

## Potential of Different Vermicast Formulations Toward Chemical Composition and Microbial Functional Diversity as Biofertilizer

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Received 27 October 2012 /Accepted 12 November 2012

**Abstract:** Three different formulations of biofertilizer - EM vermicompost, IMO vermicompost and control vermicompost were developed in this study. The macronutrients and microbiological content of each formulation were compared and field trial was conducted. The study showed that EM vermicompost had higher nitrogen, phosphorus and potassium content than IMO and control vermicomposts. Microbiological analysis results also showed that EM vermicompost had more *lactobacillus*, yeast and photosynthetic bacteria than the other two vermicomposts. This is in agreement with the field trial results where the percentage yield of the crops is higher for plants fertilized with EM vermicompost. In this study it can be concluded that EM vermicompost is a better biofertilizer than IMO and Control vermicomposts as demonstrated through its properties and performance.

**Keywords:** Vermicast, Vermicomposting, EM, IMO, Chemical and microbial analysis

### Introduction

The agriculture field has progressed dramatically since the early 1970s due to supports in research and development endeavours bestowed by policies. Nevertheless, the adverse effects of chemical pesticides on both abiotic and biotic components of the environment has been reported as well<sup>1,2</sup>. The former is exemplified by residues in soil, air, water, food and *etc.* The latter can be presented by phytotoxicity, residues, vegetation changes and *etc.* in plants as well as physiological deformities, diseases, mortality, population changes, genetic disorders and so on in mammals, avian, insects and other organisms<sup>3-5</sup>. Entry of pesticides into the food chain coupled with their bioaccumulation and biomagnification can trigger

effects of unforeseen consequences. In addition, fertilizer contamination of ground water has led to eutrophication of lake and river waters, causing depletion of oxygen that further leads to the death of aquatic life. Other related problems include nitrate pollution, increased emissions of gaseous nitrogen and metal toxicities<sup>6,7</sup>. Fortunately, with an increasing awareness about the harmful effects of synthetic plant protection and production agrochemicals, the demand for eco-friendly technologies and products based on biological processes has also been increasing steadily too.

Vermicast, similarly known as worm castings, worm humus or worm manure, is the end-product of the breakdown of organic matter by a species of earthworm during vermicomposting. Vermicast is a concentrated and rich product laden with microbacteria that is excreted by earthworms and compost worms when they digest their food. During the process, earthworms promote microbial activity greatly, which in turn accelerates the breakdown of organic matter and stabilization of soil aggregates<sup>7</sup>. Therefore, it can be said that vermicompost is also a product of an accelerated biooxydation that does not pass through the thermophilic stage<sup>8</sup>. This vermicompost has been used in the agricultural field as organic fertilizer to improve soil fertility or crop productivity<sup>4</sup>.

IMO vermicompost fertilizer is the product of composting that utilizes heterogeneous mixture of decomposing vegetable or food waste and bedding materials (IMO); sand; top soil and vermicast. In EM vermicompost, IMO is replaced with EM-1 Liquid<sup>9</sup> which contains water-soluble nutrients that can act excellently as an organic fertilizer and soil conditioner. The main objectives of the current study were to assess whether different vermicast formulations could affect the microbiological and chemical content of the vermicompost biofertilizers. These formulations were also tested for their effectiveness in promoting the emergence and growth of ladyfingers.

## Experimental

Each vermicompost was prepared with vermicast, topsoil and sand in the ratio of 2:3:1. The vermicast was supplied by Halex Biotechnologies Sdn Bhd. This basic formulation was mixed uniformly and divided into three equal portions. One of the portions was added with 3% (from total weight) of effective microorganism activated solution (EMAS) and then labelled as EM vermicompost. The second portion was added with equal percentage of heterogeneous mixtures of decomposing vegetable or food waste that had been previously prepared as well as bedding materials and was labelled as IMO. The last portion was not inoculated and was preserved as control. The temperature was measured 50 cm below the compost surface with a thermometer and was monitored daily from day 0 to day 7 throughout the fermentation period.

### *Isolation and enumeration of microorganisms*

The total microbial population in the sample was determined as described hereafter. The Dilution Plate technique was used in isolation of *Lactobacillus* sp.<sup>10</sup>, yeast and mould<sup>11</sup> as well as nitrogen fixing bacteria<sup>12</sup>. Each substrate of 10 g was suspended in 90 mL of sterilized saline and shaken thoroughly. After that, 0.1 mL of each inoculum was inoculated into acidified MRS agar (AMRS) to promote *Lactobacillus* growth. Chloramphenicol glucose yeast extract agar (CGYE) was used to promote yeast growth and nitrogen free medium (Ashby's medium) was employed to allow nitrogen fixing bacteria to grow. The samples were incubated at 37±1 °C for 48 hours; 37±1 °C for 5 days and 30±1 °C for 2 to 5 days for *Lactobacillus* sp., yeast and mould and nitrogen fixing bacteria respectively.

