Available online at AL-KAUNIYAH: Jurnal Biologi
Website: http://journal.uinjkt.ac.id/index.php/kauniyah
AL-KAUNIYAH: Jurnal Biologi, 14(1), 2021, 101-114

ALELOPATI TUMBUHAN INVASIF *Dioscorea bulbifera* L. DAN PENGARUHNYA TERHADAP PERKECAMBAHAN BIJI *Shorea selanica* (Lam.) Blume

ALLELOPATHY OF INVASIVE SPECIES *Dioscorea bulbifera* L. AND ITS EFFECT ON SEED GERMINATION OF *Shorea selanica* (Lam.) Blume

Ade Ayu Oksari[1*], Irvan Fadli Wanda[2,3], Gladys Ayu Paramita Kusumah Wardhani[4]
[1]*Biology Department, Nusa Bangsa University, Jl. KH. Sholeh Iskandar Km 4, Bogor, Indonesia. 16166*
[2]*Research Center for Plant Conservation and Botanic Gardens-Indonesian Institute of Sciences (LIPI), Jl. Ir. H. Juanda No. 13 Bogor, West Java, Indonesia*
[3]*Biology Department, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680*
[4]*Chemistry Department, Nusa Bangsa University, Jl. KH. Sholeh Iskandar Km 4, Bogor, Indonesia. 16166*
**Corresponding author:* adeayuoksari@gmail.com

Naskah Diterima: 22 Juni 2020; Direvisi: 2 Oktober 2020; Disetujui: 17 November 2020

Abstrak

Dioscorea bulbifera L. (*Dioscoreaceae*) merupakan salah satu tanaman invasif yang menciptakan masalah lingkungan. *D bulbifera* mengandung alelopati yang memengaruhi proses fisiologis pada spesies lain. Penelitian ini bertujuan untuk mengetahui pengaruh alelopati berbagai konsentrasi ekstrak daun dan umbi *D. bulbifera* L. terhadap perkecambahan biji *Shorea selanica* (Lam.) Blume. Metode yang digunakan adalah Rancangan Acak Lengkap (RAL) dengan sepuluh perlakuan dan tiga ulangan. Perlakuan yang diberikan adalah perbedaan konsentrasi ekstrak *D. bulbifera* 25, 50, 75, 100%, dan kontrol. Berdasarkan hasil uji skrining fitokimia, semua metabolit sekunder dalam ekstrak daun menunjukkan hasil yang positif. Senyawa golongan saponin, tanin, flavonoid, dan steroid/terpenoid terdeteksi dengan kuat sedangkan senyawa alkaloid kurang kuat. Senyawa fenol tersebut adalah fenol, 1,2-benzenediol (dalam ekstrak daun) dan fenol, 1,2-benzendiol, 1,4-benzendiol, dan 2-metoksifenol (dalam ekstrak umbi). Berdasarkan uji perkecambahan, ekstrak metanol umbi dan daun *D. bulbifera* berpengaruh nyata terhadap daya kecambah, koefisien kecepatan berkecambah, dan koefisien keserempakan berkecambah *S. selanica*. Konsentrasi yang menurunkan viabilitas biji terendah adalah pada konsentrasi 75% dan 100% dengan rata-rata daya kecambah 10,00 ± 6,32 % (pada ektrak umbi) dan 0,00 ± 6,32 % (pada ektrak daun).

Kata kunci*:* Alelokimia; *Dioscorea bulbifera* L.; Perkecambahan; *Shorea selanica* (Lam.) Blume

Abstract

Dioscorea bulbifera L. (Dioscoreaceae) is one of the most unutilized invasive plants. This plant contains allelopathy that affects the physiological process of native species. This study aimed to discover the type of allelochemical in D. bulbifera and its effect on seed germination of Shorea selanica (Lam.) Blume. The method used was a Completely Randomized Design with ten treatments and three replications. The treatment given was the different concentration of D. bulbifera extract of 25, 50, 75, 100%, and control. Based on phytochemical screening tests, saponin, tannin, flavonoid, and steroid/terpenoid compounds were detected in leaf and tuber extract. The total phenolic in leaf extract was more significant than that in the tuber extract of D. bulbifera but the type of phenolic compounds was lower. The phenolic compounds were phenol, 1,2-benzenediol (in leaf extracts) and phenol, 1,2-benzendiol, 1,4-benzendiol, and 2-methoxyphenol (in tuber extracts). Based on the germination test, it was found that the methanol extract from the tubers and leaf of D. bulbifera significantly affected the germination capacity, coefficient rate of germination, and simultaneity coefficient of germination of S. selanica. It was found that treatment of concentration of 75% and 100% resulted in the lowest seed viability reduction with an average germination rate of 10.00 ± 6.32% (in tuber extracts) and 0.00 ± 6.32% (in leaf extracts).

Keywords*: Allelochemical; Dioscorea bulbifera L.; Germination; Shorea selanica (Lam.) Blume*

Permalink/DOI: http://dx.doi.org/10.15408/kauniyah.v14i1.16160

INTRODUCTION

Invasive plant species have become a global problem that causes adverse impacts on ecosystems, the economy, and human health. Increased invasion is one of the leading causes of biodiversity loss (Inderjit, Seastedt, Callaway, Pollock, & Kaur, 2008; Rastogi, Rawat, & Chandra, 2015). In fact, some invasive plants containing allelopathy might affect the physiological processes of native species and soil microorganisms which eventually cause impact on biodiversity (Lorenzo, Hussain, & González, 2013).

Chemical compounds from allelopathy (allelochemistry) can be released into the environment through various mechanisms such as evaporation from leaves, roots and exudation from washing falling leaves and waste plants (Albuquerque, Santos, Lima, Filho, & Nogueira, 2011). Allelopathic compounds from a plant directly or indirectly can affect other plants' growth and production, both positively/stimulating and negatively/inhibiting, by releasing chemical compounds into the environment (Singh, Batish, & Kohli, 2003). Previous studies have shown that allelopathy in invasive plant species can affect other plants, including *Aster lanceolatus* Willd. (Nešić et al., 2016), *Clidemia hirta* (Ismaini, 2015), *Imperata cylindrica* (Yanti, Indriyanto, & Duryat, 2016), *Spartina alternifolia* (Duan, Liang, Li, & Zhou, 2015), and *Abutilon theophrasti* (Šćepanović et al., 2007). One of the invasive species that inherits native plant communities is *Dioscorea bulbifera* L.

