Malaysian Journal of Microbiology, Vol 9(1) 2013, pp. 60-67

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Agriculture wastes conversion for biofertilizer production using beneficial microorganisms for sustainable agriculture applications

Siti Zulaiha Hanapi[1], Hassan M. Awad[1,2*], Sheikh Imranudin Sheikh Ali[1], Siti Hajar Mat Sarip[1], Mohamad Roji Sarmidi[1], Ramlan Aziz[1]

[1]Institute of Bioproduct Development, Universiti Teknologi Malaysia (UTM), 81310, UTM, Johor, Malaysia.
[2]Chemistry of Natural and Microbial Products Department, National Research Centre (NRC), Dokki, Cairo, Egypt.
E-mail: hassan@ibd.utm.my

Received 27 April 2012; Received revised form 15 August 2012; Acceptance 23 August 2012

ABSTRACT

Aims: The emphasis of this study is to generate new valuable bioproducts from non-toxic cleaning waste for environmental healing technology.
Methodology and Results: Comparisons between different types of biofertilizer formulations and the field trial effectiveness were done. Results indicated that biofertilizer C contained the highest N value (1.8%) when compared with biofertilizers B and A, which only contained 1.7% and 1.4%, respectively. Biofertilizer A showed significant difference in the total count of yeast, mould, ammonia oxidizing bacteria and nitrate oxidizing bacteria compared to biofertilizer B and C. Meanwhile, biofertilizer C was found to be significantly different from others in *Lactobacillus* sp. and nitrogen-fixing bacteria count. Photosynthetic total count and *Actinomycetes* sp. were not noticed in all formulations tested.
Conclusion, significance and impact of study: The findings of this study suggest that biofertilizer A is suitable to be used as a promotional biofertilizer in flower and fruit production, biofertilizer B can be used for a leafy crop, while biofertilizer C is good for the growth of roots and stem of plants.

Key words: Biofertilizer, Beneficial microorganisms, Microbiological analysis, Chemical analysis, Field trials.

INTRODUCTION

Currently, there are more than 4.3 million hectares of oil palm plantation in Malaysia, which is equivalent to approximately 67 percent of total agricultural land in the country (DOA, 2010). Malaysia had generated in excess of 15,000 tons of solid waste per day in the form of biomass that consists of forest and mill residues, wood wastes, agricultural wastes, and municipal waste. Agricultural wastes from agro-based industries are also on the increase. State of Johore, Selangor, and Perak collectively accounted for 65.7% of the overall identified pollution sources in the agro-based and manufacturing sector (DOE, 2001).

These biomasses bear a huge potential to be applied as an alternative and beneficial invention for various applications such as in sustainable agriculture and etc. because they are high in moisture, organic matter and other minerals. Thus, they can actually be reproduced into more useful and value-added products with safety and profitability. Recently, many countries have made an effort to recycle 15 – 50% of the wastes they generated (Diza *et al.*, 1993). Weeds, stalks, stems, fallen leaves, pruning, and dead branches (Boraste *et al.*, 2009); animal manure (Bheki *et al.*, 2010); vermicompost (Warman and AngLopez, 2010); and agriculture wastes such as cornstalks, sugarcane bagasse, drops and culls from fruits and vegetables (Weber *et al.*, 2007) have long been used as the soil conditioner to fertilize the soil and plant with the cooperation of beneficial microbes.

Biofertilizers are environmental friendly fertilizers that not only prevent damages to natural sources but help, to some extent, in cleaning the nature from precipitated chemical fertilizers (Food and Agricultural Organization, 2008). The use of organic matter such as sawdust, rice bran, rice husk and shredded paper in producing biofertilizer is economical. They also act as the carrier material for nutrient and microorganisms.

The role of plant nutrients in crop production is well-established and 16 essential plant nutrients have to be available to the crops in required quantities to achieve the yield target. Many studies have also emphasized on the importance of N, P and K in enhancing the natural ability of plants to resist stress from drought and cold, pests and diseases (Debosz *et al.*, 2002; Tsai *et al.*, 2007). Essential plant nutrients such as N, P, K, Ca, Mg and S are called macronutrients, while Fe, Zn, Cu, Mo, Mn, B and Cl are called micronutrients. It is necessary to assess the capacity of a soil to supply the lacking amounts of needed plant nutrients (total crop requirement-soil supply) (Food

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

and Agricultural Organization 2008). This is also important to produce a good biofertilizer formulation and to supply nutrients that can improve soil health and fertility of plants.

Several authors such as Debosz *et al*. (2002); and Chen *et al.* (2007) are concentrating on the potential usage of nitrogen from animal manures. Nonetheless, the effort to find another source instead of animal manure needs further study. Granite powder has also been studied as a good source of slow-release K fertilizer (Chen *et al.*, 2007). Generally, the addition of nitrogen to high C: N ratio residues is capable of accelerating the microorganism activity during the fermentation process (Saratchandran *et al.*, 2001).

