Paper Pak Alri Pemprovsu

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ALLELOPATHIC EFFECTS OF *Mikania micrantha* Kunth ON BARNYARDGRASS [*Echinochloa crus-galli* (L.) Beauv] AND LOWLAND RICE

ABSTRACT

The use of secondary metabolites from weed has the potential as a natural herbicide and safe for the main crop. This study was aimed to screening of secondary metabolites in *Mikania micrantha* extract and its effect on the agronomic, physiological, and histological characteristics of barnyardgrass and lowland rice. A non-factorial Randomized Block Design was used with the extracts concentration of *Mikania micrantha* (0; 20; 40; 60; 80; 100%) and 2,4-D dimethyl amine herbicide as comparison. The *Mikania micrantha* extract had the flavonoids, tannins, alkaloids, and saponins. The concentration of 20-100% inhibited the barnyardgrass growth, but it was considered safe for lowland rice. The 20-100% concentrations proved to increase the root length and volume, while the 60% concentration increased the number of leaves and stomatal density in lowland rice. The 20-100% concentration significantly inhibited the root volume, biomass, SPAD total chlorophyll, growth reduction of barnyardgrass, and it was classified as an inhibitor. The barnyardgrass tocixity also increased as the concentration grow from 60 to 100%. Since the potency of *Mikania micrantha* extracts in reducing the highest of barnyardgrass growth by 63.5% and the result was approaching the ability of 2,4-D dimethyl amine of 65.6%. All concentration of *Mikania micrantha* has the opportunity to reduce the use of synthetic herbicides.

Keywords: barnyardgrass; extract; histological; lowland rice; physiological; tocixity

INTRODUCTION

Rice (*Oryza sativa* L.) is widely cultivated in Asia, Latin America, and Africa, and is consumed as a staple food by over 50% of the world's population (Lou et al. 2012). Globally, rice crop yield will increase by 42% to fulfill projected demand by 2050 (Ray et al. 2013). Efficient crop management, such as weed control, in rice-growing areas is needed to achieve optimal yield. Previous research showed that the loss of main crop yield due to the presence of weed reached 32% and was significantly greater than effects of pests (18%) and plant diseases (15%) (Van Evert et al. 2017). One of the common weed species that can reduce rice yield is barnyardgrass [*Echinochloa crus-galli* (L.) Beauv] and it has been reported by Ottis & Talbert (2007) that the loss of rice plants due to barnyardgrass can attain up to 70%. Wilson et al. (2014) added that the presence of *E. crus-galli* at a distance of 40 cm from lowland rice reduced yields by 27% and absorbed nitrogen availability by 60-80%. Travlos et al. (2011) added that a density of 10 plants of *E. crus-galli* per m^2 can produce 34,600 seeds. According to Moon et al. (2010) *E. crus-galli* significantly inhibited the number of tillers, reduced the grains, maturity, and 1,000 grains weight of lowland rice.

One of the weed management for *E. crus-galli* in lowland rice fields is by spraying herbicides. Although the use of chemical herbicides such as 2,4-D dimethyl amine at a dose of 1 L ha^{-1} significantly controlled the total dry weight of *E. crus-galli* by 67.37%, it inhibited the growth of the number of tillers and the total dry weight of lowland rice by 3.54% and 58.54%, respectively compared to the control (Alridiwirsah et al. 2020a). Therefore, it is necessary to have an alternative environmentally friendly method of controlling *E. crus-galli* through the use of natural resources as herbicides. According to Tampubolon et al. (2018) weeds can be used as herbicides which are abundantly available from agricultural land. Junaedi et al. (2006) also stated that secondary metabolites in weed such as phenolics, terpenoids, tannins, alkaloids, steroids, essential oils, and polyacetylene have allelopathic activity.

Previous research has shown that allelopathic compounds of weeds can suppress the growth of the targeted weed such as bitter vine (*Mikania micrantha* Kunth). It was identified that *M. micrantha* has phenolic compounds, flavonoids, alkaloids, and terpenes that influence the growth of other weeds (Ni et al. 2007). Shao et al. (2005) discovered a 50% concentration index value of dihydromicanolide compounds from *M. micrantha* extract in suppressing the growth of *Lolium perenne* roots of 230 g mL^{-1}.

Although it has been proven that *M. micrantha* extracts can inhibit the growth of *E. crus-galli*, studies on the effects of *M. micrantha* extracts on physiology, histology, agronomy of lowland rice, and *E. crus-galli* have not been reported. Therefore, this study was aimed to screening the secondary metabolites in *M. micrantha* extract, and determine the effect of *M. micrantha* extract on the characteristics of *E. crus-galli* and lowland rice.

MATERIAL AND METHODS

This study was conducted at the farmland in Padang Bulan Selayang I, Medan Selayang Sub-district, Medan City, Indonesia, from June to October 2021. The barnyardgrass seeds were taken at the lowland rice fields in Padang Bulan, Medan (98°38.601' E and 3°33.560' N). The lowland rice seeds used were the Inpari 32 variety from the Technical Implementation Unit of the Seed Supervision and Certification Center of North Sumatra. The growing media was taken from the rice fields at a depth of 0-20 cm, put into a bucket, and flooded for one week.

The planting medium for cultivating the lowland rice and barnyardgrass seeds were taken from the bucket and put into the germination tray. Subsequently, there were 100 seeds of lowland rice and barnyardgrass sown in each germination tray and maintained for two weeks after sowing. After growing to a height of 15 cm, the lowland rice seedlings were transplanted into bucket media at one seedling per bucket with the planting place in the middle. Subsequently, the barnyardgrass seedlings were transplanted after 3-4 leaves in a bucket of five seedlings per bucket in a position surrounding the lowland rice (Fig 1).

