EFFECT OF ORGANIC FERTILIZERS ON SUSCEPTIBILITY OF POTTED DURIAN SEEDLINGS TO *Phytophthora* DISEASES

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A B S T R A C T

This research was aimed on evaluating the effect of organic fertilizers on susceptibility of durian seedlings to *Phytophthora palmivora*. The green compost, chicken manure and rice husk, alone or in a mixture, significantly increased seedling survival. Therefore, such growing media composition should be taken into account as one of the elements of integrated *Phytophthora* control in durian. On the other hand, goat manure has a ruinous effect on the growth and survival of durian seedlings and shall be avoided in durian orchards.

Key words: *Phytophthora palmivora*, durian, organic fertilizers, control

INTRODUCTION

Durian is one of the important fruit in Indonesia. Its production has risen as much as 42.4% during 4 last years; from 525.064 tons in 2002 to 747.848 tons in 2006 (Anonymous, 2007). However, even though this country is one of the biggest durian producers in the world, it was reported that Indonesia imported 11.086 tons of this fruit at a cost of 11.730.903 US$. This indicates that Indonesia is still open for durian market and for developing durian orchards.

There are several constraints in developing durian orchard in Indonesia. One of the most important is *Phytophthora palmivora.* This soilborne pathogen causes many diseases in various tissues of durian, including

root rot on root, collar rot/foot rot and patch canker on stem, leaf blight on leaf, and fruit rot on fruit. (Lee and Lum, 2004). When *Phytophthora* attacks roots, the symptom will apear on upper parts of the plant such as leaf yellowing, particularly at the shoot tips, leaf shed, slowed plant growth, and possibly limb dieback occurring at early stages of the disease (Hagan, 2008).

This disease destroyed many durian orchards in Indonesia. This can be indicated by very small supply of superior durian fruits on the market even though the farmers have planted many superior durian varieties. According to Drenth and Sendall (2004), this pathogen caused losses in durian orchards in Indonesia varying from 20-25% with economic value about US$ 780.000.000. The pathogen also destroyed up to 30% durian orchards in Penang, Malaysia (Hasan and Siew, 2000) and up to 54% in Australia (Zappala et al., 2002).

Integrated management of *P. palmivora* could be reached by incorporating several control measures, such as planting resistant varieties, biological control, manipulation of the environment by reducing humidity in orchards through pruning, weeding and improving soil drainage (Purwantara et al., 2004). Poor aeration of the growth media may promote the growth of *Phytophthora* and increase vulnerability of plant to infection as well. All *Phytophthora* species grow better under water lodging. In this condition the spores germinate and produce sporangia that release zoospores which are moving actively or passively in water toward the infection zone (Drenth and Guest, 2004; Linderman, 2003; Ristaino and Johnston, 1999).

Some wild species of durian were reported to be resistant to root rot (Shamsudin et al., 2000; Kanzaki et al., 1997). Hence, these materials should be used as durian rootstock. Durian varieties with good quality are then grafted onto these rootstocks to produce marketable durian trees that are resistant to *P. palmivora*. Meanwhile, biological control can be applied by using antagonist fungi like *Gliocladium* sp., *Trichoderma* sp., *P. fluorescens*. This measure can also be incorporated with other control measures to control *P. palmivora* (Drenth and Guest, 2004; da S. Costa et al., 2000).

Phytophthora palmivora can also be controlled by using organic fertilizers such as manure and compost because they can stabilize soil pH and be appropriate for developing antagonistic microorganism (Liu et al., 2007; Drenth and Guest, 2004; Linderman, 2003; Aryantha et al., 2000). Organic matters can induce systemic resistance in plants due to the presence of microorganisms in composts (Hoitink et al., 2000). It has been reported that certain plant growth promoting microorganisms (PGPM) could enhance defensive activity and stimulate plant resistance against soil borne pathogens (Kilic-Ekici and Yuen, 2003; Zheng et al., 2005)

It is important to choose the appropriate organic fertilizer for controlling disease because composition of organic matter has important effect on

development of plant diseases. Hoitink et al. (2000) stated that each type of compost has its own properties that must be considered during its utilization.

The aim of the research was to evaluate the effect of organic matter composition in the growing media on the control of *P. palmivora* infecting durian seedling.

MATERIAL AND METHODS

The research was conducted in the screen house of Indonesian Tropical Fruit Research Institute from January to December 2007.

