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ORIGINAL RESEARCH

Investigation of pesticidal ability of humic acid derived from palm oil Empty Fruit Bunch (EFB) vermicompost

Alvyana Khiew Ai May[1], Rashid Shamsuddin[1,2*], Lew Jin Hau[1], Aqsha Aqsha[1,2], Nurlidia Mansor[1,2], Nur Izzati Mustapa[3], Murugesu Narasimha[3]

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Abstract

Purpose Humic Acid (HA), a plant's growth promoter readily available in compost was found to have insect repelling functional groups. This study was conducted to investigate the pesticidal ability of HA extracted from Empty Fruit Bunches (EFB) vermicomposts at Carbon to Nitrogen (C:N) ratio of 25, 30 and 35.

Methods The vermicomposts were prepared using EFB, a palm oil mill by-product as the base material with underutilized organic wastes (fishmeal, bonemeal, and bunch ash) as additives to enhance the macronutrients of vermicomposts. Composting was conducted for 52 days with earthworms as composting agents. HA (mass yield of 2.34 – 2.63%) was extracted from the matured vermicomposts through alkaline extraction before acid precipitation. The insect repelling effectiveness of HA, Garlic Extract (GE) and 87% GE + 13% HA was evaluated by spraying them onto soil samples in different compartments with crickets.

Results Vessel with lesser crickets indicates agent sprayed repels insect better. Results showed 87% GE + 13% HA repels insect most effectively, followed by 100% HA and lastly 100% GE. The macronutrients (Nitrogen, Phosphorus and Potassium) were improved upon mixing additive materials to the vermicompost.

Conclusion HA alone possesses some ability to repel crickets. It performs better when it is coupled with GE as HA acts as a diluent to promote GE absorption into soil thus enhancing the pesticidal effect. This demonstrates EFB has potential as a precursor material for high value vermicompost with insect repellent ability, thus presenting an opportunity for respective industries to generate value-added products while solving by-product underutilization issue.

Keywords Humic acid, Insect repellent, Empty fruit bunch, Vermicompost

Introduction

The rapid growth of global population demands higher crop production that can be achieved by the application of more effective fertiliser and pesticide. Generally, fertiliser is added onto crops to enhance the yield while pesticides act as crop's protection against any pests or harmful diseases (Kumar 2013). However, the continuous and excessive usage of modern synthetic fertiliser and pesticides in agriculture has adversely affected the environment by causing surface and ground water contamination and a decline in beneficial soil microorganisms which are important to maintain soil fertility. Apart from affecting the environment, chemical fertiliser and pesticide could also bring severe health implication on humans through the consumption of food containing toxic pesticide residue (Aktar et al. 2009). Therefore, efforts have been extended to synthesize environmental friendly fertiliser and natural pesticides which could be derived from organic wastes or natural resources.

An overabundance of biomass including Empty Fruit Bunches (EFB) from oil palm industry encouraged its exploitation as organic compost. However, the high carbon to nitrogen (C:N) ratio of raw EFB results in extremely slow decomposition rate (Nahrul Hayawin et al. 2012). Raw EFB also contains an insufficient amount of nitrogen (N), phosphorus (P) and potassium (K), which are vital macronutrients to sustain healthy growth of agricultural crops. Hence, the effort of co-composting EFB with various organic biomass which include POME, chitinous material and cow dung has been conducted to hasten the decomposition rate and to increase the nutritional content of the final compost. For instance, the quality of EFB compost in

✉ Rashid Shamsuddin
mrashids@utp.edu.my

[1] Chemical Engineering Department, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia
[2] HICoE-Centre for Biofuel and Biochemical Research, Institute of Self-Sustainable Building, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia
[3] Vata VM Synery Sdn. Bhd., Lot 5164-676, MK, Cegar Gala, Batu 29, Kg Chuar Kati, 33020 Kuala Kangsar, Perak, Malaysia

terms of N, P and K increased by +162.5%, +775%, +57.73%, respectively with the addition of POME and raw shrimp shells (Lai 2014). Another study on blending of EFB and liquid POME together with oil palm decanter cake slurry produced compost with an increase in 46.4% nitrogen, 17.9% phosphorus, 17.7% potassium and 23.1% calcium (Yahya et al. 2010).

A more superior composting method known as vermicomposting has gained significant attention in the agricultural industry owing to its advantages of faster decomposition rate, better water holding capacity of the soil and improved compost texture. In contrast to conventional composting that only relies on the natural decomposition of the organic waste by soil microorganism, vermicomposting utilizes worms to bio-transform the organic biomass into vermicompost (Lim et al. 2015). In relation to this, a study has been carried out on vermicomposting of EFB mix with solid POME and the result shows that the vermicompost achieved maturity in 45 days with the final C:N ratio which was recorded to be 20.6 (Nahrul Hayawin et al. 2012). The result of another study showed the maturation period of EFB vermicompost mixed with cow dung is achieved within 12 weeks with the final C:N ratio of 18.53 (Lim et al. 2015). Vermicomposting also promises a fast maturation rate than conventional composting. A study by (Sinha et al. 2009) showed that conventional composting requires 12-week period (including 4 weeks of curing) to achieve maturation while vermicomposting only takes up half of this time. This study also revealed that vermicomposting can produce finer compost structure that provides nutrient content similar or higher than that of conventional composting.

