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Effect of *Azadirachta excelsa* and *Melia azedarach* extracts on soybean germination

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Abstract. *Gumilar RA, Wijayanto N, Wulandari AS. 2017. Effect of* Azadirachta excelsa *and* Melia azedarach extracts *on soybean germination. Nusantara Bioscience 9: 346-351. Azadirachta excelsa* and *Melia azedarach* are potential tree species developed in agroforestry system. Both plants are fast growing species and good for timber use. However, there is an inadequacy on both species due to their allelopathic compound contents. Allelopathic compounds in the plants are distributed from root, stem, leaf, and fruit; which at certain concentrations could inhibit germination, growth, and development of other plants. The objective of this study was to analyze the effects of root, leaf, and twig extracts of *M. azedarach* and *A. excelsa* on the germination of soybean. Result of this study showed that the root, leaf, and twig extracts of *M. azedarach* and *A. excelsa* were able to inhibit the development of soybean. 5 % twig extract of *M. azedarach* significantly inhibited the germination by 77.75%. Leaf and twig extracts of *A. excelsa* at 1.25% concentration has shown to provide stimulant effect on the germination of soybean.

Keywords: Agroforestry, allelopathy, germination, inhibition

INTRODUCTION

Azadirachta excelsa and *Melia azedarach* are species of trees which have potential to be developed in agroforestry system. In Malaysia and Thailand, Azadirachta excelsa has been developed extensively for forest plantations and agroforestry. Due to conical canopy with a balanced architecture, *A. excelsa* has the potential to be developed in agroforestry systems (Wibowo 2012). On the other hand, *M. azedarach* easily grows on a variety of soil conditions without intensive maintenance. These plants are found on community forest in West Java as part of mixed cropping systems.

A. excelsa and *M. azedarach* can be combined with many agricultural commodities in agroforestry system. Agroforestry system enables a more optimal land utilization, increase soil fertility and increase the high production of timber and agricultural crops when conducted with appropriate management. However, there is an inadequacy on *A. excelsa* and *M. azedarach* due to their allelopathic compound contents within the body tissue. Allelopathy is defined as biochemical interactions either beneficial or detrimental to the crop and weeds, or plants with microorganisms through the production of chemical compounds released into the environment and further affect the growth and development of surrounding plants (Sangheeta & Baskar 2015). Allelopathy of a plant can affect growth and development of other crops through allelochemical compounds, toxic secondary metabolite which can be found in all plant tissues such as root, leaf and twig. Plant allelochemicals can lead to increased cell membrane permeability, inhibit plant root elongation, cell division, change ultra-structure, and then interfere with the normal growth and development of the whole plant.

Study on the allelopathic compound of *A. excelsa* has not been done so far. However, several studies have shown that the allelopathic compound from *M. azedarach* is capable of inhibiting the germination and growth of other plants. The objective of this study was to analyze the effect of *A. excelsa* and *M. azedarach* extracts on soybean germination. According to Lungu et al. (2011) *M. azedarach* contains allelopathic compound that may inhibit germination and growth of lettuce. It also inhibits the germination and growth of soybean and green beans. Soil from the root zone of *M. azedarach* is known to contain more allelopathic compound compared to soil treated with *M. azedarach* extract (Shapla et al. 2011). Stunted germination of plants will lead to the disruption of the growth in the later stages; besides the quality of seedlings and yield will also decrease. Therefore, the study on the effect of the allelopathic compound of *A. axcelsa* and *M. azedarach* on soybean is needed.

MATERIALS AND METHODS

Study area

This experiment was done in Seed Technology Laboratory of the Faculty of Agriculture, Bogor Agricultural University. Extract materials such as leaf, root, and twig of *A. excelsa* and *M. azedarach* were obtained from two-year-old stands in community-owned forests in

Cikabayan, Darmaga. The chemical contents of extract materials were analyzed by the method of pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) at the Laboratory of Centre for Research and Development of Forestry Engineering and Forest Products Processing Bogor.