Determination of photosynthetic bacteria<sup>13</sup> was carried out by incubating 5 g of the sample in succinate broth for 4 to 7 days at  $30 \pm 1$  °C until the emergence of red pigment (bloom); such bloom is indicative of the presence of photosynthetic microorganism. The positive broth was then inoculated anaerobically in succinate agar again at  $30 \pm 1$  °C for 4 to 7 days. Subsequent test involved the Multiple Tube method<sup>14</sup> to identify nitrifying bacteria in the sample that had been inoculated into Ammonia-oxidizing broth (AOB) and Nitrogen-oxidizing broth (NOB). The broths were kept at 25-30 °C for 23-28 days and 23-28 °C for 23 days or more. The Double Agar Layer method<sup>15</sup> was also conducted to measure the amount of *Actinomycetes*; the incubation condition was set at  $28 \pm 1$  °C for 7 days. The final experimental results were expressed in terms of colony forming unit (CFU) and most probable number (MPN) according to the method used.

#### *Chemical analysis*

The total nitrogen, phosphorus and potassium content of the samples was analysed as described below. Nitrogen was determined using the Macro Kjeldahl method in which the sample was digested with concentrated  $H_2SO_4$  (1:20, w/v) and then distilled<sup>16</sup>. The amount of phosphorus and potassium were analysed from the wet digest (tri-acid of  $HNO_3$ - $H_2SO_4$ - $HClO_4$ ) mixture which had been used for digestion<sup>17</sup>. There spective concentration was determined spectrophotometrically using NOVA Merck (Merck, Germany). The moisture content of samples with constant weight was determined using a moisture analyser (MIX-50, A&D Company, Japan). The corresponding pH level was measure in five times volume of distilled water that had been equilibrated with the sample for an hour with a pH meter (Delta 320, Mettler Toledo, USA). Ash in a dried sample was measured at 550°C (Carbolite CWF 110, England) and lastly, the carbon and nitrogen ratio was analysed using the HACH method.

#### *Field trial monitoring*

The experiment was conducted from Jan-May 2010 using field facilities of the Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, Malaysia. Lady fingers were planted at different plots and fertilized with EM, IMO and Control vermicomposts. The plants were fertilized once a week with 5 g (fresh mass) of vermicompost at 5 cm below soil surface and near to the roots before being watered. When the crops were ready to be harvested; the average plant height; root length; diameter of leaves and fruits and fruit weight were recorded.

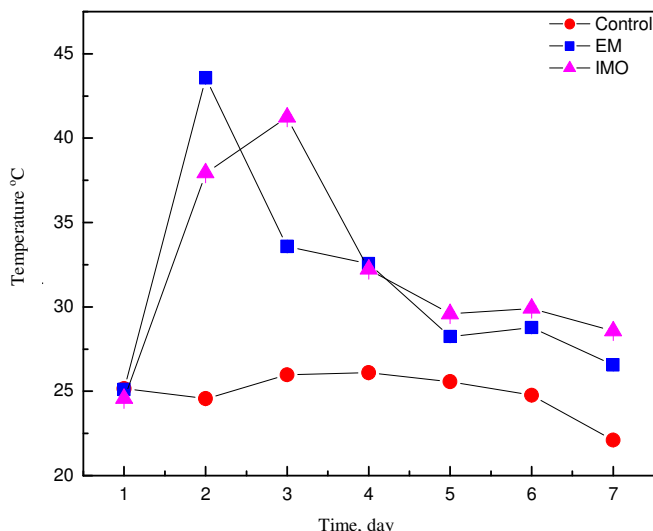
#### *Statistical analysis*

The whole experiment was repeated three times and the data were pooled before being analysed using one-way ANOVA. The factor was set as the different vermicompost formulations and the general linear model approach in SPSS (Version 15.0, SPSS Inc, USA)<sup>18</sup> was used. All ANOVA analyses were performed using Type III sums of squares before the Tukey's least squares means test was performed to allow for multiple comparisons.