The *Mikania micrantha* shoot was taken from the experimental field in the Faculty of Agriculture, Universitas Sumatera Utara, Medan (98°39.214' E and 3°33.467' N) up to 2.367 kg with 96% ethanol extracting material (ρ= 0.789 g cm^{-3} or equivalent to 789 g L^{-1}). The total of distilled water used was one liter and each treatment was given the agristick adjuvants (Dadang & Prijono 2011) at a concentration of 2% (20 ml L^{-1}) to maintain the stability of the natural herbicide formulation.

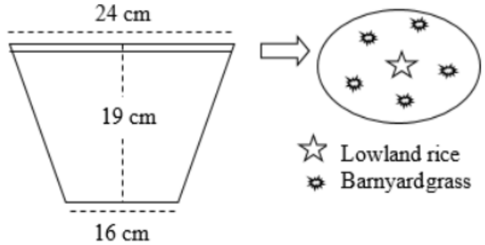

Figure 1. Position for seedlings planting of lowland rice and barnyardgrass in the bucket.

The screening of *Mikania micrantha* secondary metabolites was conducted using a qualitative method by obtaining 10 ml of the extract (Fig 2) and analyzing the content of alkaloids, tannins, saponins, flavonoids, steroids, and triterpenoids at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. The alkaloid secondary metabolites were measured by inserting 2 ml of the extract into a test tube, adding 2 drops of Bouchardat reagent, and waiting for one minute to obtain a brown precipitate when the sample is positive. Furthermore, the flavonoid was measured by inserting 2 ml of the extract into a test tube, then adding 2 drops of 5% $FeCl_3$ reagent and waiting for one minute to achieve a colloid black when the sample was positive.

The screening of saponin was conducted by soaking the sample with ethanol and putting in a test tube, a distilled water, and 96% alcohol reagent were further added and waiting for one minute to obtain a foam for a positive sample. For the measurement of secondary metabolites for steroids and triterpenoids, 2 ml of extract was inserted into a test tube, then 2 drops of Liebermann-Burchard reagent was added and allowed to stand for one minute to achieve a silver ring. Furthermore, the measurement of secondary metabolites for tannins was conducted by inserting 2 ml of the extract into a test tube and adding 2 drops of 1% $FeCl_3$ reagent and allowed to stand one minute, the sample changed to colloidal black was positive.

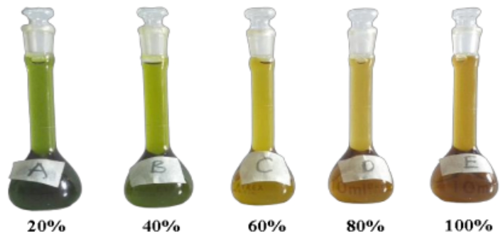

Figure 2. Samples of *Mikania micrantha* weed ethanol extract in qualitative secondary metabolite screening tests. [Photographer: Koko Tampubolon].

This study used a non-factorial Randomized Block Design (RBD) with concentration factors of *Mikania micrantha* extract such as NH_0= 0% (untreated), NH_1= 20% (157.8 g L^{-1}), NH_2= 40% (315.6 g L^{-1}). l), NH_3= 60% (473.4 g L^{-1}), NH_4= 80% (631.2 g L^{-1}), NH_5= 100% (789 g L^{-1}), and NH6= 2,4-D dimethyl herbicide amine (720 g a.i.ha^{-1}) as a comparison with three replications. The application of natural herbicides from *Mikania micrantha* extract and comparison herbicides was conducted when the barnyardgrass had 5-6 leaves. Furthermore, the manually spraying was conducted at 9:40-10:53 AM with a temperature, humidity, air and spray pressure were 29.3^{o}C, 88%, 1008 hPa, and 2-6 kg cm^{-2}, respectively. Basic fertilization was conducted at one week after transplanting the lowland rice seedlings with NPK fertilizer at a dose of 10 g per bucket which was sown evenly. Subsequently, it was sprayed with insecticide deltamethrin 25 g L^{-1} to control pests and fungicide propine 70% to control disease based on the recommended dose.

The observations measured were agronomic characters of lowland rice included phytotoxicity at 2, 7, and 14 days after spraying (DAS), the number of leaves at 7-14 DAS, root length and volume, biomass (roots and shoots) at 42 DAS. Agronomic characters for barnyardgrass included phytotoxicity level at 2, 7, and 14 DAS, root length and volume, biomass (roots and shoots), growth reduction, and Allelopathic Response Index (ARI) at 42

DAS. The measurement of the phytotoxicity of lowland rice and the weed toxicity was carried out visually and scored based on leaf color as follows: score 0= green, score 1= yellowish-green, score 2= overall yellow, score 3= yellow-brown spots, and score 4= brown and shrink (Alridiwirsah et al. 2020b). The score was calculated and was converted to a percentage using equation 1. The fresh weight was measured by cleaning the roots from the soil then drying for two hours and weighing with an analytical balance. Meanwhile, the dry weight measurement was carried out in an oven at 65^{o}C for 72 hours and the growth reduction of weed was measured based on total dry weight using equation 2. The ARI measurement was based on the dry weight of shoot using the formula from Williamson & Richardson (1988) in equation 3. When the ARI value was greater than 0 the allelopathic effect is stimulatory and if the ARI value <0, the allelopathy effect is inhibitory (Sunmonu & van Staden 2014).

$$\text{Phytotoxicity}=\frac{(\sum \text{leaves x score 4})+(\sum \text{leaves x 3})+(\sum \text{leaves x 2})+(\sum \text{leaves x 1})}{\text{Total number of leaves}} \times 100\% \quad (1)$$

$$\text{Growth reduction}= 100 - \frac{\text{Total dry weight in sprayed}}{\text{Total dry weight in control}} \times 100\% \quad (2)$$

$$\text{Allelopathic response index (ARI)}= \frac{\text{Shoot dry weight in control}}{\text{Shoot dry weight in sprayed}} - 1 \quad (3)$$