Dioscorea bulbifera L. (*Dioscoreaceae*) belongs to the Global Weed Compendium and is an invasive plant that creates environmental problems in many parts of the world. According to Santosa et al. (2014), *D. bulbifera* (a collection plant) has spread out from some Bogor Botanical Garden areas by rapidly climbing a host tree, covering its branches and twigs, and reducing photosynthetic activity of a host plant. The other studies have shown that *D. bulbifera* influenced seed germination. Extracts of tubers and leaves of *D. bulbifera* successfully inhibited the germination of *Polyalthia littoralis* seeds (Oksari, Susanty, & Wanda, 2019). *D. bulbifera* extracts could also reduce the germination rate of *Amaranthus palmeri* (Julia, 2014). Moreover, *D. bulbifera* is one of the most dominant invasive species in the Bogor Botanical Gardens which is harmful for the collection plants that become its host such as from the *Dipterocarpaceae* (Santosa et al., 2014).

The seeds of *Shorea selanica* (Lam.) Blume (*Dipterocarpaceae*) are recalcitrant (germinate quickly); hence they cannot be stored for a long time. Storage will reduce seed viability (ability to germinate) (Tata, Wibawa, & Joshi, 2008). Furthermore, *S. selanica* is a species with critical conservation status (critically endangered) (Ashton, 1998); native species of Maluku which has a distribution area from Maluku to the southwest of the region; and predominantly found at the lowland forest on well-drained soils at altitude of 150 m above sea level (Panjaitan, Wahyuningtyas, & Ambarwati, 2011). Currently, red meranti species such as *S. selanica* has become a promising type of meranti for planting programs, such as commercial planting, rehabilitation of degraded forests and reforestation of conservation area. Besides, the wood of *S. selanica* has been used as construction wood, boat decks, and torch material in the Maluku region (Panjaitan et al., 2011). The amount of wood utilization, its critical conservation status (critically endangered), the limited distribution area, and the recalcitrant nature of the seeds are reasons why the effort to support the preservation of *S. selanica* is urgently needed, namely by conserving seeds through utilization of the allelochemical of *D. bulbifera* for inhibiting its seed germination.

MATERIALS AND METHODS

This research was conducted from March 2019 until May 2020 at the Biology and Chemistry Laboratory of the University of Nusa Bangsa and the Bogor Botanical Gardens-LIPI (Seed Bank).

Materials

The materials used in this study were plant parts (tubers and leaf) of *D. bulbifera*, seeds of *S. selanica*, methanol, chloroform, ammonia, 2 N H_2SO_4, Mayer Reagent, Wagner Reagent, Dragendorff Reagent, ethanol, anhydrous acetic acid, concentrated H_2SO_4,

Mg, concentrated HCl, distilled water, 1% $FeCl_3$, tannic Acid, 5% Na_2CO_3, and Folin-Ciocalteu Reagent.

The equipment used in this study included a set of glassware, spray bottles, mortars, ovens, hot plates, water bath, binocular microscope, spectrophotometers, 60 mesh sieves, shakers, and petri-dish, growth chamber, GC-MS, and filter paper.

Procedure

1. Extraction of Leaf and Tuber of *D. bulbifera*

Fresh leaf and tuber of *D. bulbifera* (approximately 3.5 kg) were collected randomly from around Botanical Garden Conservation Center-LIPI, washed thoroughly, chopped, dried in an oven at temperature 60 °C until dry (72 hours). The dried leaf and tubers were mashed to powder and sieved with 60 mesh sieves. As much as 500 g of leaf and tuber powder was extracted with 2,000 mL of methanol at room temperature for ±3 days to be further evaporated to vaporize the remaining methanol in the extract.

2. Qualitative Phytochemicals Assessment of The Leaf and Tuber of *D. bulbifera*

Tuber and leaf extracts were tested for bioactive content based on a modified method (Harborne, 1984) and analyzed by GC-MS (phenol compound), allowing identification through a total ion chromatogram graph.

Alkaloids

A total of 1 mL of leaf and tuber extract was added with 5 mL of chloroform and 5 mL of ammonia, put into a test tube, divided into three, added with three drops of 2 N H_2SO_4, and left for several minutes to separate. The top of each filtrate was taken and tested with Mayer, Wagner, and Dragendorff Reagents. Samples contain alkaloids if they produce white, red, and brown deposits after the addition of Mayer Reagents, Dragendorff Reagents, and Wagner Reagents, respectively.

Steroids/Terpenoids

A total of 1 mL of leaf and tuber extract was added with 3 mL of ethanol and 5 mL of anhydrous acetic acid. Later, 10 drops of concentrated sulfuric acid were added (Liebermann-Burchard). Changes in color from purple to blue or green indicate the presence of steroids; the appearance of a brownish-red color between the surfaces suggests terpenoids.

Flavonoids

A total of 1 mL of leaf and tuber extract was added with 3 mL of ethanol and further heated. Then, 0.1 g of Mg and two drops of concentrated HCl were added. The red color in the ethanol layer indicates the presence of flavonoids.

Saponin

A total of 1 mL of leaf and tuber extract was put in a test tube, then heated in a water bath contained 10 mL of distilled water. The filtrate was shaken and allowed to stand for 15 minutes. Durable (stabile) formed foam indicates the presence of saponins.

Tannin

A total of 1 mL of leaf and tuber extract was added with 10 mL of distilled water, heated in a water bath, and added with 2–3 drops of 1% $FeCl_3$. The appearance of green, dark blue, or greenish-black colors indicates the presence of tannins.

Total Phenolic Content

Measurement of total phenolic content carried out in this study was a modification of the method introduced by Andarwulan, Fardiaz, Wattimerz, and Shetty (1999). Preparation of tannic acid standard was conducted by dissolving 0.1 g of tannic acid into distilled water using a 25 mL measuring flask. Standard solutions at concentrations of 0, 25, 50, 75, 100, and 125 mg/L were made. The total phenolic content test was done by dissolving 5 mL of extract in a 25 mL measuring flask filled with distilled water to be homogenized using a shaker. A total of 1 mL of the solution was taken and added with 1 mL of 50% Follin Ciocalteu, allowed to stand for 5 minutes, added with 1 mL of 5% Na_2CO_3, and homogenized in the dark for 1 hour. The absorbance value was measured at 725 nm wavelength using a UV-VIS spectro-photometer.

