

## **Effects of IMO and EM application on soil nutrients, microbial population and crop yield**

(Kesan aplikasi IMO dan EM terhadap nutrien tanah, populasi mikroba dan hasil tanaman)

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Keywords: effective microorganism, indigenous microorganism, nature farming, crop yield

### **Abstract**

The performance of indigenous microorganisms (IMO) and effective microorganisms (EM) on leafy vegetables (*Brassica alboglabra*, *Brassica chinensis* and *Lactuca sativa*) grown under rain shelter was evaluated. Results at the end of the planting season showed that microbial populations were significantly ( $p < 0.05$ ) higher in soil inoculated with EM compared to IMO, urea and normal compost. The increase in microbial populations ranged between 5.45 and 7.12  $\log_{10}$  cfu/g in line with the increase in soil pH. Assessment of the application of IMO and EM showed no significant differences in yield performance, antioxidant levels in vegetable leaf samples and soil nutrient status.

### **Introduction**

Soil fertility is equated with chemical and physical properties. The microbial component is always being neglected because of inconsistent performance in the soil. In Malaysia, most of the microbial inoculants are imported and only a few are made locally. Their performance (local and imported inoculants) varies depending on the type of crops, applications and soil types (Mahdi et al. 2010). However, the performance of imported microbial inoculants was largely influenced by the climatic similarity of the country of origin. It has been reported that the imported microbes may find difficulty in competing with indigenous microbes in tropical soil (Aini 2006). Until now, only a few reports have been found on the inoculation effects of microbes in matured compost and its effect on the microbial community (Kato and Miura 2008).

In nature farming (NF) concept, the indigenous microorganisms (IMO) preparation (Cho 1997) is based on local micro-flora which do not require a laboratory for biomass culture. The procedures and use of biomass sources as a substrate (carbohydrate source e.g. rice bran and starch) to culture microbes and transform organic wastes into valuable biofertilizers are easy for farmers to adopt. Farmers claimed that the technology is able to reduce the cost of production by 30% as compared to conventional practice (Aini 2006).

Through the NF technology, the use of IMO inoculants produced by rice fermentation technique became popular. It is a main carbon source from carbohydrate for its substrate in microbial culture. This carbon source is important for microbial multiplication before it can be applied to the soil. It is believed that these IMO

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inoculants could improve soil structure and increase nutrient uptake by the crop (Prell 2010). This IMO consists of a local consortium of microbes that will regenerate fast according to local conditions and can survive in the environment. The types and quantities of microbes depend on their origin. Even though the process of reculturing the microbes can easily be done without sophisticated laboratory facilities, the inoculants are still effective.

There is a disadvantage in using this IMO because the nature and survival of the microbial population remains unknown and in many cases questionable, especially after being applied to the compost and soil. The performances of the microbes normally depend on the 'environment' of the habitat and in many cases inefficient, probably because of competition with soil indigenous microbes (Bashan 1998). In addition, factors such as oxygen, water, storage conditions and nutrients in the media could be depleted over time, subsequently resulting in decreasing microbial population. There is lack of research to support the claim from farmers who have used this technology.

Doran and Zeiss (2000) demonstrated that the efficiency of microorganisms in the soil is of fundamental importance for an ecosystem to function by determining the nutrient cycling, organic matter decomposition and energy flow. Microbial application also promotes diversification of microbial ecology in the soil and plant surface (Baker et al. 1999). However, the survival of microbial inoculants in the compost preparation and finally to soil application is questionable because there is not much information available. The objective of this study was to evaluate the effects of microbial inoculants (EM and IMO) on soil nutrient and crop performance on leafy vegetables (*Brassica alboglabra*, *Brassica chinensis* and *Lactuca sativa*).

## Materials and methods

### *Field experiment*

The field experiment was conducted in three cropping seasons at the Integrated Organic Farm, MARDI, Serdang. The test crops, *B. alboglabra*, *B. chinensis* and *L. sativa*, were grown on mineral soil under a simple open-type rain shelter. The treatments consist of compost with microbial inoculants for IMO (T1) and EM (T2), urea (T3) and normal compost as control (T4). The crops were grown on 7 m x 1 m sized beds with a randomized complete block design (RCBD) replicated five times. Only one crop was grown in each planting season.

