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Dimension growth of *Azadirachta excelsa* and *Phyllanthus* spp. in agroforestry system

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Abstract. *Dewi N, Wijayanto N, Gusmaini. 2017. Dimension growth of* Azadirachta excelsa *and* Phyllanthus *spp. in agroforestry system. Biodiversitas 18: 494-499. Azadirachta excelsa* Jack. is one of the fast growing species which have high resistance to pest and disease, good quality of wood, and high economic value. *A. excelsa* planting can be integrated with *Phyllanthus* spp. in agroforestry system. The research about meniran and sentang in agroforestry system was conducted to analyze the influence of *A. excelsa* allelopathy towards the growth of meniran and to analyze the growth of both plants. This research was conducted for six months in Bogor, West Java. This study was divided into three parts, (i) analyze the effect of allelopathy in *A. excelsa* leaf and twig on the growth of *P. urinaria* and *Phyllanthus debilis*, (ii) analyze the growth of *A. excelsa* in monoculture and agroforestry systems and (iii) analyze the growth of meniran in monoculture and agroforestry systems. The result showed that leaf and twigs litter of *A. excelsa* did not have negative allelopathic effect for the growth of *P. urinaria* and *P. debilis.* Agroforestry system did not give significant effect to the growth of *A. excelsa* . Agroforestry systems reduced the growth of both *P. urinaria* and *P. debilis* due to light deficiency.

Keywords: Agroforestry, allelopathy, *Azadirachta excelsa, Phyllanthus debilis, Phyllanthus urinaria*

INTRODUCTION

Wood supply either from natural, plantation or community forests is decreasing (BPS 2016). This is due to many factors such as the reduction of forest land, conversion of forest to other land uses an occurrence of pests and diseases on cultivated plants, etc. Pests and diseases could reduce wood productivity as well as quality. Popular wood species such as *Swietenia macrophylla*, *Anthocephalus cadamba*, *Falcataria moluccana*, and *Acacia mangium* are attacked by pests and diseases.

Azadirachta excelsa Jack. is one of the fast growing species in the Meliaceae family and local species of Borneo Island. *A. excelsa* is classified as modest hardwood (Ching 2003). This wood is utilized for lightweight construction, furniture, panel, and veneer (Gan et al. 1999). The other part of the plant such as young shoots and flowers can be consumed as vegetables (Directorate for Forest Plants Seeds). *A. excelsa* seed contains azadirachtin (3.3-3.5 mg/g weight) which is used as an insecticide. Besides, *A. excelsa* has also potential as wood energy due to its low ash percentage and high calorific value (Hossain and Jalil 2015). Based on those uses, *A. excelsa* has potential to be developed in community and plantation forests to replace the wood attacked by pests and diseases.

Forest plantation of *A. excelsa* can be integrated into agroforestry systems along with other crops. Agroforestry system is a management of natural resources based on ecology by integrating forestry plant and crops in one landscape. Several benefits of agroforestry are an increase of soil nutrient content (N, P, K) derived from the decomposition of forest plant litter. Microbial activities within the soil in agroforestry systems are also higher than that in monoculture system although it is still lower than the one in natural forest. It indicates that agroforestry can improve soil conservation. An agroforestry system also can reduce the loss of soil nutrient due to leaching by rain than that in monoculture system (Lehmann 1999). *A. excelsa* is a potential tree grown in agroforestry systems with its cone crown in the shape of canopy architecture (Orwa et al. 2009) so the light intensity entering stands floor is high enough to support crops growth.

The crops that can be grown under *A. excelsa* stand canopy are seasonal and medicinal crops. One of the potential medicinal crops is *Phyllanthus*. Sarin et al. (2014) stated that *Phyllanthus* grow together in the same open habitats and wastelands. Half of Indonesian people have been utilizing this crop as a traditional medicinal crop. The efficacy of this crop is probably derived from its chemical compounds content such as saponins, flavonoids (Nakweti et al. 2013), phenolic, steroids and lignans (Bagalkotkar et al. 2006). Some researchers also revealed that *Phyllanthus* has property of antihepatotoxic (Ahmed et al. 2009), antibacterial (Sabir and Rocha 2008), antimicrobial (Amin et al. 2012), and antiplasmodial (Njomnang et al. 2009) activities and inhibiting virus growth that causing disease (Naik and Juvekar 2003). Several types of research have been conducted on the advantage and efficacy of *Phyllanthus* sp. However, research on the cultivation of the species especially in agroforestry systems is still limited.