Preparation of extract material

Extract materials with a concentration of 10% (w /v) were obtained by mixing 10 g of the dried plant material with 100 mL of 70% ethanol. The extract was shaken for 24 hours at 150 rpm, at room temperature ($\pm 25^0$c). The extract was then paper filtered and allowed to stand until the alcohol evaporates. The remaining residual extract was used as a stock solution by adding 100 mL of distilled water and filtered through a filter paper. Extracts with concentration of 1.25%, 2.5%, and 5% were obtained by diluting the stock solutions (Lungu et al., 2011).

Soybean germination test in the laboratory

Soybean used in this study was Argomulyo variety. Seeds used were obtained from the Center for Research and Development of Biotechnology and Genetics, Cimanggu, Bogor. Soybean germination test was conducted by rolled paper method which was then coated in plastic. Each roll was sprayed with 30 mL of the extract solution and 25 grains of soybean seeds were planted in a zigzag. Germination was observed every two days, started from 2 days after planting (DAP) to 7 DAP. The parameters observed in these experiments were: the percentage of germination, percentage of abnormal sprouts, as well as plumule and radicle length.

Germination percentage is the percentage of normal seedling produced at the optimum condition within a specified time. The criteria of normal sprouts were the long and straignt development of the root system especially the primary root, perfect hypocotyl growth, perfect plumule growth with green leaf and grow well, and intact cotyledon (Widajati et al. 2012). The characteristics of abnormal sprouts were the short primary root, no intact cotyledons, malformed shape , twisted plumule, swollen hypocotyl, and stunted sprouts. Allelopathic activities on soybean sprouts was analyzed by the following formulas:

Response index of germination (RI) (Hu et al. 2013):

$$RI = \left(\frac{The\ germination\ rate\ of\ treated\ seeds}{The\ germination\ rate\ of\ the\ control} \right) - 1$$

The inhibition percentage of radicle growth (Tsao et al. 2002):

$$Inhibition = \left(\frac{L_{control} - L_{treatment}}{L_{control}} \right) \times 100$$

$L_{control}$ is redicle length of the control sprouts, and
$L_{treatment}$ is redicle length of the treated sprouts

Experimental design

This study used a completely randomized design factorial with three factors. The first factor was the plant species as the extract materials consisted of *Azadirachta excelsa* and *Melia azedarach*. The second factor was the part of the plant consisting of root, leaf, and twig. The third factor was the concentration of the extract consisting of four levels, 0% (control), 1.25%, 2.5%, and 5%. Each treatment was repeated for 3 times.

Data analysis

Data were processed using SAS 9.1 software. To determine the effect of treatment, when significant effect of the variables was found, the analysis of variance (ANOVA) was conducted, followed by Duncan multiple range test.

RESULTS AND DISCUSSION

Chemical compounds analysis

Table 1 shows the allelochemical compounds which might provide an allelopathic effect and inhibit the germination of soybean. The results of pyrolysis GCMS analysis showed that the root and leaf of *A. excelsa* and *M. azedarach* contained 5 types of allelochemicals compounds grouped to phenols, fatty acids and terpenoids. The twig of *A. excelsa* and *M. azedarach* contained 6 types of allelochemical compounds grouped to phenols, fatty acids and alcohols. According to Harbone (1999), phenolic acids and fatty acids were the compounds which were capable of inhibiting the growth and development of other plants.

Chemical compound under phenol group are eugenol, 4-allyl-2,6-dimethoxyphenol, 2.6-dimethoxyphenol, 3,5-xylenol, p-cresol and syringaldehide. Fatty acids groups consisted of nonoic acid, stearic acid, oleic acid, lauric acid, palmitic acid and myristic acid. Alcohol groups consisted of coniferyl alcohol and 2,5-dimethoxybenzyl alcohol, whereas the phytol compound is grouped to terpenoids.

Several studies suggested that the compounds found in *A. excelsa* and *M. azedarach* could provide inhibitory effects on the growth of other plants. The study results of Ahuja et al. (2014) showed that *eugenol* could inhibit the germination and early growth of *Avena fatua*. In addition, *eugenol* is also toxic to some species of weeds such as *Taraxacum officinale, Amaranthus retroflexus, Ageratum conyzoides*, so the *eugenol* has the potential to be used as bioherbicide.

