Effects of *Piper betel* Leaf Powder on Tomato Seedling Emergence, Seedling Growth and *Pythium* Mycelial Growth

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Key words: *Piper betel*, *Pythium*, Tomato

Summary

This study investigated the most optimal mixing ratio of *Piper betle* leaf powder when mixed with peat moss for tomato seedling growth and inhibition of tomato *Pythium* mycelial growth. *Piper betel* leaves were collected from Horticulture Research Station, College of Agriculture and Natural Resources, National Chung Hsing University. *Piper betel* leaf powder and peat moss were mixed in ratio of 0%, 1.25%, 2.5% and 5% (ml/ml), respectively to test its effect on tomato seedling emergence rate and growth. Our results showed that growth substrates containing 5% and 2.5% of *Piper betle* leaf powder decrease seedling emergence rate. Seedling emergence rate was not significantly different between treatment with 1.25% and 0% *Piper betle* leaf powder. *Pythium* was inoculated in potato dextrose agar (PDA) that treated with 0%, 0.1%, 0.5%, and 1% *Piper betel* leaf powder, respectively to determine its mycelial growth. Results from this study indicated that mycelial growth of *Pythium* was most inhibited on PDA treated with 1% of *Piper betle* powder with a mycelial growth rate of 0.39 mm/hr and a mycelial growth inhibition rate of 78% after 48 hr of inoculation. *Pythium*-infested peat moss was mixed with *Piper betel* leaf powder at mixing ratios of 0%, 0.1%, 0.5%, and 1% (ml/ml), respectively. Our results demonstrated that the highest seedling emergence rate, plant height and root length were recorded at the mixing ratio of 0.5% compared to those of 0%, 0.1% and 1%. Taken together, results from this study suggested that peat moss containing 0.5% *Piper betle* leaf powder may reduce the inhibitory effects of tomato *Pythium* on tomato seedling growth.

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Introduction

Piper betle belongs to the family *Piperaceae* and is normally named as Phlu in Thai, Sirih in both Malaysian and Indonesian, Paan in both Indian and Bangladesh and Betle in English (Mahfuzul *et al*., 2011). *Piper betle* has more than 700 species around the world. They were found around Asia and East African countries (Sripradha, 2014).

Piper betle is normally used as an oral medicine by wrapping with betle nut for oral hygiene. The *betle* powder is treated as herb medicine on symptom and disease such as carminative, aphrodisiac, stimulant, analgesic and cooling properties, antiseptic, wound healing, anti-parasitic, antifungal, and antibacterial agent (Caburian and Osi, 2010). The leaf is also useful for the treatment of various diseases such as bad breath, boils, and abscesses, ulcers, conjunctivitis, constipation, headache, hysteria, itches, mastitis, mastoiditis, leucorrhoea, catarrh, diphtheria, ringworm, swelling of gum, rheumatism, abrasion, cuts ,and injuries (Chopra *et al*., 1956, Jayaweera, 1982).

Fungal disease often causes tremendous decrease in crop yield and fungal pathogens may remain in soil after harvest. Agriculturist encounters a huge problem when they find incidence of fungal infection in the field. Crude extracts of *Piper betle* were previously reported to have antibacterial and antifungal activities (Bissa *et al.* 2007; Nair and Chanda 2008). However, the effect of leaf powders derived from *Piper betle* on plant pathogenic fungi remains largely unknown. There is a possibility that leaf powders of *Piper betle* may be used as an alternative to suppress fungal disease in the field and to improve farmers' income. Therefore, this study aims to find the most suitable mixing ratio of *Piper betle* leaf powder mixed with peat moss, to observe the effect of *Piper betle* leaf powder on *Pythium* mycelial growth, and to test the potential of using *Piper betle* leaf powder containing peat moss as an antifungal agent to reduce the inhibitory effect of *Pythium* on tomato seedling growth.