The histological characters of lowland rice and barnyardgrass measured included upper and lower epidermis, mesophyll, and stomata density. The upper and lower epidermis, and mesophyll tissue were measured by taking the second leaf at 7 DAS with a transverse incision using the paraffin method (Johansen 1940). Stomata density was measured by applying clear nail polish on the underside of the leaf and allowing it to dry for a few minutes and applying the clear tape. The dried tape was pulled and placed on the surface of the preparation and the stomatal density was calculated with a microscope. The histological images were taken using the Axiovision 4.8 application with a magnification of 10x10. The physiological characters of lowland rice and barnyardgrass included the total chlorophyll at 2, 7, and 14 DAS. Subsequently, total chlorophyll was measured on the second leaf using a SPAD 502 plus chlorophyll meter. The data of lowland rice and barnyardgrass were analyzed using an ANOVA and followed by Duncan's Multiple Range Test (DMRT) at *P*<0.05 using IBM SPSS Statistics v.20 software.

RESULTS AND DISCUSSION

The results showed that there was not difference in the compounds from several concentrations of *Mikania micrantha* extract (Tab 1). The secondary metabolites from *Mikania micrantha* extract had the alkaloids, flavonoids, saponins, and tannins, but it was undetected for steroids and triterpenoids compounds. The findings of this compound in this species have also been reported by Jyothilakshmi et al. (2015), who found glycosides, terpenoids, phenolics, alkaloids, steroids, flavonoids, and tannins compounds.

Table 1. The screening of *Mikania micrantha* secondary metabolites on different extract concentrations.

Extract concentrations (%)	Secondary metabolites of *Mikania micrantha*				
	Alkaloids	Flavonoids	Saponins	Steroids and triterpenoids	Tannins
20	+	+	+	-	+
40	+	+	+	-	+
60	+	+	+	-	+
80	+	+	+	-	+
100	+	+	+	-	+

Note: (+) indicate detected; (-) indicate undetected.

The extracts of *Mikania micrantha* in any concentration and the herbicide 2,4-D dimethyl amine significantly affected the phytotoxicity of lowland rice and barnyardgrass at 2, 7, and 14 DAS (Fig 3). Herbicide 2,4-D dimethyl amine showed the highest phytotoxicity in lowland rice and barnyardgrass at 14 DAS compared to all extract concentrations. There was a phytotoxicity in lowland rice due to exposure to *Mikania micrantha* extract in the concentration of 20% at 2 DAS and a concentration of 20-40% at 7 DAS, but all extract concentrations (20-100%) were safe in lowland rice at 14 DAS. In contrast, the weed toxicity of barnyardgrass increased along with an increase in the concentration of 60-100% from *Mikania micrantha* extract at 14 DAS (Fig 3-B).

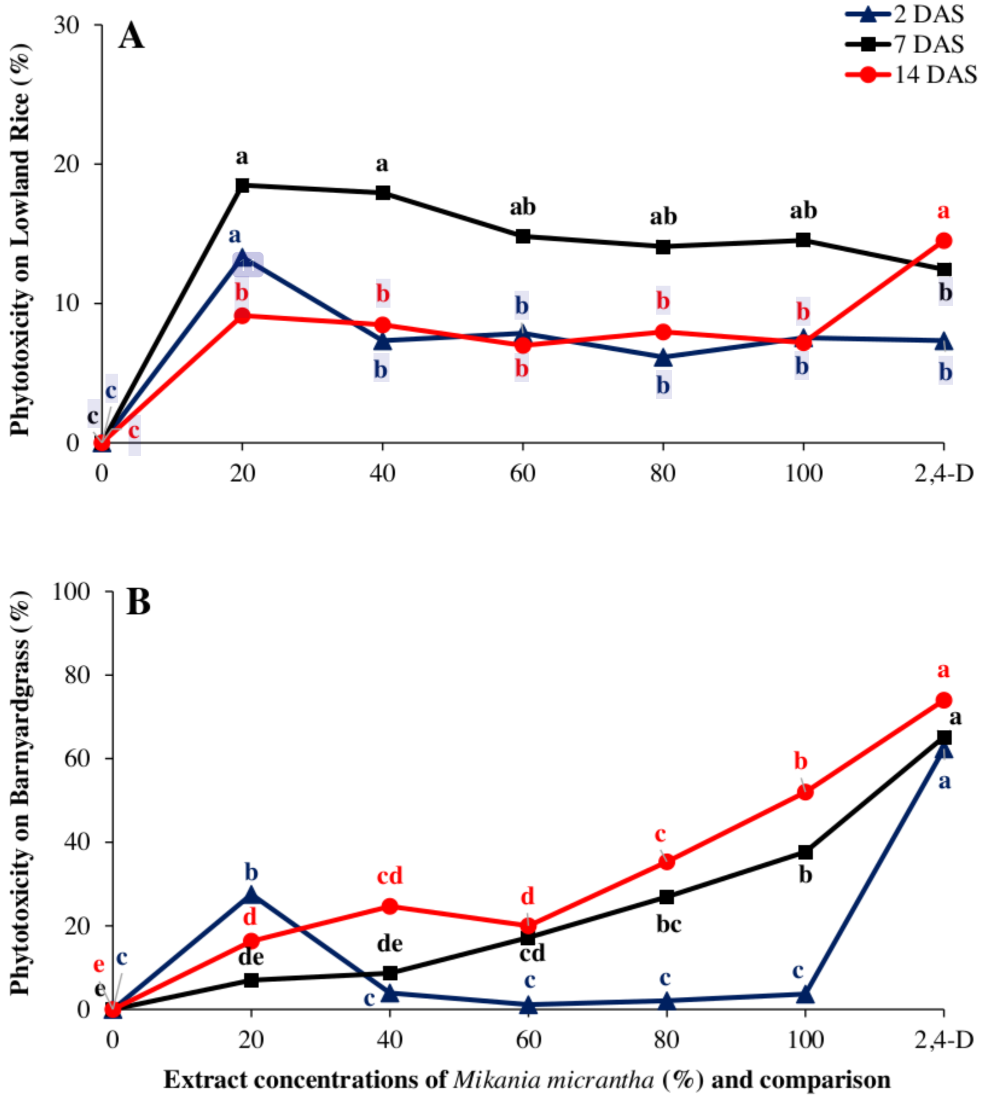

Figure 3. Effect of extract concentration of *Mikania micrantha* and comparison herbicide on the phytotoxicity of lowland rice (A) and barnyardgrass (B). Different letters on the graph indicate the significant effect by a DMRT at $P<0.05$.