The combination between *A. excelsa* and *Phyllanthus* sp. in agroforestry system will lead to some interactions such as positive, negative and neutral interaction. Negative interaction occurred when either one or both of the plant production decrease. It might be due to allelopathy compounds. Allelopathy is defined as direct or indirect effects from particular plant to another organism including microbes either positively (stimulating growth) or negatively (inhibiting growth) (Singh et al. 2003). In agroforestry systems, the allelopathy sources could derive from both forestry and agricultural plant, but the effect will be bigger from forestry plant to an agricultural crop. The study of *A. excelsa* allelopathy was performed because of neem (*Azadirachta indica*) as close genus to *A. excelsa* is one of the woody plants with allelopathy properties which can inhibit the growth of agricultural crop (Xuan et al. 2003; Kato-Noguchi et al. 2014).

Positive interaction is indicated when the system can support the growth of both forestry and agricultural plant. To obtain the information about growth response for both of *A. excelsa* and *Phyllanthus* sp. in agroforestry systems, thus this study was performed. This research aimed to (i) analyze the effect of allelopathy in *A. excelsa* leaf and twig on the growth of *Phyllanthus urinaria* L. and *Phyllanthus debilis* Klein ex Wild, (ii) analyze the growth of *A. excelsa* in monoculture and agroforestry systems and (iii) analyze the growth of *Phyllanthus* in monoculture and agroforestry systems.

MATERIALS AND METHODS

Research location

The research was conducted at the greenhouse of Silviculture Laboratory, Department of Silviculture, Faculty of Forestry, Institut Pertanian Bogor, West Java, Indonesia and land of Conservation Unit of Biopharmaceutical Research Center Cikabayan, West Java, Indonesia. Analysis of *A. excelsa* compounds content was performed by GC-MS pyrolysis test in Laboratory of Research Center and Development of Forestry Technique and Forest Products Processing (P3KKPHH) Gunung Batu, Bogor, West Java, Indonesia.

Materials

Materials used in this research were a plastic bag, soil, compost, two years old *A. excelsa* stand, *P. debilis* Klein ex Wild and *P. urinaria* seeds and seedlings, inorganic fertilizer, etc.

Research procedure and data analysis

This study was divided into three parts:

Analyze the effect of allelopathy in A. excelsa *leaf and twig on the growth of* P. urinaria *and* P. debilis

Research procedure. The seedling used must have 3-5 leaves (Oktavidiati 2012), then planted into a plastic bag with a size of 15 cm x 20 cm filled with growth medium (soil and compost 2: 1) as much as 1 kg. Plant maintenance was done such as weeding, pest and disease controlling, watering, and fertilizing. The fertilizer used was inorganic fertilizer namely Urea, SP-36, and KCL. SP-36 with a dose of 100kg/ha was given at the beginning of planting period while Urea with a dose of 200 kg/ha and KCL with a dose of 150 kg/ha was given two times at 4 weeks after planting and 6 weeks after planting (Bermawie et al. 2006). Re-planting dead plant was done until the plants 2 weeks after planting.

The method used to extract *A. excelsa* leaf and twig litter was maceration. Maceration method used sample submersion inside an organic solvent at room temperature (Darwis 2000). Ethanol solvent is better used to extract *Phyllanthus* to obtain phenolic content and antioxidant compared to water solvent with composition 60: 40 (Martinus and Riva'i 2011). Therefore, this experiment used ethanol solvent. Leaf and twig litter of *A. excelsa* were cut to a length of 0.5 cm and was oven-dried in temperature of 80^0C for 48 hours. Then, the leaf and twig were grinded to make it powder. To obtain 10% extract concentration w/v, each powder of leaf and twig were weighed and as much as 10 g of powder were extracted using 100 ml of alcohol 70%. The solution was then centrifuged at a speed of 150 rpm in room temperature (25-27^{o}C). The extract obtained was then filtered using filter paper. The extract then diluted and sprayed in the samples crop as much as 10 ml/plastic bags according to treatment. Application of *A. excelsa* leaf and twig extract was done once in three days (Solichatun and Nasir 2002) for 10 weeks. The observation of *Phyllanthus* growth was done once in two weeks for 10 weeks to the parameters observed such as height, number of composite leaves, and number of branches.

