Study on humic acid derived from EFB based vermicompost as biopesticide

A A M Khiew¹, J H Lew¹, M R Shamsuddin¹²*, Aqsha¹², N I Mustapa³ and M M Narasinha³

¹ Chemical Engineering Department, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia.
² Centre for Biofuel and Biochemical Research (CBBR), Institute for Sustainable Living, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia.
³ Vata VM Synergy Sdn.Bhd. Lot 5164-67, MK, Cegar Galah, Batu 29, Kg Chuar Kati, 33020 Kuala Kangsar, Perak, Malaysia.
*Corresponding author: mrashids@utp.edu.my

Abstract. The increment of global population by 1.1% annually demands higher agricultural yield to ensure sustainable food source which leads to intensive usage of chemical fertilizer and pesticide. However, this practice poses various environmental and health problems which ultimately encourages the development of safer organic fertilizers and bio-pesticides. One potential method is to utilize Humic Acid (HA), a natural plant’s growth promoter, present in organic compost as an environmental-friendly fertilizer as well as insect control agent. This study investigated the effectiveness of HA extracted from vermicompost of Palm Oil Empty Fruit Bunches (EFB) as biopesticide. HA was extracted from the vermicompost via alkaline extraction followed by precipitation in strong acid that resulted in HA mass yield of 2.336%. The insect repellent ability of HA, Garlic Extract (GE) and 87% GE + 13% HA was investigated by spraying the solution onto soil samples in the presence of crickets. The effectiveness of the insect control agents was determined by recording the number of crickets avoiding the infected area. The test revealed that the insects exhibited most repulsive behavior towards 87% GE + 13% HA, followed by HA and lastly GE. The results indicate that HA has the potential to be used as biopesticide, with higher prospect when combined with GE. The dual benefits of HA utilization as plant growth promoter and insect repellent promise a more sustainable approach towards modern agriculture.

1. Introduction
Rapid growth of global population by 1.1% annually leads to higher demand on food production thus exerting pressure on the intensive use of pesticides and fertilizers in agricultures industry. The term pesticide covers a wide range of compounds including insecticides, herbicides, nematicide, rodenticides, fungicides as well as plant growth regulator [1]. Generally, pesticide is applied onto agricultural crops to provide crop protection against any pests or harmful diseases and this would result in pest-free agricultural crops which promises improved crop productivity. For instance, food grain production experienced tremendous improvement from 50 million tons in 1948/1949 to 198 million tons by the end of 1996/1997, which is partially contributed by the ability of agricultural pesticides in reducing losses from weeds, diseases and insect pests [1].

However, the continuous and excessive application of modern synthetic pesticide has brought upon negative effect to the environment. Excessive pesticide usage poses the tendency of leaching into
surface water through treated plants and soil runoff and subsequently cause widespread of surface and ground water contamination. Besides, overdosage of chemical fertiliser and pesticides reduce the quantity of beneficial soil organisms that helps to retain the soil nutrients, thus deteriorating the soil fertility. Apart from affecting the environment, squandering pesticide will also cause severe health implication on humans where trace pesticide residing in food commodities might cause gradual poisoning in human body. Therefore, these problems encourage the development in environmental friendly natural pesticide or pest control agent derived from plant or natural resources.

Humic Acid (HA), a natural plant’s growth promoter present in organic compost has the potential to be used as biopesticide due to the presence of phenol and carboxyl functional group in HA. The uptake of carboxyl and phenolic compounds by the plant will make the plant tissue unpleasant to consume, hence hindering pest attack on plant and subsequently affecting pest reproduction rates and survival [2]. Therefore, this study is significant in studying the pesticidal ability of HA derived from Empty Fruit Bunch (EFB) based vermicompost blended with various organic additives. Overabundance and the drawbacks of current disposal practices of EFB (landfill, incineration) open an opportunity of EFB utilisation as feedstock in composting and hence, EFB is chosen as the base compost in this study. The research work of this project is part of collaboration between Universiti Teknologi PETRONAS with one of the agriculture eco-product manufacturing company, VATA VM Synergy (M) Sdn. Bhd. The work involved the vermicomposting of EFB with various organic wastes, extraction of HA and insect repellent test. The dual benefits of HA utilization as plant growth promoter and biopesticide promises a more sustainable approach toward modern agriculture.

