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Allelopathy Potential of *Alpinia malaccensis* (Burm. F.) Roxb. due to Seeds Germination and Growth of *Merremia peltata* (L.) Merril

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ABSTRACT

Allelopathy is a natural strategy for the protection or inhibition toward other vegetation through the release of chemicals into the environment. *Alpinia malaccensis* is thought to be capable of carrying out allelopathic mechanisms, as this species is found to grow well on land invaded by *Merremia peltata*. Invasive type control with alleopathic mechanism is an alternative to consider as it does not leave a potential residue as a contaminant of soil as it is chemically controlled. The study attempt to investigate the content of *A. malaccensis* allelochemicals and analyzed the alelopathy potential of *A. malaccensis* on seed germination and seedling growth of *M. peltata*. Research method used completely randomized design with 6 replicates for seed treatment and three replicates for seedling treatment. Seeds which have relatively similar size and weight were obtained from the field. Seeds were germinated in petri dishes that have been coated by filter paper, each petri dish contained 6 seeds of *M. peltata*. The treatment of the seeds germination was performed by giving 3 ml of rhizomes and leaves extracts of *A. malaccensis* (control, 30 g/L, 60 g/L, 90 g/L, 120 g/L, and 150 g/L). The treatment of the seedlings was performed by giving 30 ml of rhizomes and leaves extracts of *A. malaccensis* (control, 50 g/L, 100 g/L, 150 g/L, and 200 g/L). The analysis of chemical compounds of fresh rhizomes and leaves showed that *A. malaccensis* contains alcohol, amide, fatty acid, phenol, ketones, and terpenoids. Rhizomes and leaves extracts 150 g/L of *A. malaccensis* showed the highest inhibition in germination, dry weight, plumule and radicle length of *M. peltata* sprout parameters. Rhizomes and leaves extract 200 g/L of *A. malaccensis* showed the highest inhibition in tendril length, amount of leaves, length and width of *M. peltata* leaves parameters. To be more efficient, application in the field should use low concentration of extracts that can inhibit the *M. peltata*. Results of this study are expected to provide information about alternative solutions to suppress the invasion of *M. peltata* to preserve ecosystems of Bukit Barisan Selatan National Park (BBSNP) in Lampung.

Keywords: *Allelopathy, Alpinia malaccensis, BBSNP, Merremia peltata*

INTRODUCTION

Bukit Barisan Selatan National Park (BBSNP) is the forest that has a high diversity of plant species. Tampang Resort is one of BBSNP areas which its biodiversity decreases due to *Merremia peltata* invasive species [1]. Diversity of plant species need to be preserved so that forest ecosystems are maintained and balanced.

M. peltata is a species invading BBSNP. It has a woody climb of Covolvulaceae family, large and broad orbicular leaves connected to the stem in the middle of the leaves. The development of it is caused by clearing forest areas which is widely spread [1]. *M. peltata* can grow well and uncontrolled in open areas that have a high intensity of sunlight. *M. peltata* can hinder the process of photosynthesis causing the surrounding vegetation is dead. Therefore, controlling the growth of *M. peltata* is needed in order to maintain the BBSNP biodi-

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versity. The control can be done by means of eradication, improvement of biological agent's natural enemies, and allelopathy. Invasive plants can be controlled by the activity of allelopathy [2].

Allelopathy is the plant's natural strategy as the form of self-protection against enemies of the environment and competition. This process involves secondary metabolites which can suppress the growth and development of biological systems surrounding plants called allelochemicals [3]. Allelochemicals have the very good potency for raw materials of bioherbicide. Some plants contain allelochemicals, such as *Zingiberaceae*, *Poaceae*, *Pinaceae*, *Verbenaceae*, and others.