Material

Durian seedlings of five cultivars were planted in the plastic pots (10 l in volume). The experiment was arranged in a completely randomized design consisted of 12 treatments and 10 replications. The five unit samples were used in each replication. The treatments, various media compositions for potted durian seedlings, were as follows:

1) Soil + cow manure (1 : 1).
2) Soil + goat manure (1 : 1).
3) Soil + chicken manure (1 : 1).
4) Soil + green compost (1 : 1).
5) Soil + rice husk (1 : 1).
6) Soil + compost + cow manure (2 : 1 : 1).
7) Soil + compost + goat manure (2 : 1 : 1).
8) Soil + compost + chicken manure (2 : 1 : 1).
9) Soil + compost + cow manure + rice husk (3 : 1 : 1 : 1).
10) Soil + compost + goat manure + rice husk (3 : 1 : 1 : 1).
11) Soil + compost + chicken manure + rice husk (3 : 1 : 1 : 1).
12) Control (soil without organic matter).

The organic matters used in this experiment were the materials available for farmers and usually used for fertilizing their orchards.

Preparation of *P. Palmivora* inoculum and plant inoculation

P. palmivora used in the experiment was S-20-Sut strain from a collection of Indonesian Tropical Fruit Research Institute, isolated from durian root infected by *P. palmivora* in North Sumatra. The postulate Koch had also been tested to ensure that the pathogen gave the same symptom on the tested durian seedlings.

The isolate was cultured on PSM (*Phytophthora* Selective Medium) for 7 days. To multiply and sporulate the fungi, 15 Petri dishes (10 cm in diameter) of *P. palmivora* culture were mixed with 1 litre of papaya juice and shaken for 2 days to get density of spore suspension 10^7 spore/cm^3 (counted with haemocytometer). Two-month-old durian seedlings were then inoculated with 10 ml/pot of such prepared *P. palmivora* suspension by soil drenching.

Soil sampling and *Phytophthora* baiting

Soil sampling was done every month to observe the dynamic of

Phytophthora and other microbes as well in each media. Twenty five grams of media were taken from each replication and the samples taken from the same treatment were bulked. To analyse the dynamic of *Phytophthora* growth in each treatment, the baiting was done using apple fruit. The procedures of *Phytophthora* isolation from the soil were as follows: 1) The surface of apple fruits was sterilised with 70% alcohol and then air dried; 2) Three holes 5 mm in diameter were made in each apple using cork borer, where each hole represented a replication; 3) Three grams of soil sample were put into each hole and covered with cellulose tape; 4) The apples with soil samples inside were then incubated at room temperature for 3 days, 5) After incubation, usually rot symptom appeared on the edge of the inoculated holes. The edge of the rot symptom on the apple was sliced, inoculated on PSM medium and incubated for 3-5 days; eventually the cultures were observed to investigate the presence of *Phytophthora*.

Observations were also made on the presence of other microorganisms as well. For this purpose, the soil samples were cultured on the PDA medium for fungi and on the NA medium for bacteria. The procedures were as follows: 1) Ten grams of soil sample were mixed with 90 cm^3 of sterile distilled water and the mixture was gently shaken 2) One cm^3 of soil/water suspension was taken out and put into test tube containing 9 ml distilled water. The suspension was then diluted three times in a proportion of 1 : 10 to obtain final dilution 10^{-3}, 3) One cm^3 of diluted suspension was put into 9 cm Petri dish and 10 cm^3 PDA or NA media were added. After incubation at 26°C for 5 days, the morphology of developed cultures was observed under microscope in order to determine their affiliation to systematic groups.

Observations were done on**:**

1. The density of microbial population. The density of bacteria was determined based on the number of colonies per gram of soil sample (Schaad et al., 2001) while the density of the fungi was determined based on the number of spores per gram soil sample (Barnet, 1962).
2. The number of plants showing *Phytophthora* symptoms (die back, yellowing of the foliage, leaf shed) and/or dead plants due to *Phytophthora.*
3. The plant growth rate (number of leaves, diameter of stems, height of plants). The observations were made at 2 week intervals.

Statistical analysis. The data were subjected to analysis of variance and means were separated by LSD test at $p \leq 0.05$. The relationships between the parameter observed were analyzed by correlation analysis ($p \leq 0.05$) followed by analysis of regression ($p \leq 0.05$). The regression model showing the highest R^2 was chosen as the fitted model. All calculations were performed using the statistical program SPSS 2000.