## **Results and Discussion**

#### *Fermentation and temperature monitoring*

Figure 1 shows the temperature recorded from the day of production to day 8 of vermicompost fermentation. It has been observed that different formulations would result in different temperature patterns for each vermicompost. The peak temperature achieved during fermentation was recorded at 44 °C (on day 3) for IMO vermicompost, 41 °C (on day 3) for EM vermicompost, and 26 °C for Control vermicompost. The temperature dropped after day 3 to around 28 °C and 27 °C for EM and IMO vermicompost respectively. As for the Control vermicompost, the temperature stabilized around 26 °C from day 3 to day 6.



**Figure 1.** Temperature profile for different vermicompost during fermentation period

#### *Isolation and enumeration of microorganisms*

Table 1 gives an account of the free-living microbial populations assayed from all vermicomposts. The Control vermicompost was significantly different from EM and IMO vermicomposts in terms of *Lactobacillus sp.* population, yeast count and photosynthetic bacteria population. Generally, the amounts of the aforementioned microorganisms were higher in the EM vermicompost than those in the IMO vermicompost, but the data obtained were not significant for both formulations. Microbial colony counting by the method described above revealed that the total *Lactobacillus sp.* count ( $4.72 \pm 0.77 \log_{10} \text{g}^{-1}$ ) as well as yeast count ( $5.02 \pm 0.96 \log_{10} \text{g}^{-1}$ ) were the lowest in the EM vermicompost. The latter was also obviously different from that in control vermicompost, which had been reported at  $3.05 \pm 0.28 \log_{10} \text{g}^{-1}$  at  $p < 0.05$  significance level. The *Lactobacillus sp.* count in IMO formulation was at  $4.30 \pm 0.86 \log_{10} \text{g}^{-1}$  while its yeast count and photosynthetic bacteria counts were at  $4.03 \pm 0.86 \log_{10} \text{g}^{-1}$  and  $1.59 \pm 1.40 \log_{10} \text{g}^{-1}$  respectively. The *Lactobacillus sp.* and yeast count for the control vermicompost were recorded at  $3.40 \pm 0.18 \log_{10} \text{g}^{-1}$  and  $3.99 \pm 0.11 \log_{10} \text{g}^{-1}$  respectively. Absence of ammonia and nitrate oxidizing bacteria, nitrogen fixing bacteria and *Actinomyces* bacteria had also been detected in all biofertilizers tested.

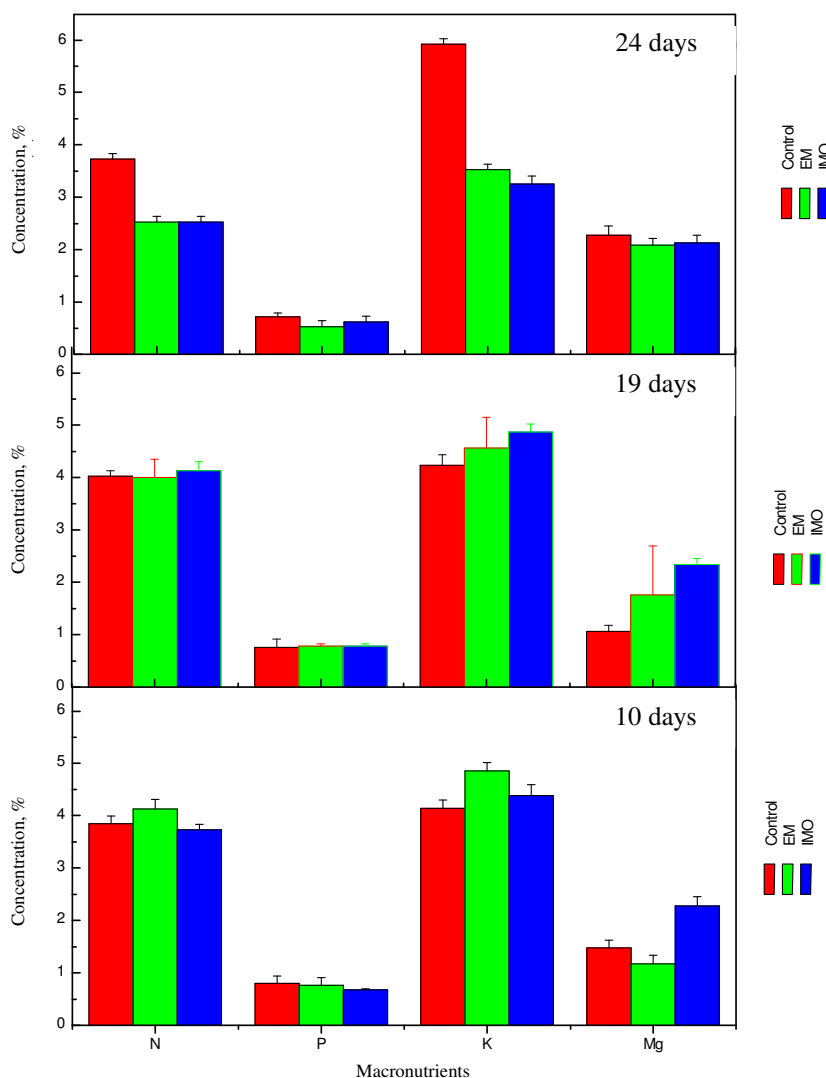
**Table 1.** The populations of microorganisms ( $\log_{10} \text{g}^{-1}$ ) examined in control, EM and IMO vermicompost

