

Effectivity of soap nuts extract (*Sapindus rarak*) as bioherbicide toward the growth of *Leptochloa chinensis* and *Fimbristylis milacea*

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Abstract. Pujisiswanto H, Mar'ah DL, Sriyani N, Yusnita, Evizal R. 2022. Effectivity of soap nuts extract (*Sapindus rarak*) as bioherbicide toward the growth of *Leptochloa chinensis* and *Fimbristylis milacea*. *Biodiversitas* 23: 1222-1230. *Fimbristylis miliaceae* and *Leptochloa chinensis* weeds were grown predominantly in rice fields which cause a decrease in production yields of up to 36%-43%. The aim of this experiment was to evaluate the method of extractions and find the best concentration of *Sapindus rarak* by Aqueous and methanol extracts against the growth of *F. miliaceae* and *L. chinensis*, which do not reduce the growth of rice. Two experiments were arranged for this research; the first was bioassay tests of weed growth of *F. miliaceae* and *L. chinensis* at the greenhouse, which were applied by aqueous and methanol *S. rarak* extracts. It was carried out using a randomized block design in factorial (2x5). The second experiment was the rice growth toxicity test which was applied *S. rarak* extracted by aqueous and methanol, analyzed to single factor randomized design with various concentrations. The results showed that the application of *S. rarak* extracted by aqueous and methanol was able to inhibit the growth of *L. chinensis* and *F. mileacea* weeds at a concentration of 25%, 50% and 75%. The application of *S. rarak* extracted by aqueous did not affect rice growth, whereas *S. rarak* extracted by methanol inhibited rice height and stover weight at a concentration of 75%, but did not inhibit root length growth and leaf number.

Keywords: Allelopathy, bioherbicide, *Fimbristylis milacea*, *Leptochloa chinensis*, *Sapindus rarak*

Abbreviations: SEBA: soap nuts (*Sapindus rarak*) extracted by aqueous; SEBM: soap nuts (*Sapindus rarak*) extracted by methanol

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the important plants in Indonesia. It contributed 45% of major carbohydrates from the total "food intake" or about 70% in society consumption pattern (Rohimat et al. 2017). Proper rice cultivation is one activity to stabilize rice productivity. However, there is a weed problem in the implementation. These weeds cause a decrease in rice production up to 76% (Sureskumar et al. 2016). Weeds are plants that are unwanted and detrimental to human interests, so they need to be controlled. Losses due to weeds in cultivated areas are related to decreased production and product quality and hampered human activities. Weed competition in rice plants is known to often occur in the critical phase of the crop. The crucial period of rice plants is 0 to 1/3 of the age of the plant (early planting until the rice is about 40 days old). In the crucial period, the canopy between plants has not closed so that weed seeds will germinate and grow faster than rice plants (Pane and Jatmiko 2009), thus causing competition for growing facilities, which will have the potential to decrease rice production and productivity. Weeds in rice fields consist of several groups of grasses, cyperuses, and broad leaves. The most common weeds species are *Monochoria vaginalis*, *Cyperus difformis*, *Fimbristylis miliacea*, *Echinochloa crus-galli* and *Leptochloa chinensis* (Fitri et al. 2014; Tjitrosemito 1994). Zarwazi et

al. (2016) stated that *F. miliacea* weed could produce large quantities of seeds, be tolerant of environmental stress, and germinate in a short time, which will dominate cultivated land. In this research, Benvenuti et al. (2004) stated that *L. chinensis* weed is an annual grass that can adapt to aquatic and semi-aquatic environments and can become an invasive weed it produces large amounts of seeds. Seeds do not experience endogenous dormancy and can germinate in anoxic conditions (low oxygen availability) and germinate quickly. As a result of the nature of the weeds, it is necessary to have effective and efficient weed control measures, especially in the pre-growing phase of weeds. In general, weed control is mostly done by using synthetic herbicides. However, the overuse and continuous use of synthetic herbicides can lead to the emergence of herbicide-resistant weeds and negatively affect the environment and human health (Estiati 2019). The alternative weed control is environmentally friendly and sounds being intensively carried out.

This control can be done by looking for secondary metabolic compounds or allelochemical compounds. Allelopathy is a phenomenon whereby other plants release chemical compounds into the environment and has any direct or indirect negative or positive effects on germination, growth and development of one plant (Bhadoria 2011; Bravo et al. 2013; Khanh et al. 2013). The negative impacts of allelochemicals are disturbances in

membrane permeability function, water and nutrient transport, respiration, protein, and nucleic acid synthesis, and photosynthesis rate (Latif et al. 2017). The soap nut (*Sapindus rarak*) contains allelochemical compounds in the form of saponin and other active substances such as alkaloids, flavonoids, polyphenols, and tannins (Syahroni et al. 2013). This compound was obtained by extraction. Soap nut extraction can be done by adding aqueous and methanol as solvents; both have the same characteristic in filtering out most secondary metabolites in simplicia (Salamah and Widyasari 2015). This experiment aimed to evaluate the method of extractions and find the best concentration of *S. rarak* by aqueous and methanol extracts against the growth of *F. miliacea* and *L. chinensis*, which do not reduce the growth of rice.

