139.2	166.6	244.7	56.3	118.6	122.3	226.6	52.2	102.5	119.8	
	B	58.3	126.6	155.1	232.1	48.3	104.2	114.4	212.5	48.7	97.4	112.6	
Hemicellulose (mg/g)													
ID	14.1	15.7	28.1	23.5	12.3	14.8	24.5	20.4	10.6	12.4	22.6	18.5	
NC	11.9	12.5	24.6	20.5	9.5	11.4	21.6	17.5	8.3	10.8	19.3	15.1	
VC	A	8.4	9.6	13.5	17.2	6.1	8.7	15.2	11.7	5.1	6.7	13.1	
	B	7.9	9.1	11.4	15.6	5.8	7.9	14.1	10.1	5.3	5.9	11.7	
Phenol (mg/100g)													
ID	29.1	39.5	49.3	56.5	24.1	36.1	45.3	54.2	21.8	32.1	43.5	53.8	
NC	24.3	35.3	41.8	50.1	19.5	30.5	40.2	49.1	17.6	28.3	38.1	47.2	
VC	A	18.6	28.6	26.1	38.5	15.2	22.1	24.4	36.3	13.3	20.12	28.4	
	B	17.1	26.1	24.5	36.2	13.9	20.3	22.1	33.8	11.4	18.2	25.5	
Humic acid (mg/5g)													
ID	6.07	4.18	3.69	1.71	5.84	4.02	3.43	1.57	5.77	3.91	2.73	1.39	
NC	7.13	5.22	4.41	2.63	6.61	5.05	4.04	2.36	6.41	4.75	3.84	2.11	
VC	A	8.18	6.36	5.88	3.41	7.21	6.11	4.95	3.29	7.03	5.75	4.32	
	B	9.11	6.68	6.03	3.69	7.38	6.38	5.21	3.54	7.24	5.91	4.77	

GL- Guava leaves, CD- Cow dung, HD- Horse dung, SD- Sheep dung, A- *Perionyx excavates*, B- *Lampito mauritti*, mean value followed by different letters in statistically different (SAS- Statistical Analysis System- ranged test, p<0.05), ID-Initial day (0-day), NC- Normal compost, VC- Vermicompost (*P. excavates* and *L. mauritti*), *-µl H/ 5g substrate

Table 2: continue,

ANOVA							
Parameters	Total Microbial Population	Dehydrogenase	Lignin	Cellulose	Hemicellulose	Phenol	Humic acid
Substrates							
Sum of Squares	15,32,448.15	188.99	14,315.84	1,68,827.08	2,961.35	11,029.30	159.547
Mean of Squares	5,10,816.05	62.997	4,771.95	56,275.69	987.115	3,676.43	53.182
F- value	1,24,779.31	1,08,177.18	9,316.89	25,152.38	78,234.83	70,737.35	35,719.42
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CD value	0.729	0.009	0.258	0.539	0.04	0.082	0.014

Treatments							
Sum of Squares	6,92,977.03	93.842	3,14,559.75	12,83,541.45	4,238.12	21,665.34	614.373
Mean of Squares	62,997.91	8.531	28,596.34	1,16,685.59	385.284	1,969.58	55.852
F- value	15,388.78	14,649.50	55,832.32	52,152.54	30,536.08	37,896.13	37,512.52
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CD value	1.263	0.015	0.447	0.934	0.07	0.142	0.024
Interactions							
Sum of Squares	43,291.56	6.623	3,262.02	35,071.76	436.142	1,105.25	7.556
Mean of Squares	1,311.87	0.201	98.849	1,062.78	13.216	33.493	0.229
F- value	320.455	344.628	192.996	475.009	1,047.48	644.421	153.795
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CD value	2.526	0.03	0.894	1.868	0.14	0.285	0.048