3. The Effect of Tuber and Leaf Extracts of *D. bulbifera* L. on *S. selanica* Seed Germination Test

Preparation, Characterization, and Measurement of Seed Moisture Content

The fruits of *S. selanica* that have been ripe physiologically were selected and further extracted. Moreover, the uniform seeds were selected, washed with clean water, and dried. Characterization of fruits and seed was descriptively described based on direct observation. The seed characters observed using a binocular microscope were the number of wings, color, weight, length, width, the thickness of seeds, and internal seed structure. Measurement of moisture content of seeds was done using the International Seed Testing Association (ISTA) of Constant Temperature Oven Method (2015). The formula applied to determine the seed moisture content (MC) is MC= (M3-M2)/(M2-M1) x 100%. In which M1 is the weight of the container used, M2 is the weight of the seed and the container before being placed in the oven, and M3 is the weight of the seed and container after being placed in the oven.

Seeding

The seeds were soaked in leaf and tuber extract of *D. bulbifera* for 60 minutes. Seeding was carried out in the growth chamber, and filter paper was used as the germination media. After soaking in the extracts, the seeds were taken and planted to result in ten seeds/petri dish. Observations of variable were conducted every two days.

Variables observed in this study were germination capacity, first-day germination, last day germination, germination rate, and coefficient of simultaneity of germination. The formula used to calculate germination capacity (GC) is GC= n/N x 100%. In which, n= seeds that germinate; N= seeds that are germinated.

The formula applied to calculate the germination rate (x) is $x= \sum n \times 100/\sum(t \times n)$. In which, n= seeds that germinate, t= day when the seeds germinate. The formula used to calculate the coefficient of simultaneity of germination= $\sum n \times 100/\sum\{(T-t)^2 \times n)\}$; where $T= \sum(t \times n)/\sum n$; Σn= the total number of seeds germinate; and t x n= n seeds that germinate on day t.

Research Design

The research applied a completely randomized design. Storage treatment of *S. selanica* (P) seeds: immersion of seeds to be stored with leaf and tuber extract of *D. bulbifera* (concentrations of 0%, 25%, 50%, 75%, and 100%) for 60 minutes with three replications. Each experimental unit consisted of 10 seeds, with a total of 300 observation units.

Data analysis

The data obtained were analyzed using the Statistical Tool for Agricultural Research (STAR) program for variance testing with (F test) at 5% significance level. If the F test shows a significant effect, then the mean is further tested by Duncan's Multiple Range Test (DMRT) at the 5% level.

RESULTS

Secondary Metabolites in *Dioscorea bulbifera* L. Extract

In this study, the source of allelopathy used was the leaves and tubers of the invasive plant *D. bulbifera.* Phytochemical screening tests were applied to identify secondary metabolites in the tubers and leaves of *D. bulbifera* extracted in methanol (Table 1).

Table 1. Phytochemical screening test results

Name of test	Results		
	Leaf	Tuber	Method
Saponin	+	+	Froth test
Tannin	+	+	$FeCl_3$
Flavonoid	+	+	
Steroid/terpenoid	+	+	Liebermann-Burchard
Alkaloid	+	-	Mayer
	+	-	Wagner
	+	-	Dragendorff

Description : (+) = the presence of phytochemicals; (-) = the absence of phytocemicals

Phenolic Compounds in Methanol Extracts of Leaves and Tubers of *Dioscorea bulbifera* L.

Phenolic compounds in the methanol extract of leaves and tubers of *D. bulbifera* were determined quantitatively and qualitatively. Quantitative determination was expressed in total phenolic value, while qualitative determination was expressed in the type of phenolic group of compounds. The total phenolic value of the extract was expressed as a tannic acid equivalent (TAE).

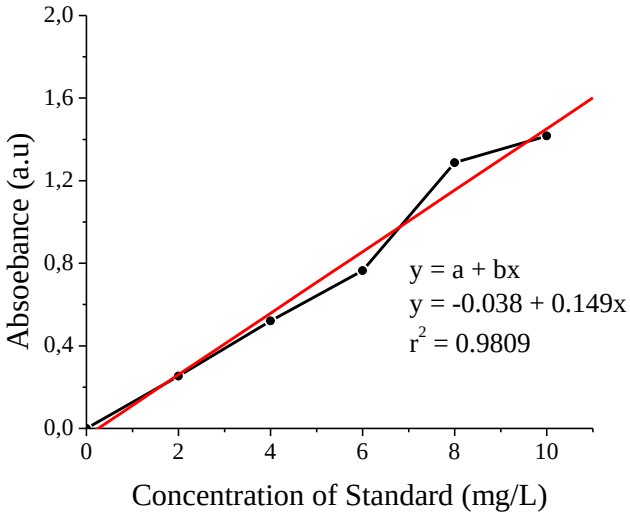

Figure 1. Tannic acid standard calibration curve tanat

Total phenolics in leaf and tuber extracts were tested using tannic acid standards at a wavelength of 725 nm. The regression value obtained from the tannic acid standard curve was 0.9809, with a linear equation y = 0.149x - 0.038 (Figure 1). Based on the calculations using a standard calibration curve of tannic acid, the results obtained are shown in Table 2.

Table 2. Results of total phenolic calculations

Sample	Absorbance	Concentration (mg/L)	Total phenolic (mg TAE/g extract)
Leaf	0.6160	4.389	2.19 ± 0.13
Tuber	0.2475	1.916	0.96 ± 0.01

The phenolic compound in the methanol extract of leaf and tuber of *Dioscorea bulbifera* L. was determined using Gas Chromatography-Mass Spectroscopy (GC-MS). Some phenolic compounds detected in GC-MS are shown in Table 3. Thus, the content of phenolic compounds obtained in this study was found to have allelopathic activity potential as seen from its effect on the germination of *S. selanica* seeds (Table 5).

Characterization of Seeds of *Shorea selanica* (Lam.) Blume

Seed characterization aims to provide information about the characteristics or morphological characters of germinated seeds (Figure 3). Information on seeds' moisture content of seeds can indicate the group or type of seed germinated.