The extracts of *Mikania micrantha* in any concentration and the herbicide 2,4-D dimethyl amine significantly affected the number of leaves in lowland rice plants aged 7-14 DAS (Tab 2). The results showed that exposure to *Mikania micrantha* extract at a concentration of 60% increased the number of leaves by 35.0%, and the herbicide 2,4-D dimethyl amine inhibited leaf growth of the lowland rice by 25.2% at 14 DAS compared to the control.

Table 2. Effect of extract concentration of *Mikania micrantha* and comparison herbicide on the number of leaves in lowland rice at 7-14 DAS

Treatments	Number of leaves in lowland rice		
	Before spraying	7 DAS	14 DAS
0%	30.7	37.0 ± 1.0 cd	43.7 ± 1.2 bc
20%	36.7	40.0 ± 2.6 bcd	45.0 ± 2.3 bc
40%	36.3	38.0 ± 1.0 cd	41.0 ± 0.8 bc
60%	52.7	56.7 ± 2.2 a	59.0 ± 2.1 a
80%	46.0	51.7 ± 2.5 ab	52.7 ± 2.5 ab
100%	40.0	44.0 ± 1.0 abc	45.3 ± 1.1 bc
2,4-D	28.3	29.7 ± 0.5 d	32.7 ± 0.3 c

Note: different lowercase letters in a column are significant by a DMRT at $P<0.05$ ± standard error.

The extracts of *Mikania micrantha* in any concentration and the herbicide 2,4-D dimethyl amine significantly affected the root volume of barnyardgrass, but it was insignificant on the root length and volume for lowland rice and root length of barnyardgrass (Fig 4). Furthermore, exposure to *Mikania micrantha* extracts caused a decrease in the root volume of barnyardgrass from 22.2 to 42.1% and herbicide 2,4-D dimethyl amine by 55.2% with the highest decrease was found at 100% concentration. This indicated that 100% exposure to *Mikania micrantha* extracts inhibited the growth and development of barnyardgrass roots.

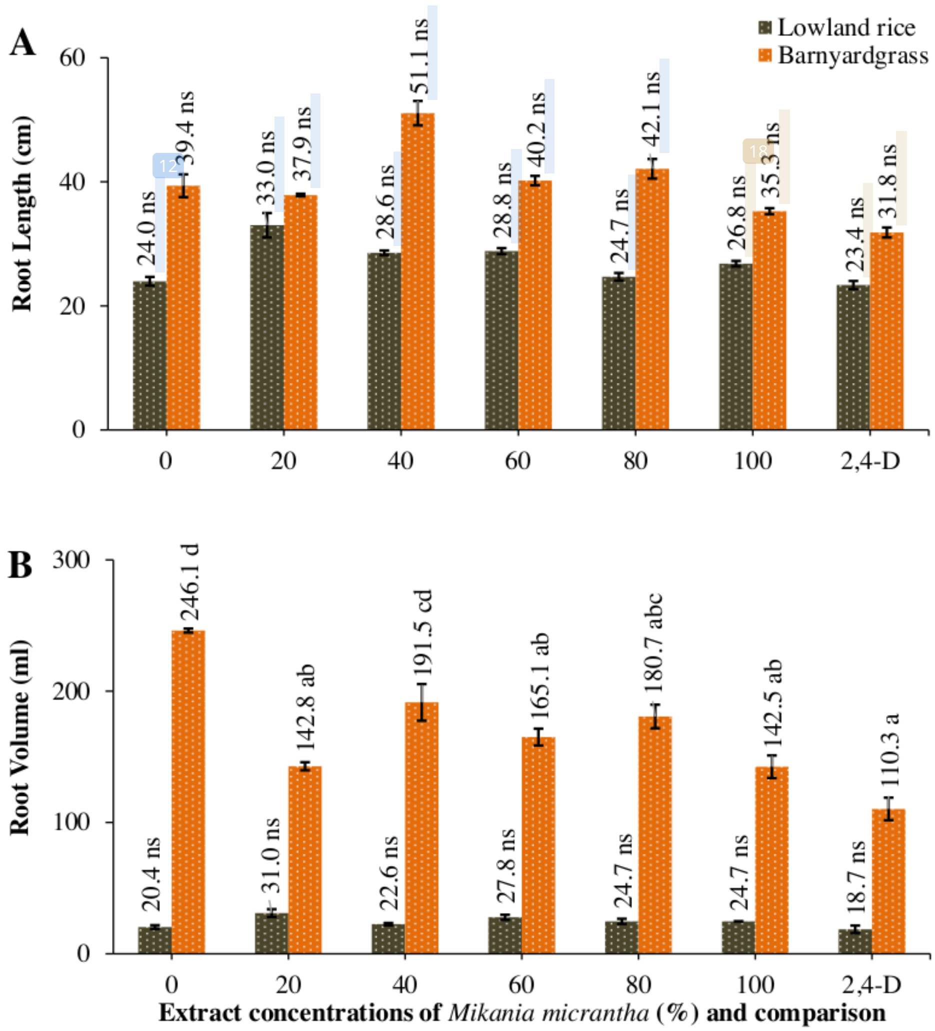

Figure 4. Effect of extract concentration of *Mikania micrantha* and comparison herbicide on root length (A) and root volume of lowland rice and barnyardgrass (B). Different letters on the graph indicate the significant effect by DMRT at $P<0.05$. ns= not significant. The vertical line on the graph indicates the standard error.