The number of microorganisms and the level of macro- and micronutrient obviously affect the growth of plants (Coroneos *et al.*, 1995). One of the benefits of fertilizers is that they contribute to the availability of microorganism population (Marrs, 1993). Having a higher initial count of appropriate microbes in ready biofertilizer right after the fermentation is essential. One of the ways to increase the number of selected microorganisms is by using the concept of an effective microorganism (EM) as introduced by Higa and Wididana (1991).

Field experiments are needed to determine the nutrient availability and efficacy of most organic fertilizers. Such an experiment is important because the nutrient content of organic fertilizers varies widely (Parr *et al.*, 1998). The quality is directly governed by the number of selected microorganisms in the active form per gram and their capability to promote plant growth and soil fertility.

The aim of the work is to investigate the conversation and different formulation of the agriculture wastes for biofertilizer production by beneficial microorganisms.

MATERIALS AND METHODS

Raw materials

The raw materials used for the bioorganic fertilizer production were obtained from a local manufacturer in Kulai, Johor. The waste was in the form of granules with chemical characteristics as shown in Table 1.

Table 1: Biofertilizer type combination

Ingredients (%)	Type of biofertilizers		
	A	B	C
1- Burned soil	41	46	39
2-Nitrogen source meal	7	10	7
3- Saw dust	15	30	30
4- Burned rice husk.	15	-	-
5- EM	3%	3%	3%
6-Gibberelic Acid	10 ppm	10 ppm	10 ppm

(-) = without addition of burn rice husk, EM= Effective microorganisms

Biofertilizer preparation

Generally, the ingredients of each biofertilizers differed in or without following the addition of burned soil, nitrogen source meal, saw dust and burned rice husk. The formulation of these biofertilizers types is shown in Table 2. Each of biofertilizers was inoculated with 3% of effective microorganisms (EM) before the fermentation proceeds.

Table 2: Microorganisms and specific media used for isolation and identification.

No.	Microorganism	Specific medium	Reference
1	*Lactobacillus* sp.	Acidified MRS	(Institute,2009)
2	Yeast and Mold	CGYE	(Leuschner *et al.*, 2003)
3	N_2 fixing bacteria	Ashby's medium	(Ashby, 1907, Harunor *et al.*, 2008)
4	Photosynthetc bacteria	Mineral salts-Succinate Broth	(Prasertsan *et al.*, 1993)
5	Nitrifying bacteria	AOB, NOB	(Bhuiya and Walker, 1977)
6	*Actinomycetes*	Actinomycetes isolation agar	(Awad *et al.*, 2009, Shirling and Gottlieb, 1966)

CGYE = Glucose Yeast Extract Agar, AOB: Ammonia-oxidizing broth and Nitrogen-oxidizing broth (NOB)

Fermentation and temperature monitoring

The starting fermentation was performed for biofertilizer A, B and C in different proportions of ingredients, which differed in or without the addition of burned soil, nitrogen source meal, saw dust and burned rice husk. The formulations of these biofertilizer types are shown in Table 1. All biofertilizers were inoculated with 3% of effective microorganisms (EM) before the fermentation proceeds. The substrate temperature was measured daily from Day 1 until Day 7 at a depth of 50 cm with a thermometer.

Isolation and enumeration of microorganisms

The total microbial population of the sample was determined using the following methods and the specific media for each strain were according to the literatures as shown in Table 2. The media composition and the preparation methods which were used in this study are also listed in Table 2.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Isolation and enumeration of *Lactobacillus* sp. by dilution plate technique

Man Rogosa Sharpe (MRS) medium was used to encourage the growth of lactic acid bacteria such as *Lactobacilli, Enterococci* and *Pediococci*. Selection of *Lactobacilli* was carried out using the pH selection method (pH 5.5 to 6.2) with *Enterocci* and *Pediococci* growing best in this range. For this purpose, acidified MRS agar medium (Merk, Darmastdt, Germany) was used (Institute, 2009).

Standard method for determining number of yeasts and molds

A pour plate method following (International Standards Organization) ISO 7954 (ISO, 1987) using chloramphenicol glucose yeast extract (CGYE) was used. The CGYE agar medium contained (g/L): dextrose, 20; yeast extract, 5.0; chloramphenicol, 0.1; and agar, 15.0. The medium was adjusted to pH 6.6. ± 0.2 prior autoclaving. Each substrate of 10 g was suspended in 90 ml sterile saline, shaken thoroughly and 0.1 mL of each inoculum was inoculated with AMRS and incubated at 25 °C ± 1 °C for 48 hours. At the same time, the CGYE medium was incubated at 25 °C ± 1 °C for five days (Leuschner *et al.*, 2003).