Table 3. Types of phenolic compounds in leaf and tuber extracts

No.	Sample extract	Types of phenolic compounds
1	Leaf	Phenol
		1,2-Benzenediol
2	Tuber	2-Methoxyphenol
		Phenol
		Hidroquinon (1,4-Benzenediol)
		1,2-Benzenediol

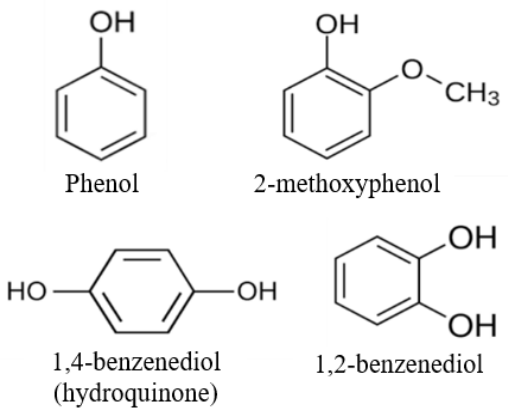

Figure 2. Chemical structure of phenolic compounds in methanol extracts of leaf and tuber of *D. bulbifera*

Figure 3. *Shorea selanica* (Lam.) Blume Seed

Table 4. Recapitulation of the effects of concentration, extracts and their interactions on several *Shorea selanica* germination parameters

Parameters	Factors		
	Concentration extract	Source extract (leaf and tuber)	Interaction
Germination capacity	*	*	*
Coefficient of rate of germination	*	*	*
Coefficient of simultaneity	*	*	*

Note: *= Significantly different (F <0.05); ns= not significantly different (F >0.05)

Table 5. Recapitulation of test result of the effect of leaf and tuber extracts of *D. Bulbifera* on some germination parameters of *S. selanica*

Concentration of tuber and leaf extract	Germination capacity (%) F= 7.28 p= 0.0009	Coefficient rate of germination F= 11.51 p= 0.0001	Coefficient of simultaneity of germination F= 7.21 p= 0.0009
Source extract: tuber *D. Bulbifera*			
0	$90.00^{a} \pm 6.32$	$0.1700^{bc} \pm 0.0334$	$0.8042^{a} \pm 0.1228$
25	$50.00^{b} \pm 6.32$	$0.1584^{bc} \pm 0.0334$	$0.8596^{a} \pm 0.1228$
50	$36.67^{c} \pm 6.32$	$0.2244^{ab} \pm 0.0334$	$0.6080^{ab} \pm 0.1228$
75	$10.00^{d} \pm 6.32$	$0.2500^{a} \pm 0.0334$	$0.5376^{b} \pm 0.1228$
100	$10.00^{d} \pm 6.32$	$0.1310^{c} \pm 0.0334$	$0.4878^{b} \pm 0.1228$
Source extract: leaf *D. Bulbifera*			
0	$93.33^{a} \pm 6.32$	$0.1621^{a} \pm 0.0334$	$0.8417^{a} \pm 0.1228$

Concentration of tuber and leaf extract	Germination capacity (%) F= 7.28 p= 0.0009	Coefficient rate of germination F= 11.51 p= 0.0001	Coefficient of simultaneity of germination F= 7.21 p= 0.0009
25	$16.67^{a} \pm 6.32$	$0.1429^{a} \pm 0.0334$	$0.9259^{a} \pm 0.1228$
50	$0.00^{c} \pm 6.32$	$0.0000^{b} \pm 0.0334$	$0.0000^{b} \pm 0.1228$
75	$0.00^{c} \pm 6.32$	$0.0000^{b} \pm 0.0334$	$0.0000^{b} \pm 0.1228$
100	$0.00^{c} \pm 6.32$	$0.0000^{b} \pm 0.0334$	$0.0000^{b} \pm 0.1228$

Note: Numbers followed by the same letter are not significantly different at p <0.05 using DMRT

Effect of Leaf and Tuber Extracts of *Dioscorea bulbifera* L. on *Shorea selanica* (Lam.) Blume Seed Germination

Based on the results of the analysis of variance (Table 4), it is known that the concentration of the extract, allelopathic sources, and interactions significantly affected the germination capacity, coefficient of the rate of germination, and coefficient of simultaneity of germination. To say, all concentrations of methanol extract from the leaf and tuber of *D. bulbifera* influenced the germination of *S. selanica* seeds.

Based on the result of DMRT test at 5% significance level, methanol extract from the tubers and leaf of *D. bulbifera* significantly affected the germination capacity, coefficient of germination rate, and coefficient of simultaneity of germination. Furthermore, leaf methanol extract was observed to have greater effect on *S. selanica* seeds than tubers (Table 5).

DISCUSSION

Secondary Metabolites in *Dioscorea bulbifera* L. Extract

Secondary metabolites, including allelochemicals, are found in nature, one of which is obtained from the release of chemical compounds in plant tissues such as leaves, stems, roots, flowers, seeds, pollen, rhizomes shoots, and tree bark (Weston & Duke, 2003). In this study, the source of allelopathy used was the leaves and tubers of the invasive plant *D. bulbifera.* Phytochemical screening tests were applied to identify secondary metabolites in the tubers and leaves of *D. bulbifera* extracted in methanol. According to Torres, Velázquez, and Brito-Arias (2011), methanol is used as a solvent in extraction because of its polar nature with a polarity index of 5.1. Therefore, methanol can extract other compounds with a more diverse polarity. Secondary metabolites include saponins, tannins, flavonoids, steroids/terpenoids, and alkaloids. Junaedi, Chozin, and Kim (2006) reported that phenolic compounds, terpenoids, alkaloids, and steroids have allelopathic activity.

Based on the phytochemical screening tests (Table 1), all secondary metabolites in leaf extract obtained positive result. Saponin, tannin, flavonoid, and steroid/terpenoid compounds were strongly detected, while alkaloid compounds were less strong. This result was indicated by the intensity of the concentration of different solutions. In tuber extracts, saponin, flavonoid, and steroid/terpenoid compounds were strongly detected, while tannins were less strong. Alkaloid compounds was not yet found in the tuber extract. Secondary metabolites found in the tuber and leaf of *D. bulbifera* have the potential as allelochemicals. As confirmed by Walton and Brown (1999) and Rice (1974), plant metabolites and allelochemicals can be further divided into three major groups: phenolic, terpenoids, and alkaloids.

This is consistent with the results of Adeosun, Arotupin, Toba, and Adewole (2016) which showed that the ethanol extract of the tuber of *D. bulbifera* did not contain alkaloids. In the study of Oksari et al. (2019), *D. bulbifera* leaf and tuber extracted in distilled water also did not contain alkaloids. Those previous studies had similar results depicting that alkaloids are not synthesized as secondary metabolites in the leaves and tubers of *D. bulbifera*. The compounds detected strongly in the leaf and the tubers of *D. bulbifera* were flavonoid compounds. Flavonoids are one of the largest classes of phenolic compounds. In plants, phenolic compounds can be simple phenols, anthraquinones, phenolic acids,

coumarin, flavonoids, lignin, and tannins (Harborne, 1987). The existence of this phenolic compound needs to be further investigated concerning its role as an allelochemical compound.

Phenolic Compounds in Methanol Extracts of Leaves and Tubers of *Dioscorea bulbifera* L.