MATERIALS AND METHODS

Study area

The study was conducted from September to December 2019. The research was conducted at the Integrated Laboratory of the Technology Innovation Centers, University of Lampung, Indonesia; Greenhouse of Botanical Garden of Lampung State Polytechnic, Indonesia, and the Weed Science Laboratory, Faculty Agriculture, University of Lampung, Indonesia.

Experimental design

This study consists of two experiments, involving two types of weeds, there are grassess (*L. chinensis*) and sedge (*F. miliaceae*). Experiment 1: Bioassay test on the growth *F. miliaceae* and *L. chinensis* weeds applied with *S. rarak* extracted by aqueous (SEBA) and *S. rarak* extracted by methanol (SEBM) in a greenhouse. It was carried out using a randomized block design in factorial (2x5) and experiment 2: Toxicity test of rice growth that has been applied to extract of *S. rarak* using SEBA and SEBM. It was analyzed to single factor randomized design. There are two extraction methods, (SEBA) and (SEBM) solvents. Then the extract is qualified into several concentration levels, consisting of 0 (control), 25% soap nuts extract, 50% soap nuts extract, 75% soap nuts extract and 5% saponins. The first and second experiments were replicated six times.

The planting experiment was arranged in a randomized block design. Weed seeds and rice plants were sown first, then seedlings with the same height and number of leaves were transplanted into plastic pots (12 cm diameter size of plastic pots) with rice media. They are maintained until the vegetative phase.

Soap nuts (*Sapindus rarak*) extraction methods

The fresh *S. rarak* fruits were cleaned, and the seeds were removed. The fruits were dried in an oven at 80°C for three days. Dried *S. rarak* were ground into simplicia powder.

Sapindus rarak extracted by aqueous solvent (SEBA)

The simplicia powder was soaked in aqueous (the amount of aqueous is adjusted to the concentration to be

made) for 24 hours. The preparation of the concentration solution can be adjusted according to the following conditions: 25% (25 g simplicia powder + 100 mL aqueous); 50% (50 g simplicia powder + 100 mL aqueous); 75% (75 g simplicia powder + 100 mL aqueous); 5% of saponin (5 g pure saponin powder + 100 mL aqueous). The filtrate can be applied in accordance with the provisions of the concentration that has been made.

Sapindus rarak extracted by methanol solvent (SEBM)

The extract was obtained by the maceration method with 96% methanol solvent. As much as one kg of simplicia powder was put into a beaker glass, poured with 3000 mL of the methanol, stirred, and covered. After 24 hours, the mixture was filtered. Then the powder was soaked again in 3000 mL of 96% methanol solution; after 24 hours, the mixture was filtered again. All collected filtrates were concentrated using a rotary evaporator at 60°C to obtain the thick liquid (Ahmed et al. 2012).

Bioherbicide treatment on weed and rice plant

Bioherbicide application

Application of *S. rarak* extract on weeds and rice was carried out one week after planting (WAP) using a knapsack sprayer. Before applying bioherbicides, weeds and rice plants were selected and calibrated. Weeds and rice are planted and maintained with the same degree of uniformity. Calibration was carried out by an extensive method using a red nozzle with a spray field width of 2 m. The applied weeds were arranged randomly on an area of 10 m². Experiment 1 was applied once at the beginning of seeding, while experiment 2 was carried out once seven days after planting.

Observation

The variables observed in the first experiment were weed height (measured from the base of the stem to the tip of the highest leaf), root length (measured by using a ruler starting from the base of the growing stem to the longest root), plant dry weight (was obtained by inserting weeds into envelopes and then in the oven at 80°C for a day), the percentage of damage, level of damage (the level of damage of weeds and rice plants is seen visually at 1,2,3 and 4 WAA) and weed growth rate (observation of weed growth rate was carried out at 2 and 4 WAA) (Pujisiwanto et al. 2017):

$$WGR = \frac{1}{Ga} \times \frac{W_2 - W_1}{T_2 - T_1} \text{ (g/m}^2\text{/week)}$$

Where:

Ga : area of land shaded by weeds (m²)

W1 : dry weight of weeds at the first observation (g)

W2 : dry weight of weeds in the second observation (g)

T1 : time of first observation (weeks)

T2 : second observation time (weeks)

While those observed in the second experiment were plant height (measurements were carried out at 4 WAA from the rootstock close to the soil surface to the tip of the

leaf), the number of duns (calculated on fully developed leaves at 4 WAA), Dry weight of rice plant (such as stems, leaves and plant roots are part of the rice plant. The grains were dried after harvesting and their wet weight was weighed using an oven at 80°C for 3x24 hours) and Phytotoxicity (level of damage) seen visually in 4 WAA with the rules from the Directorate of Fertilizers and Pesticides (2012) which are classified using numbers, which are as follows: (i) 0 = No damage, 0-5% abnormal shape and or color of young leaves. (ii) 1 = Mild toxicity, >5-10% abnormal shape and/or color of young leaves. (iii) 2 = moderate toxicity, 10-20% abnormal shape and/or color of young leaves. (iv) 3 = Severe damage, >20-50% abnormal shape and/or color of young leaves. (v) 4 = Very severe damage, >50% the shape and or color of the young leaves is not normal until it dries and falls off, the plant dies.

