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IDENTIFICATION OF MICROORGANISM Composition in Indigenous Microorganisms (IMO) SOLUTIONS OF VARIOUS FRUIT PEELS AND THEIR UTILIZATION IN ORGANIC PEANUT (*Arachis hypogaea* L.) PRODUCTION

Elisabeth Sri Pujiastuti[*)], Juli Ritha Tarigan, Yanto Raya Tampubolon, Ferlist Rio Siahaan, Susana Tabah Trina Sumihar
Agroecotechnology Department, Faculty of Agriculture, Universitas HKBP Nommensen
Jl. Sutomo No. 4, Medan, Indonesia
[*)]*Correspondence author*: puji_purba@yahoo.com

Abstract

This paper consists of two researches. A research to identify the microbial composition and density of various types of indigenous microorganisms (IMO) solutions was carried out descriptively. Another research aimed to study the effect of the types of IMO on the growth and yield of peanut was carried out using a randomized block design with three replications and two treatment factors, namely the types of IMO consisting of IMO of tamarillo, pineapple and orange peels, and the dose of chicken manure with the levels of 0, 10, 20 and 30 tons/ha. The IMOs were applied at a concentration of 45 ml/liter, each. The results showed that the IMO of tamarillo, pineapple and orange peels contained different densities of at least six types of microbes, namely: Pseudomonas sp*; Azotobacter* sp*; Bacillus* sp*; Actinomycetes* sp*; Streptomyces* sp *and P (phosphate) solubilizing microbes, but the type of IMO had no significant effect on plant height and stem diameter at 6 WAP as well as the number of filled pods and the yield per plot. The three types of IMO gave the same good effect, as indicated by the production of 197 percent compared to the yield potential of Gajah variety.*

Keywords*: Fruit waste, indigenous microorganisms, microbial composition.*

INTRODUCTION

The issue of food security had been rolling since the 1980s. This issue arose because of the imbalance between the growing world population and the availability of food, so it was feared that there would be food shortages. The current world population (in the year of 2021) was estimated to be 7,854,965,732 people or 7.85 billion people. Indonesia was in the fourth rank, with a population of 273.523615 people (Zulfikar, 2021). The fulfillment of food needs encouraged the use of land that exceeded its capacity and the growing use of chemical fertilizers and pesticides that threatened the sustainability of our environment Awareness of environmental sustainability gave place to sustainable agricultural systems. Sustainable agriculture was referred as the use of land, water and plant resources in order to maintain sustainable production businesses that produced agricultural products economically and profitably (Sudaryanto et al., 2018). Sustainable agriculture was applied in several cultivation systems, including organic farming systems. Organic farming was defined as a comprehensive production management system that made maximum use of plant residues, livestock feces and urine, organic waste, organic pesticides, and others (Mayrowani, 2012).

Environmental protection was an aspect that became more and more important in mankind life nowadays. Nature farming had a principal goal of producing plentiful and healthy crops without using chemical fertilizers and pesticides and without causing negative effects on the natural environment (Higa and Wididana, 1991). In pursuing technologies that naturally available for agricultural waste management and at the same time improving agriculture yields, scientists found. Indigenous Microorganisms (IMO's) based technology as one such great technology (Kumar and Gopal, 2015). IMO solution is a liquid fermented from certain substrates or media available in the environment, such as: orange peel, pineapple peel, tamarillo peel, rice, urine, banana roots, vegetable waste and other fruit waste (Handayani et al., 2015) that was useful for increasing the rate of organic materials decomposition or as decomposers or as organic fertilizer for plants. These materials were thought to be substances that were able to stimulate plant growth (known as phytohormones), such as: gibberellins, cytokinins, auxins, and inhibitors. IMO based technology had been practiced as an excellent approach of organic farming in more than 30 countries. It was used for subsistence and commercial purposes (Kumar and Gopal, 2015). The benefits of effective microorganisms in improving crop yields and quality, and in protecting plants from pests and disease had been demonstrated on various crops and in the wide range of soil conditions (Higa and Wididana, 1991).