### *IMO and EM preparation and application*

IMO was prepared according to the Natural Farming Manual from the Department of Agriculture, Malaysia (Anon. 2006). One kg of cooked rice was allowed to ferment under the bamboo tree for 4 days. Molasses was then added into the fermented rice with a ratio of 1:1 (now known as IMO2). A sample of 10 g of IMO2 was then diluted with water and mixed into 5 kg of rice bran to become IMO3 which was then left for 5 days to allow the surface to be covered with whitish spores (now known as IMO4). Fermentation was completed when the temperature stopped increasing.

About 700 g of fermented IMO4 was mixed into the soil on each bed as treatment T1 at 1 week before planting. Plants planted on soil with treatment T1 were also supplemented with a foliar spray of 2% diluted fermented plant juice (FPJ) at every 2 weeks. The FPJ was a filtered juice from 1 week fermentation of papaya fruit mixed with brown sugar.

For treatment T2, the EM was purchased from Pertubuhan Peladang Negeri Johor, the distributor for dormant EM<sup>®</sup> product from Japan. The EM has to be activated before application which involved the addition of 7 litres of chlorine-free water and 1.5 kg of molasses to 3 litres of EM, 1 week prior to application (Anon.

1995). The activated EM was applied during preparation of normal compost.

NPK Green 15:15:15 was used as treatment T3 (urea) as a conventional fertiliser based on nutrient requirements for vegetables recommended by MARDI (Anon. 2005). The normal compost without EM was used as control (T4). It was made from a combination of composted materials consisting of chicken dung, empty oil palm fruit bunches and rice bran at a ratio of 2:3:1. The EM treatment (28 kg of compost plus activated EM liquid) and normal compost (28 kg) were applied into the soil by mixing it at 2-week intervals until harvesting.

#### ***Soil sampling and laboratory analyses***

Soil sampling was carried out at 0 – 20 cm depth at three points on each bed before the application of compost (initial) and after harvest (30 days) of each cropping season for soil nutrient status and soil microbial population analyses. The soils were air dried and ground to pass a 2 mm sieve.

Total nitrogen in the soils was determined using Micro Kjeldahl digestion followed by distillation and titration with 0.1 M HCl (Bremner and Keeney 1966). Organic carbon was determined by employing rapid titration method (Nelson and Sommers 1982). Available phosphorus in soils was extracted based on the Bray and Kurtz no. 2 procedure (Bray and Kurtz 1945). Soil pH was measured at 1:2.5 ratios with deionized water using a glass electrode (McLean 1982). The exchangeable cations were extracted by leaching with 1 M ammonium acetate and their concentration was determined using Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) (Thomas 1982).

As for microbial population determination, a serial dilution technique was used with 10 g of wet soil samples diluted in 90 ml of saline water. Three aliquots (replicates) of 100 µl from each dilution were then spread on nutrient agar (Oxoid). After incubation for 48 – 72 h at

28 °C, the microbial colonies growing on the plates were counted for the microbial population in total colony forming units (cfu-log<sub>10</sub>) (Wollum 1982).

#### ***Crop Performance***

After one month, the crop was harvested and weighed at the end of each season. Only the fresh weight of the whole plant was taken. Data on yield collection and comparison of economic yield to normal practices were based on yield per ha.

#### ***Antioxidant activity***

Sample extraction for all vegetables was done using the method described by Peschel et al. (2006). Prior to the analysis, the vegetable samples were dried at 60 °C for 48 h before they were ground and extracted twice with methanol (10:1 solvent to raw material) in a closed vessel, stirred at room temperature for 4 h, and left for another 4 h before filtration. The filtrate was concentrated in the rotary evaporator to yield the sample extract for antioxidant assay using 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

Later, 1.5 ml of the extract (0.001 g/ml) was added into 3.0 ml of freshly prepared DPPH solution in methanol. The solutions were stirred and then left to stand in the dark for 30 min before measuring the absorbance with UV spectrophotometer (UV-1700 PharmaSpec, Shimadzu) at 517 nm. Butylated hydroxytoluene (BHT) was used as the positive control. Inhibition of free radical DPPH in percent (*I* %) was calculated using the following formula:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

where  $A_{blank}$  is the absorbance of the control sample and  $A_{sample}$  the absorbance of the extract.