Microorganism count	Control	EM	IMO
<i>Lactobacillus sp.</i>	$3.40 \pm 0.18^b$	$4.72 \pm 0.77^a$	$4.30 \pm 0.86^a$
Yeast	$3.99 \pm 0.11^b$	$5.02 \pm 0.96^a$	$4.03 \pm 0.35^a$
Photosynthetic bacteria	NG	$3.05 \pm 0.28^a$	$1.59 \pm 1.40^b$
Ammonia oxidizing bacteria	NG	NG	NG
Nitrate oxidizing bacteria	NG	NG	NG
Nitrogen-fixing bacteria	NG	NG	NG
<i>Actinomyces</i>	NG	NG	NG

'a' and 'b' are significant difference within the group  $P < 0.05$  (Tukey LSD test), NG= No growth

### Chemical analysis

The nutrient status of the vermicomposts as presented in Figure 2. Among all the macronutrients, the availability of potassium ( $4.81\pm1.05\%$ ) was highest in the control vermicompost while EM vermicompost showed higher concentrations of available nitrogen ( $4.25\pm0.35\%$ ) and phosphorus ( $0.81\pm0.18\%$ ). The next highest concentration was recorded by magnesium ( $2.34\pm0.10\%$ ) in the EM vermicompost.



**Figure 2.** Macronutrients of EM, IMO and control vermicompost

### Ladyfingers biomass production

The effect of bacteria inoculation of EM and IMO vermicomposts is as shown in Table 2. Both EM and IMO vermicomposts performed significantly better than the Control vermicompost. Ladyfingers fertilized with EM and IMO vermicomposts had taller plant

height, longer root length, bigger leaves and fruits diameter. Even the weight of fresh fruits was heavier. The physical biomass monitoring of EM vermicompost was identical to those fertilized with IMO vermicompost.

**Table 2.** Biomass production during field trial for ladyfingers fertilized with control, EM and IMO vermicompost

Biomass production (mean)	EM vermicompost	IMO vermicompost	Control
Plant height (cm plant <sup>-1</sup> )	189.9±1.89 <sup>b</sup>	151.8±7.45 <sup>b</sup>	82.0±3.54 <sup>a</sup>
Root length (cm plant <sup>-1</sup> )	29.7±1.30 <sup>b</sup>	26.0±1.04 <sup>b</sup>	14.8±2.74 <sup>a</sup>
Leaves diameter (cm plant <sup>-1</sup> )	35.5±1.47 <sup>b</sup>	33.7±0.92 <sup>b</sup>	16.7±0.97 <sup>a</sup>
Fruits diameter (cm plant <sup>-1</sup> )	3.0±0.06 <sup>b</sup>	2.9±0.00 <sup>b</sup>	1.8±0.06 <sup>a</sup>
Fruits weigh (g plant <sup>-1</sup> )	25.0±0.31 <sup>b</sup>	24.8±0.56 <sup>b</sup>	11.7±0.31 <sup>a</sup>

'a' and 'b' are significant difference within the group  $P < 0.05$  (Tukey LSD test), NG= No growth

To determine the quality of all vermicomposts, all related parameters have to be considered, particularly those related to the microbiological, chemical and physical characteristics of the vermicomposts. These characteristics can also be used for classification purposes. In other studies, the admissibility criteria that had been studied include the heavy metals<sup>7</sup> and pathogenic content<sup>1</sup>. Conventionally, the vermicomposting process takes about 70-85 days to complete<sup>8</sup>. However, with the inoculation of microbes, the completion period can be reduced to 7-8 days only. This has been supported by Pramaniket *al.*<sup>19</sup> who stated that a pre-composting period of seven days is enough to improve the enzymatic and microbial activities in the succeeding vermicomposting process. Furthermore, compared to conventional compost, such vermicompost is also better since it has a finer structure and larger surface. This means that it is capable of providing stronger absorptivity and retention of nutrients<sup>4</sup>. It has also been reckoned that by inoculating some microorganisms, the process can be expedited<sup>20,21</sup>. Observation on vermicomposts obtained through inoculation with EM showed the highest abundance of *Lactobacillus sp.*, yeast and photosynthetic bacteria count, but the values are actually statistically on par with those of IMO vermicompost.