Another benefit of vermicomposting is the high content of humic compounds particularly Humic Acid (HA) which is a product of decomposition of organic matter. HA is an effective plant's growth promoter that enhances nutrients' uptake from the soil, boosts root growth and helps plants to overcome stress (Sinha et al. 2010). HA is insoluble in water at the acidic condition of pH less than 2. The hetero-cyclic structure of HA which comprises of carboxylic, quinone and phenol functional groups were claimed to have a pesticidal ability (Seenivasan and Senthilnathan 2018).

Currently, less work has been done on the application of HA as a pest control agent (biopesticide) in agricultural industry. The available related research focuses on the interaction of synthetic pesticide with humic compounds. HA consists of both hydrophilic and hydrophobic sites whereby the former is originated from the oxygen-containing functional group and the hydrophobic site is formed due to the presence of aromatic rings, aliphatic hydrocarbon and fatty acid esters (Helal et al. 2006). Hence, HA is an excellent natural chelator as it is capable of absorbing both the hydrophilic and hydrophobic compound. This large exchange capacity of hydrophilic and hydrophobic compound allows HA to effectively absorb pesticides (Helal et al. 2006). This alone, however, will not solve the dependency on synthetic pesticide to control pest activities in agriculture.

Various research has been conducted to produce natural insect control agents which are derived from plants or organic resources. Garlic, for example has been studied extensively on its antimicrobial properties and utilized as an insect repellent for a small scale usage. However, garlic extract (GE) is not successfully commercialized as an agricultural pesticide due to its relative low efficiency (Valencia and Luis 2010). The author proposed the combination of GE and HA in the ratio of 87:13 to enhance the pesticidal ability of GE. GE is utilised as an active repellent ingredient whereas HA in water acts as a diluent to improve the absorption of GE into the vascular system of the plant. It is also reported that the addition of HA into GE yields higher repellent effect by increasing the permeability of the membranes, allowing greater penetration of GE into plant, thus stimulating the biochemical processes in the plant (Valencia and Luis 2010).

On the other hand, there is also claims made on the potential use of HA alone as natural insect control agent. (Pathma and Sakthivel 2012) reported that the uptake of phenolic compound present in HA will make the plant tissue unpleasant to consume hence hindering pest attack on plant and affects pest reproduction rates and survival. A study on the effect of HA on *Meloidogyne incognita* (a type of nematode that protrudes the growing root tips of banana) found that HA is toxic to plant-feeding parasites, which causes a reduction in growth of the nematode. It was predicted that the nematocidal efficacy against HA is contributed by the functional groups of the acid which includes carboxyl and phenolic groups (Seenivasan and Senthilnathan 2018).

This study focuses on the extraction of HA derived from EFB-based vermicompost and examines its pesticidal ability against crickets. Three vermicomposts pile were prepared by mixing EFB as the base material with underutilized organic wastes (fishmeal, bonemeal, and bunch ash) to achieve C:N ratio of 25, 30 and 35. The C:N ratio range is widely accepted as the ideal composition of carbon and nitrogen sources in compost. The value of less than 25 and greater than 35 signifies rapid nitrogen mineralization and microbial immobilization respectively (Brust 2019). The composting process was conducted for 52 days with earthworms as composting agents.

Materials and methods

Materials

EFB, bunch ash and liquid POME were supplied by VATA VM Synergy (M) Sdn. Bhd. Fishmeal was provided by Dinding Poultry Manjung. Bonemeal was purchased from Promise Earth (M) Sdn. Bhd. Sawdust was obtained from Wan Sang Sawmill Enterprise Sdn. Bhd. Two different commercial composts, Compost 1 and Compost 2 were obtained from garden nursery for comparison purpose in terms of C, N, P and K value. Earthworm, *Eisenia fetida* obtained from local fishing shop was selected as the composting agent due to the wide range of temperature and high metabolic rate tolerance.

Analytical-grade sodium hydroxide, NaOH pellets, (Sigma Aldrich) hydrochloric acid, HCl (Sigma Aldrich, 37%) and other lab materials and consumables were provided by Universiti Teknologi PETRONAS research lab.

A 35 L HDPE multipurpose container was modified into a composting bin. Adequate aeration holes were provided on the bin with plastic mesh cover to protect from insects.

Vermicompost preparation

Three batches of 3 kg vermicomposts were prepared by mixing EFB + liquid POME (1:1 ratio), fishmeal, bonemeal, bunch ash and sawdust at specific mass weight determined using Equation (1) to yield initial carbon to nitrogen ratio (C:N) of 25, 30 and 35. Individual carbon and nitrogen values of each feedstock material is shown in Table 1. Carbon and nitrogen contents of bonemeal were provided by the supplier while the rest of the values were taken from our previous work (Lew et al. 2020). Prior to mixing, the feedstock materials were ground to less than 3 mm.

$$C{:}N_{avg} = \frac{\sum Q_i (C_i \times (100 - M_i)}{\sum Q_i \,(N_i \times (100 - M_i)} \quad (1)$$

where $C{:}N_{avg}$ is the average C:N ratio of the mixture, Q_i is the mass of each feedstock material (kg), C_i is the carbon content of material (%), N_i is the nitrogen content of the material (%) and M_i is the moisture content of material (%).