Nonanoic acid or *pelargonic acid* is a potential bioherbicide that can control duckweed growth in aquatic environments. According to Webber (2009), *pelargonic acid* was able to control the growth of duckweed up to 99% or more at a concentration of 0.00975% (v / v) after three days of application. *Arachidic acid, behenic acid, palmitic acid* and *stearic acid* were allelopathic to the rice plant. At a concentration of 250 ppm, the chemical compounds can inhibit the germination of rice (Xuan & Tsuzuki 2004). In addition to the rice plants, *palmitic acid* with *oleic acid* can also inhibit the germination and root growth of *Arabidopsis thaliana* (Angelini et al. 2014).

Table 1. The result of allelochemical analysis of *Azadirachta excelsa* and *Melia azedarach*

Chemical compound	Root		Leaf		Twig	
	A. excelsa	*M. azedarach*	*A. excelsa*	*M. azedarach*	*A. excelsa*	*M. azedarach*
	%					
Eugenol	2.69	5.59	-	-	0.84	-
Nonoic acid	8.15	5.56	-	-	-	-
4-Allyl-2,6-dimethoxyphenol	4.17	4.60	-	-	7.82	8.22
2.6-Dimethoxyphenol	3.00	3.58	-	-	3.60	2.77
Stearic acid	-	2.91	6.17	-	-	-
Oleic acid	1.26	-	4.71	4.07	-	0.83
Palmitic acid	-	-	-	-	0.48	-
Lauric acid	-	-	3.90	3.20	-	-
p-cresol	-	-	-	1.22	-	-
Phytol	-	-	6.01	4.82	-	-
3,5-xylenol	-	-	-	1.18	-	-
Coniferyl alcohol	-	-	-	-	4.92	4.42
2,5-Dimethoxybenzyl alcohol	-	-	-	-	5.28	4.31
Syringaldehide	-	-	-	-	-	1.48
Myristic acid	-	-	4.68	-	-	-

Table 2. The effect of root, leaf and twig extracts of *Azadirachta excelsa* and *Melia azedarach* on germination rate and percentage of abnormal sprouts of soybean

Part of plant	Concentration (%)	Germination rate (%)		Abnormal sprouts (%)	
		A. excelsa	*M. azedarach*	*A. excelsa*	*M. azedarach*
Root	0.00	84.00^{ab}	72.00^{abcd}	16.00^{fg}	28.00^{defg}
	1.25	84.00^{ab}	68.00^{bcde}	16.00^{fg}	32.00^{cdef}
	2.50	78.67^{abc}	65.33^{cde}	21.33^{efg}	34.67^{cde}
	5.00	13.33^{g}	38.67^{f}	86.67^{a}	61.33^{b}
Leaf	0.00	74.67^{abcd}	72.00^{abcd}	25.33^{defg}	28.00^{defg}
	1.25	77.33^{abcd}	52.00^{ef}	22.67^{defg}	48.00^{bc}
	2.50	77.33^{abcd}	69.33^{bcd}	22.67^{defg}	30.67^{def}
	5.00	38.67^{f}	21.33^{g}	61.33^{b}	78.67^{a}
Twig	0.00	72.00^{abcd}	86.67^{a}	28.00^{defg}	13.33^{g}
	1.25	74.67^{abcd}	80.00^{abc}	25.33^{defg}	20.00^{efg}
	2.50	65.33^{cde}	61.33^{de}	34.67^{cde}	338.67^{cd}
	5.00	66.67^{cde}	6.67^{g}	33.33^{cde}	93.33^{a}

Note: The numbers followed by the same letter in the same column are not significantly different at 5% level test (Duncan multiple range test)

Coniferyl alcohol is the defense compound of a phytoalexin type included in propanoid phenyl group. It has the allelopathic potential with a weak inhibitory effect. According to Kalinova et al. (2011), *coniferyl alcohol* showed a weak inhibitory effect on the germination of *Plantago lanceolata L*. compared with *eugenol. Lauric acid* identified from the plant *Rehmannia glutinosa* was proven to provide inhibitory effect on germination, root length and fresh weight of sesame plants. Phenols such as *p-cresol* and *syringaldehide* are able to inhibit the growth of other plants. *P-cresol* can disrupt the photosynthesis process and respiration of water plants and can also inhibit the pollen germination of *Impatiens sultanii* at concentration of 100-125 ppm (Bilderback 1981). *Syringaldehide* can provide inhibitory effect on germination of *Echinochola cruss-galli*.