2. Material and Methods

2.1. Material
Empty Fruit Bunches (EFB), Palm Oil Mill Effluent (POME) and Bunch Ash were provided by VATA VM Synergy Sdn. Bhd., Saw Dust was supplied in-kind by Wan Sang Sawmill Enterprise S/B, Fishmeal was obtained from Dinding Poultry Manjung and Bonemeal was purchased from Promise Earth (M) Sdn. Bhd. 0.1 M sodium hydroxide, NaOH, 6 M hydrochloric acid, HCl and other lab materials and consumables were provided by Universiti Teknologi PETRONAS research lab.

2.2. Methods

2.2.1. Determination of Moisture Content. Initial moisture content of individual materials was analysed using HX-240 Moisture Analyser by Mettler Toledo. The overall moisture content of C:N 25, C:N 30 and C:N 35 vermicompost was determined by applying Equation (1).

\[
MC_{\text{avg}} = \frac{\sum m_i(MC_i)}{m_T}
\]

where \(m_i\) is the individual weight of the sample, \(MC_i\) is the individual moisture content and \(m_T\) is the total weight of the compost.

2.2.2. Vermicompost Preparation. 3 kg EFB vermicomposts with C:N ratio of 25, C:N 30 and C:N 35 were prepared by mixing the raw materials in different proportion as shown in table 2 based on theoretical C and N values tabulated in table 1. The theoretical C and N value of raw materials were mainly obtained from literatures except for Bonemeal, where it was provided by manufacturer, and Saw Dust which was obtained from the result of previous research in UTP. EFB and POME was mixed in 1:1 ratio before other organic additives were added as 1:1 mass ratio is reported to be the best initial mass ratio [3]. Furthermore, the practice of mixing EFB and POME is also conducted by the collaborated company. Note that 3 kg soil was used as compost bedding and 50 earthworms, *Eisenia Fetida* were added in each batch of vermicompost.
The composition of raw materials of each batch shown in Table 2 was designed based on Equation (2).

\[ C : N_{\text{avg}} = \frac{\sum Q_i (C_i \times (100-M_i))}{\sum Q_i (N_i \times (100-M_i))} \]  

(2)

whereby C:N_{avg} represents the average mixture C:N ratio, Q represents mass of raw material, C, represents carbon percentage of raw material, N, represents nitrogen percentage of material and M, is the moisture content of material.

2.2.3. pH and Temperature Determination. Throughout the vermicomposting activity, the pH and temperature of vermicompost was determined in triplicate by using HANNA Instruments Waterproof Tester in every three to four days. The readings were collected between 10.00 A.M. to 12.00 P.M. The average values were used to plot pH and temperature profile to determine compost’s maturation point. Noted that the vermicomposts were also constantly moisturised with POME and shovelled for aeration after the readings were recorded.

2.2.4. Humic Acid Extraction. After the vermicompost reached maturation (Day 40 as per discussed in section 3.1), Humic Acid (HA) was extracted from the vermicomposts via alkaline extraction followed by precipitation in strong acid [6]. 200g of dried vermicompost was weighed and 0.1 M NaOH was added until final volume was made to 1 L in a beaker. The mixture was stirred for 6 hours to ensure complete dissolution of HA and Fulvic Acid (FA). Then, the mixture was centrifuged at 4000 rpm for 10 minutes and the resultant insoluble materials was discarded while the alkaline supernatant was collected. The alkaline supernatant was proceeded with precipitation in strong acid by adding 6 M HCl to flocculate the HA until it achieved the pH of 1. The mixture was stirred for an hour with constant pH monitoring before it is centrifuged for 10 minutes (4000 rpm) to separate HA precipitate. HA slurry was collected and washed with distilled water and neutralised to pH 7 by adding NaOH and HCl. Finally, HA was dried in an oven at 105°C overnight. HA yield from each batch of compost was calculated by using Equation (3).

\[ HA \text{ yield} = \frac{m_{HA}}{200g} \times 100\% \]  

(3)

where \( m_{HA} \) is the mass of dried Humic Acid extracted.

HA extracted from this procedure was analysed analytically through Fourier-Transform Infrared Spectroscopy (FTIR) to verify the identity of HA extracted.
2.2.5. Insect Repellent Test. Three different insect repellent agents which are 100% HA, 100% GE and 87% GE + 13% HA were prepared for the purpose of insect repellent test. Four different vessels layered with 5cm soil with different insect repellent agent sprayed were also prepared and named as A: Void, B: 100% HA, C: 100% GE and D: 87% HA + 13% GE. The insect repellent agent was sprayed evenly six times (approximately 20 mL) on the soil bedding. First, two of the vessels were combined and 10 crickets were released at one side of the vessel as illustrated in figure 1.