Each batch of vermicomposts with the composition shown in Table 2 were placed on top of a 3 kg soil bedding in separate bins. Fifty numbers of *Eisenia fetida* earthworms that act as composting agent were released in each batch of vermicompost. The compost bins were located at an outdoor compound under a covered shelter.

pH and temperature monitoring

The pH and temperature of vermicompost were recorded every 3 days in triplicate by using HANNA Instruments waterproof tester. The readings were recorded at the same time of the day at about 10:00 A.M. The average values were used to plot a graph of pH and temperature profile in order to determine the vermicompost's maturation point.

Moisture content and mass yield

The initial moisture content of the feedstock and the mass of each batch of vermicompost were measured at the beginning of the vermicomposting activity. The percentage of moisture of the feedstock was measure using HX-240 moisture analyser. The formula to calculate the initial moisture content of vermicomposts is as shown in Equation (2).

$$MC_{avg} = \frac{\sum m_i (\, MC_i)}{m_T} \quad (2)$$

Table 1 Carbon and nitrogen value of feedstock materials

Feedstock	Composition		
	(%) Carbon	(%) Nitrogen	C:N ratio
EFB + POME	38.60	1	38.60
Fishmeal	37.79	11	3.44
Bonemeal	15.75	4.5	3.50
Bunch Ash	0.55	0.08	6.88
Sawdust	45.93	0.125	367

Table 2 Initial feedstock composition of vermicompost piles

C:N	Composition (kg)				
	EFB: POME	Fishmeal	Bonemeal	Bunch Ash	Sawdust
25	0.850	0.150	0.150	0.150	1.700
30	1.300	0.100	0.100	0.100	1.400
35	1.450	0.075	0.075	0.075	1.325

where m_i is the individual mass of the sample (kg), MC_i is the individual moisture content (%) and m_T is the total mass of the compost (kg).

Noted that liquid POME was used as the moisturization medium instead of water. This approach provided natural bacteria for the composting pile and as a sustainability strategy on palm oil processing waste. Upon maturation, the mass yield was calculated using Equation (3).

$$MY = 100 - \left[\frac{m_i\,(100 - MC_i) - m_f\,(100 - MC_f)}{m_i\,(100 - MC_i)}\, x\, 100\%\right] \quad (3)$$

where MY is the mass yield of compost, m_i and m_f are the initial and final mass of vermicompost (kg), and MC_i and MC_f are the initial and final moisture content (%) of vermicompost.

Analytical test for macronutrient –C, N, P, K

Carbon (C) and nitrogen (N) content of feedstock materials and vermicompost were determined using Perkin Elmer CHNSO Analyser 2400 Series. Phosphorus (P) and potassium (K) content were conducted by an external laboratory, ERALab Sdn. Bhd. using the MS 417:1994 standard.

Humic acid extraction

Humic Acid (HA) was extracted from the matured vermicomposts through alkaline extraction followed by precipitation in strong acid (Lamar et al. 2014). A 200 g of dried vermicompost was mixed with 1 L sodium hydroxide (NaOH) solution of 0.1 M in a beaker. The mixture was stirred for 6 hours to completely dissolve the HA. Next, the mixture was centrifuged at 4000 rpm for 10 minutes. The solid pellet containing humic substance was removed and the supernatant containing dissolved HA was collected for alkaline precipitation. A 6 M hydrochloric acid (HCl) solution was then added to the supernatant continuously and mixed using a stirrer until it reached pH 1 where HA precipitated out of the mixture. It was later centrifuged at 4000 rpm for 10 minutes to obtain the HA in the pellet while supernatant containing fulvic acid was discarded. The HA pellet was rinsed using excess distilled water and neutralized to pH 7 by adding 6 M NaOH and 6 M HCl. The HA slurry was dried overnight in an oven at 105 °C. The HA yield from 200 g of vermicompost was calculated by applying Equation (4).

$$HA\ yield = \frac{m_{HA}}{200g}\, x\, 100\% \quad (4)$$

Where m_{HA} is the mass (g) of dried HA collected.

Humic acid verification

HA extracted from vermicomposts were verified by analysing the spectra obtained from Fourier-Transform Infrared Spectroscopy (FTIR) using a Spectrum 1000 spectrophotometer (Perkin Elmer) with 2 cm^{-1} resolution in the range of 500–4000 cm^{-1}. Around 1 mg of HA was milled with high purity infrared grade KBr powder and compressed into small pellets for measurement.

Insect repellent test

Three different insect control agents (diluents) were prepared from humid acid (HA) and garlic extract (GE). The diluents prepared were 100% HA, 100% GE and 87% GE + 13% HA. Four different vessels layered with soil bedding were sprayed with different insect control agent and labelled as A (void), B (100% HA), C (100% GE) and D (87% HA + 13% GE). For insect repellent test, two of the vessels were combined and 10 crickets were released at one side of the vessel as illustrated in Fig. 1. After 30 minutes, the number of crickets at each side of the vessel was counted and recorded. The reduction in the number of crickets represents the pesticidal ability of the insect repellents. The experiment was repeated with a different set of vessel combinations as shown in Table 3. All the tests were done twice and the average results are reported.