Being beneficial for plant growth, IMO processed from organic wastes was a solution to the problem of accumulating organic waste from various sources. Organic wastes presented a

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number of problems. According to Ministry of Environment and Forestry (2021), waste production in Indonesia in 2020 reached 33,171,983.20 tons/year, and only 59.41% of them were managed well. The composition of waste based on the source was from households (38.3%), followed by traditional markets (17.2%). Based on the type of waste, the largest was food waste (40.3%), followed by plastic waste of (17.1%). When organic wastes were not managed and allowed to decompose naturally, it caused several problems, such as: aesthetic problems, including odor pollution, health problems because they were sources of various human diseases, and produced CH_4 which was one of the greenhouse gases that contributed to global warming.

The positive effect of IMO on the production of various plants observed in several studies was thought to be caused by its microbial content (Jumriani et al, 2018; Tambunan, 2018). It was expected that by giving IMO to agricultural soil, the microorganisms in IMO would enrich the diversity and number of soil microorganisms, wherein these microorganisms was able to decompose organic matter in the soil into humus and available nutrients. This encouraged the research to identify the types of microbes in IMO sourced from the peels of various types of fruit. The three types of IMO identified were tested on peanut plants to study their effect on peanut growth and production. Some of the problems faced in increasing the national peanut were poor tillage resulting in poor drainage and solid soil structure, as well as the low use of biological and organic fertilizers (Directorate General of Food Crops, 2013).. It was expected that the use of IMO in organic peanut production was able to increase the crop yield and quality.

This study was to identify the microorganism composition and density in the IMO of pineapple (*Ananas comosus*), tamarillo (*Solanum betaceum*) and orange (*Citrus sinensis*) peels and their effect on the growth and yield of peanut plant.

MATERIALS AND METHOD

The study was conducted on Ultisol soil with a pH of 5.5 – 6.5 and a loamy sand texture (Lumbanraja, 2013) in Simalingkar B Village, Medan, at the altitude of 33 m above sea level. It was held in August 2019 to January 2020

Production of IMO

The materials prepared were: 5 kg of fruit peels (for example: pineapple peels), 1 litre of cow urine, 2 kg of palm sugar, 2 litres of coconut water and 10 litres of water. The peels of the fruit were crushed using a blender and then were put into a plastic bucket with a capacity of 20 litres. Cow urine and coconut water were added to the plastic bucket. The palm sugar that had been melted in 1 litre of water was put into the same plastic bucket. Eventually, water was added up to about 5 cm from the surface of the bucket, then all the ingredients in the bucket were stirred evenly. The plastic bucket containing the IMO materials was closed then the lid was strengthened with a rope and an air hole was made by inserting a plastic hose into the bucket lid and connect it to a bottle filled with water. All the mixture was left for 21 days, but every 4 days it must be stirred well and then the lid was put again on the bucket. The process was finished after 21 days when the IMO solution showed its clear yellowish colour and had a sweet sour aroma of alcohol.

Determination of Biological Properties of IMO

To determine the biological properties of IMO, namely: the content of *Pseudomonas* sp, *Bacillus* sp, *Streptomyces* sp, *Azotobacter* sp, Actinomycetes and P solubilizing microbes, each 500 ml of samples of the three types of IMO were packed in dark plastic bottles and sent to Environmental Biotechnology Laboratory, PT Biodiversitas Biotechnology Indonesia in Bogor.

The identified species of the microbes were selected from the types of microbes contained in the EM-4 bio fertilizer which were considered to play an important role in plant growth. (Kumar and Gopal, 2015) stated that the term ‘‘indigenous microorganisms’’ referred to a group of beneficial microbes that were native to the area; so the name indigenous meant locally existing, or not imported. On the other hand, EMs or effective microorganisms referred to a laboratory-cultured mixture of microorganisms. Although IMOs were naturally made, while EMs were man-made, these two types were basically the same in many aspects. That was the main consideration that the microbes found in EM were used as a reference to determine the species or groups of microbes expected to be found in IMOs.