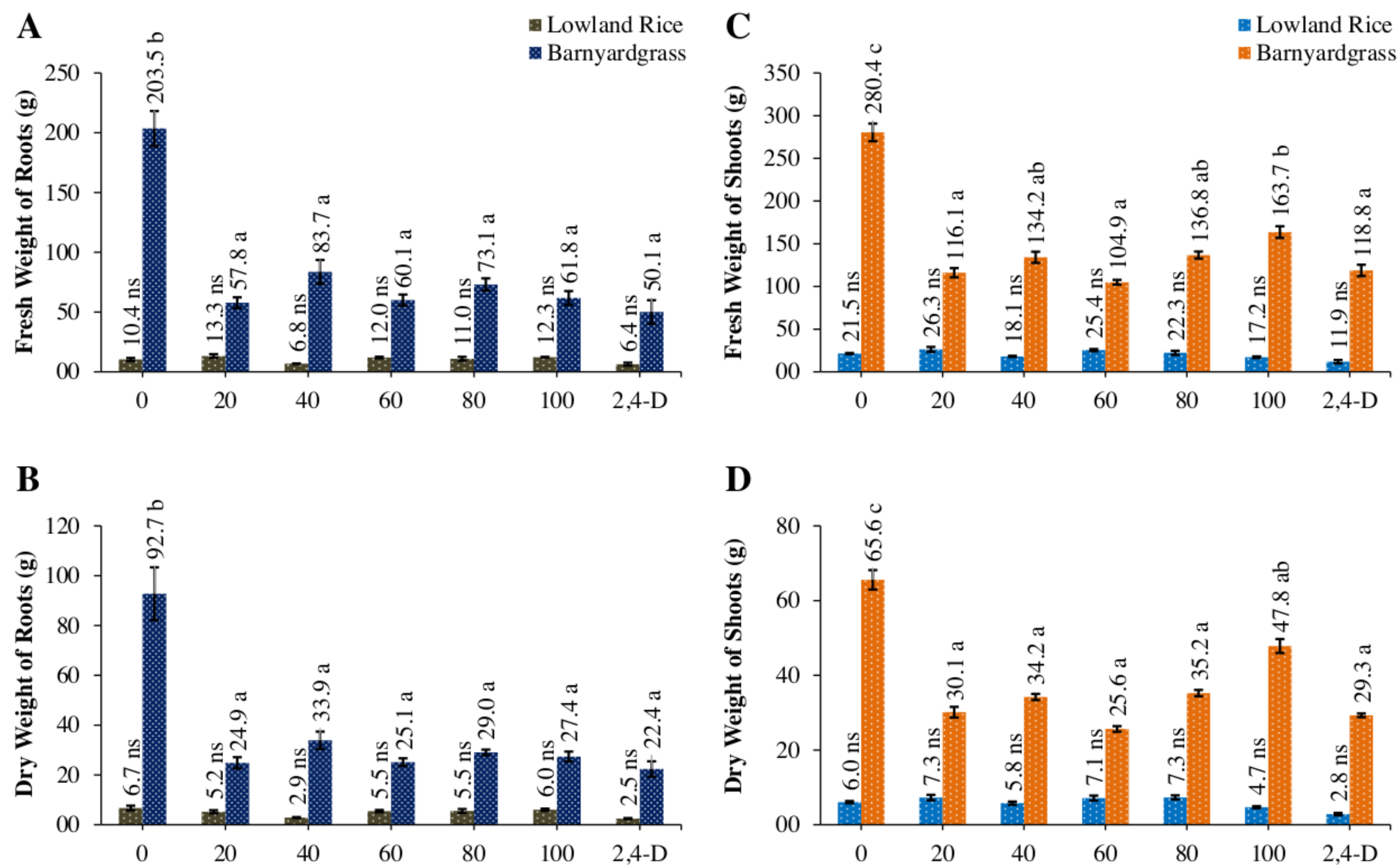
A
Lowland Rice
Barnyardgrass
Fresh Weight of Roots (g)
250
200
150
100
50
00
10.4 ns
203.5 b
13.3 ns
57.8 a
6.8 ns
83.7 a
12.0 ns
60.1 a
11.0 ns
73.1 a
12.3 ns
61.8 a
6.4 ns
50.1 a
0
20
40
60
80
100
2,4-D
B
Dry Weight of Roots (g)
120
100
80
60
40
20
00
6.7 ns
92.7 b
5.2 ns
24.9 a
2.9 ns
33.9 a
5.5 ns
25.1 a
5.5 ns
29.0 a
6.0 ns
27.4 a
2.5 ns
22.4 a
C
Lowland Rice
Barnyardgrass
Fresh Weight of Shoots (g)
350
300
250
200
150
100
50
00
21.5 ns
280.4 c
26.3 ns
116.1 a
18.1 ns
134.2 ab
25.4 ns
104.9 a
22.3 ns
136.8 ab
17.2 ns
163.7 b
11.9 ns
118.8 a
D
Dry Weight of Shoots (g)
80
60
40
20
00
6.0 ns
65.6 c
7.3 ns
30.1 a
5.8 ns
34.2 a
7.1 ns
25.6 a
7.3 ns
35.2 a
4.7 ns
47.8 ab
2.8 ns
29.3 a

Figure 5. Effect of extract concentration of *Mikania micrantha* and comparison herbicide on fresh weight of roots (A), dry weight of roots (B), fresh weight of shoots (C), and dry weight of shoots (D) in lowland rice and barnyardgrass. Different letters on the graph indicate the significant effect by a DMRT at $P<0.05$. ns= not significant. The vertical line on the graph indicates the standard error.