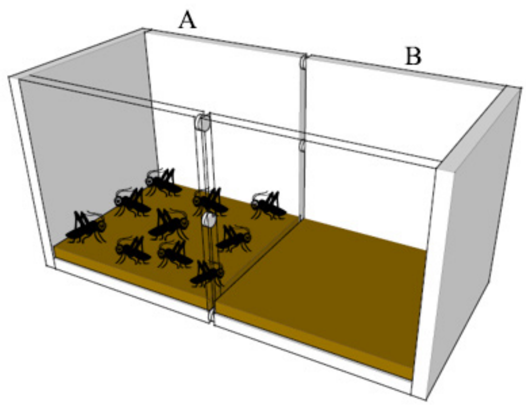

Fig. 1 Example of setup for insect repellent test

Table 3 Combination of insect vessel for insect repellent test

Test	Combination of vessel
(1)	A versus B*
(2)	A versus C*
(3)	A versus D*
(4)	B versus C*
(5)	B versus D*
(6)	C versus D*

* Vessel where the crickets were initially released.

* A= void, B= 100% HA, C= 100% GE and D= 87% HA + 13% GE.

Result and discussion

pH and temperature profiling

Fig. 2 shows the pH and temperature profile from Day 1 to Day 52 of C:N 30 vermicompost. Since all three batches of vermicomposts display similar trend, the C:N 30 profile was selected to represent the overall vermicomposting setups as C:N 30 compost is often regarded as a healthy and balanced compost.

Theoretically, each compost should exhibit four stages of temperature change which are mesophilic, thermophilic, cooling and curing stage (Jenkins 1999). Mesophilic stage (temperature up to 44 ˚C) begins when the organic materials undergo composting process and heat is released due to microbial breakdown of organic matters by composting bacteria. In the thermophilic phase (second stage), thermophilic bacteria become very active and will increase the temperature of the compost to as high as 70 ˚C. During the cooling stage, the microorganism that was present during the mesophilic stage will reactivate and feed on undigested organic materials until a complete degradation is achieved. This final stage (curing) is marked by constant temperature and pH of the pile (Jenkins 1999).

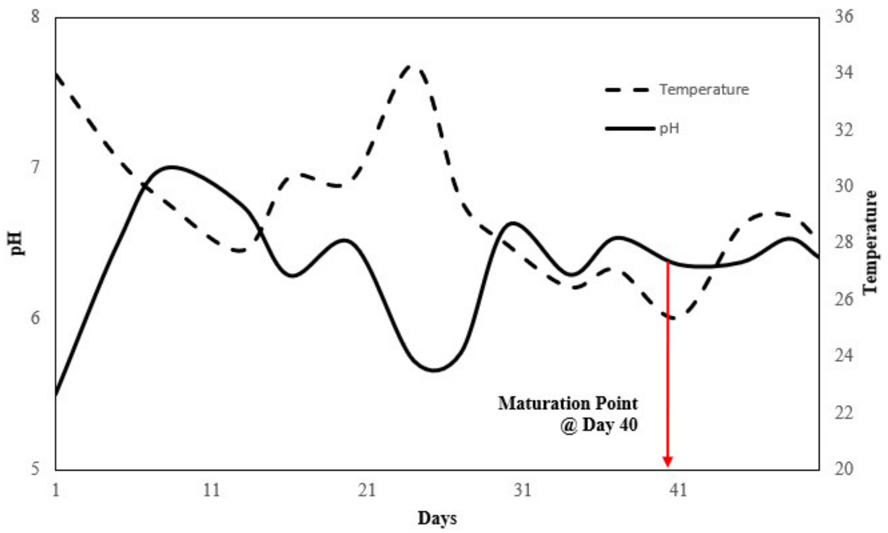

Fig. 2 pH and temperature profiling of C:N 30 vermicompost from Day 1 to Day 52

Based on Fig. 2, it is observed that the reading of the temperature fluctuates between 25 ˚C to 34 ˚C throughout the composting period due to the weather effect as the vermicomposts were placed under an outdoor shelter. The temperature profile gave no clear indication on the change of composting stages. Therefore, it can be concluded that the compost maturation for an outdoor com posting cannot be determined on the temperature profile alone. Similar result was obtained in another study on dried leaves based composting (Shamsuddin et al. 2017). Beside this shortcoming, the temperature trend shows the general temperature trend of composting process whereby the temperature started to increase from Day 13 to Day 24 with the highest temperature of 34˚C. The temperature rises after Day 13 implied the start of mesophilic phase where microbial activities on organic matters are rapid and as the result, more heat is released. After Day 24, the temperature of the vermicompost started to decrease indicating lower microbial activities.

For pH profile, the vermicompost increased in alkalinity at the start due to the release of ammonium ion from the biochemical reaction of nitrogen-containing materials (Nahrul Hayawin et al. 2012). After Day 10, there is a drop of pH value as the result of active degradation of organic matters that is responsible for the production of organic and inorganic acid (Oluchukwu et al. 2018). Accumulation of acid in composting pile inhibits bacterial growth, reducing bacterial population and as the pH improved the bacteria was thriving again (Shamsuddin et al. 2017). This cyclic trend continues until the pH stabilises, which occurs at around Day 40 where it is taken as the maturation period with the final pH of 6.3. The lowest pH recorded was 5.5 while the highest pH was 7.0 which are within a healthy pH range of 5.5 to 9.0 for composting (Oluchukwu et al. 2018). Healthy pH is required for soil microorganism and earthworms to function.