Determination of nitrogen-fixing bacteria by the spread plate method

This method is based on the ability of nitrogen-fixing bacteria to grow in a nitrogen-free medium. The total N_2-Fixing bacteria were counted using Ashby's medium after an incubation period (Ashby, 1907). Ashby's medium composed of (g/L): mannitol, 20; K_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.2; NaCl, 0.2; K_2SO_3, 0.1; $CaCO_3$, 5.0; and agar, 15.0. One gram of sample was transferred into 50 ml Ashby's medium and incubated at 30 °C ± 1 °C for 2-5 days. Then, the broth surface was examined and using serial dilution, it was streaked to nitrogen-free medium agar for enumeration. The colonies that grew on the medium appeared as white, off white, gray and gray to white. They were circular, flat, raised, serrate in elevation, and small and pinpoint in size (Ashby, 1907; Harunor *et al.*, 2008).

Isolation and enumeration of photosynthetic bacteria

The current method is based on the ability of photosynthetic bacteria to assimilate CO_2 and use light as their energy source during incubation under bright and dark conditions. Determination of photosynthetic bacteria was carried out by incubating 5 g of sample in succinate broth. It consisted of three media as follows: Mineral Salts-Succinate Broth medium (1) made up with (g/L): K_2HPO_4, 0.33; $MgSO_4.7H_2O$, 0.33; NaCl, 0.33; NH_4Cl, 0.50; $CaCl_2.2H_2O$, 0.05; sodium succinate, 1.0; yeast extract, 0.02; and agar, 15 g, at pH 6.8-7.2 using 5M NaOH.

Trace element's medium (2) with (mg/L): $ZnSO_4.7H_2O$ 10; $MnCl_2.7H_2O$, 3; H_3BO_3, 30; $CoCl_2.6H_2O$, 20; $CuCl_2.2H_2O$, 1; $NiCl_2.6H_2O$, 2; and Na_2MoO_4, 3 mg, the solution was adjusted to pH 3-4 using 5M HCl. Medium (3) composed of 0.02% $FeSO_4.7H_2O$. The isolates were incubated for four to seven days at 30 °C ± 1 until the appearance of red pigment (bloom) which indicated the presence of photosynthetic microorganisms. Positive control (*Rhodopseudomonas palustris* NRRL B-4267) was incubated under the same conditions (Prasertsan *et al.*, 1993).

Isolation and detecting of nitrifying bacteria (Multiple Five Tube method)

Multiple Five Tube method (Bhuiya and Walker, 1977) was used in detecting nitrifying bacteria using ammonia-oxidizing broth (AOB) and nitrogen-oxidizing broth (NOB). These media were composed of the following constituents: the AOB-medium (g/L): $MgSO_4.7H_2O$, 0.04; $(NH_4)_2SO_4$, 0.50; KH_2PO_4, 0.20; $CaCl_2.2H_2O$, 0.04; and phenol red, 0.001 and the NOB medium (g/L): KNO_3, 0.30; $MgSO_4.7H_2O$, 0.1875; $KHCO_3$, 1.5; K_2HPO_4, 0.5; KH_2PO_4, 0.5; NaCl, 0.1875; $CaCl_2.2H_2O$, 0.0125; and $FeSO_4.7H_2O$, 0.01. Each set of AOB and NOB tubes was inoculated with 1 ml of sample suspended and incubated at 25-30 °C for 23-28 days in case of (AOB) and for 23 days or more for (NOB).

After the end of incubation, one drop of sulfanilic acid and N, N-dimethyl-1-naphthylamine were added into AOB and NOB media in tubes. Red color indicates the presence of active AOB while an absence of any color changes is a positive result for NOB. A confirmation test for nitrite/nitrate was carried out by added one drop of diphenylamine to a drop of sample on a clean spot plate. Positive tubes or wells were identified by the development of a blue color and the absence of color is scored negatively. All results were computed into the MPN table.

Isolation and enumeration of actinomycete's colonies by dilution plate technique

Isolation and enumeration of actinomycetes colonies were performed by a soil dilution plate technique using two different media: the first medium is an actinomycete's isolation agar medium (Difco, NJ, USA) at pH 7.0. The second medium, *Streptomyces* medium, consisted of (g/L): glucose, 5; L-glutamic, 4; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.7; NaCl, 1; $FeSO_4.7H_2O$, 3 mg; and agar, 25. This medium was supplemented with 50 µg/L cycloheximide (Sigma-Aldrich Corp., MO, USA) (Awad *et al.*, 2009). The isolates were incubated at 28 ± 0.5 ºC for 7-10 days. The results obtained were expressed as the colony forming unit (CFU).