Observation of Peanut Growth and Yield

In order to observe the effect of IMO types on the growth and yield of peanut plant, the three IMO types were applied to peanut plants grown on Ultisol Simalingkar. The study used a factorial randomized design with 3 replications and 2 treatment factors, namely the types of IMO and the doses of chicken manure. The types of IMO used were: IMOs sourced from pineapple, tamarillo and orange peels, while the doses of chicken manure were: 0, 10, 20 and 30 tons/ha. The variables observed were: plant height and stem diameter at the age of 6 WAP, as well as

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the number of filled pods and dry seed production per plot. To determine the effect of the treatment, the data were analyzed using analysis of variance. The treatment that had a significant effect was then analyzed using the Duncan's mean difference test. In this article, what would be discussed was the effect of IMO types on the growth and production of peanut plants.

The steps of the research at the field were as follows:

a. Land preparation: The land was cleared from weeds and was processed using a hoe to a depth of 20-30 cm, then plots of the size of 150 cm x 100 cm were made, with a bed height of 40 cm, then the surface of the beds was loosened and leveled. The distance between plots/beds was 50 cm and the distance between blocks was 100 cm.
b. Planting: Prior to planting, seeds of high-yielding peanut varieties of Gajah were selected by soaking them; the submerged seeds were selected. Planting was done using a sharpened wooden stick. The planting hole depth was 3-5 cm with a spacing of 25 cm x 25 cm. The seeds were put into the planting hole, then the hole was covered with loose soil. In each planting hole, 2 seeds were planted and after 2 WAP, the better plant was selected. With a spacing of 25 cm x 25 cm, the number of rows per plot was 6 rows, the number of plants per row was 4 plants, so 24 plants/plot were obtained. The number of sample plants per plot was 5 plants.
c. Treatment application: The dose of chicken manure was 20 tons/ha and was applied 1 week before planting by spreading it evenly on the surface of the plot, and then covered it with soil. The concentration of IMO was 45 ml/liter of water, given at 1 week before planting, 1 WAP, and 2 WAP. After being dissolved in water (concentration of 45 ml/liter of water), each type of IMO was added to the water sprinkler, then sprinkled evenly over the soil surface in the plot until it was wet. The required volume of the solution was measured by the calibration method using fresh water.
d. Plant maintenance: Plant maintenance included: watering every morning and afternoon, weeding at 3 and 6 WAP and controlling plant pests and diseases manually which was started at 3 WAP with one week intervals.
e. Harvesting: Harvesting was done on 86 days after planting. Harvesting was done by pulling the plant at the base of the stem carefully. To facilitate harvesting, the land surface was watered first.

RESULTS AND DISCUSSION

Identification of Microbial Composition in IMO Solution Made of Tamarillo, Pineapple and Orange Peels

The IMO solution of tamarillo, pineapple and orange peels showed the physical properties of a yellowish color and a typical odor of alcohol. The IMOs biological property of the total population of bacteria and fungi was analyzed using Plate Count method, and the results were presented in Table 1.

Table 1. Microbial Composition in IMO Solution Made of Tamarillo, Pineapple and Orange Peels

No	Mikrobes	Method	Unit	Types of IMO		
				Tamarillo Peels	Pineapple Peels	Orange Peels
1.	*Pseudomonas* sp	Plate Count	CFU/ml	3.34×10^{7}	2.50×10^{2}	$< 1 \times 10^{*}$
2.	*Azotobacter* sp	Plate Count	CFU/ml	2.55×10^{2}	$1.40 \times 10^{2**}$	$1.05 \times 10^{2**}$
3.	*Bacillus* sp	Plate Count	CFU/ml	1.89×10^{3}	$1.80 \times 10^{2**}$	1.78×10^{3}
4.	*Actinomycetes*	Plate Count	CFU/ml	$< 1 \times 10^{*}$	$< 1 \times 10^{*}$	$< 1 \times 10^{*}$
5.	*Streptomyces* sp	Plate Count	CFU/ml	$< 1 \times 10^{*}$	$< 1 \times 10^{*}$	$< 1 \times 10^{*}$
6.	P solubilizing microbes	Plate Count	CFU/ml	1.86×10^{7}	1.99×10^{7}	$< 1 \times 10^{*}$