Physical observation of vermicompost

The change in the physical appearance of C:N 30 vermicompost from Day 1 to Day 52 is shown in Fig. 3. Throughout the vermicomposting period, the colour of the vermicompost changed from light brown to dark brown, while the texture of the compost became fluffy and porous. This is consistent with the study by (Alkoaik 2019), where the increase in colour change and crumby structure is an indication of compost maturation (Hau et al. 2020). On Day 13, it was observed that mushrooms started to grow on the compost of C:N 30, attributed to its optimal carbon to nitrogen content. During the decomposition process, microorganism normally utilizes carbon and nitrogen in a 30 to 1 part ratio as they grow and reproduce (Cornell 1996). Therefore, the conducive environment of C:N 30 has supported the mushrooms to grow on the compost pile (Hau et al. 2020). Fewer mushrooms were observed on C:N 25 and C:N 35 vermicomposts. It was also observed that all the setups did not release any unpleasant smell during the composting process and the earthworms were alive at the end of the work.

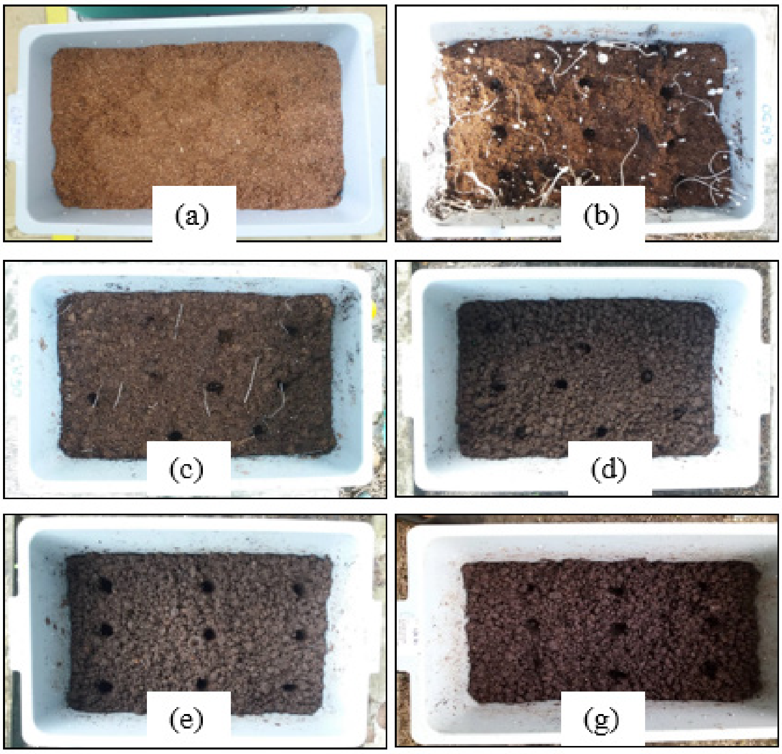

Fig. 3 Physical appearance of C:N 30 vermicompost on (a) Day 1; (b) Day 13; (c) Day 20; (d) Day 34; (e) Day 41; (f) Day 52

Produced vermicomposts were compared with commercial composts obtained from local garden nursery. Compost 1 is derived from EFB by utilizing effective microorganism (EM) as the composting agent while Compost 2 is derived from household and garden waste without the addition of composting agent. The physical appearance of the matured vermicomposts and commercial composts were illustrated in Fig. 4.

As can be observed, all three batches of final vermicomposts have a fine and porous structure with earthy smell upon maturation. These are attributed to earthworms' movement within the compost pile and their activities in breaking down organic matters (Khiew et al. 2020). In contrast, Compost 1 has fibrous and larger particles whereas Compost 2 contains small grits and even plastic pieces which could have resulted from the inefficient sorting practice of household waste.

Characterization of vermicompost

Vermicompost and commercial composts characterization summary is reported in Table 4. The ratio of carbon to nitrogen indicates the maturation index for the compost. The C:N ratio of vermicomposts at maturation were between 16 to 22. This is in accordance with literature suggestion that C:N less than 20 signifies compost stabilization (Yahya et al. 2010). Vermicompost of C:N 35 had slower decomposition rate due to higher carbon content and therefore has final C:N of 22. The ratio is expected to decrease to less than 20 over a longer period.

These C:N values are between the values of commercial Compost 1 (26.91) and Compost 2 (7.15). The high C:N ratio of Compost 1 is due to its main composition of EFB that naturally has high carbon content. Since Compost 2 is derived from household and garden wastes such as food scraps and leaves, these contributed to high nitrogen content, hence resulted in low compost C:N ratio. Compost 1 and 2 are expected to have a final C:N ratio of less than 20 in the long run. However, from a business standpoint, generating compost within a very short period of time is important for profit. That could explain why the commercial composts have a non-ideal ratio of final carbon to nitrogen contents.