Actinomycete colonies were characterized morphologically and physiologically following the directions given by the International *Streptomyces* project (ISP) (Shirling and Gottlieb, 1966).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Chemical analysis

The total nitrogen, phosphorus and potassium (NPK) content of samples were analyzed according to the following methods. Nitrogen was determined by the acid combustion elemental analysis method using the macro kjeldahl system (Gerhadt, German) (Tandon 1993). The phosphorus, potassium and other micronutrients were digested using the acid digestion method and analyzed spectrophotometrically (Spectroquant NOVA 60, Merck, USA) using EPA method 3050B (Tandon, 1993). Moisture content of the samples was determined using the moisture analyzer (MX-50, A&D Company Ltd, Japan) to a constant weight. The pH value was measured in a 5-fold dilution of distilled water equilibrated with the sample for an hour with a pH meter (Delta 320, Mettler Toledo, Germany). Ash content in a dried sample was determined at 550°C for 24 hours using (CWF 110, Carbolite, England). C % was determined by APHA 5310 B method according to APHA (2005). N % was determined by APHA 4500-N org B (Mod) according to APHA (2005).

Efficacy of biofertilizers

Efficacies for the biofertilizers were carried out for six months. Soil for this experiment was natural silt loam with a pH of 7.3 and moisture content of 14.4%. A local variety of ladyfingers was used as test plant. The experimental design was the completely randomized design with three replicates. Four different soil beds at a size of 15 feet x 4 feet (LxW) were prepared for each treatment as followed: Plot 1 (Biofertilizer A), Plot 2 (Biofertilizer B), Plot 3 (Biofertilizer C) and Plot 4 Controls (without Biofertilizer). Before the planting started, each plot was treated by spreading a total of 200 g of the respective biofertilizers into the loose soil. The plots were then watered regularly for 14 days. After the soil treatment, a seeding inoculation was performed. The seeds of ladyfingers were soaked in water for 10 minutes, and good qualities seeds were taken out for seeding; good seeds will sink underneath the water and vice versa. For seeding, about 3-4 seeds were pressed 1-2 cm into the soil bed. One tablespoon of the respective biofertilizer (about 14 g) was then dispersed on the soil surface surrounding the planted seed and water was applied. This was done weekly and continued to twice a month until the day of harvesting. During harvesting, the plants were carefully uprooted from each plot and the plant height, length of roots, diameter of leaves, fruits and fruit weight were recorded.

Statistical Analysis

All experiments were carried out in triplicate. All data are reported as means ± SD (standard deviation). The results were analyzed statistically by a one-way ANOVA using (SPSS Inc. 2006) (Levesque, 2007) with the results: microbiological, chemical and field trial with biofertilizers as the main factors. The mean of each measurement parameter was separated statistically using Tukey's and Dunnet's multiple range tests with the plants grown without the aid of biofertilizer set as control. Significance was defined as $P<0.05$, unless otherwise indicated.

RESULTS

Fermentation and temperature monitoring

The temperature was recorded from the first day of production until the eighth day of biofertilizer fermentation. The temperature increased rapidly during fermentation, peaking at 71 °C on Day 4 and then decreased gradually until Day 8 when the biofertilizers achieved maturity. The result is as shown in Figure 1.

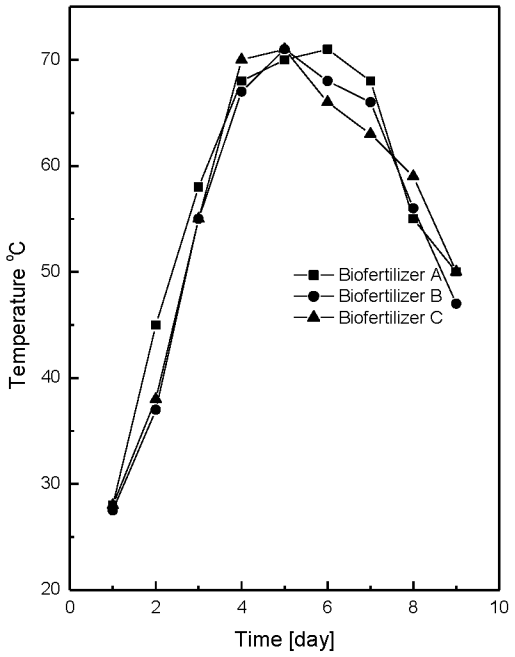

Figure 1: Temperature profile of different types of biofertilizers during fermentation period

Isolation and enumeration of microorganisms

During fermentation, the results of total microbial population in different biofertilizers of A, B and C measured using the CFU/g biofertilizer are as shown in Table 3.

In all biofertilizers tested, *Lactobacillus sp.* was the major population while the nitrogen-fixing bacteria were the minority population. The results showed that the total count of *Lactobacillus sp* was 3.3×10^5 CFU g/L, 4.9×10^5 CFU g/L, and 2.3×10^4 CFU g/L in biofertilizer A, B and C, respectively. Meanwhile, biofertilizer B and C showed the greatest growth of yeast total count, which were 3.0×10^7 CFU g/L and 3.5×10^7 CFU g/L and the lowest total count of yeast, 2.4×10^5 CFU g/L, was recorded in biofertilizer A.