A. malaccensis is one of *Zingiberaceae* members. Habitus of *A. malaccensis* is herb, upright, 1-4 meters in height and grows in dense clumps. The uses of *A. malaccensis* are such as traditional medicine, cooking, drinks, soap, and allelopathy. Plants of the genus *Alpinia* contain antimicrobial compound [4], antioxidants [5], and essential oil [6]. Essential oils are compounds that have function as allelopathy against the other plants. The study attempts to investigate the content of *A. malaccensis* allelochemicals and analyzed the alelopathy potential of *A. malaccensis* on seed germination and seedling growth of *M peltate*.

MATERIALS AND METHODS

The research was conducted in May 2014 to September 2015. *A. malaccensis* allelochemicals analysis was conducted at Laboratory of Forest Engineering Research Center for Forestry and Forest Products Processing, Bogor. The experiment of germination response and growth of the *M. peltate* seedling on the effect of allelopathy was conducted at the Laboratory of Biology Bogor Agricultural University and Forest Engineering Research Center for Forestry and Forest Products Processing, Bogor.

Analysis of chemical compounds used instrumental methods of pyrolysis gas chromatography mass spectrometry (Py-GC-MS) toward samples of fresh rhizomes and leaves of *A. malaccensis* was conducted at Laboratory of Forest Engineering Research Center for Forestry and Forest Products Processing Bogor. Fresh samples used as much as 1 mg in the form of thin slice. The sample was put into the quartz chamber pyrolysis unit and heated in an oxygen-free environment at a temperature of 610°C for 10 seconds. The chemical composition indicating specific types of macromolecules progressed along the column GC-MS analysis then performed data storage.

Germination response of *M. peltate* seeds was analyzed by using Completely Randomize Design. It was done for 6 repetitions in 10 days. Seeds having a relatively similar size and weight were obtained from the field. Seeds were germinated in a petri dishes that has been coated with filter paper, each petri dish contained 6 seeds of *M. peltate*. Germination test was done by giving 3 mL rhizomes and leaves extract of *A. malaccensis* (control, 30 g/L, 60 g/L, 90 g/L, 120 g/L, and 150 g/L) toward seeds of *M. peltata*. The crude extracts were precipitated for 24 hours and filtered by using filter paper. The parameters observed were germination ability, dry weight of sprout, and length of plumule and radicle. Allelopathy Potential of *A. malaccensis* can be seen by a large or small value of inhibition (*Inhibition Rate* (IR)) of plumule and radicle of *M. peltate*.

The germination is calculated using the following formula [7]:

$$Germination\ (\%) = \frac{A \times 100\%}{B}$$

Note: A: The number of seeds that germinated
B: Total of seeds that germinated

IR of plumule and radicle is calculated using the following formula [8]:

$$IR\ (\%) = \frac{Plumule\ or\ Radicle\ Treatment}{Plumule\ or\ Radicle\ Control} \times 100\%$$

Note: IR: Inhibition of germination (%)

Seedlings response of *M. peltate* was analyzed using Completely Randomize Design (CRD). It was done for 3 repetitions in one month. Seedlings used were the one which was grown for eight weeks with an average height of 25 cm. Allelopathy test was done by giving 30 mL rhizomes and leaves extract of *A. malaccensis* (control, 50 g/L, 100 g/L, 150 g/L, and 200 g/L) toward seedlings of *M. peltate*. The parameters observed were tendrils length, amount of leaves, length and width of *M. peltate* leaves.

Data of *M. peltate* seeds responses were analyzed using analysis of variance (ANOVA), if the result of treatment is significant then conducted a further test Tukey 95% confidence level (software *Minitab 16*). Data of *M. peltate* seedlings response were analyzed using t-test.