Materials and Methods

1. Plans materials

Piper betle leaves were collected from Horticulture Research Station, College of Agriculture and Natural Resource, National Chung Hsing University. *Piper betle* leaves were incubated in 45 °C until water was eliminated. Dry *piper betle* leaves were then ground to powder by a grinding machine and stored at 1°C

2. Evaluation of tomato seedling emergence rate and seedling growth

Growth substrates for tomato seedling emergence rate and seedling growth tests were prepared by mixing peat moss with four different proportions of *piper betle* leaf powder with final concentrations of 0%, 1.25%, 2.5% and 5% (ml/ml), respectively. Tomato seeds were planted on the above-mentioned mixed substrates. Tomato seedling emergence rate, plant height and root length were measured one week after planting.

3. Preparation of *Piper betle* leaf powder-containing PDA

Two hundred grams of unpeeled potato was cut into small cubes, mixed with one liter of distill water and boiled for 30 minutes, then filtered through cheesecloth. The effluent was saved as potato infusion.

One liter of potato infusion was mixed with 20 g sucrose and 20 g agar, then incubated in autoclave at 121 °C for 15 minutes. Potatoes dextrose agar (PDA) was treated with 4 different proportions of *Piper betle* leaf powder at final concentrations of 0%, 0.1%, 0.5% and 1%, respectively by dilution and dispensing into petri dishes.

4. Measurement of *Pythium* mycelial growth

Pythium was obtained from the Department of Horticulture, Naitonal Chung Hsing University. The pathogenic fungus was sampled by cutting from the margin of an actively growing fungal culture and put on the above-mentioned PDA media. *Pythium* mycelial growth was defermined daily by measuring the diameter of the fungal colony until mycelium spread throughout the plate.

5. Preparation of *Pythium*-infested and *Piper betle* leaf powder-containing peat moss for measurement of tomato seedling emergence rate and seedling growth tests

One-kilogram peat moss was added with 200 ml distill water in a sterile plastic bag and then autoclaved for sterilization. After the temperature of the peat moss decreased, the sterile peat moss was infested with *Pythium*.

Pythium-infested peat moss was mixed with 4 different proportions of *Piper betle* leaf powder with final concentrations of 0%, 0.1%, 0.5% and 1% (ml/ml), respectively then incubated at 25± 2 °C for 1 week. After that, tomato seeds were planted in the growth substrate. Seedling emergence rate, plant height and root length were recorded two weeks after planting.

6. Statistical analysis

The statistical analysis was performed by using SAS 9.0 (Institute Inc., 2000) and the data was subjected to one-way analysis of variance (ANOVA) for a completely randomized design (CRD) statistical model. Mean values among treatments were compared by least significant difference (LSD) range test at the 5% ($\rho \leq 0.05$) level of significance.

Results

Our results showed that treatment with 2.5% *Piper betle* leaf powder has the lowest seedling emergence rate and treatment with 1.25% *Piper betle* leaf powder has the highest seedling emergence rate. In addition, no significant difference in plant height was noticed among all treatments. On the other hand, root length is similar between treatment with 1.25% *Piper betle* leaf powder and the control (0%) and a significant reduction in the root length was recorded in treatment with 2.5% and 5% *Piper betle* leaf powder (Table 1). Taken together, peat moss containing 1.25% *Piper betle* leaf powder may be used as a suitable growth substrate for tomato seedling growth.

Results from mycelial growth analysis indicated that mycelial growth of *Pythium* spp. on PDA containing 1% *Piper betle* leaf powder was minimal with a colony diameter of 18.9 mm after 48 hours of inoculation and a mycelial growth rate of 0.39 mm/hr (Table 2).

After 48 hr of inoculation, the inhibition rate of *Pythium* mycelial growth was maxial with a value of 78% on PDA containing 1% of *Piper betle* leaf powder compared to that of 0%, 0.1% and 0.5 % (Table 3, Fig. 1).