On the other hand, the population of nitrifying bacteria in terms of biofertilizer A was significantly different from biofertilizer B and C and ranged between

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 3: The populations of Effective Microorganisms (EM) examined in Biofertilizer A, B and C in CFU/g.

Microorganism Total Count		Biofertilizers		
		A	B	C
Lactobacillus sp.		$3.30 \times 10^{5\,a}$	$4.90 \times 10^{5\,a}$	$2.30 \times 10^{4\,b}$
Yeast		$2.40 \times 10^{5\,b}$	$3.00 \times 10^{7\,a}$	$3.50 \times 10^{7\,a}$
Nitrifying Bacteria	AOB	$>1.60 \times 10^{3\,b}$	$3.3 \times 10^{2\,a}$	$3.5 \times 10^{2\,a}$
	NOB	$6.20 \times 10^{2\,b}$	$3.2 \times 10^{2\,a}$	$3.6 \times 10^{2\,a}$
Photosynthetic bacteria		NG	NG	NG
Nitrogen-fixing bacteria		$4.5 \times 10^{1\,b}$	$5.2 \times 10^{1\,b}$	$1.4 \times 10^{1\,b}$
Actinomycetes		NG	NG	NG

Significance difference ($P<0.05$) NG= no growth, AOB=Ammonia-oxidizing bacteria, NOB= Nitrite-oxidizing bacteria

3.20×10^2 to 3.60×10^2 CFU g/L for AOB and NOB, respectively. Concurrently, the results showed that no significant difference had been observed for all types of biofertilizer formulation in the case of total nitrogen-fixing bacteria count. Also, the results indicated that no growth was detected in both photosynthetic bacteria and *Actinomycetes* in all biofertilizers examined.

Chemical analysis

The results in Table 4 show that the pH value of all formulated biofertilizers was slightly alkaline and ranged between 8.2 - 8.5 due to the degradation of nitrogen-containing materials to soluble organic nitrogen. The moisture content for each formulated biofertilizer differed from one another based on the formulation composition and ranged from 16.60 to 22.30%.

In addition, the ash content of all biofertilizers ranged between 0.42-0.61 percent, depending on the formulation composition. The stability of ash content can be used as the parameter of compost maturity. The total organic carbon and nitrogen content was determined and the results showed that biofertilizer B possessed the highest content (19.2) followed by biofertilizer C (12.0) and lastly biofertilizer A (6.0). The results also indicated that the macro- and micronutrients content of biofertilizer B was the highest followed by biofertilizer A and C.

Efficacy of biofertilizers

The field trail results in Table 5 show that the plants treated with biofertilizer C grew more vigorously than the other plants grown in different treatments. On the other hand, the plants treated with biofertilizer A and contained burned rice husk exhibited the largest fruit diameter as well as the fruit weight. The plants treated with biofertilizer B grew better than the plants treated with biofertilizer A and C for the diameter of leaves recorded. Overall, it was observed that the plants treated with biofertilizer A, B and C were growing well and had better yields than the plants which grew without any treatment (control).

DISCUSSION

During biofertilizer preparation, microbes decompose the organic matter and release the fermentation heat (Yang, 2003). Temperature changes have to be recorded during fermentation to monitor the activity of the microbes. The results showed that the temperature increased from 41 °C to 71 °C at Day 4 and gradually decreased to 50 °C at Day 8, indicating that the biofertilizers had achieved maturity. The increasing temperature during fermentation occurs due to the active microbial growth. The temperature changing patterns in this work are similar to the commercial composting process made by Pai *et al.* (2003).

Proper fermentation also will effectively destroy pathogens and weeds through the metabolic heat generated by the microorganisms (Yang 2000; Nakasaki *et al.*, 1996). These results are in accordance to those obtained by Tsai *et al.* (2007) who found that the inoculation of appropriate microbes during fermentation will shorten the period of maturity and thus improve the quality of biofertilizers. Nevertheless, there is a lack of reported studies in the number of actinomycetes and photosynthetic bacteria present in biofertilizer samples. Many of the literatures only showed that the isolation of these microorganisms from environmental samples such as oil was noticeable (Fuentes *et al.*, 2010). In order to prepare a multi-functional biofertilizer, thermo-tolerant phosphate-solubilizing microbes, including bacteria, actinomycetes and fungi have to be isolated from different compost plants and biofertilizers (Chang and Yang, 2009).

Biofertilizers of three different formulations were analyzed for their microbiological, chemical and physical components. The presence of certain microorganisms and the nutrient mineralization are favorable to support plant growth and yields (Parthasarathi and Ranganathan, 1999). Another study was done by Edward and Fletcher (1988); they stated that the increase of microbial populations increases the performance of biofertilizer microbiologically, chemically, and physically.