Notes :
*) Number of colonies less than one time of the lowest dilution level (No growth at the lowest dilution level)
**) Total colony count outside less than 25 - 250 CFU/ml

Indigenous microorganisms (IMO) contained not a single but a multiple cultures of beneficial microorganisms; then mixture of different good microorganisms were living together in harmony with the surrounding nature (Kumar and Gopal, 2015). It was predicted that the microorganisms contained in the tamarillo, pineapple and orange peels were more than the six types that had been identified.

Based on Table 1, six types of beneficial microbes in the form of bacteria and actinomycetes were identified in both tamarillo, pineapple and orange IMOs. For the sake of discussion, the total for each microbe was simplified as shown in Table 2.

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Table 2. Simplification of the Total Microorganism Results in the Three Types of IMO

No	Microbes	Types of IMO		
		Tamarillo Peels	Pineapple Peels	Orange Peels
1	*Pseudomonas* sp.	+++++++	++	<<
2	*Azotobacter* sp.	++	+	+
3	*Bacillus* sp.	+++	+	+++
4	*Actinomycetes* sp.	<<	<<	<<
5	*Streptomyces* sp.	<<	<<	<<
6	P solubilizing microbes	+++++++	+++++++	<<

Indigenous microorganisms (IMOs) were a group of indigenous microbial community that lived in the soil and on the surfaces of living things that had the potential ability in organic matter decomposition, nitrogen fixation, soil fertility improvement and in plant growth hormones production (Kumar and Gopal, 2015).

In the three types of IMOs several beneficial microbes in agriculture were identified, namely: *Pseudomonas* sp., *Azotobacter* sp., *Bacillus* sp., *Actinomycetes* sp., *Streptomyces* sp. and P (phosphate) solubilizing microbes. The functions of the microbes were as follows:

1. Organic matter decomposer/soil fertilizer or Plant Growth Promoting Rhizobacteria, namely *Pseudomonas* sp., *Bacillus* sp., *Actinomycetes* sp. and *Streptomyces* sp.)
2. Nitrogen fixation, namely *Azotobacter* sp., *Pseudomonas* sp. dan *Bacillus* sp. which were classified as non-symbiotic nitrogen fixers.
3. P- solubilizing microbes, whose function was to release P adsorbed in soil colloids, namely, *Pseudomonas* sp., *Bacillus* sp. dan *Actinomycetes* sp.
4. Biological control/protecting plants from diseases, namely *Pseudomonas* sp. dan *Bacillus* sp (Nasahi, 2010).

The identification of microorganisms content in IMO solution was carried out by several researchers. (Mahmuda et al., 2020) identified microbes in IMO made of beef rumen contents, gold snails, banana roots, and bamboo shoots in the form of *Rhizobium* sp. and Phosphate Solubilizing Bacteria. In banana roots IMO there were *Bacillus* sp., *Aeromonas* sp. and *Aspergillus niger*, in the golden snail IMO there were *Staphylococcus* sp. dan *Aspergillus niger*, while rabbit urine IMO contained *Bacillus* sp., *Rhizobium* sp., *Pseudomonas* sp., *Aspergillus niger* and *Verticillium* sp. (Suhastyo et al., 2013). (Astriani et al., 2020) found that *Lactobacillus plantarum* isolated from the IMO of cow rumen in East Java was identified as one of the phosphate solubilizing bacteria that was of a good potential in developing bio fertilizer. The application of phosphate solubilizing microbes can be used to increase the soil fertility.