#### ***Statistical analysis***

The data were analysed statistically using the Statistical Analysis System 9.1 (SAS). Following the analysis of variance

Table 1. Soil nutrient status at the end of third planting season

	EM	IMO	Urea	Control	Initial
pH (H <sub>2</sub> O)	5.70b	4.81b	5.58ab	6.19a	6.32a
Total N (%)	0.18a	0.14ab	0.11b	0.08c	0.05c
Organic C (%)	0.97a	1.03a	0.91a	1.08a	1.41a
Available P (ppm)	99.94b	82.31c	167.72a	107.20b	n.a.
K (meq/100 g)	0.80a	0.35b	0.68a	0.41b	0.52ab
Na (meq/100 g)	0.27a	0.28a	0.31a	0.27a	0.57a
Ca (meq/100 g)	4.48b	2.26c	5.06b	6.51a	2.71c
Mg (meq/100 g)	2.20a	1.8b	0.34c	0.85c	1.03b

Values are given as a mean of five replicates. Means with the same letter in a row are not significantly different at  $p < 0.05$

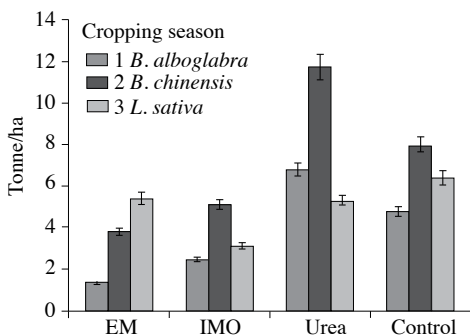


Figure 1. Yields of leafy vegetables harvested in three planting seasons (Season 1: *Brassica alboglabra*, 2: *Brassica chinensis* and 3: *Lactuca sativa*)

(ANOVA), differences between treatment means were determined using Duncan multiple range test (DMRT).

## Results and discussion

### Soil nutrient status

Results showed higher values in total N, organic carbon, K and Mg in EM and IMO treatments as compared to urea treatment (Table 1). At the early stages of planting season, the application of IMO and EM decreased the soil pH. This could be due to the decomposition of organic matter which helps to increase the soil acidity. Similarly, the ammonium or urea in the chemical fertilizer speeds up the rate at which acidity develops (Crozier and Hardy 2003).

It was also revealed that the relationship between the availability of the soil nutrient component and the outcome

of yield was not significant (Figure 1).

The period of the experiment was only 9 months; hence the changes may not be significant at this point of time. Perhaps the changes would be significant with a longer period of time as the nutrients build up and accumulate in the soil. This is because the organic matter in the compost serves as the basis for soil fertility through the breakdown of nutrients such as nitrogen, phosphorus and a range of the other nutrients (Chan 2008).

### Crop performance

There was no significant difference for EM and IMO treatments in terms of yield performance (Figure 1). Urea treatment gave the highest yield in the first two seasons of planting for *B. alboglabra* and *B. chinensis*. The yields were almost double compared to the other treatments. However, in the third season, there was a decreasing trend in the crop yields for all treatments with IMO having the lowest yield. This was probably because the application of IMO solely depends on the ability of microbes to act as a nutrient synthesizer in nutrient uptake by the plants. The amount was insufficient for cell building and other micronutrients were also needed by the plants.

Sharon et al. (2009) also found that microbial application with EM did not improve spinach yield regardless of fertilizer or management regime used and suggested crop cultivation to be continued at the same location with longer growth period.

However, treatment with normal compost using chicken dung (control) showed an increase in yield performance during the third season. For short term crops such as leafy vegetables, compost containing chicken dung gave sufficient basic nutrients for plant uptake. It also revealed better nutrient availability compared to the nutrients synthesized by the microbes.