The extracts of *Mikania micrantha* in any concentration and the herbicide 2,4-D dimethyl amine significantly inhibited the biomass of barnyardgrass, but it was insignificant on the biomass of lowland rice (Fig 5). Furthermore, exposure to *Mikania micrantha* extracts caused a decrease in the fresh and dry weight of roots for barnyardgrass from 58.9 to 71.6% and 63.4 until 73.2%, with the highest decrease at a concentration of 20%. This was also discovered in the treatment of herbicide 2,4-D dimethyl amine, which caused a decrease in the fresh and dry weight of roots for barnyardgrass by 75.4% and 75.9%, respectively. There was a decrease in fresh and dry weight of shoots for barnyardgrass due to exposure to *Mikania micrantha* extract, which ranged from 41.6-62.6% and 27.0-60.9%, respectively, with the highest decrease at 60% concentration. This was similar to the treatment of herbicide 2,4-D dimethyl amine, which caused a decrease in fresh and dry weight of shoots for barnyardgrass by 57.6% and 55.3%, respectively.

The concentration of *Mikania micrantha* extract and the herbicide 2,4-D dimethyl amine significantly affected leaf stomata density of lowland rice and barnyardgrass, but it was insignificant on upper and lower epidermis, and mesophyll tissue (Tab 3 and Fig 6). The extract concentration of 60% and the herbicide 2,4-D dimethyl amine increased the highest stomatal density of lowland rice by 96.9% and 91.1%, respectively, compared to the control. However, the extract concentration of 100% *Mikania micrantha* significantly increased stomatal density of barnyardgrass by 81.7% compared to the control.

Table 3. Effect of extract concentration of *Mikania micrantha* and comparison herbicide on histological characteristics of lowland rice and barnyardgrass.

Extracts concentration	Histological characteristics			Stomatal density (n/μm)
	Upper epidermis	Lower epidermis	Mesophyll	
	-------------------------thickness (μm)---------------------			
Lowland rice				

0%	43.5 ± 1.9 ns	28.1 ± 2.5 ns	138.1 ± 6.8 ns	71.1 ± 0.8 c
20%	36.3 ± 3.0 ns	23.6 ± 0.6 ns	137.0 ± 3.5 ns	67.4 ± 2.8 c
40%	35.4 ± 1.8 ns	28.8 ± 1.0 ns	172.4 ± 0.8 ns	104.5 ± 2.1 b
60%	54.7 ± 1.7 ns	28.9 ± 2.6 ns	121.3 ± 10.0 ns	140.0 ± 3.4 a
80%	41.0 ± 1.2 ns	31.9 ± 3.0 ns	164.3 ± 5.0 ns	66.9 ± 1.6 c
100%	49.1 ± 4.9 ns	36.7 ± 1.7 ns	123.0 ± 8.1 ns	92.0 ± 1.6 b
2,4-D	59.7 ± 1.2 ns	41.6 ± 2.6 ns	164.8 ± 14.0 ns	135.9 ± 4.0 a
Barnyardgrass				
0%	44.3 ± 3.0 ns	40.8 ± 3.3 ns	155.6 ± 10.6 ns	69.0 ± 2.4 c
20%	39.9 ± 3.7 ns	36.9 ± 4.1 ns	142.9 ± 4.0 ns	66.9 ± 3.4 c
40%	44.2 ± 2.3 ns	25.2 ± 2.2 ns	170.6 ± 15.6 ns	79.4 ± 2.9 bc
60%	39.3 ± 2.3 ns	34.2 ± 1.7 ns	128.7 ± 2.1 ns	96.2 ± 2.1 b
80%	33.6 ± 0.9 ns	24.1 ± 1.8 ns	109.4 ± 0.7 ns	64.8 ± 3.4 c
100%	29.3 ± 0.9 ns	24.8 ± 1.6 ns	116.2 ± 3.9 ns	125.4 ± 2.7 a
2,4-D	42.1 ± 2.9 ns	25.8 ± 2.2 ns	112.7 ± 4.9 ns	56.4 ± 3.6 c

Note: different lowercase letters for each column and species mean significant by a DMRT at P<0.05 ± standard error. ns= not significant.

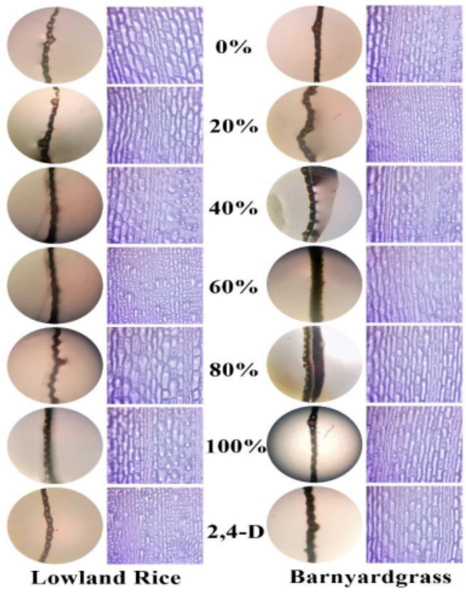

Figure 6. Histological tissue of lowland rice and barnyardgrass due to exposure of *Mikania micrantha* extract and comparison herbicides.

The concentration of *Mikania micrantha* extract and the herbicide 2,4-D dimethyl amine significantly inhibited the total chlorophyll of barnyardgrass at 2, 7, and 14 DAS as well as the total chlorophyll in lowland rice at 2 and 14 DAS, but it was insignificant effect at 7 DAS (Fig 7). There was a decrease in total chlorophyll of SPAD in lowland rice due to exposure to *Mikania micrantha* extract ranging from 13.0 to 28.0% than the control and the highest decrease was found in a concentration of 40% at 14 DAS. The herbicide 2,4-D dimethyl amine also slightly decreased the total chlorophyll content of SPAD in lowland rice by 7.5%. On the other hand, the concentration of *Mikania micrantha* extract had uneffected in decreasing the total chlorophyll of SPAD for barnyardgrass at 2 DAS, but the chlorophyll decreased from 7 to 14 DAS. The highest decrease in total chlorophyll was found in a 100% concentration of *Mikania micrantha* extract by 28.4% compared to the control at 14 DAS. Furthermore, the herbicide 2,4-D dimethyl amine also decreased the SPAD total chlorophyll for barnyardgrass by 26.1% at 14 DAS.