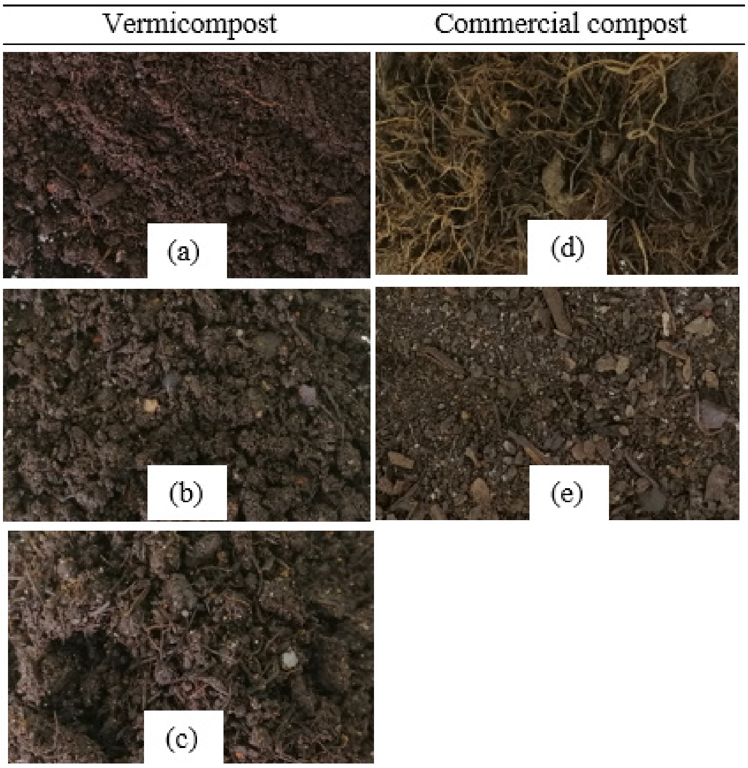

Fig. 4 Physical appearance of vermicomposts at (a) C:N 25; (b) C:N 30; (c) C:N 35, in comparison to commercial composts of (d) Compost 1 and (e) Compost 2

In term of macronutrient values, the nitrogen (N), phosphorus (P) and potassium (K) content of the three batches of vermicomposts showed an increment especially in K. The slight increase in the N content is due to the losses of organic carbon (Varma et al. 2016) and the released of excretory products from earthworms (Nahrul Hayawin et al. 2012). The increase in P is due to the release of phosphorus from earthworm gut phosphates and the total phosphorus released by P-solubilizing microorganisms in worm cast during the consumption of organic matter by the worms (Nahrul Hayawin et al. 2012). The potassium (K) content, on the other hand, is mainly contributed by the acid produced from microorganism activities that are vital in the solubilization of insoluble potassium. It is suggested that earthworms in vermicompost enhance the rate of mineralization by enhancing the microbial activities which resulted in a high concentration of exchangeable K through the action of residue processing (Batham 2014).

All the three batches of vermicomposts produced have higher nutritional contents than commercial composts except for N and P of Compost 2. The greater N:P:K values of the mixed vermicompost implies that the addition of organic additives such as fishmeal, bonemeal and bunch ash are able to enhance the nutrient contents in EFB compost. Compost 2 has a different trend in the nutrient content where it has the highest P but the lowest K due to its high content of perishable wastes such as food waste and garden leaves.

As for moisture content, all batches of vermicompost showed an increment in moisture content due to constant moisturization. The optimum moisture content of 50% to 75% should be maintained as it acts as the transporting medium of nutrient required by the microorganism (Liang et al. 2003; Yahya et al. 2010). A low moisture content of compost pile would cause the biological process to be halted due to the dehydration of microorganisms. In contrast, the excessively high moisture will cause water logging in the compost, creating an anaerobic condition which will affect the composting activity (Liang et al. 2003). The final moisture of the vermicomposts is between 59% to 73% that is within the recommended moisture range, suggesting a conducive environment for biological activity inside the compost pile.

Generally, the mass yield of vermicomposts is observed to reduce by 10% as compared to the initial mass of the compost pile. This reduction in mass is due to the decomposition process within the compost. Reduction of 10% however is not significant for vermicomposting since in this study highly cellulosic materials are used such as EFB and sawdust that are considerably difficult to decompose in nature. For comparison, mass reduction of almost 59% were obtained for vermicomposting of chicken manure (Tiquia and Tam 2000).

Humic acid yield

Humic Acid for vermicomposts and commercial composts were extracted using alkaline extraction and acid precipitation on 200 g samples and the mass yield is displayed in Fig. 5.

Table 4 Summary of compost characterization

Parameter	Vermicompost			Commercial compost	
	C:N 25	C:N 30	C:N 35	Compost 1	Compost 2
Initial					
Carbon to nitrogen ratio (C:N)	25.00	30.00	35.00	-	-
Moisture content (wt%)	39.15	41.80	42.94	-	-
Carbon, C (wt%)	42.6	42.93	43.21	-	-
Nitrogen, N (wt%)	1.21	1.02	0.91	-	-
Phosphorus, P (wt%)	0.54	0.36	0.27	-	-
Potassium, K (wt%)	3.31	3.24	3.16	-	-
Final					
Carbon to nitrogen ratio (C:N)	16.01	17.96	22.33	26.91	7.15
Moisture content (wt%)	59.32	72.44	64.71	-	-
Carbon, C (wt%)	24.07	21.54	33.06	32.59	22.62
Nitrogen, N (wt%)	1.50	1.20	1.48	1.21	3.16
Phosphorus, P (wt%)	1.22	2.72	2.73	1.05	7.09
Potassium, K (wt%)	12.05	11.71	12.43	5.54	1.68
Mass yield (%)	89.38	88.85	89.93	-	-