Tomato seedlings cultivated in *Pythium*-infested peat moss containing 0.5% and 1% *Piper betle* leaf powder showed higher seedling emergence rate, plant height and root length compared to that of 0 and 0.1%. Treatment with 0.5% *Piper betle* leaf powder had significantly higher root length compared to that of 1%, however, no significant difference on plant height was observed. Treatment with 0.1% of *Piper betle* leaf powder had significantly lower plant height and root

Table 1. Effect of *Piper betle* leaf powder on seedling emergence rate and seedling growth of tomato cultivated in peat moss for one week.

Treatment	Emergence Rate (%)	Plant Height (cm.)	Root Length (cm.)
0%	87.0	3.3±0.3A[z]	5.5±0.5A
1.25%	98.2	3.5±0.1A	5.5±0.2A
2.50%	77.8	3.1±1.0A	3.7±0.4B
5%	87.0	2.6±0.3A	2.9±0.4B

[z]Values with different letters in the same column are not significantly different at $P < 0.05$ by LSD test.

Table 2. Effect of *Piper betle* leaf powder on mycelial growth of *Pythium* spp.

Treatment	Colony Diameter (mm)			Mycelial[y] Growth Rate
	24[z]	48	72	(mm/hr)
0%	63.7A[x]	85A	85A	1.77A
0.1%	62.1B	85A	85A	1.77A
0.5%	40.6C	85A	85A	1.77A
1%	0D	18.9B	18.9B	0.39B

[z] Representing the time period (hr) during *Pythium* inoculation.

[y] Mycelial growth rate was calculated by using the colony diameter (mm) when it first reaches the maximum value divided by the time period (hr) during inoculation.

[x]Values with different letters in the same column are not significantly different at $P < 0.05$ by LSD test.

Table 3. Inhibitory effect of *Piper betle* leaf powder on mycelial growth of *Pythium* spp..

Treatment	Inhibition Rate of Mycelial Growth (%)[z]
0%	0B[y]
0.1%	0B
0.5%	0B
1%	78A

[z] Inhibition rate of mycelial growth (%) = (colony diameter of the control (0%) – colony diameter of the treatment) / colony diameter of the control x 100

[y] Values with different letters in the same column are not significantly different at $P < 0.05$ by LSD test.

Data are collected and calculated 48 hr after inoculation.

length compared to that of 0%, 0.5% and 1%. Treatment with 0.5% *Piper betle* leaf powder displayed the highest seedling emergence rate with a value of 79.63%, follow by 1%, 0% and 0.1% with values of 70.37%, 40.74%, and 22.22%, respectively (Table 4). Overall, these results suggested that peat moss containing 0.5% *Piper betle* leaf powder has the potential to be considered as a suitable growth substrate for tomato seedling growth against tomato *Pythium* infection.

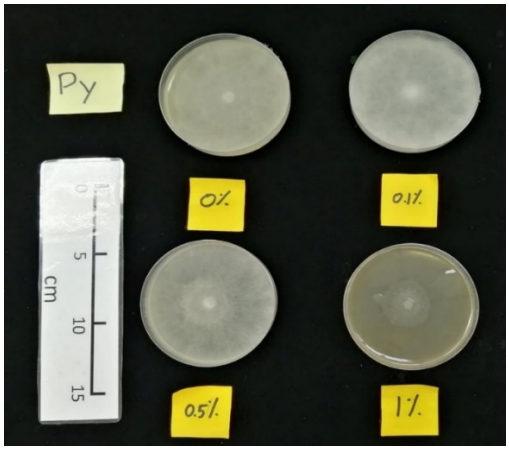

Fig. 1. Mycelial growth of *Pythium* spp. on PDA containing different proportions of *Piper betle* leaf powder. The picture was taken 72 hours after inoculation.

Table 4. Effect of *Piper betle* leaf powder on seedling emergence rate and seedling growth of tomato cultivated in *Pythium*-infested peat moss for two weeks.