Data analysis. The research used split plot design with 14 treatments and three replications for each treatment. The main plot was *Phyllanthus* species, while the subplot was extracted concentration. *Phyllanthus.* species consisted of *P. urinaria* and *P. debilis* while the subplot consisted of seven extracts concentration.% *A. excelsa* leaves extract, P3 = 5% *A. excelsa* leaves extract, P4 = 1.25% *A. excelsa* twigs extract, P5 = 2.5% *A. excelsa* twigs extract, and P6 = 5% *A. excelsa* twigs extract. Data was analyzed using analysis of variance (ANOVA) with 5% degree to identify the difference among treatments. Duncan's multiple range test of 5% level was conducted when there was a significant difference among observed parameters.

Growth of A. excelsa *in monoculture and agroforestry systems*

Research procedure. *A. excelsa* stands in this research was 2 years old with planting space 2.5 m x 2.5 m in two cropping pattern namely monoculture and agroforestry. Plant maintenance was conducted in monoculture stand and in agroforestry stand. Measurement of *A. excelsa* dimension included height (cm) and stem diameter (cm) (Wijayanto and Hidayanti 2012).

Data analysis. The design used in this part was complete randomize design with one factor and two treatment which was monoculture (P0) and agroforestry (P1) system with 14 replications for each treatment. Data was analyzed using analysis of variance (ANOVA) with 5% degree to identify the difference among treatments.

Duncan's multiple range test of 5% level was conducted when there was a significant difference among observed parameters.

Growth of P. urinaria *and* P. debilis *in monoculture and agroforestry systems*

Research procedure. The area used was open area and area with *A. excelsa* stand. Before planting, tillage was done in 30 cm depth to make the soil loose. Weed under *A. excelsa* stand was cleared. The soil was ripped and plotted with a size of 3 m x 1 m. There were 4 plots for each treatment. Every agroforestry plot was surrounded by *A. excelsa* stand with a spacing of 2.5 m x 2.5 m.

Phyllanthus seedling used was collected from a natural area with 2-5 leaves both for *P. urinaria* and *P. debilis*. After that, the seedling planted based on the most similar character to obtain the same age range of the seedling. The seedling was planted with a spacing of 20 cm x 20 cm. Number of seedlings planted was 4 plots with 12 individuals for each plot. Next, the observed plant was 10 sample plants which were obtained based on randomization and not included edge crop.

Plant maintenance was fertilizing, weeding, pest and disease control, spraying and replanting of death plant. Fertilizer used was manure and inorganic fertilizer (Urea, SP-36, KCL). Manure with dose of 20 ton/ha and SP-36 with dose of 100kg/ha was given in the beginning of planting while urea with dose of 200 kg/ha and KCL with dose of 150 kg/ha was given two times in 4 weeks after planting and 6 weeks after planting (Bermawie et al. 2006).

Phyllanthus harvesting was done in 14 weeks after planting (before flowering). Harvesting was done by pulling out the plant when optimum vegetative phase before flower initiation occur (Bermawie et al. 2006). The growth parameters of *Phyllanthus* observed were height (cm), number of complex leaves, and number of branches.

Data analysis. This experiment used Complete Randomized Block Design with one factor. The treatment used were monoculture of *P. urinaria* (P0Mm), monoculture of *P. debilis* (P0Mk), agroforestry of *P. urinaria* and *A. excelsa* (P1Mm), and agroforestry of *P. debilis* and sentang (P1Mk). The group consisted of four groups namely cropping pattern. Data was analyzed using analysis of variance (ANOVA) with 5% degree to identify the difference among treatments. Duncan's multiple range test of 5% level was conducted when there was a significant difference among observed parameters.

RESULTS AND DISCUSSION

Analyze the effect of allelopathy in *A. excelsa* leaf and twig on the growth of *P. urinaria* and *P. debilis*,

The result of analysis of variance (Table 1) shows that treatments did not significantly affect the growth of *Phyllanthus* for all parameters. The treatments from the lowest concentration (1.25%) to the highest one (5%) did not give different growth to *Phyllanthus* when it is compared to control. It indicated that the leaves and twigs extract of *A. excelsa* were not allelopathy negative to *Phyllanthus* growth.

Based on GC-MS pyrolysis test, litter extract of leaves and twigs of *A. excelsa* have compound content that may probably be allelopathy such as diterpene, phenolic, and fatty acid (Li et al. 2010), however, the content was quiet low so they did not inhibit the growth of *Phyllanthus*. Besides, the number of leaves and twigs of *A. excelsa* that fell to the ground was lower than neem which in some papers reported having allelopathy properties. This condition is beneficial in agroforestry systems because the integration of two plants did not give negative effect to both plants based on allelopathy properties.