Data analysis

The data obtained will be tested for homogeneity using the Bartlett test and Tukey's test to test the additivity of the data. If the data meet the assumptions, then proceed with the analysis of variance to determine the difference in the mean value between treatments using the Least Significant Difference Test (BNT) at the 5% level.

RESULTS AND DISCUSSION

Experiment 1: Bioassay test on the growth of *F. miliaceae* and *L. chinensis* weeds applied with *S. rarak* extract with SEBA and SEBM in a greenhouse.

Effect of soap nut fruit extract with SEBA and SEBM on the height of *F. miliaceae* and *L. chinensis* weeds at 4 WAA

Figure 1a shows the interaction of the two factors (weed type and concentration), which applied with the soap nut fruit extract with the extraction method using SEBA and SEBM at 4 WAA, affected the height of *L. chinensis* and *F. miliaceae* weeds. Application of soap nut fruit extract with SEBA at concentrations of 25%, 50%, and 75% caused the stunted growth of *L. chinensis* weed height there

are 21,20 cm, 15,19 cm, and 12,89 cm, respectively. Application of soap nut fruit extract with SEBA in *F. miliaceae* weeds at concentrations of 50% and 75% caused stunting of weed height growth to 21,28 cm and 18,37 cm, respectively. If the concentrations of soap nut extract using SEBA applied to two types of weeds were compared, the results showed that concentrations of 25%, 50%, and 75% were more effective in inhibiting the height of both *L. chinensis* and *F. mileacea* weeds.

Figure 1b also shows the application of soap nut fruit extract with SEBM at concentrations of 25% and 50% affected the height of *L. chinensis* weeds which caused a decrease in weed height to 6,53 cm and 3,7 cm, respectively. Increasing the concentration of soap nut fruit extract to 75% caused the stunted growth of weed height to 2,33 cm, no different from the application of 5% pure saponins, which was 4,54 cm. Application of soap nut fruit extract with SEBM on *F. miliaceae* weeds at concentrations of 25%, 50%, and 75% resulted in stunted growth to 11.18 cm, 7.37 cm, and 6.59, respectively. If the concentration of soap nut extract using SEBM applied to both types of weeds was compared, it showed that concentrations of 25%, 50%, and 75% were more effective in inhibiting the height of *L. chinensis* weeds, while the height of *F. mileacea* weeds could be inhibited at concentrations of 50% and 75%.

Inhibiting weed height growth can be caused by the content of allelopathic compounds in soap nut fruit extracts such as saponin or saponins containing sugar chains that cause changes in membrane permeability, leakage, and hemolysis. Changes in cell membrane permeability facilitate the release of cell substances such as proteins and nucleic acids so that cause cell damage as well as inhibition of transport and diffusion of the reshuffled food reserves that pass through the cell membrane so that these conditions cause cell growth to be inhibited. In addition, the presence of phenolic content in soap nut fruit extract inhibits cytokinin activity and ketoglutaric acid synthesis. These barriers cause disruption and disrupt cells in the shoot meristem, thereby inhibiting weed growth (Pebriani, 2013).

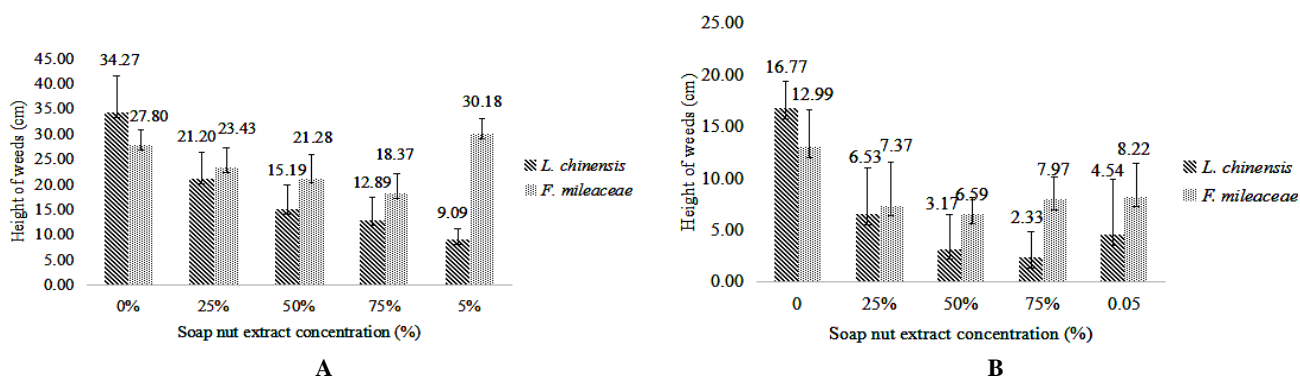


Figure 1. Height (cm) of *L. chinensis* and *F. mileacea* weeds applied soap nut fruit extract with SEBA (A) and SEBM (B) at 4 WAA