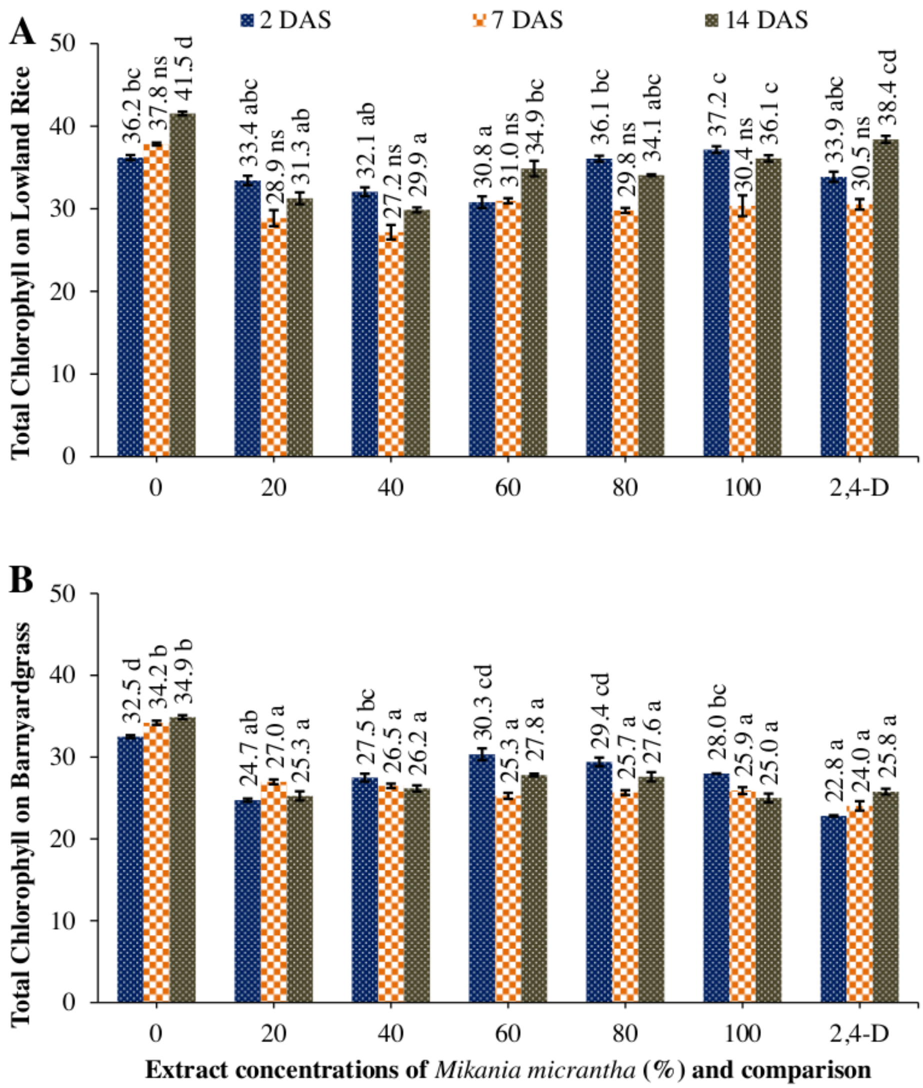

Figure 7. Effect of extract concentration of *Mikania micrantha* and comparison herbicide on total chlorophyll SPAD in lowland rice (A) and barnyardgrass (B). Different letters on the graph indicate the significant effect by DMRT at $P<0.05$. ns= not significant. The vertical line on the graph indicates the standard error.

The extracts of *Mikania micrantha* in any concentration and the herbicide 2,4-D dimethyl amine significantly reduced the growth of barnyardgrass and it were classified as inhibitor (Fig 8). The results showed that the highest growth reduction of barnyardgrass was found in exposure to the herbicide 2,4-D dimethyl amine by 65.6%. Meanwhile, the ability of the extract to reduce the growth of barnyardgrass ranged from 46.0 to 63.5%, with the highest was found in a concentration of 60%. Also, the ARI of all concentrations of *Mikania micrantha* extract and the herbicide 2,4-D dimethyl amine ranged from -0.6 to -0.3. This indicated that the extract concentration of 20-100% and the herbicide 2,4-D dimethyl amine inhibited the growth and biomass of barnyardgrass (ARI<0).