Treatment	Emergence Rate (%)	Plant Height (cm.)	Root Length (cm.)
0%	40.74	1.27±0.2B[z]	2.12±0.4C
0.1%	22.22	0.61±0.1C	0.81±0.2D
0.5%	79.63	2.58±0.2A	3.90±0.3A
1%	70.37	2.43±0.2A	3.16±0.3B

[z]Values with different letters in the same column are not significantly different at $P < 0.05$ by LSD test.

Discussion

Singha *et al*. (2011) reported that tomato plants cultivated in soil containing 0% and 1% chloroform extract of *Piper betle* demonstrate no significant difference in plant height and root length. This report is consistent with our results in which peat moss treated with 0% and 1.25% *Piper betle* leaf powder showed no significant difference in plant height and root length (Table 1). Nevertheless, treatment with 1.25% *Piper betle* leaf powder had the highest seedling emergence rate one week after planting among all treatments tested (Table 1) and may be suitable for tomato seedling growth.

On the other hand, results from PDA test indicated that treatment with 1% *Piper betle* leaf powder displays the highest inhibition rate of mycelial growth in *Pythium* (Tables 2, 3 and Fig. 1). These results were similar to Singha *et al*. (2010) who reported that petroleum ether, chloroform and methanol extract of *Piper betle* may inhibit mycelial growth in *Rhizoctonia solani, Fusarium oxysporum* and *Curvularia sp*. Future research may involve various extraction methods of *Piper betle* to evaluate its inhibitory effect on mycelial growth of different fungal species.

In addition, growth analysis of tomato seedlings cultivated in *Pythium*-infested peat moss treated with different proportions of *Piper betle* leaf powder showed that treatment with 0.5% and 1% *Piper betle* leaf powder, especially 0.5%, have higher seedling emergence rate and seedling growth compared to 0% and 0.1% (Table 4). These results were closely related to the findings of Singha *et al*. (2011) who revealed that tomato cultivated in *Fusarium*-infested soil containing 1% or 2% chloroform extract of *Piper betle* has significant higher plant height and root length compared to *Fusarium*-infested soil without chloroform extract of *Piper betle*.

Taken together, our results suggested the potential of mixing *Piper betle* leaf powder with peat moss for fungal inhibition during tomato seedling growth. These results may be applied to other vegetables, however, suitable mixing ratio of *Piper betle* leaf powder should be tested. Although the use of *Piper betle* may be considered to be an alternative way to inhibit the mycelial growth of various fungal pathogens, further research should involve more growth substrates and various extraction methods of *Piper betle*.

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苎葉葉片粉末對番茄幼苗出土率、幼苗生長及腐霉菌菌絲生長之影響

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關鍵字：苎葉、番茄、腐霉菌

摘要：本實驗探討在泥炭土中拌入苎葉葉片粉末對番茄幼苗生長及抑制番茄腐霉菌菌絲生長之最適混合比例。從國立中興大學農資學院園藝試驗場採集苎葉葉片乾燥製成粉末後和泥炭土混合，分別配製含 0%、1.25%、2.5%和 5%苎葉葉片粉末之混合介質，用於測試番茄的幼苗出土率及生長。結果顯示，含 5%和 2.5%苎葉粉末的介質會降低幼苗出土率。幼苗出土率在含 1.25%的苎葉粉末和不含苎葉粉末的處理組間相比並無顯著差異。腐霉菌分別接種於含 0%，0.1%，0.5%和 1%苎葉粉末之 PDA 中測量菌絲生長狀況。結果顯示，經過 48 小時，在含 1%苎葉粉末之 PDA 中有最佳之腐霉菌菌絲生長抑制率，其菌絲生長速率為 0.39 mm/hr，生長抑制率為 78%。將腐霉菌接種於混入不同比例苎葉粉末的泥炭土裡，混合比例分別為 0%，0.1%，0.5%及 1% (ml / ml)。結果顯示，與 0%，0.1%及 1%相比，含 0.5%苎葉粉末的介質具有最高之幼苗出土率，株高及根長。因此，實驗結果顯示含 0.5%苎葉粉末之泥炭土可減少腐霉病菌對番茄幼苗生長之抑制作用。

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