Effect of soap nut fruit extract using SEBA and SEBM on the root length of F. miliaceae and L. chinensis weeds at 4 WAA

This experiment showed that weed type and concentration significantly affected weed root length, but the interaction between the two factors did not significantly affect weed root length. Table 1 shows that the application of soap nut fruit extract using SEBA and SEBM at concentrations of 25% to 75% was able to inhibit the growth of root length of *L. chinensis* and *F. miliaceae* weeds. The inhibition of root length was thought to result from the phytotoxic effect of the application of soap nut fruit extract containing allelochemical compounds, causing cell division disorders and inhibition of meristematic cell proliferation in the mitotic phase. This is supported by Koodkaew et al. (2018) in their research which found that mitotic disruption from the application of *Mimosa pigra* L extracts containing chemical compounds with soap nut fruit (*S. rarak*) caused inhibition of root and shoot growth of lettuce and popping pod (*Ruellia tuberosa* L). In addition, the presence of allelochemical compounds instance of phenol interferes with the transport of auxin as the promoter of root elongation and interferes with the synthesis of cytokinin in the root part which functions for root division (Tetelay 2003).

Effect of soap nut fruit extract using SEBA and SEBM on the dry weight of F. miliaceae and L. chinensis weeds at 4 WAA

Application of soap nut fruit extract using SEBA at 50% and 75% caused the growth of dry weight of *L. chinensis* weeds to 14,57 cm and 11,16 cm. It also happened to *F. miliaceae* weed which caused a decrease in dry weight of weeds to 41,18 cm and 31,31 cm (Figure 2a). The application of soap nut fruit extract with SEBM at concentrations of 25%, 50%, and 75% caused a decrease in the dry weight of *L. chinensis* weeds to 6,37 cm; 4,07 cm and 1,69 cm, which did not differ from the application of 5% pure saponins, namely 5,24 cm (Figure 2b). The same thing happened to *F. miliaceae* weed which showed that the administration of soap nut extract at the concentration of 25%, 50%, and 75% caused a decrease to 7,71 cm; 5,82 cm,

and 3,06 cm. The dry weight variable indicates the nutrient content that will affect plant growth and development because dry weight reflects the accumulation of organic compounds that have to be successfully synthesized by plants, Sitorus et al. (2014). The decrease in the dry weight of weeds compared to the control indicates inhibition of photosynthesis in plants.

Growth rate of F. miliaceae and L. chinensis weeds applied soap nut fruit extract with SEBA and SEBM at 4 WAA

The results showed that the treatment of soap nut fruit extract with the extraction method using SEBA and SEBM at a concentration of 25% to 75% was able to reduce the growth rate of *L. chinensis* weeds compared to the control and comparison of synthetic saponins from 1 WAA to 4 WAA. Figure 3a and 3b shows that the growth rate of *L. chinensis* and *F. miliaceae* weeds applied by soap nut extract with SEBM was lower than SEBA. The lowest growth rate of the two weeds was at 75% soap nut extract concentration.

Table 1. Effect of soap nut fruit extract using SEBA and SEBM on the root length of *F. miliaceae* and *L. chinensis* weeds at 4 WAA

Treatment	Weeds root length (cm)	
	Aqueous	Methanol
Weed		
<i>L. chinensis</i>	15.39 b	7.03 b
<i>F. mileaceae</i>	23.55 a	13.05 a
BNT 5%	1.63	0.88
Concentration		
Control	9.93 a	5.01 a
25% soap nuts extract	8.11 ab	3.62 ab
50% soap nuts extract	6.43 bc	3.50 bc
75% soap nuts extract	5.10 cd	2.14 c
5% pure saponins	2.89 d	2.47 bc
BNT 5%	2.58	1.39

Note: The average value in each column followed by the same letter is not significantly different according to the BNT test at the 5% level

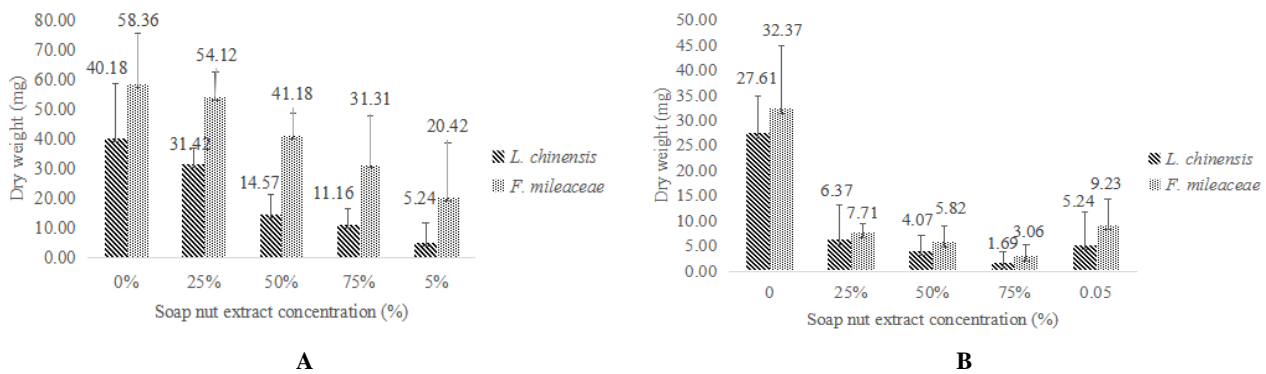


Figure 2. Dry weight (mg) of *L. chinensis* and *F. mileaceae* weeds applied soap nut fruit extract with SEBA (a) and SEBM (b) at 4 WAA