RESULTS AND DISCUSSION

Addition of goat manure to the growing media resulted in a strong increase of durian seedling mortality (Tab. 1). This phenomenon may be explained by the lowest microbe diversity in the media containing goat manure. The regression analysis showed that there is a significant positive correlation between the number of microbes present in the media and the survival rate of the seedlings (Fig. 1).

The high microbe diversity present in the media tended to promote high competition among the microbes, hence, the ability of *Phytophthora* to infect plant became lower. This fact is in accordance with the principle of biological control of *Phytophthora*, that the improvement of the environment, especially of the rhisosphera, is important to support the antagonistic microorganisms in suppressing *Phytophthora* infecting the plants. Adding organic matter to the soil is the key factor to stimulate antagonistic activity (Linderman, 2003; Bulluck et al., 2002).

The use of goat manure mixed with soil for durian media not only caused the highest mortality of durian seedling but also was unfavourable for their growth. The most favourable media for durian growth was the soil mixed with green compost, chicken manure, and rice husk, since the highest of leaves number, stem diameter and plant height as well as 100% seedling survival were observed in these treatments (Fig. 2-4). This might be explained by the fact that the better growth of plant led to the better defence against pathogen infection (induced resistance). Similar results were obtained by Tan et al. (2008) who reported that the incorporation of chicken manure into the potting mix resulted in enhanced survival of *P. palmivora*-infected papaya plants, stimulation of root regeneration, and reduction of *P. palmivora* to undetectable levels within 4 weeks. The enhanced survival of infected papaya and durian plants grown in chicken manure-amended potting mix was attributed to the higher levels of antagonistic microorganisms, such as *Actinomycetes*, which led to the suppression of the pathogen.

There are several ways in which organic matters in the soil can indirectly control *Phytophthora*, namely: 1) Increasing the activity of the indigenous microflora, resulting in suppression of pathogen population through competition or specific inhibition (Konam and Guest, 2002; Broadbent and Baker, 1974); 2) Releasing degrading compounds such as carbon dioxide, ammonia, nitrites, saponins or enzymes that are generally toxic to *Phytophthora* (Tsao and Oster, 1981); 3) Acting as a trap, since *Phytophthora* will be attracted to and encyst on organic matter (Grant et al., 1985); 4) Inducing plant defence mechanisms (Gilpatrick, 1969); and 5) Creating an environment that stimulates root development by physically inhibiting *Phytophthora* (Turner and Menge, 1994).

Experiments with the soil baiting have shown that the organic matters

Table 1. Effect of growth media composition on survival of durian seedlings

Treatment	Mean percentage of dead plant [%]
1. Soil + cow manure (1 : 1)	2 a*
2. Soil + goat manure (1 : 1)	18 b
3. Soil + chicken manure (1 : 1)	0 a
4. Soil + green compost (1 : 1)	0 a
5. Soil + rice husk (1 : 1)	0 a
6. Soil + compost + cow manure (2 : 1 : 1)	4 a
7. Soil + compost + goat manure (2 : 1 : 1)	4 a
8. Soil + compost + chicken manure (2 : 1 : 1)	2 a
9. Soil + compost + cow manure + rice husk (3 : 1 : 1 : 1)	0 a
10 Soil + compost + goat manure + rice husk (3 : 1 : 1 : 1)	0 a
11. Soil + compost + chicken manure + rice husk (3 : 1 : 1 : 1)	0 a
12. Control (soil without organic matter)	4 a

*Means followed by the same letter are not significantly different by LSD test at $p \leq 0.05$