Apart from providing the inhibitory effect, the chemical compounds contained in *A. excelsa* could also provide stimulant effect on the germination of soybean at a very low level. The root, leaf, and twig of *A. excelsa* contained *ammonium carbamate* compound. This compound is generally used as a raw material for urea fertilizer. Therefore, the stimulant effects of plant extracts of *A. excelsa* might arise because of the presence of *ammonium carbamate* compound.

Normality of Soybean Sprouts

The application of *A. excelsa* and *M. azedarach* extracts significantly affected the germination rate and the percentage of abnormal soybean sprouts. The percentage of germination of soybean control ranged from 72-86.67% and decreased along with the increasing of the extract concentration. The germination percentages of soybean sprouts with the extract (1.25% of *A. excelsa* twig, 1.25% and 2.5% of *A. excelsa* leaf) were greater than the control. The greatest inhibitory effect on the germination of soybean occurred in the soybean treated with 5% root extract of *A. excelsa*, 5% leaf extract of *M. azedarach*, and

5% twig extracts of *M. azedarach* (Table 2). Abnormal germination percentage was negatively related to soybean germination. The higher the concentration applied, the lower the rate of soybean germination and the higher percentage of abnormal germination.

The decrease of germination rate of soybean as a result of the extract of *A. excelsa* and *M. azedarach* at various concentrations indicated the presence of allelopathic effect. The effect of allelopathic arose because of the interaction of several allelochemical compounds such as phenols, fatty acids, and alcohols. According to Aisah (2016), the interactions between the allelochemical compounds would produce optimal inhibition compared with inhibition by a single allelochemical.

The previous study has proven that allelochemical compounds released into the environment might affect the germination of surrounding plants. According to Hong et al. (2003), extracts of *M. azedarach* could provide a significant inhibition of *Raphanus sativus L.* germination with a percentage of 58.1%.

The analysis results of the chemical compound showed that the part of *A. excelsa* and *M. azedarach* contained allelochemical compounds of phenols, fatty acids, and alcohols which were capable of inhibiting the germination of plants. According to Einhellig (1996), the inhibitory mechanism of phenolics has begun with the structure degradation of the plasma membrane, membrane channel modification, or the loss function of the ATPase. Phenolic allelochemical can also lead to increased cell membrane permeability. Consequently, cell content spill and there is increased lipid peroxidation. Finaly, there is slow growth leading to death of plant tissue (Li et al. 2010). According to Aisah (2016), phenol might cause the biosynthesis disruption of nucleotides and prevent gibberellin biosynthesis, thereby inhibiting the germination of soybean.

Supriatno (1995) stated that the inhibition during early germination phase was generally followed by the failure of the mobilization of food reserves to the growing point of sprouts. Inhibition of soybean germination by *M. azedarach* and root extract of *A. excelsa* produced abnormal sprouts with low quality. The results showed the abnormal germination conditions such as the imperfect appearance of the radicle, twisted root, and nonsimultaneous germination. The abnormal germination of soybean would result in lower soybean germination rate.

The effect of phenol on the seed germination process depended on its concentration. The higher concentration of phenol in the water, the greater allelopathic effect. This research showed that the application of the root, leaf, and twig extracts of *A. excelsa* and *M. azedarach* at concentrations of 5% showed the greater inhibition of soybean germination rate than that concentration of 1.25% and 2.5%.

Table 2 show that the germination percentages of soybean sprouts with the extract (1.25% of *A. excelsa* twig, 1.25% and 2.5% of *A. excelsa* leaf) were greater than the control and provide stimulant effect on the germination of soybean. When a number of allelopathic compound are released into the environment, the addition of a number of nutrients has occured and had a stimulant effect on the germination of other plants (Hong et al. 2003). This is thought to be caused by the ammonium carbamate compound contained in the extract of *A. excelsa*. This compound is commonly used as a raw material for urea fertilizers which are able to provide the needs of nitrogen for plants. It is possible that the other compounds can also provide stimulant effect on the soybean germination. According to Rice (1984), inhibiting organic compounds at a concentration level can provide stimulation effects at other concentrations.