Percentage of visual toxicity of F. miliaceae and L. chinensis weeds applied with soap nut fruit extract with SEBA and SEBM

Four weeks after application (WAA), visual observations showed that soap nut fruit extract with SEBA and SEBM at specific concentrations caused poisoning in *L. chinensis* weeds. Symptoms of severe poisoning were seen in applications with concentrations of 50% and 75%, which caused abnormal shapes and colors to experience chlorosis (Figure 4), and inhibited weed growth (Figure 5).

Visual observations at 1 to 4 weeks after application (WAA) showed that extracts of soap nut fruit with aqueous and methanol extraction methods at certain concentrations caused poisoning in *F. mileacea* weeds. Symptoms of severe poisoning were seen on application at concentrations of 50% and 75%, which caused inhibited weed growth (Figure 6).



Figure 4. Symptoms of poisoning caused by applying soap nut fruit extract with SEBA and SEBM against *L. chinensis* weeds

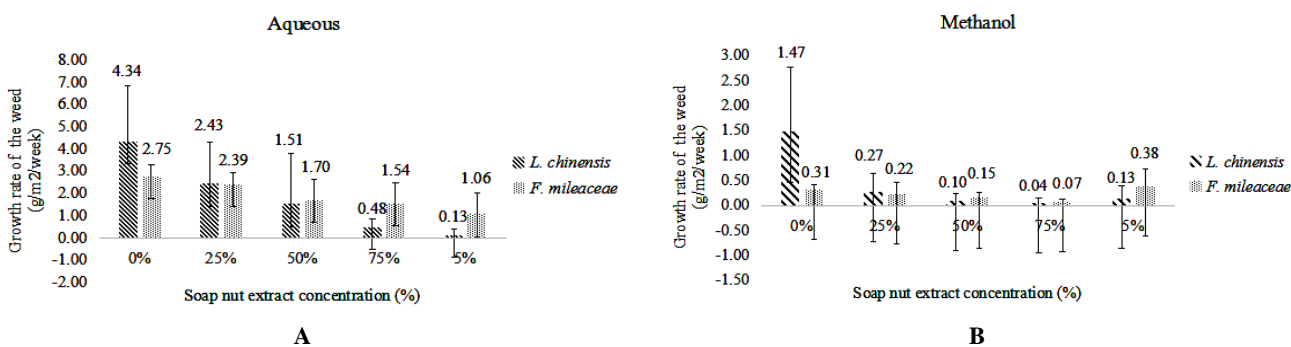


Figure 3. Standard error of growth rate of *L. chinensis* and *F. mileacea* weeds applied soap nut fruit extract with SEBA (A) dan SEBM (B)

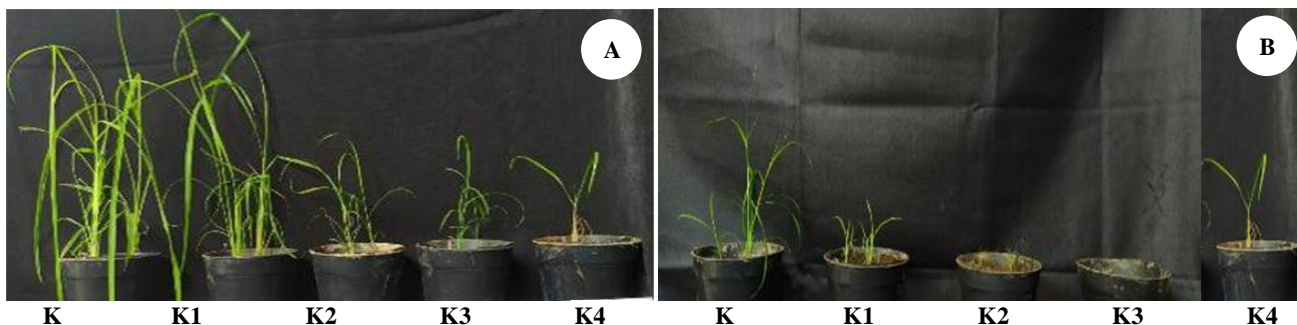


Figure 5. Effect of soap nut extract with SEBA (A) and SEBM (B) on *L. chinensis* weed at 4 WAA. Note: Control (K), 25% soap nuts extract (K1), 50% soap nuts extract (K2), 75% soap nuts extract (K3), 5% pure saponin (K4)