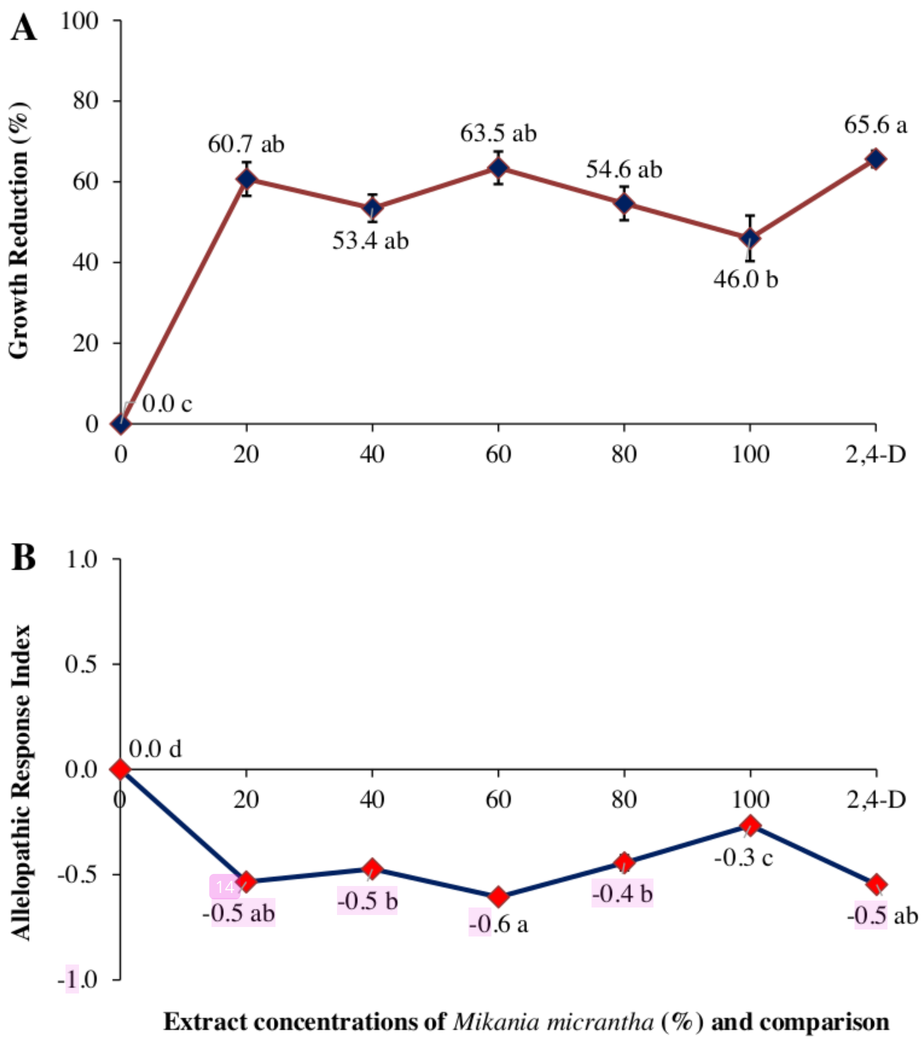

Figure 8. Effect of extract concentration of *Mikania micrantha* and comparison herbicide on the growth reduction (A) and allelopathic response index (B) in barnyardgrass. Different letters on the graph indicate the significant effect by DMRT at $P<0.05$.

In general, the concentration of *Mikania micrantha* effect is less toxic or safe for the growth of lowland rice plants. This is supported by the concentration of 60% which increased the number of leaves and the highest stomatal density of lowland rice (Tab 2-3). Furthermore, the length and volume of lowland rice root at all extract concentrations (20-100%) were higher than the control (Fig 4). These parameters is possibly used as the basis to determine the mechanism of plant growth disorders due to allelopathy. According to Chon et al. (2005) the effect of allelopathy is more visible in the inhibition of root growth than the shoot because the roots are more sensitive to phytotoxic compounds. Similarly, Li et al. (2010) also emphasized that roots are the first organs directly related to allelochemistry, the absorption of water and ions is disrupted, while the accumulation of abscisic acid increases, which affects the stomata opening width and the photosynthesis process.

The extracts of *Mikania micrantha* in any concentration significantly affected the characteristics of barnyardgrass weed. The concentration of 60-100% increases the toxicity of barnyardgrass, and 20-100% concentration could inhibited the growth of root volume, biomass (root and shoot), and also total chlorophyll of SPAD. However, 100% concentration increased stomatal density by 81.7% compared to the control. This is due to the flavonoids, tannins, alkaloids, and saponins from *Mikania micrantha* (Tab 1) could inhibited chlorophyll biosynthesis and have an affected the establishment of lower weed biomass compared to the unspraying. According to Li & Jin (2010) that the roots, stem and leaf extracts from *Mikania micrantha* reduced POD enzyme activity in *Coix lacryma-jobi* seedlings by 27%, 52%, and 34%. Shajib et al. (2012) reported that isoflavones had a role as phytotoxic allelochemicals against *Echinochloa crus-galli*. Jali et al. (2021) also stated that the *Mikania micrantha* extract up to 200 mg mL^{-1}, the toxicity increase to 329%, but reduced the root and shoot length, and the biomass of *Macrotylama uniflorum*.

The extracts of *Mikania micrantha* in any concentration reduced the growth of barnyardgrass from 46.0 to 63.5%, with the highest ability found at 60% concentration. Meanwhile, all concentrations from 20 to 100% had negative allelopathic response index or were classified as inhibitors (ARI<0). The highest 63.5% was close to the effect of 2,4-D (65.6%). The results obtained are in line with Ma et al. (2020) that a concentration of 50-400 mg mL^{-1} from *Mikania micrantha* extract had a negative allelopathic response index.

The herbicide 2,4-D dimethyl amine had a less safe impact on the growth of lowland rice plants due to a higher level of phytotoxicity, a lower number of leaves, inhibiting root and shoot. Furthermore, the use of *Mikania micrantha* extract is considered safe for the growth of lowland rice, which potential to reduce the use of synthetic herbicides and save costs for farmers.

CONCLUSIONS

Mikania micrantha extract contains the flavonoids, tannins, alkaloids, and saponins. Concentrations from 20 to 100% were safe for the growth of lowland rice than the use of the herbicide 2,4-D dimethyl amine. Furthermore, a concentration of 60% showed the highest number of leaves and stomatal density on lowland rice. The 20-100% concentration of *Mikania micrantha* also significantly inhibited the barnyardgrass growth in the range from 46.0-63.5% with a negative allelopathic response index. Specifically, it reduced total chlorophyll of SPAD, root volume, fresh and dry weight of roots, and shoots. There were an increase in the weed toxicity of barnyardgrass along the concentration increased from 60 to 100%. The ability of *Mikania micrantha* extract in reducing the growth of barnyardgrass was nearby to the herbicide 2,4-D dimethyl amine.

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