In the three types of IMO the same six types of microbes were found, but in the different rate of density (Tables 1 and 2). IMO of tamarillo had microbial colonies of *Pseudomonas* sp., *Azotobacter* sp., *Bacillus* sp. and P Solubilizing Microbes with the largest number (10^2 to 10^7 cfu), followed by IMO of pineapple, and then IMO of orange. *Actinomycetes* sp. and *Streptomyces* sp. were found in all three IMO solutions and both were in a very small amounts, but they were still able to grow and reproduce when the abiotic environment supports such as temperature, humidity, pH and food were available. The conditions of temperature and humidity as well as the availability of food/energy sources from chicken manure supported the growth of these bacteria, fungi and actinomycetes. As stated by (Miller and Donahue, 1990), there were many factors that influenced the activity of soil microorganisms. Climate, soil and vegetation were the strong factors. It was not easy to determine the conditions that were suitable for all microbes in the soil because each group of the microbes had different growth requirements. Generally, many microbes developed well in soils with the field capacity of moisture, pH around neutral, high nutrient content, and temperatures around 30^{o}C.

Effect of The Types of IMO on Peanut Growth and Yield

To study the effect of different types of IMO on the growth and yield of peanuts, a study was conducted with the treatment of IMO types and doses of chicken manure. The results of the analysis of variance showed that the IMO type treatment had no significant effect on all observed variables. The effect of IMO types on the mean plant height and stem diameter at 6 WAP as well as the number of filled pods per plant and dry seed production per plot were presented in Table 3.

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Table 3. The Effect of Types of IMO on Average Growth and Yield of Peanut

Types of IMO (45ml/liter air)	Average Growth and Yield of Peanut Plant			
	Plant Height at 6 WAP (cm)	Stem Diameter at 6 WAP (mm)	Filled Pod (pods/plant)	Yield Per Plot (g/plot)
M_1 (Pineapple)	38,09	3,99	97,83	174,60
M_2 (Orange)	38,35	4,30	96,91	179,63
M_3 (Tamarillo)	38,28	4,23	98,08	181,03

The differences in the types of IMO and the number of colonies in the three types of IMO solutions had no different effect on growth and yield of peanut. Both microbes with small or large numbers were able to grow and multiply (proliferate) in a suitable environment. It was proven that IMO with any type of fruit peels material from the three IMOs had the same good results when applied to peanut plants (Table 4). IMO of tamarillo, pineapple and orange peels had the same benefits. This indicated that the environment during the growing season was suitable for the beneficial microorganisms to grow and play their roles effectively. According to Kumar and Gopal (2015), the rate of biodegradation potential was affected by many environmental factors, both physical and chemical ones. Some of the factors were temperature, pH, organic matter content, and the availability of oxygen and nutrients.

Azotobacter sp bacteria had a dual role as a provider of N and as promoter of plant development by producing growth hormones. *Azotobacter* was an N-fixing bacterium capable of producing growth-promoting substances of gibberellins, cytokinins, and indole acetic acid, so that they stimulate root growth.

The genus of *Bacillus* had several abilities, such as: to decompose organic compounds, produce antibiotic compounds, involve in nitrification and denitrification and also were included in the group of phosphate solubilizing bacteria. In addition, it was shown that *Bacillus* sp. had a potential as a good biological control agent against pathogenic bacteria, *Ralstonia solanacearum* for example (Soesanto, 2008).

Phosphate solubilizing microorganisms consisted of bacteria, fungi and a few of actinomycetes. Microorganisms belonged to the group of phosphate solubilizing bacteria included *Pseudomonas striata, P. diminuta, P. fluorescens, P. cerevisia, P. aeruginosa, P. putida, P. denitrificans, P. rathonis, Bacillus polymyxa, B. laevolacticus, B. megatherium, Thiobacillus* sp. The fungi that dissolved phosphate generally came from the Deuteromycetes group, such as *Aspergillus niger, A. awamori, Penicillium digitatum, P. bilaji, Fusarium, Sclerotium,*, and others. The dominant phosphate solubilizing fungi in soil were *Penicillium* and *Aspergillus.* The dominant phosphate solubilizing fungi found in Indonesian acid soils were *Aspergillus niger* dan *Penicillium* (Ginting et al., 2011).