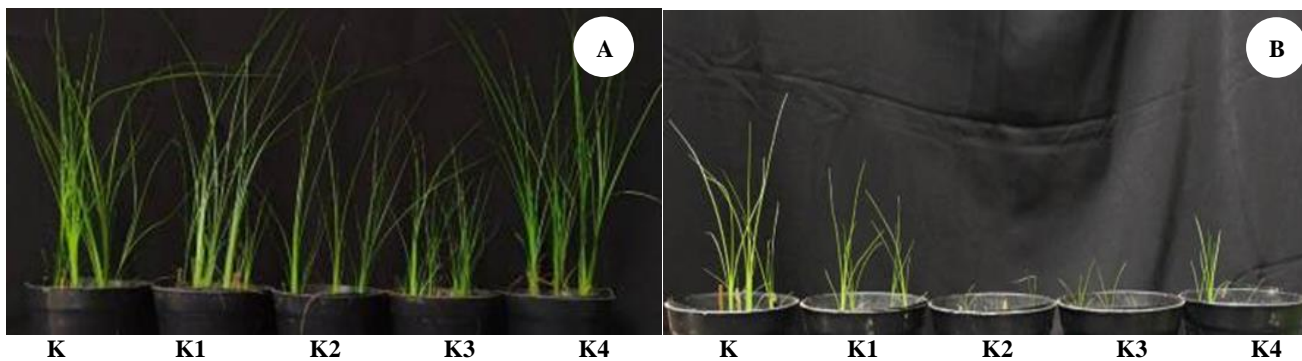


Figure 6. Effect of soap nut extract with SEBA (A) and SEBM (B) on *F. mileacea* weed at 4 WAA. Note: Control (K), 25% soap nuts extract (K1), 50% soap nuts extract (K2), 75% soap nuts extract (K3), 5% pure saponin (K4)

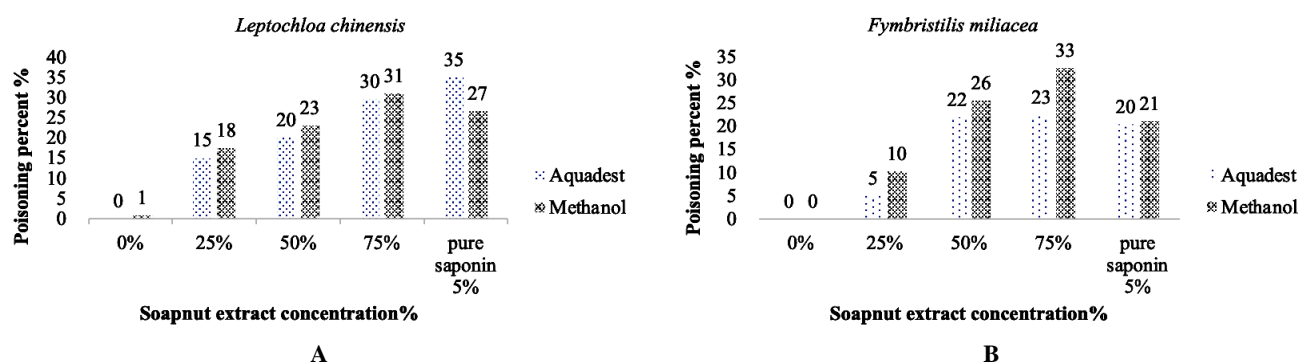


Figure 7. Percentage of visual *Leptochloa chinensis* (A) and *Fymbristilis miliacea* (B) weed poisoning due to the application of soap nut fruit extract extracted using SEBA and SEBM at 4 WAA

The percentage variable for weed damage showed that the application of *S. rarak* extract with SEBA and SEBM at a concentration of 25% to 75% caused poisoning in *L. chinensis* and *F. mileacea* weeds. Saponin compounds in *S. rarak* extracts are believed to be the cause of plant damage or toxicity, resulting in yellowing symptoms, abnormal growth, tissue death and chlorosis. The mechanism of chlorosis symptoms that occurs due to inhibition of lipid synthesis by inhibiting the action of the enzyme Acetyl-CoA carboxylase (ACCase), which is similar to the research of Ramprakash et al. (2015) stated that the herbicide metamifop causes poisoning symptoms in the form of chlorosis in developing leaves and inhibits growth. In addition, allelochemical compounds are also capable of causing membrane damage which causes loss of function of the ATPase enzyme that functions in the respiration process. The obstacles pioneered by the compounds in the lerak extract eventually lead and combine to disrupt the cell division process, leading to damage or even death of the plant.

Figure 7a shows that the application of soap nut extract extracted using SEBA and SEBM caused poisoning in *L. chinensis* weeds compared to controls at 4 WAA. The application of soap nut extract, extracted using SEBA at a concentration of 25%-50%, showed a percentage value of 15 to 20% and was classified as mild poisoning. The concentration of 75% and application of 5% synthetic saponins showed the percentage value of poisoning was 30% and 35% and were classified as severe poisoning. The application of soap nut extracts extracted using SEBM at a concentration of 25% showed a percentage value of 18% and was classified as moderate poisoning. The concentration of 50%-75% indicates the percentage value of poisoning is 23% and 31% were classified as severe poisoning.