Inhibition mechanism of allelopathy occurs through complex metabolism process including cell division and elongation, growth regulation by inhibiting the growth regulator substances, nutrient uptake, photosynthesis, respiration, stomata opening, protein synthesis, carbon storage and pigment synthesis, membrane permeability, and alter the function of specific enzyme (Einhellig et al. 1985). The addition of *A. excelsa* leaf and twig litter extract to yellow and *P. urinaria* did not affect significantly due to the good defense mechanism of *Phyllanthus.* Either morphologically or physiologically to the inhibition mechanism of *A. excelsa* allelopathy. Astutik et al. (2016) explained that green beans do not experience growth inhibition caused by beluntas leaf because green beans cell is covered by lignin as a morphological defense system. *Phyllanthus* also has lignin content in the entire stem which might prevent *A. excelsa* allelopathy effect (Gupta et al. 1984).

Phyllanthus species influenced significantly the number of leaves and number of branches (Table 1). This result shows that both species have different morphology. *P. urinaria* and *P. debilis* had relatively the same height. *P. urinaria* had more number of branches which made the number of leaves also higher than *P. debilis* (Table 2). This is because the stem leaf segment of *P. urinaria* is shorter and the leaf is denser. The number of leaves and branches for the *P. urinaria* reached approximately 70 and 8 respectively while *P. debilis* had only 30 leaves and 5 branches.

Height, number of leaves and number of branches are the factors which influenced biomass component of a plant. Biomass also can be estimated from wet weight and dry weight of the plant. *P. urinaria* has higher biomass than the yellow one. Part of *Phyllanthus* used to make traditional medicine is the biomass due to the higher compounds content in the biomass. Therefore, the higher biomass of *Phyllanthus*, the more compounds content will be obtained so it is best to use as medicine. *P. urinaria* which have high biomass is recommended to be cultivated.

Growth of *A. excelsa* in agroforestry system

Cultivation in agroforestry systems will create a process that mutually influences components and it is called as interaction. The interaction can be positive or negative. Therefore, choosing plants to be cultivated in agroforestry must be done carefully by considering to create no harm on the cultivation. One of the interactions occurs can be seen from the growth of components planted.

Table 1. Recapitulation of analysis of variance of *A. excelsa* extract influence to *Phyllanthus.*

Parameters	F test	Treatments	
		Agroforestry	Monoculture
Height	ns	ns	ns
Number of leaves	*	ns	ns
Number of branches	*	ns	tn

Note: ns: not significantly different, (*): significantly different on test-level 5%

Table 2. Duncan's Multiple Range Test of *Phyllanthus*

Phyllanthus species	Height	Number of leaves	Number of branches
P. urinaria	27.62a	70.21a	8.00a
P. debilis	33.56a	31.33b	5.38b

Numbers followed by the same letters and columns are not significantly different (Duncan's MRT)

Table 3. Recapitulation of analysis of variance of *A. excelsa*

Parameters	F test	Treatments	
		Agroforestry	Monoculture
Height	ns	160.71^{a}	153.79^{a}
Stem diameter	ns	1.599^{a}	1.331^{a}

Note: ns: not significantly different. Numbers followed by the same letters and rows are not significantly different

Table 4. Analysis of variance of *Phyllanthus*

Parameters	Treatments	Block
Height	*	ns
Number of leaves	*	ns
Number of branches	*	ns

Note: ns: Not significantly different, (*): significantly different on test-level 5%

Table 5. Duncan's Multiple Range Test of *Phyllanthus* growth

Treatments	Height	Number of leaves	Number of branches
P0Mm	30.97^{b}	380.95^{a}	17.75^{a}
P0Mk	49.84^{a}	84.86^{b}	11.06^{b}
P1Mm	29.80^{b}	38.90^{b}	5.20^{c}
P1Mk	21.73^{b}	36.39^{b}	3.74^{c}

Note: Numbers followed by the same letters and columns are not significantly different

A. excelsa is local species of Borneo that, grows better from 350 m above sea level with a temperature between 22 and 27 ^{0}C and rainfall around 2000 mm/year (Joker 2000). It is matched to the condition of the research site, namely 193 m above sea level with an average temperature of 26.2-27.1 °C and average rainfall of 329.7-373.0 mm/months. *A. excelsa* is very potential to be implemented in agroforestry systems due to its cone and balance architecture.