The total phenolic in leaf extract was more significant than that in the tuber extract of *D. bulbifera* (Table 2). Hence, the leaf extract contains higher total phenolic content. It is possibly due to the existence of organ that plays an essential role during photosynthesis in the leaves since leaves contain chloroplasts that function to capture sunlight. Exposure to sunlight will increase the production of secondary metabolites; however, excessive sun exposure will decrease ~~causes~~ the production of secondary metabolites (Ibrahim & Jaafar, 2012).

Oksari et al. (2019) reported that the leaves and tubers of *D. bulbifera* extracted in distilled water had a smaller value compared to the results of this study. This difference might be caused by environmental factors, solvent factors, and the amount of extract used. Borges et al. (2013) mentioned that environmental factors, such as soil composition, temperature, rainfall, and ultraviolet radiation can affect the concentration of phenol components. Also, polar solvents like water could attract more polar phenolic compounds than non-polar phenolic compounds. According to Abdelwahab et al. (2009), the total phenolic content will be different if extracted with other solvents.

The GC-MS is a method of separating organic compounds using two compound analysis methods (Table 3). Gas chromatography is a spectroscopic technique which separates mixtures based on the different migration rates of constituent components. Gas chromatography is commonly used to identify a compound in a gas mixture and determine the compound concentration in the gas phase. Mass spectroscopy is a method for obtaining molecular weight by finding the ratio of mass to charge of ions whose charge is known by measuring the circular orbit radius in a uniform magnetic field (Pavia, Lampman, Kriz, & Engel, 2006).

In this study, phenolic compounds in leaf extracts consisted of only two types, namely phenol and 1,2-benzenediol. However, total phenolic content in leaf was higher than that in tuber. Tuber extract had 4 types of phenolic compounds with two compounds that were similar to phenolic compounds in leaf extracts, namely phenol and 1,2-benzenediol, while the other compounds were hydroquinone (1,4-benzenediol) and 2-methoxyphenol (Figure 2). The mechanism of allelopathy will be related to the content of phenolic compounds in a plant. It will be closely related to the enzymes released by the plant and its physiological processes (Einhellig, 2004).

Characterization of Seeds of *Shorea selanica* (Lam.) Blume

Based on observations of morphological characters, *S. selanica* seed is an oval-ellipse shape with ptero (wing) (Figure 3). The seeds' length ranged from 17.40 to 20.05 mm with an average of 18.6625 ± 0.759523 mm; seed diameter ranged from 9.20 to 11.60 mm with an average of 9.991667 ± 0.754933 mm, while seed weight ranged from 0.5399 to 0.9665 g with an average of 0.697883 ± 0.123719 mm. The average moisture content of *P. littoralis* after direct harvesting was 14.40759 ± 2.485845%. According to Hong, Linington, and Ellis (1998), *S. selanica* seeds are recalcitrant seeds since *S. selanica* seeds have several morphological and physiological characteristics as a sign of recalcitrant seeds.

Effect of Leaf and Tuber Extracts of *Dioscorea bulbifera* L. on *Shorea selanica* (Lam.) Blume Seed Germination

The response of these chemical compounds depends on the concentration of the extract used (Einhellig, 1986); because, in some cases, allelochemicals can delay plant growth at high concentrations and increase plant growth at low concentrations (Subtain et al., 2014). In this study, it was seen that the higher the concentration of the extract, the more it inhibited seed germination (Table 5). Oksari et al. (2019) showed a similar result of extract of the tubers and leaves of *D. bulbifera* at concentrations of 20, 60, 80, and 100% which had a significant effect on the

germination of *Polyalthia littoralis* seeds. Previous studies of Ahmed, Mashaly, Ziada, and Deweeb (2015), Sharma, Rathore, Srinivasan, and Tyagi (2014) also indicated that a plant extract would significantly affect other plants. Simply stated, allelopathy can inhibit plant growth due to the release of these chemicals (Willis, 2007).

Allelopathic sources also influence plant growth, especially for seed germination. According to Rice (1995), some organic compounds that are inhibiting at one level of concentration can provide a stimulatory effect at another concentration level. Hence, different sources of allelopathy will lead to different impact caused. The source of allelopathy in this study is the invasive plant of species *D. bulbifera* L. This plant is known to cause many environmental problems expectedly due to the release of chemical compounds which affect the growth and development of surrounding plant. This condition is supported by its rapid growth to cover the branches and twigs of its host plant which can interfere with the photosynthetic activity of the host plant (Santosa et al., 2014).

Furthermore, the source of allelopathy found in a plant also shows differences in allelochemicals obtained and their effects. In this study, the leaves of *D. bulbifera* produced two phenolic compounds, while the tuber produced four phenolic compounds (Table 3). However, at the same concentration, phenolic compounds in leaves had a significant effect while those in tubers did not (Table 5) due to higher phenolic content in leaves than in the tubers (Table 2). Research conducted by Oksari et al. (2019) also showed similar results (phenolic levels in *D. bulbifera* leaves were higher compared to tubers) despite different solvents (distilled water) used.

Allelopathic interaction could be one of the significant factors that contribute to species distribution and abundance in plant communities besides playing important role in the success of invasive plants (Chou, 1999; Mallik, 2003; Field, Jordán, & Osbourn, 2006; Inderjit, Callaway, & Vivanco, 2006; Zheng et al., 2015). In this case, there is a relationship to interactions that give rise to competition in terms of taking and reducing several growth factors (water, nutrients, light) from the environment (Rice, 1995; Qasem & Foy, 2001). This interaction is seen from the effect of allelopathy, which is thought to be influenced by several factors, including extract solvents and test species. This statement was supported by Oksari et al. (2019) that distilled water extracts from tuber and leaves of *D. bulbifera* on *Polyalthia littoralis* seed only significantly affected germination rate and germination speed coefficient; while allelopathic sources (leaves and tubers) and interactions obtained from *D. bulbifera* did not affect germination capacity, germination rate coefficient, and germination simultaneity coefficient. Furthermore, allelochemicals are selective in inhibiting seed germination and will also have different effects on a species' seeds (Einhellig, 1994). In the study of Nornasuha et al. (2012), methanol extracts from the tubers of *Dioscorea hispida* Dennst showed a different response in each test species where only one test species was inhibited by germination, namely *Amaranthus* sp. Meanwhile, in general, species other than *Amaranthus* sp. (*Brassica* sp., *Cucumis* sp., and *Raphanus* sp.) were stimulated by germination of methanol extracts from the tubers of *Dioscorea hispida* Dennst. However, the allelochemical effect had a significant impact on root length in all test species.