Figure 7b shows the application of soap nut extract with SEBA and SEBM caused poisoning in *F. miliacea* weeds compared to controls at 4 WAA. The application of soap nut fruit extract, extracted using SEBA, at a concentration of 25%, showed a poisoning percentage value of 5% and was classified as mild poisoning. If the concentration was increased to 50% and 75%, the poisoning percentage values were 22% and 26% and were classified as severe poisoning. While the application of soap nut fruit extract, extracted using SEBM, at a concentration of 25% showed a

poisoning percentage value of 10% and was classified as moderate poisoning. The concentration of 50%-75% indicates the poisoning percentage value of 23% and 33% was classified as severe poisoning.

Experiment 2. Phytotoxicity test on rice plant growth applied soap nut extract by extraction method using aqueous (SEBA) and methanol (SEBM)

The analysis of variance showed that the group had no significant effect on the variables of crown height, number of leaves and root length in rice plants. However, the concentration treatment had a significant effect on the variables of plant height and stover weight on rice plants.

Effect of soap nut extract with SEBA and SEBM on rice plant height

Table 2 shows that the application of soap nut fruit extract using SEBA did not affect rice plant height, while SEBM affected rice plant height. Without treatment or control, the height of rice plants at the age of 4 WAA was 68,94 and 63,67 cm. The application of soap nut fruit extract with SEBA at 25%, 50%, and 75% did not affect rice plant height, while SEBM at a 25-50% concentration did not affect rice plant height. After being given an increase in the concentration of soap nut extract to 75% causing plant height rice was lower (54,76 cm) compared to the control and 5% synthetic saponins.

Effect of soap nut extract with SEBA and SEBM on the weight of rice plant stover

Table 3 shows that the application of soap nut extract using SEBA did not affect rice plant height, while SEBM affected rice plant height. The application of soap nut fruit extract with SEBA and SEBM on the weight of rice stover at concentrations of 25% and 50% did not affect the weight of rice stover, but increasing the concentration of soap nut extract with SEBM to 75% caused the weight of the rice stover to be lower (3,34 g) compared with control and 5% synthetic saponins. The plant toxicity seen in the variable height of rice plants was caused by applying soap nut fruit extract with SEBM at a concentration of 75%. It is presumably because the methanol solvent can filter out most of the desired secondary metabolites in simplicia and has high free radical scavenging activity in organic solvents

and can attract most of the chemical compounds in simplicia (polar and non-polar), one of which is saponins (Salamah and Widayarsi 2015). However, at a lower concentration of 25%-50%, the rice plant did not show toxicity. This is presumably due to the presence of the aryl acylidase enzyme, which can convert toxic compounds into non-toxic compounds contained in the soap nut extract. Sembodo (2010) stated that rice has 40 times greater resistance to the herbicide propanyl due to the high content of the enzyme aryl acylidase to hydrolyze propanyl to 3,4-dichloroaniline and propanyl, which are non-toxic.

Effect of soap nut extract with SEBA and SEBM on number of rice clumps

The number of rice clumps applied by soap nut fruit extract with SEBA and SEBM at a concentration of 25%-75% did not affect the number of leaves compared to the control treatment and 5% synthetic saponins (Table 4).

Effect of soap nut extract with SEBA and SEBM on rice root length

Root length of rice plants applied with soap nut fruit extract with SEBA and SEBM at a concentration of 25%-

75% did not affect root length compared to the control treatment and 5% synthetic saponins (Table 5).

Figures 9 show the effect of applying soap nut fruit extract using methanol extractor on the growth of rice plants at 4 WAA. The application of soap nut fruit extract using SEBA extractor at a concentrations of 25%, 50%, and 75%, as well as 5% saponins, did not affect or poison the growth of rice plants. The application of soap nut fruit extract using SEBM at a concentration of 25%-50% showed no symptoms of poisoning, but at a concentration of 75%, there were symptoms of poisoning with a percentage value of 20% poisoning and was classified as moderate poisoning.

Visual observations at 1 to 4 weeks after application (WAA) showed that the application of soap nut fruit extract with SEBA at all concentrations tested did not cause toxicity to the number of leaves, root length, and weight of stover in rice plants, while the fruit extract application soap nut with SEBM caused poisoning at a concentration level of 75%. Symptoms of poisoning caused by the application of soap nut fruit extract with the SEBM extraction method were in the form of leaf necrosis, dry leaves, and stunted growth of rice plant height (Figure 10).