A. excelsa growth planted in agroforestry did not show the significant effect to the ones in monoculture systems in all growth parameter observed (Table 3). It indicated that there was no inhibition or stimulation mechanism from agroforestry systems to *A. excelsa* growth. This condition does not match with research by Puri et al. (2016) which stated that the height and stem diameter of *A. excelsa* in agroforestry system were higher than the ones in monoculture. The difference was maybe due to a short period of *A. excelsa* measurement which was only 3 months.

Growth of *P. urinaria* and *P. debilis* in monoculture and agroforestry systems

Cultivation in agroforestry systems will also influence the crop under shade. *P. urinaria* and *P. debilis* cultivated under *A. excelsa* stand had very different growth of height, number of leaves, and number of branches compared to monoculture, but no significant difference was recorded in each planting blocks (Table 4).

Based on ANOVA results in Table 5, the height of monoculture of *P. debilis* had the highest value compared to other treatments. Height growth in plant occurs because of elongation of stem segment as a result of the increase in a number of cell in intercalary meristem of the stem (Gardner et al. 2008). The height increase of *P. debilis* between monoculture and agroforestry was different due to a light deficiency in agroforestry system. The shade from *A. excelsa* stand probably has exceeded the maximum compensation light of *P. debilis*.

Height growth of a plant in abiotic stress condition of light deficiency depends on the adaptation capability of the species. Shading influences the photosynthesis and respiration process which affect the productivity and growth (Heddy 1987). *P. urinaria* is considered to have more adaptive properties than *P. debilis*. It was indicated by the height growth of *P. urinaria* both in agroforestry and in monoculture system which did not show significant different. *P. urinaria* can grow under *A. excelsa* stand well. On the other hand, agroforestry of *P. debilis* had a lower height than the ones in monoculture. Monoculture of *P. urinaria* also showed lower growth than a monoculture of *P. debilis*. It indicated that *P. debilis* had higher height growth properties than *P. urinaria*.

Light intensity in agroforestry systems was around 100 lux, while in monoculture was 500 lux. Light intensity influences significantly to the photosynthesis rate and process in which shaded leaf will have lower photosynthesis rate than the one which is not shaded (Taiz and Zeiger 2002). *Phyllanthus* as C3 plant will not undergo CO_2 fixation process under light deficiency condition that will inhibit the photosynthesis process (Lakitan 2013). This condition will affect the plant growth including cell division in meristem tissue which leads to the height growth of the plant.

Besides the height, the light deficiency also affects the growth of another parameter such as number of leaves, and number of branches. Number of leaves in monoculture of

P. urinaria was highest than the other treatments with 390.69 leaves. Parman (2010) also stated that reduce in light intensity can reduce the number and width leaf of radish. The number of *Salvia officinalis* leaves responded similarly to light intensity (Zervoudakis et al. 2012).

P. urinaria had more number of leaves than *P. debilis*. It was due to the shorter stem segment of *P. urinaria* which makes the denser leaf and lead to the more number of leaves. The number of leaves is correlated to the number of branches in which the more number of branches, the more number of leaves. Monoculture of *P. urinaria* showed the highest number of branches than the other treatments. It led to more leaf of *P. urinaria* than the *P. debilis*.

Monoculture of *P. urinaria* showed the best growth compared to other treatments in all parameters except for height and root dry weight. Agroforestry system of *P. urinaria* had better growth than agroforestry system of *P. debilis*. It indicated that *P. urinaria* had better adaptation than *P. debilis*. This condition matched to Oktavidiati (2012) that *P. urinaria* is more stress resistant compare to green *Phyllanthus.* Either for water content stress, pest resistance, or anthocyanin content. It is predicted because *P. urinaria* has trichrome. *P. urinaria* also has high adaptability to high light intensity and under shade. Based on the growth properties, *P. urinaria* can be integrated in agroforestry system.

Height, number of leaves and number of branches are factors that affect biomass component of plant. *Phyllanthus* biomass is used as raw material for traditional medicine. Therefore, high biomass content in *Phyllanthus* will produce more chemical compounds and goods for medicine. *Phyllanthus* growth in monoculture was better to gain biomass than in agroforestry system. Therefore, *Phyllanthus* is better to be cultivated in monoculture or in agroforestry system when the stands are still young and creates a thin canopy.

In conclusion, *A. excelsa* did not have allelopathy properties to *Phyllanthus* growth when they were planted together. Agroforestry systems did not affect the growth of *A. excelsa*. Agroforestry systems reduced the growth of both *P. urinaria* and *P. debilis* due to light deficiency.

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