### **Microbial population in the soil**

Table 2 shows the fluctuation in soil microbial populations in the first season of planting due to the microbial adaptation to the local soil climatic conditions. For this reason, soils formed under high rainfall conditions are more acidic than those formed under arid (dry) conditions as rainfall also affects soil pH (McFee et al. 1977). Water passing through the soil leaches basic nutrients such as calcium and magnesium from the soil. They are replaced by acidic elements such as aluminum and

Table 2. Microbial population in the soil at initial and end of three planting seasons

Treatment	Microbial population (log <sub>10</sub> cfu)			
	Initial	1 <sup>st</sup> season	2 <sup>nd</sup> season	3 <sup>rd</sup> season
T1 : EM	4.33b	6.33a	6.89b	7.12a
T2 : IMO	4.01c	5.01b	5.23c	5.45d
T3 : Urea	4.68a	5.89a	7.23a	6.34c
T4 : Control	4.82a	6.01a	6.79b	7.01b

Means with the same letter in a column are not significantly different at  $p < 0.05$

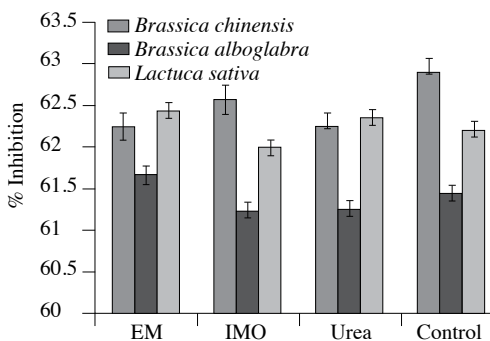


Figure 2. Cumulative antioxidant activity of all vegetable samples

iron (Crozier and Hardy 2003). However, there was a significant ( $p < 0.01$ ) increasing trend of microbial population towards the end of the planting season in all treatments as the pH in the soil increased.

Soil microorganism are undeniably important for their role and significance in driving the processes of nutrient cycling and degradation of organic matter. However, there are other microorganisms present and the numbers are usually much more than the applied IMO and EM itself. Because of this, the efficacy will not always prove to be true as stiff competition will prevail between the autochthonous microorganisms and the introduced ones (Van Vliet et al. 2006).

### **Antioxidant level in vegetable leaf samples**

Statistically there were no significant differences ( $p < 0.05$ ) in the antioxidant activities of *B. chinensis*, *B. alboglabra* and *L. sativa* treated with IMO, EM, normal compost (control) and even urea (Figure 2). The antioxidant activities ranged from 61.23 – 62.91% of inhibition. No differences in antioxidant activities for all vegetable samples were also found in other organic amendments such as goat dung and chicken dung (Faridah et al. 2008). This finding was similar to a report published by Organic Retailers and Growers Association of Australia (ORGAA) which also found no major differences in antioxidant contents of vegetables produced organically or conventionally (Amin and Cheah 2003). Leclerc et al. (1990) also reported no significant difference in  $\beta$ -carotene between lettuces grown with or without organic fertilization due to the composition of the treatment used which contained quite similar amounts of nitrogen (N), the main contributor for the synthesis of plant antioxidants (Daniel et al. 1999).

### **Conclusion**

Application of EM and IMO showed no effect on yields and antioxidant levels in vegetable leaf samples. There was an increase in microbial populations in soil

with EM application as the microbes adapt to their new environment at the end of the third season. Higher values in total N, organic carbon, K and Mg in EM and IMO treatments were observed. However, the status of some of the soil nutrients did not change from the initial soil samples. It was also revealed that the relationship between the availability of soil nutrients and the yield was not significant.

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### Abstrak

Prestasi mikroorganisma asli (IMO) dan mikroorganisma berkesan (EM) terhadap sayur-sayuran berdaun (*Brassica alboglabra*, *Brassica chinensis* dan *Lactuca sativa*) yang ditanam di dalam rumah pelindung hujan telah dinilai. Keputusan menunjukkan populasi mikrob dalam tanah yang diinokulasi dengan EM meningkat secara signifikan ( $p < 0.01$ ) di akhir musim menanam berbanding dengan rawatan IMO, urea dan kompos biasa. Peningkatan populasi mikrob adalah diantara 5.45 dan 7.12  $\log_{10}$  cfu/g selari dengan peningkatan nilai pH tanah. Penilaian terhadap aplikasi EM dan IMO juga menunjukan tiada perbezaan ketara dalam prestasi hasil, paras antioksidan dalam sampel daun dan status nutrien dalam tanah.