![Figure 1. Insect repellent test set-up](image)

After 30 minutes, the number of crickets at each side of the vessel was counted and recorded. The test was repeated with different test combination as tabulated in Table 3. The whole test (Test 1 to Test 6) was repeated twice to obtain more accurate result.

| Test | Combination of vessel |
|------|-----------------------|
| 1    | A vs B*               |
| 2    | A vs C*               |
| 3    | A vs D*               |
| 4    | B vs C*               |
| 5    | B vs D*               |
| 6    | C vs D*               |

*: Vessel where the crickets were initially released.

3. Result and Discussion

3.1. pH and Temperature Profiling

Figure 2 shows the pH and temperature profile of C:N 30 vermicompost from Day 1 to Day 52. pH and temperature profile of C:N 30 vermicompost is selected to represent the overall maturation point of vermicompost as C:N ratio of 30 was generally reported as the optimum carbon to nitrogen ratio for a compost pile.

Theoretically, there are four temperature stages of composting process which include mesophilic phase (44 – 52 °C), thermophilic phase (> 70 °C), cooling phase and curing phase [7]. From Figure 2, it is observed that the temperature fluctuates slightly between 25 °C to 35 °C which could be due to the weather as the vermicomposts were placed outdoor. Since there is no clear indication in the change of composting temperature stages, temperature profile was not a reliable indicator of vermicompost’s maturation point [8]. Nevertheless, C:N 30 vermicompost still shows the general trend of composting stages where there is an increased in temperature on Day 13 to Day 24 and then gradually decrease toward the end of composting process. The temperature rises after Day 13 implied rapid microbial activities of organic matter which generated heat. After Day 24, the temperature started to decrease showing lower microbial activities.
On the other hand, the fluctuations in pH is contributed by bacterial activity within the compost pile. Initially, the pH value of the vermicompost increased due to the increased in alkaline ammonium ion in the form of ammonia generated by biochemical reaction of nitrogen-containing materials [3]. However, after Day 10, there is a drop in pH which is contributed by the active degradation of organic matter that resulted in the production of organic and inorganic acid [9]. The pH was then increased again, promoting the regeneration of bacteria and the cycle repeats until Day 40. From Day 40 onwards, the pH of the vermicompost started to stabilize, signifying the maturation point of the vermicompost is at Day 40 with the pH value of 6.3. It is also observed that the lowest pH recorded was 5.5 while the highest pH was 7.0 where optimum pH was reported to be between 5.5 and 9.0 [9]. The optimum pH is vital for soil microorganism and earthworms to survive. Figure 3 shows the physical appearance of matured vermicompost on Day 40.

As shown in Figure 3, all three batches of vermicomposts have fine and porous structure. The earthworms consumed the large organic matter in the compost, therefore breaking them down into smaller structure. Besides, active movement of these earthworms create pathway in the compost, making it porous. The vermicomposts also do not released unpleasant smell throughout the composting process and the earthworms added inside the compost on Day 1 were found to be still alive, thus indicating a balanced and healthy decomposition process.

3.2. Humic Acid Yield
Humic Acid (HA) yield from C:N 25, C:N 30 and C:N 35 vermicompost was calculated using equation (3) and the results was shown in Figure 4.
From Figure 4, it is shown that C:N 25 yield the most HA of 2.63% follow by C:N 35, 2.55% and lastly C:N 30, 2.34%. Nevertheless, the mass yield of (HA) in all three batches were almost the same, signifying different proportion of raw materials would not significantly affect the HA mass yield. HA is the by-product of microbes (microorganism) during the decomposition of organic matter. As reported, the species of microorganisms which lives within the alimentary canal of earthworms are commonly identical to those in the soils where the worms live [10]. Therefore, since the type and quantity of earthworms added into each batch of vermicompost were same, the HA yield from each compost pile would be almost similar.