The large number of *Lactobacillus* sp. and yeast isolated from the final product of biofertilizer indicated the success of fermentation. The total number of *Lactobacillus sp.* and yeast were in between $5.00 \times 10^{5\text{-}8}$ CFU g/L. These results are consistent with the microbial analysis results from liquid biofertilizers produced by several authors such as Ngampinol and Kunathigan (2008); and Department of Agriculture (2004).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 4: Macro- and micronutrients and other chemical analysis for Biofertilizer A, B and C.

Biof. Form	pH	Mois. (%)	Ash (%)	C (%)	N (%)	C:N Ratio	Percentage (%)					Concentration (mg/L)				
							N	P	K	Mg	Ca	B	Fe	Mn	Na	Mo
A	8.5±0.01[a]	19.24±0.21[a]	0.61±0.11[a]	7.2	0.94	6.0	1.4±0.15[a]	<0.001[a]	4.7±0.11[a]	2.9±0.38[a]	<0.5[a]	0.6±0.05[a]	16.3±0.21[a]	0.8±0.04[a]	22.0±0.95[a]	0.1±0.11[a]
B	8.2±0.5[a]	16.60±0.01[b]	0.42±0.12[b]	20.5	0.81	19.2	1.7±0.25[b]	0.001[a]	6.6±0.20[b]	4.7±0.17[b]	0.8±0.02[b]	1.8±0.01[b]	<0.0001[b]	4.9±0.06[b]	8.5±0.12[b]	1.1±0.11[b]
C	8.2±0.12[a]	22.30±0.11[c]	0.45±0.19[b]	18.9	0.0001	12.0	1.8±0.11[c]	<0.001[a]	4.9±0.11[a]	8.5±0.10[c]	1.1±0.05[b]	0.5±0.05[a]	1.8±0.11[c]	0.2±0.03[a]	9.0±0.17[b]	1.0±0.00[b]

Significance difference (P<0.05)

Table 5: Physical analysis during field trial for ladyfingers fertilized with Biofertilizer A, B and C.

Physical analysis	Biofertilizer A	Biofertilizer B	Biofertilizer C	Control
Plant height (cm)	185.0±7.00[a]	217.5±6.93[b]	237.6±4.96[c]	79.9±3.53[d]
Root length (cm)	34.4±0.57[a]	36.7±0.47[a]	41.8±1.68[b]	17.1±0.85[c]
Leaves diameter (cm)	34.8±1.50[a]	44.4±0.50[b]	41.8±1.68[a]	17.3±0.32[c]
Fruits diameter (cm)	3.2±0.12[a]	2.8±0.15[a]	2.6±-0.47[a]	1.5±0.38[d]
Fruits weigh (g)	38.5±0.70[a]	36.2±3.63[b]	28.0±2.11[c]	11.4±0.95[d]

Significance difference (P<0.05)

ISSN (print): 1823-8262, ISSN (online): 2231-7538

The nitrifying and nitrogen-fixing bacteria total counts were low (< 1.00x 10^3 CFU/g) in all formulations prepared.

The chemical analysis results derived from the composting process showed that the inoculated biofertilizers with tested microbes had a significantly higher temperature, ash content, pH, total nitrogen, and soluble phosphorus content. Adding these microbes can shorten the period of maturity, improve the quality, increase the soluble phosphorus content, and enhance the populations of phosphate-solubilizing and proteolytic bacteria in the biofertilizers (Chang and Yang, 2009). The pH value of each biofertilizer in these experiments was in the range of 8.20 - 8.50 which is slightly alkaline than other solid biofertilizers as reported by many authors such as Debosz *et al.* (2002); and Tsai *et al.* (2007). The slightly alkaline pH is beneficial because this will contribute to the neutralization of acidic agricultural soil (Fageria and Baligar, 2001).

The moisture content of compost decreased during the incubation period because the inoculation of the biofertilizer with EM increased the temperature and decreased the moisture content of biofertilizer. The same phenomena has also observed in open field composting (Pai *et al*., 2003).

Total ash content in the biofertilizer samples was determined. The stability of ash content can be used as a parameter of compost maturity. The ash content significantly increased during preparation since the organic materials were decomposed to form the metabolic gases (Yang, 2003; Chang and Yang, 2009). Total organic carbon content (C:N ratio) decreased from 19.2 in case of biofertilizer B to 12.0 and 6.0 for biofertilizer A and C, respectively. Total organic carbon content significantly decreased during composting due to the degradation of organic matter. These results are in accordance with those results obtained by Chang and Yang (2009). It has been noted that the properties of the initial material, in particular is affecting the C:N ratio of the biofertilizers. Higher C:N ratio (>30%) contributed longer composting process to occur (Tiquiaa and Tam, 2000). Other factors such as aeration condition, moisture content, and temperature are also affected by the degree of N loss.