Allelopathic activity of the extract solution was measured using the response index value (RI) of soybean germination (Hu et al. 2013). RI value < 0 indicated that the extract solution provided inhibitory effect, while the value of RI > 0 indicated that the extract solution provided stimulant effect on soybean plants. 2.5% root extract of *A. excelsa*, 1.25% and 2.5% root extract of *M. azedarach*, 2.5% leaf extract of *M. azedarach* and 1.25% twig extract of *M. azedarach* provided a lower inhibitory effect on the soybean germination (Figure 1). This might be caused by the low concentration of allelochemical substances contained in the extract solution. The lowest response index was found in *A. excelsa* root extract and *M. azedarach* twig extract at a concentration of 5%. At this concentration, the root extract of *A. excelsa* provided the greatest inhibitory effect on soybean germination.

Sprouts growth

The extracts of *A. excelsa* and *M. azedarach* significantly affected the soybean radicle length but did not significantly affect the length of soybeans plumule. Soybean treated with extracts of *A. excelsa* and *M. azedarach* showed a lower radicle length than the control. The greatest inhibition of radicle growth was found in soybeans treated with extracts of *A. excelsa* and *M. azedarach* at concentration of 5% (Table 3). Extracts of *A. excelsa* and *M. azedarach* did not significantly affect the plumule length of soybean sprouts, the plumule length tended to decrease along with the increasing of extract concentration provided.

Extracts of *A. excelsa* and *M. azedarach* at each concentration showed different inhibitory effects on the radicle growth of soybean sprouts. The greater the concentration applied, the higher the inhibitory effect and the shorter radicle length of soybean. The greatest inhibition percentage was found in root extract of *A. excelsa* at concentration of 5% (Figure 2). Leaf extract of *M. azedarach* at a concentration of 5% also provided large percentage of radicle growth inhibition, by 77.75%. The smallest inhibition percentage was found in root and twig extracts of *A. excelsa* at concentration of 1.25%.

Table 3. The effect of root, leaf, and twig extracts of *Azadirachta excelsa* and *Melia azedarach* on soybean sprouts radicle length

Part of Plant	Concentration (%)	Radicle lenght (cm)		Plumule lenght (cm)	
		A. excelsa	*M. azedarach*	*A. excelsa*	*M. azedarach*
Root	0.00	14.02^{a}	12.88^{ab}	3.28^{a}	3.23^{a}
	1.25	13.52^{ab}	11.12^{bcde}	1.99^{a}	2.44^{a}
	2.50	8.38^{fghi}	7.46^{ghi}	1.25^{a}	1.55^{a}
	5.00	2.54^{mn}	5.01^{jkl}	0.57^{a}	0.93^{a}
Leaf	0.00	12.81^{ab}	12.38^{abc}	3.54^{a}	3.03^{a}
	1.25	11.43^{bcde}	9.49^{efgh}	2.81^{a}	2.99^{a}
	2.50	10.36^{cdef}	7.18^{hij}	2.31^{a}	1.99^{a}
	5.00	3.87^{lmn}	4.66^{klm}	0.98^{a}	1.22^{a}
Twig	0.00	12.28^{abc}	11.65^{abcde}	4.15^{a}	1.83^{a}
	1.25	12.08^{abcd}	9.72^{defg}	3.61^{a}	1.61^{a}
	2.50	8.62^{fghi}	6.37^{ijk}	1.97^{a}	1.18^{a}
	5.00	6.42^{ijk}	2.35^{n}	1.70^{a}	0.70^{a}

Note: The numbers followed by the same letter in the same column are not significantly different at 5% level test (Duncan multiple range test)