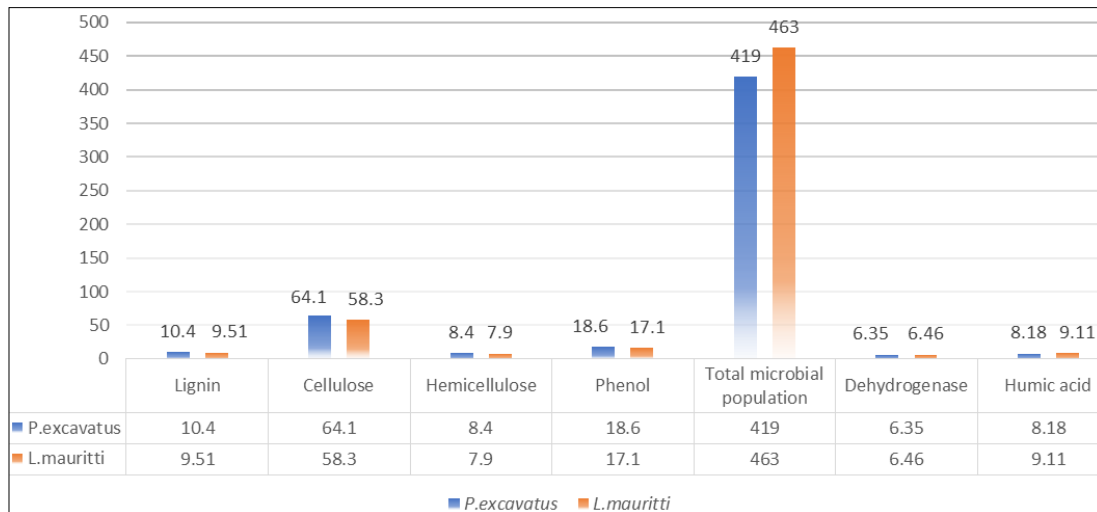


Fig 2: Comparison of biological parameters in *perionyx excavatus* and *lampito mauritti*

Table 3: Earthworm (*Perionyx excavatus* and *Lampito mauritti*) activity during in the vermicomposting of guava leaves (n=5)

Experiments (Vermibeds)	Biomass (g)				Cocoon production (N)				Hatchling (N)				Recovery of vermicompost (g)			
	Initial (0 day)		Final (60 days)		Initial (0 day)		Final (60 days)		Initial (0 day)		Final (60 days)		Initial (0 day)		Final (60 days)	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B		
4:0 (CD)	15.4	15.6	38.7	45.1	0	148.5	31.7	0	224.5	58.5	0	688.5	680.1			
3:1 (CD:GL)	15.5	15.3	36.3	41.6	0	132.5	28.5	0	193.8	54.2	0	667.1	677.5			
2:2 (CD:GL)	15.3	15.1	37.4	44.1	0	141.5	30.6	0	206.8	56.1	0	676.7	678.4			
1:3 (CD:GL)	15.5	15.6	35.2	39.8	0	126.6	27.1	0	182.4	50.4	0	653.4	662.1			
4:0 (HD)	15.8	15.6	37.2	43.5	0	126.2	29.4	0	196.5	55.1	0	661.1	670.7			
3:1 (CD:GL)	15.5	15.2	35.4	40.4	0	118.6	26.4	0	161.8	51.5	0	645.5	652.8			
2:2 (CD:GL)	15.4	15.6	36.6	42.4	0	101.5	28.1	0	173.2	53.1	0	652.4	660.1			
1:3 (CD:GL)	15.2	15.4	34.5	38.1	0	98.9	25.3	0	155.5	49.8	0	638.1	645.9			
4:0 (SD)	15.6	15.5	36.7	42.5	0	112.9	27.1	0	182.9	53.4	0	644.5	651.4			
3:1 (CD:GL)	15.6	15.2	33.7	39.1	0	104.5	23.7	0	153.4	48.1	0	619.9	628.1			
2:2 (CD:GL)	15.5	15.3	34.5	40.9	0	97.3	25.7	0	168.2	50.7	0	631.2	644.7			
1:3 (CD:GL)	15.1	15.8	32.6	37.5	0	93.1	21.8	0	144.6	45.4	0	607.1	618.5			

CD- Cow dung, HD- Horse dung, SD- Sheep dung, GL- Guava leaves, A- *Perionyx excavatus* and B-*Lampito mauritti*

Discussion

Vermicomposting is an effective, energy-efficient recycling process. This study measured and documented the biological parameters (total microbial population, dehydrogenase, lignin, cellulose, hemicellulose, phenol, and humic acid) found in compost and vermicompost made from different compositions of cattle dung. Lignocellulose, the predominant constituent of agricultural residue and the most plentiful replenishable organic material in soil is composed of polymers such as cellulose (40%), hemicellulose (20–30%), and lignin (20–30%) (Sjostrom, 2013) [26]. When compared to the initial day and vermicompost, the amounts of lignin, cellulose, hemicellulose, and phenol were lower in the vermicompost obtained from each experiment. Patidar *et*

al., (2014) [21] have reported, cellulolysis and lignolysis may have been enhanced by the combined action of lignocellulolytic fungi and microflora in the worm's gut. Additionally, the experiments modest decreases in cellulose, hemicellulose, lignin, and phenol content. According to Baldrian *et al.*, (2005) [1], *Pleurotus spp.* possess two extracellular enzyme systems that break down hemicellulose, cellulose, and lignin, respectively. These systems are called the hydrolytic and oxidative lignolytic systems.