3.3. Fourier Transform Infrared (FTIR) analysis

The FTIR spectra of HA extracted from C:N 25, C:N 30 and C:N 35 (Figure 5) were obtained to consolidate the identity of HA extracted before it is proceeded for insect repellant test. Besides, FTIR spectra allows better analysis on the structural group of HA obtained and any structural differences among the HA yield from different batches of vermicompost. As shown in Figure 5, FTIR spectra of HA from vermicomposts were largely similar with only difference in their relative intensity. C:N 25 exhibit lower intensity of absorption band while C:N 30 and C:N 35 showed almost similar intensity of absorption band. The lower intensity of absorption band of HA observed from C:N 25 might be due to lower concentration of HA in the FTIR sample. Generally, HA contains many functional chemical groups that aids in modifying and improving the soil’s chemical properties as well as stimulate plant’s growth [11]. The significant functional groups are aromatic backbone, carboxyl (R-COOH), hydroxyl (R-OH), ketone (R-C=O-R’), amines (R-NH$_2$), quinone and catechol.

The major FTIR absorption bands and assignment for Humic Acid extracted from vermicompost are tabulated in Table 4 with reference to [12] and [13]. Hence, from Table 4, it is concluded that the black solid extracted from all three batches of vermicompost was HA as the FTIR spectra revealed they contain the expected major functional group in HA structure.
Figure 5. FTIR spectra of HA extracted from C:N 25, C:N 30 and C:N 35 vermicompost

Table 4. Major FTIR absorption bands and assignments for Humic Acid extracted [12, 13]

| Wavelength (cm⁻¹) | Assignment                                                                 | Possible source                          |
|-------------------|---------------------------------------------------------------------------|------------------------------------------|
| 3400 – 3300       | O-H stretching, N-H stretching (minor), hydrogen bonded OH                | Alcohol, phenol, carboxyl (COOH), and N-H (amide) groups |
| 2900 – 2930       | asymmetric and symmetric C-H stretching of CH₂ group                      | C-H asymmetric, C-H stretch of -CH aliphatic |
| 1610 – 1590       | 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) |
| 1430 – 1420       | O–H deformation and C–O stretching of phenolic OH                        | Phenol                                   |
| 1390 – 1370       | C–H bending of CH₂ and CH₃ groups, COO– anti-symmetric stretching         | Ammonium carbonate formed by reaction of ammonia and CO₂ |
| 1220 – 1210       | C–O stretching of aryl ethers and phenols                                | Aryl ethers and phenols                  |
| 1110 – 1090       | C–O stretching of secondary alcohols and/or others                       | Secondary alcohols and/or others         |
| 1040 – 1025       | C-O stretch of polysaccharides, Si-O asymmetric stretch of silicate impurities | Polysaccharides, silicate impurities     |
| 960 – 910         | Aromatic rings and halogens (chloro-compounds)                           | Aromatic rings and halogens (chloro-compounds) |
| 800 – 790         | Aromatic rings and halogens (chloro-compounds) and Si-O asymmetric stretch | Aromatic rings, halogens, Si-O asymmetric stretch |
| 695 - 675         | Aromatic rings and halogens (chloro-compounds)                           | Aromatic rings, halogens                 |
3.4. Insect Repellent Test

Table 5 summarizes the results recorded from insect repellent test. The number of crickets was recorded after 30 minutes the cricket is added into the vessel sprayed with different pest control agent.

| Test     | Number of Crickets |           |           |
|----------|--------------------|-----------|-----------|
|          |                   | Trial 1   | Trial 2   |
| 1: A vs B* | A                  | 5         | 5         |
|          | B                  | 6         | 4         |
| 2: A vs C* | A                  | 8         | 2         |
|          | C                  | 6         | 4         |
| 3: A vs D* | A                  | 6         | 4         |
|          | D                  | 8         | 2         |
| 4: B vs C* | B                  | 3         | 7         |
|          | C                  | 4         | 6         |
| 5: B vs D* | B                  | 7         | 3         |
|          | D                  | 3         | 6         |
| 6: C vs D* | C                  | 7         | 3         |
|          | D                  | 6         | 4         |

*: Vessel where the crickets were initially released.