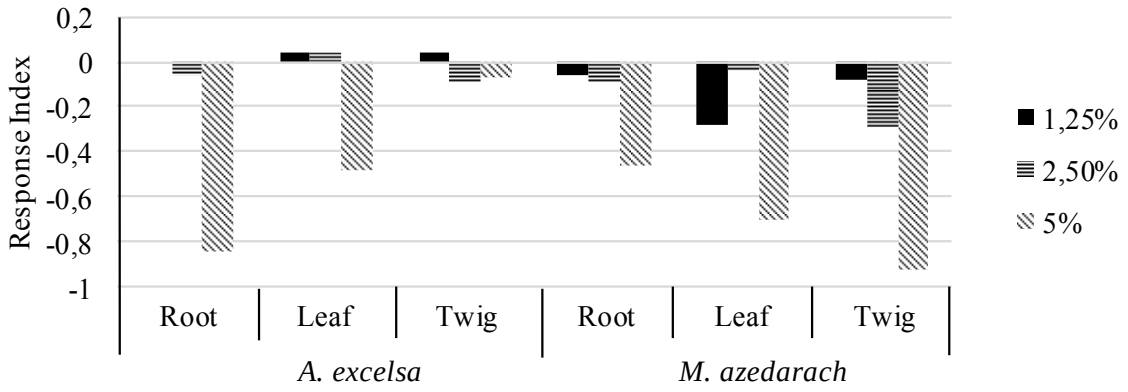

Figure 1. Response index of soybean germination to the extract of *Azadirachta excelsa* and *Melia azedarach*

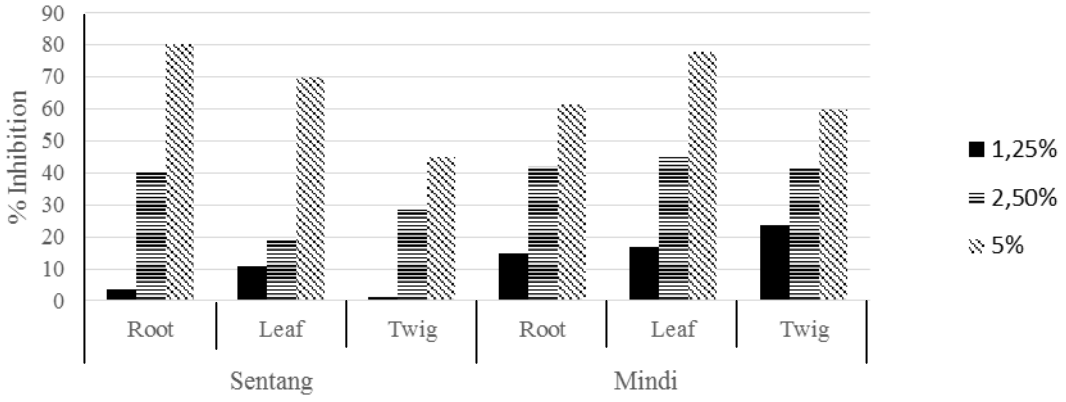

Figure 2. Inhibition percentage of *Azadirachta excelsa* and *Melia azedarach* extract solution

The lower radicle length of soybean sprouts treated with *A. excelsa* and *M. azedarach* extracts than the control indicated the presence of the allelopathic effect. Allelochemical compounds can interfere phytohormones system, whereas growth hormones such as auxin, cytokinin, and gibberellin are very important during the cell division and cell elongation (Aisah 2016). According to Pebriani et al. (2013), phenolic compounds can inhibit the cytokines activity which play significant role in the mitosis process. Growth Inhibition of soybean sprouts caused by the failure of the food reserves mobilization to growing point on plumule and radicle.

In conclusion, root, leaf and twig of *A. excelsa* and *M. azedarach* contained allelochemicals compounds grouped to phenols, fatty acids, alcohol and terpenoids which might provide an allelopathic effect and inhibit the germination of soybean. *A. excelsa* and *M. azedarach* extract at 5% concentration provide inhibitory effect on soybean germination, but also could provide stimulant effect at lower concentration. It is necessary to screen for allelopathic compound contained in Root, leaf and twig of *A. excelsa* and *M. azedarach* and to be tested separately so that it can be known which chemical compounds will have a significant inhibitory effect.

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