The use of vermicompost in agriculture increases the amount of nutrients in the soil and decreases the uses of artificial fertilizers. When hemicellulose and cellulose are broken down during the composting process, different

compounds can be produced by the metabolism of carbohydrates (Wang *et al.*, 2018) [31]. For instance, the microbes transfer and secrete enzymes that break down lignin outside of cells (Zhang *et al.*, 2022) [34]. In general, compost made from various cattle dungs has a reduced level of cellulose and lignin Table 2. Sijinamanoj *et al.*, (2021) [25] have reported, for a *Phanerochaete chrysosporium* strain (41.75%), in particular, the cleaving reaction of laccase, a ligninolytic enzyme, on lignin is widely recognized. (Liu *et al.*, 2020) [14]. Also, phenol content was found to decrease. Yahana *et al.*, (2017) have reported, vermicomposted EFBs had very low levels of total phenolic compounds and no phenol, indicating a very low lignin content.

Additionally, when compared to the initial day and vermicompost, the total microbial population, dehydrogenase, and humic acid contents increased in the vermicompost obtained from each experiment. Increased HA concentrations in microorganism-inoculated vermicompost corroborated research by Gaur (1987) [9] and Tiwari (1989) [28], which showed that composting of microorganism-inoculated organic substrates resulted in a faster rate of decomposition. The findings indicated that microbial inoculation further accelerated the rate of humification and enzyme activity, and vermicomposting raised the amount of humic acid and acid phosphatase activity in organic substrates (Pramanik *et al.*, 2009) [24]. Parthasarathi, (2010) [19] has reported, the increase in HA found in the vermicompost across all vermibeds in this study may be primarily attributed to the activity of numerous microbes as well as the earthworm's gut-associated process. In both composting methods, higher activity of dehydrogenase was noticed during the initial stage than in the final mature compost (Lakshmi *et al.*, 2014) [13]. Based on the study, the total microbial population size was also observed to decrease slightly compared with cow dung experiments. Similarly, furthermore, during the first 30 days of vermicomposting, a higher temperature dramatically increased microbial activity; at 25 °C, this activity also showed the fastest stabilization. (Zhang *et al.*, 2020) [33]. Additionally, different proportions of lignocellulosic waste in livestock manure increase nutrients and increase the total microbial population.

Additionally, compost and vermicompost biomass, cocoon production, hatchling number, and vermicompost recovery were measured and presented in Table 3. The number of doses examined was slightly higher at the end than at the beginning. Vermicomposting is also taken into account when analyzing patterns of earthworm biomass production, cocoon, and hatchling counts, and vermicompost yield (Soniya and Dhanasekaran, 2015) [27]. Debnath and Chaudhuri., (2020) [6] have reported to, *P. excavatus* and *D. bolau*, two epigeic earthworm species, were continuous breeders with low incubation periods (13 and 14.2 days) and high fecundity (187 and 92 cocoons per worm per year). Endogeic *M. posthuma* and anecic earthworm *L. mauritii* were discrete and semi-continuous breeders, respectively. According to Galanakis, (2018) [8], the temperature above the optimum for growth decreases the incubation period of earthworms and increases hatchling production. When it comes to recovering nutrients from organic biomass residues, *P. ceylanensis* is just as effective as the commonly used *P. excavatus* (Birintha *et al.*, 2020) [4]; thus, *P. excavatus* and *P. ceylanensis* are equally effective when it

comes to producing vermifertilizer and reclaiming nutrients from agricultural and urban biomass residues. Zziwa *et al.*, (2021) [35] have reported, the study examined the efficacy of batch (B) and continuous (C) vermicomposting systems over 60 days about waste reduction and nutrient recovery. At the end of the study, the contribution of *perionyx excavatus* and *lampito mauritti* is high in manure prepared from different types of cattle dung. Therefore, correct interpretations are given in the study.

Conclusion

At the end of the study, cow dung and guava leaf tests dominated the compost (1:1) made from different cattle dung. Therefore, the study aims to show that the use of compost prepared with earthworms of Indigenous species *Perionyx excavatus* and *Lampito mauritti* can improve soil structure and increase yield in agriculture. As a result, adding vermicompost to the soil increases its nutrient content. Hence, it increases soil fertility and protects against soil erosion, and regular application of vermicompost to agriculture can also increase the earthworm population. Also, the use of artificial fertilizers limits the amount of oxygen that is available to plants. Thus, increasing the quantity of natural fertilizers can increase the amount of oxygen and nutrients that plants can access.

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