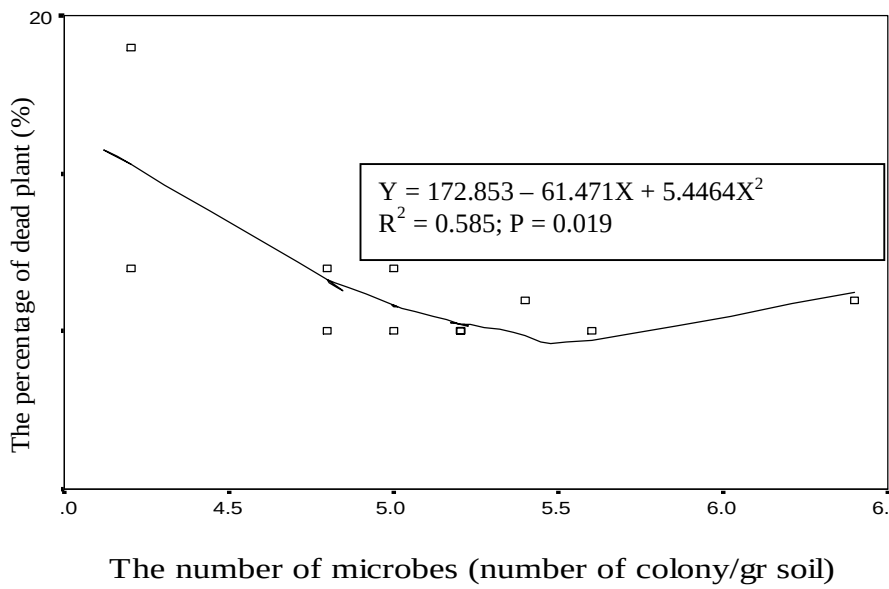

Figure 1. Relationship between the number of microbes in the growing media and the survival of durian seedlings

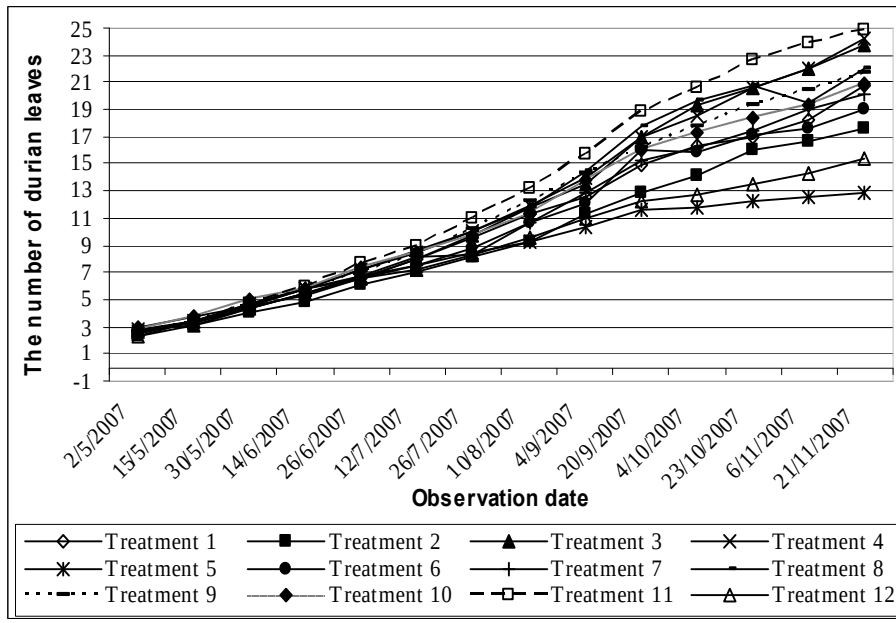

Figure 2. Effect of growth media composition on development of leaves on durian seedling

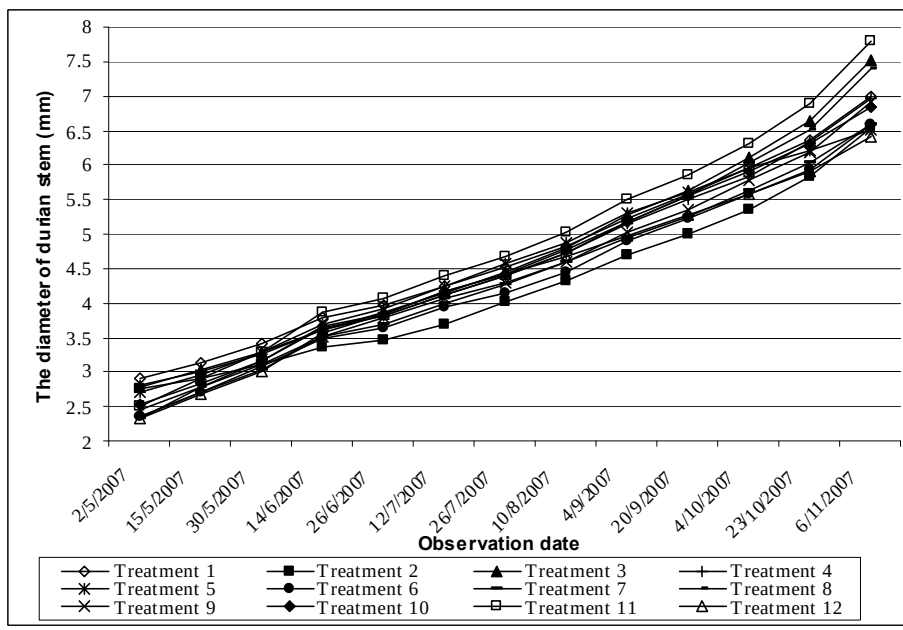

Figure 3. Effect of growth media composition on stem diameter of durian seedlings