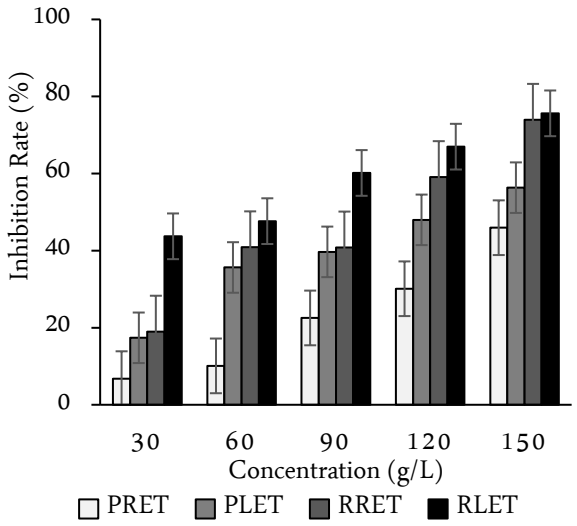

Figure 1. Inhibition rate rhizomes and leaves of *A. malaccensis* (PRET: Plumule Rizomes Extract Treatment; PLET: Plumule Leaves Extract Treatment; RRET: Radicle Rizomes Extract Treatment; RLET: Radicle Leaves Extract Treatment)

(a)

(b)

Figure 2. Plumule and radicle length of *M. peltata*: (a) Extract rhizome of *A. malaccensis* treatment; (b) Extract leaves of *A. malaccensis* treatment (from left to right: control, 50 g/L, 100 g/L, and 150 g/L)

RESULTS AND DISCUSSION

Allelochemical of A. malaccensis

Results of the chemical compounds analysis indicate that the rhizomes and leaves of *A. malaccensis* have 47.86% and 55.44% allelochemicals, respectively (Table 1). The content of chemical compounds besides allelochemicals of *A. malaccensis* is a group of organic compounds that have a function as a component for growth and development of plants.

The most widely chemical compounds contained in rhizome are amides and phenols, on the other hand, leaves contain alcohols, ketones, and terpenoids. The other group of secondary metabolites that act as self-defense and competition against other plant is essential oils. Essential oils components including alcohols, ketones, and terpenoids [9]. Additionally, *Alpinia* sp. contains essential oils which have a main component of terpenoids and small portion of shikimate acid derivatives [6]. Phenols and terpenoids are allelochemicals which have negative influence or as a self-defense against other plants [10]. Secondary metabolites such as terpenoids, phenols, fatty acids, alkaloids, steroid serve as allelochemicals [11].

Seeds germination response of M. peltata

The lowest average germination seeds of *M. peltata* was significantly different ($p < 0.05$) is in the treatment of rhizomes and leaves extract *A. malaccensis* 150 g/L. Extract of rhizomes and leaves of *A. malaccensis* 30 g/L began to show the inhibition causing the average germination percentage was lower than the control (Table 2).

The lowest average dry weight of *M. peltata* sprouts was significantly different ($p < 0.05$) is in the treatment of rhizomes extract *A. malaccensis* 150 g/L, but not significantly ($p > 0.05$) on the treatment of *A. malaccensis* leaves extract. Rhizomes and leaves extract of *A. malaccensis* 30 g/L began to show the inhibition causing average dry weight of *M. peltata* sprouts lower than the control (Table 2).

Germination values indicate the ability to germinate *M. peltata* seeds as a result of the treatment allelochemicals of *A. malaccensis*. There are a number of factors that affect the germination of M. peltata seeds including thick and hairy seed coats. The mechanism that occurs is to inhibit the absorption of water from the surrounding environment so that it will affect the period of dormancy. The average successful seed germination rate is 72.8%. The seeds have variant germination ability depending on the character of morphology, physiology, and genetics. The ability of invasive plant seed germina-

Table 1. Allelochemicals of *A. malaccensis*

No.	*Allelochemicals*	*Rhizomes (%)*	*Leaves (%)*
1.	Alcohol	3.12	26.95
2.	Amide	12.42	-
3.	Ketones	5.46	11.07
4.	Phenol	17.7	7.51
5.	Terpenoids	5.35	9.03
6.	Fatty acid	2.94	0.88
Total		47.86	55.44

tion is approximately 60 – 80% [12]. Research results in Baluran and Gunung Merapi National Park showed the successful germination of Acacia seeds as one type of invasive in the region to reach 75% [13].