The field trial study was conducted to monitor and observe the differences in the biofertilizers' effectiveness regarding their abilities to encourage plant growth. Significant reduction of all physical properties in the case of non-treatment plant can be explained by lack of or low soil fertility. The plant height, length of roots, diameter of leaves and fruits as well as the ripe fruit weight increased when the plants were treated with biofertilizers.

It has been noted that the addition of burned rice husk in biofertilizer B provided a higher percentage of potassium (6.6%), which contributed to extra growth in fruit diameter and weight compared to other biofertilizers (without burned rice husk). These results are in agreement with Seripong (1989) who mentioned that the dry weight of shoots and fruits significantly increased as the burned rice husk was added. Similarly, with the addition of more than 3% nitrogen source meal from a total of 7% in biofertilizer A and C gave a good yield of leaves diameter for plant treated with biofertilizer B.

On the other hand, the plants treated with biofertilizer C recorded the highest root and stem lengths (237.6 ± 4.96 cm) in comparison to plants treated with biofertilizer A and B, which were 185.0 ± 7.00 cm and 217.5 ± 6.93 cm, respectively. Total nitrogen content for all biofertilizers (> 1%) had no effect on populations of total bacteria, yeast, as well as ammonia and nitrite utilizing bacteria. These results are in accordance with those results obtained by Sarathchandran *et al.* (2001) who reported that the nitrogen content in biofertilizer around 048 - 0.69% did not give any significant difference to the total count of microbes studied.

CONCLUSION

In conclusion, the microbiological, chemical and physical properties of biofertilizer A, B, and C were determined. Based on these properties, we suggest that biofertilizer A is the best in encouraging flower and fruit growth, while biofertilizer B is superior in leaf production and biofertilizer C is good for the strength development of roots and stems. Furthermore, the formulated biofertilizers in this study was prepared from an economical and low-cost raw material with the inoculation of special microorganisms, which is a feasible and potential market for the commercialization as well as to promote environmental friendly technology. This will reduce the country's reliability on chemical fertilizers in a way to produce, increase and sustain food production. Therefore, the utilization of agricultural waste converted to biofertilizer can be one of the successful alternative ways of optimizing the use of resources and to generate income.

REFERENCES

Ashby, S. F. (1907). Some observation on the assimilation of atmospheric nitrogen by a free living soil microorganisms: *Azotobacter chrococum* of Beijerinck. *Journal of Agricultural Science,* **2, 35-51.**

APHA (2005). Standard Methods for the Examination of Water and Wastewater; 21st Edition; American Public Health Association, Washington, D.C.

Awad, H. M., El-Shahed, Y. I. K. and El-Nakkadi, A. M. (2009). Isolation, screening and identification of newly isolated soil *Streptomyces* (*Streptomyces* sp. NRC-35) for β-lactamase inhibitor production. *World Applied Scince Journal,* **7, 637-646.**

Bheki, G. M., Alpheus, M. Z. and Stefan, J. S. (2010). Poutry manure enhances grass establishment at a quarry rehabilitation site insubtropical South Africa. *African Journal of Agricultural Research*, **6 (1), 46-50.**

Bhuiya, Z. H. And Walker, N. (1977). Autotrophic nitrifying bacteria in acid tea soild from Bangladesh and Sri Lanka. *Journal of Applied Bacteriology*, **42, 253-257.**

Boraste, A.V., Jhadar K. K, Khainar, A., Gupte Y., Trivedi N., Patil S., Gupta P., Gupta G., Mujapara A. K. and Joshi, B. (2009). Biofertilizer: A novel tool for Agriculture. *International Journal of Microbiology Research*, **1 (2), 23-31.**

Chang, C.-H. and Yang, S.-S. (2009). Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. *Bioresource Technology*, **100 (4), 1648-1658.**

Chen, K. S., Lin, Y. S. and Yang, S. S. (2007). Application of thermotolerant microorganisms for biofertilizer preparation. *Journal of Microbial Immunology Infection*, **40, 462-473.**

ISSN (print):1823-8262, ISSN (online): 2231-7538

Coroneos, C., Hinsinger, P. and Gilkes, R. J.(1995). Granite powder as a source of potassium for plants: a glasshouse bioassay comparing two pasture species. *Nutrient Cycle in Agrosystem*, **45 (2), 143-152.**

Debosz, K., Petersen, S. O., Kure, L. K. and Ambus, P. (2002). Evaluating effects of sewage sludge and household compost on soil physical, chemical and microbiological properties. *Application of Soil Ecology*, **19 (3), 237-248.**

Deparment of Agriculture. Agriculture, D. O. (2004). Culture Collection. Scientific information of liquid biofertilizer (part 1). Bangkok.

Diaz, L. F., Golueke, C. G., Savage, G. M. and Eggerth, L. L. (1993). Composting and recycle municipal solid waste Lewis Publisher.