Concentration of 75% and 100% was found to result in the lowest seed viability reduction with an average germination rate of 10.00 ± 6.32% (in tuber extracts) and 0.00 ± 6.32% (in leaf extracts) (Table 5). As confirmed by Usman, Taiwo, Ogono, and Osoniyi (2014), methanol extracts from the tubers of *Dioscorea dumetorum* Kunth affected seed germination by 25% (20 mg/mL) to 75% (80 mg/mL). Similarly, the study of Sitthinoi, Lertmongkol, Chanprasert, and Vajrodaya (2017) found that the methanol extract of *Echinochloa colona* L. (Link) had a 50% significant effect on germination and growth of rice tillers compared to hexane and dichloromethane solvents. Therefore, methanol has a considerable impact on inhibiting seed germination.

Moreover, as presented in Table 5, leaf methanol extract is seen to have more effect on *S. selanica* seeds because the leaves have higher phenol content than tubers (Table 2).

This can affect germination speed, hence inhibiting or slowing down metabolism during germination (Ferreira & Borguetti, 2004). Based on these studies, the extract of leaves and tubers of *D. bulbifera* could influence the germination of plants with recalcitrant seeds. A study conducted by Oksari et al. (2019) showed that extracts of the leaves and tubers of *D. bulbifera* had effect on *P. littoralis* seeds. Therefore, slow germination of seed might indicate the presence of allelochemicals which could reduce the speed of distribution and translocation of nutrients of endosperm components to the embryo. Rice (1974) reported that allelopathic effects on plant germination and growth can occur through a variety of mechanisms including reduction of mitotic activity in roots and hypocotyls, suppression of hormone activity, reduction of ion absorption rates, inhibition of photosynthesis and respiration, inhibition of protein formation, and decreased permeability of cell membranes, also inhibition of enzyme action.

Phytochemical results of allelopathy can inhibit plant germination, thus interfering with cell division and the mechanism of energy transfer besides limiting the absorption of water and nutrients (Abu-Romman, Shatnawi, & Shibli, 2010). Inhibition of seed germination in *S. selanica* plants was resulted from inhibition caused by phenolic compounds (flavonoids) in the extract of *D. bulbifera*. Phenolic is the metabolite most frequently reported to play a role in higher plants' defense mechanisms, with varying toxicity, and influences cell function (Haig, 2008). Thus, seed germination can be inhibited by plant tissue's natural substances (Devlin & Witham, 1983; Abozeed, 1990; Gill, Nyawuame, & Ehikhametalor, 1992). In addition, phenolics, terpenoids, and alkaloids released from these plants have an inhibitory effect on germination and sapling growth (Sahoo, Jeeceelee, Lallinrawna, & Muthukumaran, 2015). This is consistent with the study results where three chemical compounds were found in *S. selanica* leaves, while no alkaloids were observed in *S. selanica* tubers.

CONCLUSION

In this study, the methanol extract of *Dioscorea bulbifera* L. leaves contained steroids/terpenoids, alkaloids, flavonoids, saponins, and tannins. In contrast, alkaloids were not found in the methanol extract of *Dioscorea bulbifera* tubers. Total phenolic in leaf extract reached 2.19 ± 0.13 mg TAE/g extract, while the tuber extract had a total phenolic of 0.96 ± 0.01 mg TAE/g extract. Phenolic compounds in leaf extracts were observed to consist of two types, namely phenol and 1,2-benzenediol. Moreover, tuber extract had four types of phenolic compounds. Two compounds of the same type as the phenolic compounds in leaf extracts were found to be phenol and 1,2-benzendiol, while the other compounds identified were hydroquinone (1,4-benzendiol) and 2-methoxyphenol. Leaf extract was considered more preferable than tuber extract for germination *S. selanica* seed. Methanol extract from the tubers and leaves of *D. bulbifera* significantly affected the germination capacity, coefficient rate of germination and coefficient of simultaneity of germination. Concentration of 75% and 100% was found to result in the lowest seed viability reduction with an average germination rate of 10.00 ± 6.32% (in tuber extracts) and 0.00 ± 6.32% (in leaf extracts).

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia. We would like to thank the Director of Research Center for Plant Conservation and Botanic Gardens - LIPI and the Rector of Nusa Bangsa University for supporting this research. We are highly thankful to Dr. Dian Latifah for giving permission to use the Seed Bank of Bogor Botanic Gardens for conducting research. We are also grateful for the generous assistance from all team members of LIPI Botanic Gardens Seed Bank: Mr. Harto and all team members of Biology and Chemistry and Biology Laboratory: Asep, Mona, Lesty, Ridwan, Anugerah, Emil, Satria Pertiwi.

REFERENCES

Abdelwahab, S. I., Abdul, A. B., Elhassan, M. M., Mohan, S., Al-zubairi, A. S., AlHaj, N. A., … Mariod, A. A. (2009).

Biological and phytochemical investigations of *Goniothalamus umbrosus* leaves hexane extract. *Journal of Medicinal Plants Research*, *3*(11), 880-885.

Abozeed, A. N. (1990). *Plant hormones and agricultural applications*. Cairo: Maddpoly liberary.

Abu-Romman, S., Shatnawi, M., & Shibli, R. (2010). Allelopathic effects of spurge (*Euphorbia hierosolymitana*) on wheat (*Triticum durum*). *Journal of Agricultural and Environmental Sciences*, *7*(3), 298-302.

Adeosun, O. M., Arotupin, D. J., Toba, O. A., & Adewole, A. (2016). Antibacterial activities and phytochemical properties of extracts of *Dioscorea bulbifera* Linn (air potatoe) tubers and peels against some pathogenic bacteria. *The Journal of Phytopharmacology*, *5*(1), 20-26.

Ahmed, M. A. E., Mashaly, I. A., Ziada, M. E. A., & Deweeb, M. R. (2015). Phytotoxicity of three *Plantago* species on germination and seedling growth of hairy beggarticks (*Bidens pilosa* L.). *Egyptian Journal of Basic and Applied Sciences, 2,* 303–309. doi: 10.1016/j.ejbas.2015.07.003.

Albuquerque, M., Santos, R., Lima, L., Filho, P. M., & Nogueira, R. (2011). Allelopathy, an alternative tool to improve cropping systems: A review. *Agronomy for Sustainable Development*, *31*(2), 379-395. doi: 10.1051/agro/2010031.