Kale *et al.*<sup>22</sup> reported that the presence of total bacteria, yeast and actinomycetes recorded at  $6.72 \log_{10} \text{g}^{-1}$ ,  $3.39 \log_{10} \text{g}^{-1}$  and  $3.54 \log_{10} \text{g}^{-1}$  respectively contradicted with our study. This is because our results indicated that increase in the density of microbes (*Lactobacillus sp.*, yeast and photosynthetic bacteria) after the inoculation of EM has resulted in the sustenance of these populations in the vermicompost throughout the fermentation process. In addition, the temperature achieved ( $>45^\circ\text{C}$ ) in the EM and IMO vermicomposts would directly affect the survival of pathogenic bacteria in the vermicompost<sup>1</sup>.

A study by Jourbet and Fair<sup>25</sup> has indicated that vermicast itself contains about 1.8-2.05%; 1.32-1.93%; 1.28-1.50% and 0.4-0.7% of nitrogen, phosphorus, potassium and magnesium respectively. However, results of Control vermicast obtained in this study showed a doubled percentage of macronutrients and the amount of nitrogen exhibited by the EM vermicompost was considerably higher than that of IMO and control. It seems that EM has a more profound influence on the rate of vermicomposition and amount of macronutrients in the final vermicompost. This might be due to the nitrogenous metabolic process of the microbes which successively returned the final products to the soil through casts, urine, muco-protein and earthworms tissue<sup>22,19</sup>. Moreover, acid produced during the organic matter decomposition by microorganisms is also a major mechanism that allows

insoluble phosphorus and potassium to become soluble<sup>9</sup>. This plays an important role in increasing the content of phosphorus and potassium in vermicompost<sup>25</sup>. This is also in agreement with the study reported by Khwairakpam and Bhargava<sup>26</sup> which indicated that higher content of potassium in vermicompost can enhance the degradation of ingested substrate and release of easily assailable metabolites. Higher phosphorus content can also be found in *B. Polymyxa* treated vermicompost and this can be explained by the higher adsorption rate of  $\text{NO}_3^-$  anions that had replaced the  $\text{PO}_4^-$  ions from humic colloids<sup>27</sup>. Thus, more  $\text{PO}_4^-$  ions were released into the systems. In addition, nutrients in vermicompost can be instantly up-taken by plants and contains substances that can stimulate and regulate plant growth<sup>28,29</sup>. A study by Tripathi and Bhardwaj<sup>30</sup> has stated that the process of mineralization and mobilization of phosphorus by bacterial and faecal phosphatase activity of earthworms could be the main reason of phosphorus increase in vermicompost. Vermicompost is usually more stable than their parent materials with increased availability of nutrients and improved physicochemical and microbiological properties<sup>7,30</sup>.

Various green-house and field studies have examined a variety of vermicomposts on a wide range of crops including cereals and legumes<sup>22</sup>. Generally, the results of this study showed that the usage of EM and IMO vermicomposts differed greatly in their biomass production of ladyfingers than those that had not been fertilized. The enhancement observed in biomass might be due to increased macronutrient (nitrogen, phosphorus, potassium and magnesium) uptake of the lady fingers. This is supported by previous studies that have used chickpea seedlings<sup>23,24</sup>. Results also showed that the plant height, root length, leaves, fruits diameter and fruit weight were significantly different for ladyfingers fertilized with EM and IMO vermicomposts from those without vermicompost (Control vermicompost). In addition, even though there were no significant difference between those fertilized with EM and IMO vermicomposts, plants treated with EM vermicompost grew better than IMO treated plant. These remarkable results indicated that EM has great potential as a plant growth media<sup>32</sup>. The enhancement in plant growth has also been attributed to various mechanisms, such as modification in soil structure, changes in water availability, addition or increased availability of macro and micronutrients, stimulation of microbial activity, augmentation of the critical enzymes activities and others<sup>32,33</sup>.

## Conclusions

This work concerns the production of vermicompost from three different formulations and the vermicomposts were assessed microbiological and chemically through the biomass of ladyfingers produced. Out of the three formulations, EM vermicompost was the better biofertilizer, presumably because of its superior biological and macronutrients content. It also demonstrated that vermicomposting with EM is an alternative technology for the management of biodegradable organic wastes.

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