The results of this study indicate that the presence of chemical compounds *Alpinia malaccensis* able to reduce germination of *M. peltata* seed compared with control. Allelochemicals inhibit the activity of enzymes involved in the degradation of food reserves so that the energy available for germination is very low.

The concentration level of the extract showed different resistance to the germination response. *A. malaccensis* extract treatment with a high concentration gave significant effect ($p < 0.05$) to germination of *M. peltata*, but treatment with low concentrations had not significant effect. This result is in line with the allelopathic effect of *Brassica tournefortii* on the mine reclamation process. Treatment of extracts with low concentrations did not provide significant inhibition, but the treatment of extracts with high concentrations gave effective inhibition [14]. The allelopathic properties of *B. turnefortii* are influenced by the content of phenol, tannin, alkaloids, flavonoids and saponins,

Phenol can cause disruption of the biosynthesis of nucleotides and prevents the biosynthesis of gibberellins. This condition causes the inhibition of *M. peltata* seed germination process resulting in the average percentage of germination decreased. Phenol can interfere seed germination and plant growth [15].

High concentration of *A. malaccensis* extract led to the average dry weight of sprouts was lower than the control. Allelochemicals can alter the activity and function of certain enzymes that inhibit the syntheses of proteins and carbohydrates, therefore reducing dry weight and growth of a plant [16].

The lowest average radicle length of *M. peltata* sprouts was significantly different ($p < 0.05$) on extract treatment of rhizomes 150 g/L and leaves extract 90 – 150 g/L, but the average plumule length of *M. peltata* sprouts was not significant different ($p > 0.05$) (Table 3). The average of plumule and radicle lengths began to decrease in the extract of 30 g/L *A. malaccensis* (Figure 1 and 2).

A. malaccensis contained allelochemicals that inhibited plumule and radicle length of *M. peltata*. The allelochemicals compounds including phenols and terpenoids were identified quite a lot in *A. malaccensis*. Phenolics and terpenoids compounds interfere phytohormone system. Plant hormones such as auxin, cytokinin, and gibberellin play an important role at the time of germination to assist the process of division and elongation of the cell. Phenolic and terpenoids compounds can interfere the activity of plant hormones such as cytokines that play a role in the process of mitosis [17]. The compounds damage the spindle threads during cell division. In addition, inhibition occurs during the transport of food reserves overhaul diffusion of endosperm towards growth dots on plumule and radicle length. Secondary metabolites such as phenolic compounds, terpenoids, alkaloids, steroids, polyacetylene, and essential oils have allelopathy activity [18].

Phenolic compounds with high solubility in water have a low allelopathy activity, otherwise phenolic compounds with low solubility in water were a high allelopathy activity [19]. A higher allelochemical concentration will increase germination inhibition activity, causing the length of plumules and radicle and the production of fresh and dried weights lower than that of control [20].

Seedlings response of M. peltata

T-test analysis results indicates that the treatment of rhizome extract of *A. malaccensis* was not significantly different ($p > 0.05$) to the average improvement of tendrils length and amount of leaves, but significantly different ($p < 0.05$) to the average of the length and width of *M. peltata* leaves compared to controls. Treatment of *A. malaccensis* leaves extract did not give significant effect to leaves mean number, but significant to mean increase of tendrils length, length and width of *M. peltata* leaves compared to control. Treatment extracts 50 g/L of *A. malaccensis* showed the initial inhibition of the tendrils length, amount of leaves, length and width of *M. peltata* leaves (Table 4).