Andarwulan, N., Fardiaz, D., Wattimerz, G., & Shetty, K. (1999). Antioxidant activity associated with lipid acid phenolic during seed germination of *Pangium edule* Reiw. *Agriculture Food Chemical*, *47*(18), 3158-3163. doi: 10.1021/jf981287a.

Ashton, P. (1998). *Shorea selanica*. The IUCN red list of threatened species 1998: E.T33146A9762519. (2019, March 08). Retrieved from: https://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33146A9762519.en.

Borges, L. L., Alves, S. F., Sampaio, B. L., Conceição, E. C., Bara, M. T. F., & Paula, J. R. (2013). Environmental factors affecting the concentration of phenolic compounds in *Myrcia tomentosa* leaves. *Brazilian Journal of Pharmacognosy*, *23*(2), 230-238. doi: 10.1590/S0102-695X2013005000019.

Chou, C. (1999). Critical reviews in plant sciences roles of allelopathy in plant biodiversity and sustainable agriculture. *Critical Reviews in Plant Sciences*, *18*(768420433), 609-636. doi: 10.1080/07352689991309414.

Devlin, R. M., & Witham, F. H. (1983). *Plant physiology (4th revise)*. New Delhi: Division of wadsworth, Inc.

Duan, L., Liang, S., Li, F., & Zhou, Q. (2015). Comparison of the leaf allelopathic potential of the invasive wetland plant *Spartina alterniflora* and three native mangrove plants. *Journal of Guangxi Normal University: Natural Science Edition*, *33*(2), 109-114. doi: 10.16088/j.issn.1001-6600.2015.02.017.

Einhellig, F. A. (1986). Mechanisms and modes of action of allelochemicals. In A. R. Putnam, & C. S. Tang (Eds.), *The science of allelopathy* (pp. 171–188). New York, USA: John Wiley & Sons.

Einhellig, F. A. (1994). Mechanism of action of allelochemicals in allelopathy. In W. Mushtaq, M. B. Siddiqui, & K. R. Hakeem (Eds.), *Allelopathy: Potential for green agriculture* (pp. 61-66). Switzerland: SpringerBriefs in Agriculture.

Einhellig, F. A. (2004). Mode of allelochemical action of phenolic compounds. In F. A. Macias, J. C. G. Galindo, J. M. G. Molinillo, & H. G.Cutler (Eds.), *Allelopathy: Chemistry and mode of action of allelochemicals* (pp. 217-238). Boca Raton, Florida: CRC Press.

Ferreira, A. G., & Borguetti, F. (2004). Germinação: Do básico ao aplicado. Artmed. (2019, October 12). Retrieved from http://biblioteca.usac.edu.gt/tesis/08/08_2469_C.pdf

Field, B., Jordán, F., & Osbourn, A. (2006). First encounters-deployment of defence-related natural products by plants. *New Phytologist*, *172*(2), 193-207. doi:

10.1111/ j.1469-8137.2006.01863.x.

Gill, L. S., Nyawuame, H. G. K., & Ehikhametalor, A. O. (1992). Effect of crude oil on the growth and anatomical features of *Chromolaena odorata* (L.) K. & R. *Chromolaena odorata Newsletter, 6,* 1-6.

Haig T. (2008). Allelochemicals in plants. In R. S. Zeng, A. U. Mallik, & S. M. Luo (Eds.) *Allelopathy in sustainable agriculture and forestry*. New York, USA: Springer.

Harborne, J. B. (1984). *Phytochemical methods second edition*. London and Newyork: Chapman and Hall Ltd.

Harborne, J. B. (1987). *Metode fitokimia: Penuntun cara modern menganalisis tumbuhan*. Bandung: ITB Press.

Hong, T. D., Linington, S., & Ellis, R. (1998). *Compendium of information on seed storage behaviour*. Kew: The Trustees, Royal Botanic Gardens.

Ibrahim, M. H., & Jaafar, H. Z. E. (2012). Primary, secondary metabolites, H_2O_2, malondialdehyde and photosynthetic responses of *Orthosiphon stimaneus* Benth. to different irradiance levels. *Molecules*, *17*(2), 1159-1176. doi: 10.3390/molecules17021159.

Inderjit., Callaway, R. M., & Vivanco, J. M. (2006). Can plant biochemistry contribute to understanding of invasion ecology?. *Trends in Plant Science*, *11*(12), 574-580. doi: 10.1016/j.tplants.2006.10.004.

Inderjit., Seastedt, T. R., Callaway, R. M., Pollock, J. L., & Kaur, J. (2008). Allelopathy and plant invasions: Traditional, congeneric, and bio-geographical approaches. *Biological Invasions*, *10*(6), 875-890. doi: 10.1007/s10530-008-9239-9.

International Seed Testing Association (ISTA). (2015). International rules for seed testing 2015. (2019, March 08). Retrieved from https://www.seedtest.org/upload/cms/user/2015-SH-7-019a.pdf

Julia. (2014). Weed warfare: Investigating allelopathy: Year iv. (2019, October 12). Retrieved from https://www.amnh.org/learn-teach/curriculum-collections/young-naturalist-awards/winning-essays/2013/weed-warfare-investigating-allelopathy.

Junaedi, A., Chozin, M. A., & Kim, K. H. (2006). Perkembangan terkini kajian alelopati. *HAYATI Journal of Biosciences*, *13*(2), 79-84. doi: 10.1016/S1978-3019(16)30386-2.

Lorenzo, P., Hussain M. I., & González L. (2013) Role of allelopathy during invasion process by alien invasive plants in terrestrial ecosystems. In Z. Cheema, M. Farooq, & A. Wahid (Eds.) *Allelopathy: Current trends and future applications* (pp. 3-21). Berlin, Heidelberg: Springer.

Mallik, A. U. (2003). Conifer regeneration problems in boreal and temperate forests with ericaceous understory: Role of disturbance, seedbed limitation, and keytsone species change. *Critical Reviews in Plant Sciences*, *22*(3-4), 341-366. doi: 10.1080/713610860.

Nešić, M., Obratov-Petković, D., Skočajić, D., Bjedov, I., Đukić, M., & Đunisijević-Bojović, D. (2016). Allelopathic potential of the invasive species aster lanceolatus willd. *Periodicum Biologorum*, *118*(1), 1-7. doi: 10.18054/pb.2016.118.1.2816.

Nornasuha, Y., Nashriyah, M., Hasbullah, M., 'Ain, K. M., Nor, Z. M., Adilah, N., & Azhar, M. (2012). Allelopathic effects of *Dioscorea hispida* Dennst. *(Dioscoreaceae)* tuber from Terengganu, Peninsular Malaysia. *Journal of Agricultural Technology, 8*(5), 1855-1865.