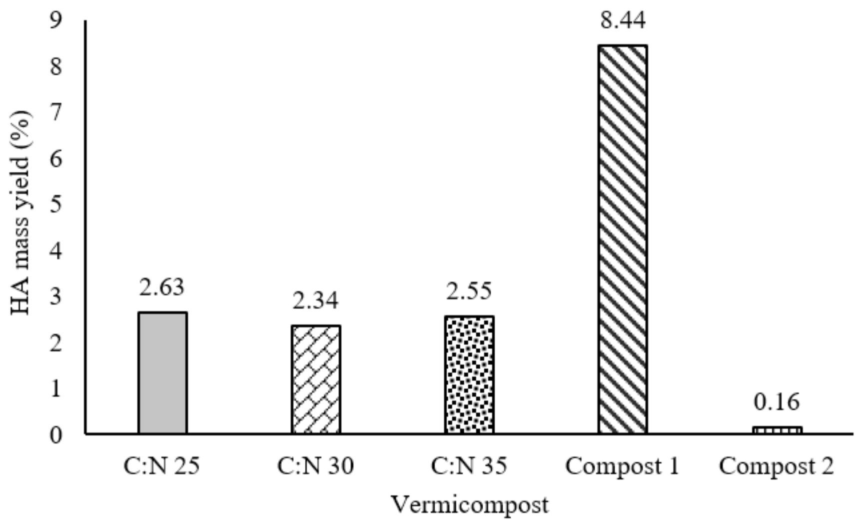

Fig. 5 Humic Acid mass yield

It is observed that C:N 25 yield the most HA at 2.63% followed by C:N 35 at 2.55% and lastly C:N 30 at 2.34%. These small differences in the HA yields imply that different proportion of raw materials added initially did not significantly affect the HA content of the vermicompost. Humic Acid is a by-product of microbial breakdown of organic materials. A literature study states that the species of microorganisms which lives within the alimentary canal of earthworms are commonly identical to those in the soils where the worms live (Edwards 1977). Since the type and quantity of earthworms added into each batch of vermicompost were similar, the HA yield from each compost pile should be almost comparable (Khiew et al. 2020).

Comparing with the commercial composts, HA yield is the highest in Compost 1 and lowest in Compost 2. The high yield of HA from Compost 1 might be due to the type of composting agent used that is effective microorganism (EM). Details on the EM species and composting approach of Compost 1 are beyond reach as all the sensitive information are considered company's trade secrets, hence limiting our comparison study on the composting technique. On the other hand, Compost 2 is derived from household domestic wastes without applying any composting agents. Without composting agents such as earthworms and microorganisms that can enhance the production of Humic Acid, the yield of Humic Acid in Compost 2 is significantly lower compared to our Vermicomposts and commercial Compost 1.

Fourier-Transformed Infrared (FTIR) analysis

The FTIR spectra of HA extracted from all Vermicomposts are shown in Fig. 6. The spectra are used to identify the key components of HA and their intensities for different formulations of Vermicomposts.

From Fig 6, all spectra display almost similar absorption bands, with only difference in their relative intensity. Humic Acid contains many functional chemical groups that aid in modifying and improving soil's chemical properties as well as stimulating plant's growth (Asing et al. 2009). The significant functional groups are the aromatic backbone, carboxyl (R-COOH) (3300-2500 cm^{-1}), hydroxyl (R-OH) (3400-3200 cm^{-1}), ketone (R-C=O-R') (1610 – 1590 cm^{-1}), amines ($R-NH_2$) (1610 – 1590 cm^{-1}) and quinone (1610 – 1590 cm^{-1}). The major FTIR absorption bands and assignments for HA extracted from vermicomposts are tabulated in Table 5 (Enev et al. 2014). Based on the peak analysis, the dark pellet particles obtained after acid precipitation were proven as HA as they contain the expected major functional groups of HA (Khiew et al. 2020).

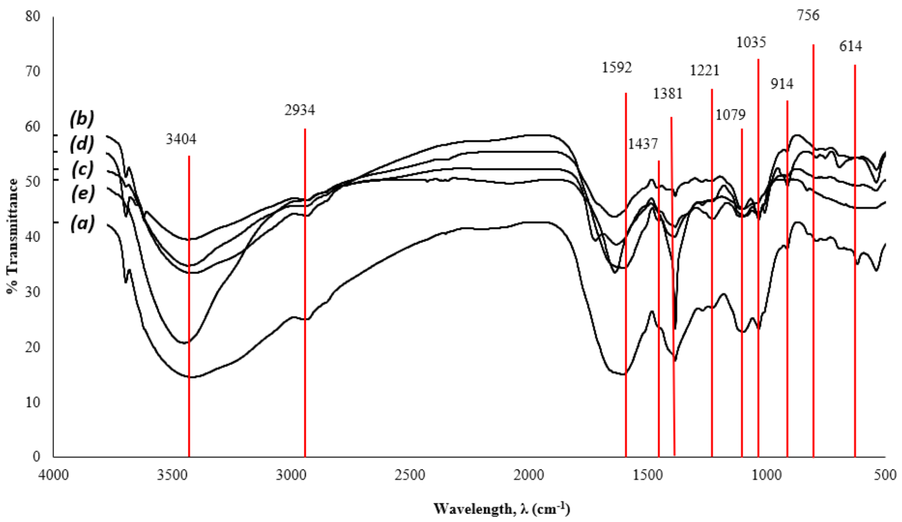

Fig. 6 FTIR spectra of HA extracted from (a) C:N 25, (b) C:N 30, (c) C:N 35 vermicompost, (d) Compost 1 and (e) Compost 2