Table 2. Effect of soap nut fruit extract with SEBA and SEBM on rice plant height

Treatment	Plant height (cm)	
	Aqueous	Methanol
Control	68.94 a	63.67 a
25% soap nuts extract	68.81 a	61.07 a
50% soap nuts extract	67.40 a	60.74 a
75% soap nuts extract	66.42 a	54.76 b
5% pure saponins	68.79 a	64.79 a
BNT 5%	3.21	4.08

Note: The average value in each column followed by the same letter is not significantly different according to the 5% BNT test

Table 4. Effect of soap nut fruit extract with SEBA and SEBM on number of rice clumps

Treatment	Number of rice clumps	
	Aqueous	Methanol
Control	20.50 a	19.39 a
25% soap nuts extract	21.39 a	20.39 a
50% soap nuts extract	18.22 a	19.78 a
75% soap nuts extract	19.94 a	21.50 a
5% pure saponins	16.17 b	19.28 a
BNT 5%	3.31	2.79

Note: The average value in each column followed by the same letter is not significantly different according to the 5% BNT test

Table 3. Effect of soap nut fruit extract with SEBA and SEBM on rice stover weight

Treatment	Stover weight (g)	
	Aqueous	Methanol
Control	4.08 a	4.48 a
25% soap nuts extract	4.00 a	4.18 a
50% soap nuts extract	3.92 a	4.17 a
75% soap nuts extract	3.91 a	3.34 b
5% pure saponins	3.65 b	3.58 b
BNT 5%	0.27	0.36

Note: The average value in each column followed by the same letter is not significantly different according to the 5% BNT test

Table 5. Effect of soap nut fruit extract with SEBA and SEBM on rice root length

Treatment	Root length(cm)	
	Aqueous	Methanol
Control	31.06 a	30.16 a
25% soap nuts extract	29.71 a	30.74 a
50% soap nuts extract	29.96 a	30.34 a
75% soap nuts extract	27.03 a	30.08 a
5% pure saponins	30.16 a	31.55 a
BNT 5%	5.01	3.61

Note: The average value in each column followed by the same letter is not significantly different according to the 5% BNT test

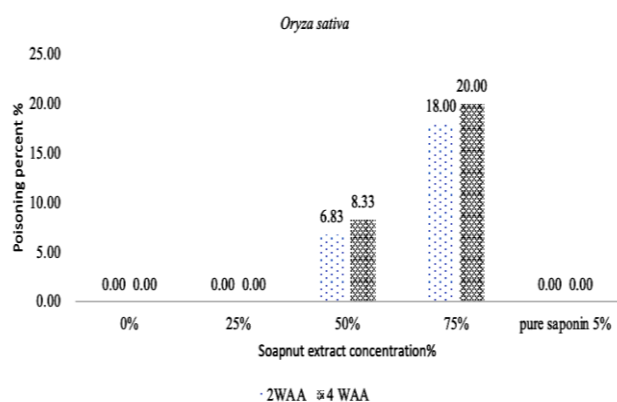


Figure 8. Percentage of visual rice poisoning due to the application of soap nut fruit extract using SEBM



Figure 9. Symptoms of poisoning caused by the application of soap nut fruit extract with SEBM at concentration of 75% on rice plants (*Oryza sativa*)

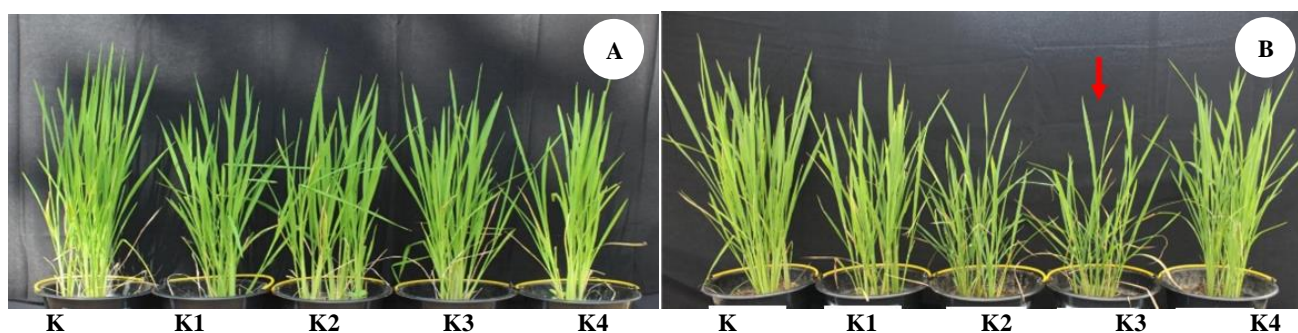


Figure 10. Effect of soap nut extract with SEBA (Figure A) and SEBM (figure B) on rice plant at 4 WAA. Note: Control (K), 25% soap nuts extract (K1), 50% soap nuts extract (K2), 75% soap nuts extract (K3), 5% pure saponin (K4)

In conclusion, this study clearly showed that the application of soap nut fruit extract with SEBA was able to inhibit the growth of *L. chinensis* and *F. mileacea* weeds at concentrations of 50% and 75%, while the application of soap nut fruit extract with SEBM was more effective in inhibiting the growth of *L. chinensis* weeds (25%, 50% and 75% concentration) compared to *F. mileacea* weeds (50% and 75% concentrations). Application of soap nut fruit extract with SEBA did not affect rice growth, while SEBM inhibited rice plant height and stover weight at a concentration of 75%, but it did not inhibit the growth of root length and number of leaves.

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