Oksari, A. A., Susanty, D., & Wanda, I. F. (2019). Allelopathic effect of invasive species air potato (*Dioscorea bulbifera*) on seeds germination of *Polyalthia littoralis*. *Nusantara Bioscience, 11*(1), 63-70. doi: 10.13057/nusbiosci/n110111.

Panjaitan, S., Wahyuningtyas, R. S., & Ambarwati, D. (2011). Pengaruh naungan terhadap proses ekofisiologi dan pertumbuhan semai *Shorea selanica* (DC.) Blume di persemaian. *Jurnal Penelitian Dipterokarpa*, *5*(2), 73-82. doi: 10.20886/jped.2011.5.2.73-82.

Pavia, D., Lampman, G., Kriz, G., & Engel, R. (2006). *Introduction to organic laboratory techniques (4th ed.)*. Canada: Thomson Brooks/Cole.

Ismaini, L. (2015). *Pengaruh alelopati tumbuhan invasif (Clidemia hirta) terhadap germinasi biji tumbuhan asli (Impatiens platypetala).* Paper presented at Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia (PSNMBI), Surakarta, Indonesia. Retrieved from https://www.researchgate.net/publication/300783068_Pengaruh_alelopati_tumbuhan_invasif_Clidemia_hirta_terhadap_germinasi_biji_tumbuhan_asli_Impatiens_platypetala.

Qasem, J. R., & Foy, C. L. (2001). Weed allelopathy, its ecological impacts and future prospects: A review. *Journal of Crop Production*, *4*(2), 43-119. doi: 10.1300/J144v04n02_02.

Rastogi, J., Rawat, D. S., & Chandra, S. (2015). Diversity of invasive alien species in Pantnagar flora. *Tropical Plant Research*, *2*(3), 282-287.

Rice E.L. (1974) Allelopathy. Physiological Ecology. New York,

NY: Academic Press

Rice E.L. (1974) Allelopathy. Physiological Ecology. New York,

NY: Academic Press

Rice, E. L. (1974). *Allelopathy, Physiological Ecology*. New York: Academic Press.

Rice, E. L. (1995). *Biological control of weeds and plant diseases: Advances in applied allelopathy*. Norman, Oklahoma: University of Oklahoma Press.

Sahoo, U. K., Jeeceelee, L., Lallinrawna, S., & Muthukumaran, R. B. (2015). Effect of *Citrus reticulata* blanco leaf extract on seed germination and initial seedling growth parameters of five home garden food crops. *Journal of Experimental Biology and Agricultural Sciences*, *3*(6), 517-528. doi: 10.18006_2015.3(6).517.528.

Santosa, E., Widiyanto, G., Lontoh, A. P., Agustin, E. K., Mine, Y., & Sugiyama, N. (2014). Invasive weeds in Bogor Botanic Gardens, Indonesia and its implication on surrounding landscapes. *Buletin Kebun Raya*, *17*(2), 113-126.

Šćepanović, M., Novak, N., Barić, K., Ostojić, Z., Galzina, N., & Goršić, M. (2007). Allelopathic effect of two weed species, *Abutilon theophrasti* Med. and *Datura stramonium* L. on germination and early growth of corn. *Agronomski Glasnik*, *69*(6), 459-472.

Sharma, A. D., Rathore, S. V. S., Srinivasan, K., & Tyagi, R. K. (2014). Comparison of various seed priming methods for seed germination, seedling vigour and fruit yield in okra (*Abelmoschus esculentus* L. Moench). *Scientia Horticulturae*, *165*, 75-81. doi: 10.1016/j.scienta.2013.10.044.

Singh, H. P., Batish, D. R., & Kohli, R. K. (2003). Allelopathic interactions and allelochemicals: New possibilities for sustainable weed management. *Critical Reviews in Plant Sciences*, *22*(3-4), 239-311. doi: 10.1080/713610858.

Sitthinoi, P., Lertmongkol, S., Chanprasert, W., & Vajrodaya, S. (2017). Allelopathic effects of jungle rice (*Echinochloa colona* (L.)Link) extract on seed germination and seedling growth of rice. *Agriculture and Natural Resources*, *51*(2), 74-78. doi: 10.1016/j.anres. 2016.09.004.

Subtain, M. U., Hussain, M., Tabassam, M. A. R., Ali, M. A., Ali, M., Mohsin, M., & Mubushar, M. (2014). Role of allelopathy in the growth promotion of plants. *Scientia Agriculturae*, *2*(3), 141-145. doi: 10.15192/pscp.sa.2014.2.3.141145.

Tata, H. L., Wibawa, G., & Joshi, L. (2008). *Petunjuk teknis: Penanaman meranti di kebun karet*. Bogor: World Agroforestry Centre (ICRAF).

Torres, L. G., Velázquez, A., & Brito-Arias, M. A. (2011). Ca-alginate spheres behavior in presence of some solvents and water-solvent mixtures. *Advances in Bioscience and Biotechnology*, *02*(01), 8-12. doi: 10.4236/abb.2011.21002.

Usman, K., Taiwo, O. E., Ogono, T., & Osoniyi, O. (2014). An investigation of allelopathic, genotoxic and cytotoxic effects of *Dioscorea dumetorum* Kunth tuber extracts. *Agriculture and Biology*

Journal of North America, *5*(5), 183-192. doi: 10.5251/abjna. 2014.5.5.183.192.

Walton, N., & Brown, D. (1999). *Book review: Chemicals from plants: Perspectives on plant secondary products.* London: Imperial College press.

Weston, L. A., & Duke, S. O. (2003). Weed and crop allelopathy. *Critical Reviews in Plant Sciences*, *22*(3-4), 367-389. doi: 10.1080/713610861.

Willis, R. (2007). *The history of allelopathy*. Australia: Springer.

Yanti, M., Indriyanto., & Duryat. (2016). Pengaruh zat alelopati dari alang-alang terhadap pertumbuhan semai tiga spesies akasia (the effect of allolepathy from blady grass to three species of *Acacia* seedlings growth). *Jurnal Sylva Lestari*, *4*(2), 27-38. doi: 10.23960/jsl2427-38

Zheng, Y. L., Feng, Y. L., Zhang, L. K., Callaway, R. M., Valiente-Banuet, A., Luo, D. Q., … Silva-Pereyra, C. (2015). Integrating novel chemical weapons and evolutionarily increased competitive ability in success of a tropical invader. *New Phytologist*, *205*(3), 1350-1359. doi: 10.1111/nph.13135.