DOA. (2010). Statistik Asas Sektor Agrimakanan.

DOE. (2001). Malaysia: Environmental Quality Act Report, Ministry of Science, Technology and the Environment, Putrajaya, Malaysia.

Fageria, N. K. and Baligar, V. C. (2001). Improving nutrient use efficiency of annual crops in Brazilian acid soils for sustainable crop production. *Communications in Soil Science and Plant Analysis,* **32, 1303-1319.**

Food and Agricultural Organization, F. FAO. (2008). Guide to laboratory establishment for plant nutrient analysis Rome.

Fuentes, M. S., Benimelli, C. S., Cuozzo, S. S. and Amoroso, M.J. (2010). Isolation of pesticides regarding actinomycetes from a contaminated site: Bacterial growth, removal and dechlorination of organochlorine pesticides. *International Biodeterioration & Biodegradation*, **64, 434-441.**

Harunor, R. K. M., Mohiuddin, M., Rahman, M. (2008). Enumeration, isolation and identification of nitrogen-fixing bacteria strains at seedling stage in rhizophere of rice grown in non-calcereous grey flood plain soil of Bangladesh. *Journal of Faculty of Environmental Science and Technology,* **13, 97-101.**

Leuschner, R. G. K., Bew, J. and Bertin, G. (2003). Validation of an official control method for enumeration of authorised probiotic yeast in animal feed. *Systematic and Applied Microbiology,* **26, 147-153.**

Levesque, R. (2007). SPSS Programming and data management: A guide for SPSS and SAS Users SPSS Inc.

Marrs, R. H. (1993). Soil fertility and nature conservation in Europe: theoretical considerations and practical management solutions. *Advances in Ecological research*, **24, 241-300.**

Nakasaki, K., Uehara, N., Kataoka, M. and Kubota, H. (1996). The use of *Bacillus licheniformis* HA1 to accelerate composting of organic wastes. *Compost Science and Utilization*, **4, 47-51.**

Ngampinol, H. and Kunathigan, V. (2008). The study of shelf life for liquid biofertilizers from vegetables waste. *Assumption University Journal Technology,* **11, 204-208.**

Pai, C. R., Wu, C. F., Sun, R. Y., Wei, C. B. And Yang, S. S. (2003). Composition analysis of livestock and poultry waste during composting. *Journal Biomass Energy Society of China,* **22, 57-72.**

Parr, J. P., Harrick, S. B. and Papendick, R. I. (1998). Transition from conventional agriculture to natural farming systems. The role of microbial inoculants and biofertilizer. In: *Proceedings of the Fourth International Conference on Kyusei Nature Farming*, **pp. 56-63**.

Parthasarathi, K. And Ranganathan, L. S. (1999). Lonngevity of microbial and enzyme activity and their influences on NPK content in pressmud vermicasts. *European Journal of Soil Biology,* **35, 107-113.**

Prasertsan, P., Choorit, W. and Suwanno, S. (1993). Isolation, identification and growth conditions of photosynthetic bacteria found in seafood processing wastewater. *World Journal of Microbiology and Biotechnology*, **9, (5), 590-592.**

Sarathchandra, S. U., Ghani, A., Yeates, G. W., Burch, G. and Cox, N. R. (2001). Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. *Soil Biology and Biochemistry*, **33, (7-8), 953-964**.

Shirling, E. B. and Gottlieb, D. (1966). Methods for Characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, **16, 313-340.**

Tandon, H. L. S. (1993). In: Tandon, H.L.S. (Eds.), Methods of analysis of soils, plants, waters and fertilizers. Fertilizer Development and Consultation Organization, New delhi, India, **pp. 36-48.**

Tiquiaa S.M. and Tam, N.F.Y. (2000). Fate of nitrogen during composting of chicken litter. Environmental Pollution **110: 535-541**

Tsai, S.-H., Liu, C.-P. and Yang, S.-S. (2007). Microbial conversion of food wastes for biofertilizer production with thermophilic lipolytic microbes. *Renewable Energy,* **32, (6), 904-915**.

Warman, P. R. and AngLopez, M. J. (2010). Vermicompost derived from different feedstocks as a plant growth medium. *Bioresource Technology*, **101, (12), 4479-4483**.

Weber, J., Karczewska, A., Drozd, J., Licznar, M., Licznar, S., Jamroz, E. and Kocowicz, A. (2007). Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. *Soil Biology and Biochemistry,* **39, (6), 1294-1302**.

Yang, S. S. (2003). Application of microbial fertilizers on the three objectives agriculture Bestwise Pulishing Co.

Yang, S. S. (2000). Recent advance in composting. In: Proceedings of the International Seminar on Issues in the Management of Agricultural Resources, **pp. 166-185.**

ISSN (print):1823-8262, ISSN (online): 2231-7538