Allelochemicals of *A. malaccensis* is thought to inhibit the activity of gibberellin. As the result cell division in the intercalary meristem was disrupted causing tendrils elongation of *M. peltata* delayed. The elongation of stem segments was influenced by the activity of the hormone gibberellin [21]. Allelochemicals affect elongation and cell division activity, photosynthesis, respiration,

Table 2. Average germination and dry weight of *M. peltata*

Consentration (g/L)	Germination (%)		Dry weight (mg)	
	Rhizomes extract	Leaves extract	Rhizomes extract	Leaves extract
0	72.78 [a]	72.78 [a]	31.54 [a]	31.54 [a]
30	55.56 [ab]	58.33 [ab]	22.57 [ab]	24.93 [a]
60	52.78 [ab]	44.44 [ab]	19.27 [ab]	18.98 [a]
90	38.89 [ab]	36.11 [ab]	18.63 [ab]	18.97 [a]
120	38.89 [ab]	36.11 [ab]	18.36 [ab]	18.87 [a]
150	30.56 [b]	27.78 [b]	13.22 [b]	10.40 [a]

Note: The numbers followed by the same letter in the same column showed not significant difference in the level of 95% Tukey test

Table 3. Average plumule and radicle length of *M. peltata*

Consentration (g/L)	Rhizome extract		Leaves extract	
	Plumule (cm)	Radicle (cm)	Plumule (cm)	Radicle (cm)
0	4.833 [a]	4 [a]	4.833 [a]	4 [a]
30	4.506 [a]	3.24 [ab]	3.998 [a]	2.251 [ab]
60	4.346 [a]	2.37 [ab]	3.115 [a]	2.094 [ab]
90	3.744 [a]	2.37 [ab]	2.919 [a]	1.594 [b]
120	3.378 [a]	1.636 [ab]	2.517 [a]	1.321 [b]
150	2.612 [a]	1.042 [b]	2.113 [a]	0.975 [b]

Note: The numbers followed by the same letter in the same column showed not significant difference in the level of 95% Tukey test

Table 4. Average improvement of tendrils length, amount of leaves, length and width of *M. peltata* leaves

Extract	Concentration (g/L)	Tendrils length (cm)	Amount of leaves	Leaves length (cm)	Leaves width (cm)
Rhizomes	0	51.67 [a]	9 [a]	1.97 [a]	1.33 [a]
	50	29.97 [a]	6 [a]	1.43 [b]	0.60 [b]
	100	25.83 [a]	4 [a]	0.90 [b]	0.47 [b]
	150	16.37 [a]	3 [a]	0.87 [b]	0.43 [b]
	200	0.63 [a]	2 [a]	0.17 [b]	0.13 [b]
Leaves	0	51.67 [a]	9 [a]	1.97 [a]	1.33 [a]
	50	20.13 [b]	6 [a]	1.83 [b]	0.80 [b]
	100	10.30 [b]	5 [a]	0.70 [b]	0.17 [b]
	150	5.83 [b]	3 [a]	0.33 [b]	0.13 [b]
	200	1.07 [b]	1 [a]	0.03 [b]	0.07 [b]

Note: The numbers followed by the same letter in the same column showed not significant difference in the level of 95% Tukey test

membrane permeability, stomatal opening, mineral ion absorption, metabolism of proteins and nucleic acids [22]. Fatty acids (FAs) are categorized into compounds that inhibit the growth and development of plants [23].

Amide compounds contained in extracts of *A. malaccensis* are suspected causing inhibition of the absorption of water and nutrients by the roots. Nutrients play a role in the formations of proteins and chlorophyll, therefore the process of photosynthesis of *M. peltata* is disturbed. Amide compounds affect the photosynthesis process of plants in that they affect glucose levels as well [24]. Dissolved water and nutrients were inhibited because of the decrease of membrane permeability [25]. Allelochemicals cause a decrease in the permeability of the cell membrane. The decrease in the permeability of the cell causes inhibition of the transport and diffusion of the results of an overhaul of food reserves across the cell membrane. These conditions resulted in cell growth become stunted [26].

CONCLUSION

The content of alcohols, amides, fatty acids, phenols, ketones, and terpenoids from *A. malaccensis* is a potential chemical compound in the allelopathic process. Extract of rhizomes and leaves of *A. malaccensis* could inhibit seeds germination and seedlings growth of *M. peltata*.

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