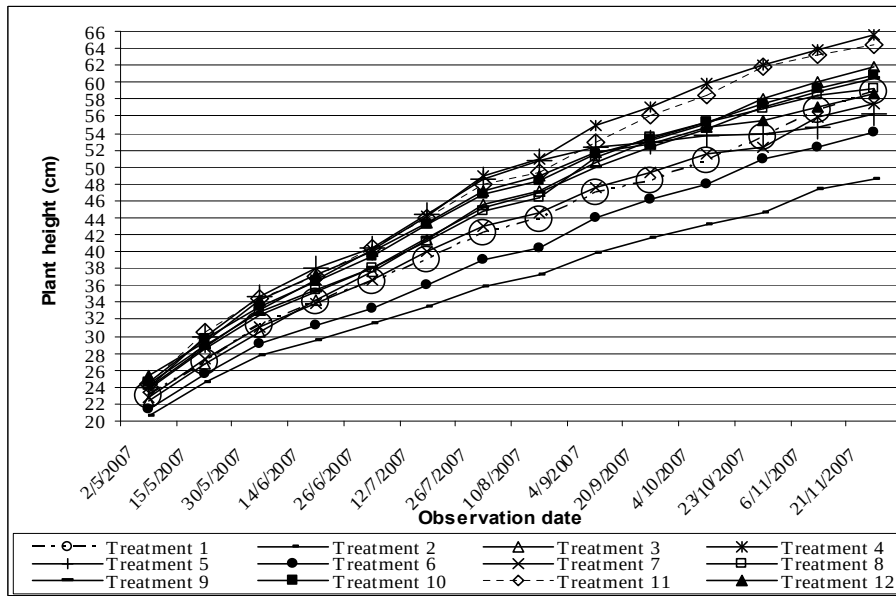

Figure 4. Effect o growth media composition on height of durian seedlings

Table 2. *Phytophthora* propagules isolated from growth media before and after artificial inoculation

Treatment	Propagule incidence				
	one month before inoculation	after inoculation (months)			
		1	2	3	4
1	–	+	+	–	–
2	–	+	+	–	–
3	–	+	+	–	–
4	–	+	+	+	–
5	–	+	–	–	–
6	–	+	+	–	–
7	–	–	+	–	–
8	–	+	+	–	–
9	–	+	+	–	–
10	–	+	+	–	–
11	–	+	+	–	–
12	–	+	+	–	–

Note: – = absent; + = present

did not affect the *Phytophthora* incidence in the soil (Tab. 2). However, the presence of organic matter could generate a favourable condition for increasing diversity of microorganism in the soil, which in turn hinters *Phytophthora* activity due to competition mechanism. In other words,

in the suitable plant growth media, *Phytophthora* does not threaten the plant because of existing competitors and increased plant defence reaction.

CONCLUSION

1. Growing media supplemented with compost, chicken manure and rice husk were the best compositions for promoting durian seedling's growth and limiting the incidence of *P. palmivora*-related plant mortality. This composition is, therefore, recommended as a component of the integrated durian orchard management components.
2. Goat manure has a ruinous effect on the growth of durian seedling and does not provide protection against *P. palmivora* attack.

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WPŁYW NAWOŻENIA ORGANICZNEGO NA WRAŻLIWOŚĆ SIEWEK DURIANA NA CHOROBY POWODOWANE PRZEZ *Phytophthora*

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S T R E S Z C Z E N I E

Celem badań było określenie wpływu różnego organicznego nawożenia na wrażliwość siewek duriana na *Phytophthora palmivora*. Kompost z roślin, nawóz kurzy i łuski ryżu, zastosowane osobno lub w ich mieszankach, istotnie podwyższały zdrowotność siewek duriana. Tak więc te nawozy organiczne powinny być wzięte pod uwagę jako czynniki integrowanej ochrony siewek duriana przed *Phytophthora*.

Słowa kluczowe: *Phytophthora palmivora*, durian, nawozy organiczne, ochrona