Based on Table 5, the results obtained in both trials shows similar trend in term of number of crickets. From test 1, 2 and 3, it is proven that sample B, C and D are able to repel insect as the number of crickets in vessel A is always higher than the opposite vessel. The comparison of HA and GE was presented by Test 4, where HA was proven to be more effective insect repellent agent than GE. This might be due to the presence of phenolic and carboxyl functional group in HA which hindered the cricket to move to vessel B [2]. Nevertheless, the combination of HA and GE in 87:13 ratio is found to be the most effective insect repellent agent where the number of insects stayed in vessel D was less than that in vessel B and C, indicating the crickets dislike the environment in vessel D. GE is known as an active repellent ingredient while HA diluent promotes the absorption of GE thus, gives higher insect repellent effect [14]. Overall, it is concluded that the most effective insect repellent agent is 87% GE + 13% HA, followed by 100% Humic Acid and lastly 100% GE.

4. Conclusion

EFB vermicomposts of C:N ratio 25, 30 and 35 reached maturation in 40 days with pH of 6.3 and yielded 2.3% to 2.6% (in mass) Humic Acid (HA). The FTIR spectra obtained shows HA extracted contained major functional groups such as phenol, carboxyl, ketone, amines, quinone and catechol. As for insect repellent test, it is revealed that the combination of HA and GE in 87:13 ratio showed the most effective pesticidal ability followed by 100% HA and lastly 100% GE. This concluded that HA has the potential to be utilized as biopesticide, while exhibiting more superior insect repelling ability when it is used together with GE. Future work will be focused on methods to maximise HA yield to ensure an economically sustainable usage of HA as biopesticide.

Acknowledgment

This work was conducted under a consultation grant from Vata VM Synergy Sdn. Bhd. (015QB0-014). The author also would like to acknowledge Centralised Analytic Laboratory of UTP for the technical assistance on FTIR analysis and Centre for Biofuel and Biochemical Research (CBBR) of UTP on the assistance in Moisture Analyser.
References

[1] Aktar M W, Sengupta D and Chowdhury A 2009 Impact of pesticides use in agriculture: their benefits and hazards Interdisciplinary Toxicology 2 pp 1-12
[2] Pathma J and Sakthivel K 2012 Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential SpringerPlus 1 pp 26
[3] Nahrul H Z, Astimar A A, Anis M, Ibrahim M H, Khalil H P S A and Ibrahim Z 2012 Vermicomposting of empty fruit bunch with addition of palm oil mill effluent solid Journal of Oil Palm Research 24 pp 1542-49
[4] Barlow P and McCurran A n.d. The Nutrient Value and Effect of Fish Meal as a Fertiliser for Hayward Kiwifruit Grown Under an Organic Regime
[5] Adjei-Nsiah S 2012 Response of Maize (Zea mays L.) to Different Rates of Palm Bunch Ash Application in the Semi-deciduous Forest Agro-ecological Zone of Ghana Applied and Environmental Soil Science 2012
[6] Lamar R T, Olk D C Mayhew L and Bloom P R 2014 A new standardized method for quantification of humic and fulvic acids in humic ores and commercial products J AOAC Int 97 pp 721-30
[7] Jenkins J 1999 The Humanure Handbook, A Guide to Composting Human Manure, ed J Jenkins (Grove City: Jenkins Publishing)
[8] Shamsuddin, R M, Borhan A and Lim W K 2017 Humic acid batteries derived from vermicomposts at different C/N ratios IOP Conf Series: Materials Science and Engineering 206 p 012067, 2017/06 2017
[9] Oluchukwu C A, Nebechukwu, A G and Egbuna S O 2018 Enrichment of nutritional content of sawdust by composting with other nitrogen rich agro wastes for biofertilizer synthesis Journal of Chemical Technology and Metallurgy 53 pp 430-436
[10] Edward C A and Lofty J R 1977 Biology of Earthworms ed C A Edwards and J R Lofty (Boston: Springer US) pp 182
[11] Asing J, Wong N C and Lau S 2009 Optimization of extraction method and characterization of humic acid derived from coals and composts Journal of Tropical Agriculture and Food Science 37 pp 211-223
[12] Enev V, Pospíšilová L, Klucakova M Liptaj T and Doskočil L 2014 Spectral Characterization of Selected Natural Humic Substances Soil & Water Res. 9 pp 9-17
[13] Liu Z, Yang X, Zou A and Luan Y 2014 Analysis of garden waste composting and the effects of humic acid content using near-infrared spectroscopy BioTechnology: An Indian Journal 10 pp 16291-98
[14] Valencia M and Luis J 2010 Botanical repellent composition containing allium sativum and humic acid and intended for pest insect control, method for producing same, and uses thereof French Patent