The availability of different microorganisms had a better effect on plant growth. Co-inoculation of *Bacillus megaterium var. phosphaticum* and *Bacillus mucilaginosus* consistently increased K and P mineral availability and the nutrients uptake and, consequently, the growth of pepper and cucumber plants (Han et al., 2006). Kumar et al. (2012) reported that a combination of two *Fluorescent pseudomonas* strains increased *Vigna mungo* yield by 300 % compared to the control crop. These results indicated that a combination of beneficial microorganisms increased the nutritional status of plants and soils.

The good effects of IMO due to the presence of various microbes that benefit the plant were shown by peanut production that exceeded the potential yield. According to Balitkabi (2016), peanut productivity in Indonesia in 2016 was 13.21 ku/ha. In this study, the yield of peanut per plot due to IMO of pineapple, orange, and tamarillo was 174.60, 179.63 and 181.03 g per plot, or 3.49, 3.58 and 3.61 tons/ha, respectively. This yield exceeded the potential production of peanuts for the Gajah variety, which was 1.8 t/ha (Balitkabi, 2016), and the increase was as high as 197 percent or almost doubled. In addition to satisfactory quantity, the quality of pods and seeds was also very good (the number of filled pods was >95 pods/plant, and each pod generally contained three seeds, the seeds were pithy and crunchy, sweet and savory).

Although there was a satisfactory yield in using a concentration of 45 ml/L of different types of IMO, the research did not study the difference between control and the IMO treatments. A research to study the effect of different concentration of IMO was also needed, since the concentration used in the study was relatively low compared to the concentration used on other plants, such as 300 ml/L in soybean (Zamriyetti et al., 2021) and 180 ml/L in raddish (Barus et al., 2020).

The improvement in quantity and quality of the peanut yield may due to the following roles of the IMOs (Kumar and Gopal, 2015):

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1. Protecting the normal host from invasion by causing disease microorganisms. There was a competition between the IMOs and the pathogens for essential nutrients that would repress the growth of the pathogens. The IMOs also produced inhibitory substances that had the same negative effect on the pathogens.
2. Increasing the availability of nutrients to host plants
3. Increasing the water-holding capacity that ensure the suitable soil moisture for the host plants all the time
4. Improving the aeration to the plant roots so that the exchange of gases occurs effectively
5. Preventing soil erosion.

Furthermore, Kumar and Gopal (2015) concluded that IMOs created the optimum and favorable environment for soil flora and soil fauna to develop well, which in turn would support the quality life of higher plants and animals including the human. The soil structure was improved to provide sufficient air and water, which in turn created a suitable environment for other symbiotic microbes.

Based on the result of this study, it is recommended to use IMOs, no matter the fruit peels sources of the IMOs. The natural farming method in growing peanuts resulted in a high yield and good quality of peanuts. Using no chemical fertilizers and pesticides during the growing season helped in creating a better environmental condition for microorganisms growth and activity. (Pujiastuti et al., 2018) stated that the use of pesticides during the experiment on kale might limit, or even terminate, the activity of some or all of the microbes applied through EM-4 addition. Miller and Donahue (1990) added that when organic fertilizers ware added to the soil, it would correct many soil problems at the same time. The organic fertilizers played a role in: supplying organic matter and plant nutrients, providing appropriate moisture and air movement in the root area, and acting as micro nutrient carriers. According to Kumar and Gopal (2015), the benefits of using the Natural Farming methods include the following:

1. Lower costs by as much as 60 percent, meaning saving cost for the farmers
2. Better crops, and stronger, healthier and more nutritious plants
3. Higher and better quality of yield
4. Farmer and consumer friendly
5. Zero waste emission.

The inputs were made from organic materials, which were friendly to the farmers, consumers and environment, and activate and rehabilitate the ecology as well.

CONCLUSIONS

1. IMO derived from various fruit peels contained the same number of microbes but with different densities.
2. IMO derived from various fruit peels had the same effect on plant height and stem diameter at 6 WAP as well as on the number of filled pods and dry seed production of peanuts.
3. IMO sourced from pineapple, tamarillo and orange peels both had the same good effect on the growth and production of peanuts. Peanut production reached 197% of the potential production.

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