Table 5 Major FTIR absorption bands and assignments for Humic Acid (Enev et al. 2014; Zhen Liu et al. 2014)

Wavelength (cm^{-1})	Assignment	Possible source
3300 – 3400	O-H stretching, N-H stretching (minor), hydrogen bonded OH	Phenol, carboxyl (COOH), hydroxyl, alcohol and N-H (amide) groups
2930 – 2900	asymmetric and symmetric C–H stretching of CH_2 group	C-H asymmetric, C-H stretch of -CH aliphatic
1590 – 1610	aromatic C=C skeletal vibrations, C=O stretching of amide groups (amide I band), C=O of quinone and/or H-bonded conjugated ketones	Aromatic structure, COO- and C= O groups (e.g., amides, ketones and quinones)
1420 – 1430	O–H deformation and C–O stretching of phenolic OH	Phenol
1370 – 1390	C–H bending of CH_2 and CH_3 groups, COO− anti-symmetric stretching	Ammonium carbonate generated by reaction of ammonia and CO_2
1210 – 1220	C–O stretching of aryl ethers and phenols	Aryl ethers and phenols
1090 – 1110	C–O stretching of secondary alcohols and/or ethers	Secondary alcohols and/or ethers
1025 – 1040	C-O stretch of polysaccharides, Si-O asymmetric stretch of silicate impurities	Polysaccharides, silicate impurities
910 – 960	Aromatic rings and halogens (chloro-compounds)	Aromatic rings and halogens (chloro-compounds)
790 – 800	Aromatic rings and halogens (chloro-compounds) and Si-O asymmetric stretch	Aromatic rings, halogens, Si-O asymmetric stretch
675 - 695	Aromatic rings and halogens (chloro-compounds)	Aromatic rings, halogens

Insect repellent test

Table 6 summarizes the insect repellent test results. The numbers were recorded in every 30 minutes after the crickets were released into the vessel.

Trial 1 and Trial 2 test results are similar but had a different number of crickets. For test (1), test (2) and test (3), it is proven that sample B (100% HA), sample C (100% GE) and sample D (87% GE + 13% HA) are able to repel insect as the number of crickets in vessel A (void) is always higher than the opposite vessel. This indicates that the crickets prefer void (soil bedding) alone as compared to either Humic Acid, garlic extract or combination. The comparison of HA and GE was presented by Test (4), where HA was proven to be a more effective insect repellent agent than GE. This might be due to the presence of phenolic and carboxyl functional group in HA (Seenivasan and Senthilnathan 2018). The combination of HA and GE (87% GE+ 13% HA) was found to be the most effective insect repellent agent where the number of insects remained in vessel D was consistently less than those in vessel B and C. These findings indicate that the crickets dislike the duo combination of HA and GE more than HA and GE alone. This is due to GE known as an active repellent ingredient while HA diluent is able to promote the absorption of GE thus giving a higher pesticidal effect (Valencia and Luis 2010). Overall, it is concluded that the most effective insect repellent agent is 87% GE + 13% HA, followed by 100% Humic Acid and lastly 100% garlic extract.

Table 6 Insect repellent test result

Test	Number of crickets			
	Trial 1		Trial 2	
(1) A versus B*	**A**	**B**	**A**	**B**
	5	5	6	4
(2) A versus C*	**A**	**C**	**A**	**C**
	8	2	6	4
(3) A versus D*	**A**	**D**	**A**	**D**
	6	4	8	2
(4) B versus C*	**B**	**C**	**B**	**C**
	3	7	4	6
(5) B versus D*	**B**	**D**	**B**	**D**
	7	3	6	4
(6) C versus D*	**C**	**D**	**C**	**D**
	7	3	6	4

*Vessel where the crickets were initially released

Conclusion

The EFB based vermicompost of C:N ratio 25, 30 and 35 reached maturation in 40 days with the final pH of 6.3 and final C:N ratio obtained between 16 to 22 that is around the healthy range of C:N 20. The addition of nitrogen (N), phosphorus (P) and potassium (K) contributors are able to enhance the final NPK and HA contents of the vermicomposts. FTIR spectra confirmed the HA presence in vermicomposts by showing the major peaks of the chemical functional groups. Lastly, the insect repellent test revealed that insect exhibited most repulsive behaviour toward 87% GE + 13% HA, followed by 100% HA and 100% GE. This finding strongly suggests that HA has the potential to be utilized as a biopesticide, with superior insect-repelling ability can be achieved when it is coupled with GE. This work could promote circular economy activity on common industrial by-products in Malaysia such as EFB, sawdust, fishmeal, bonemeal and bunch ash that are typically considered as wastes.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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