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Research Article

Allelopathic effects of *Mikania micrantha* Kunth on barnyardgrass and lowland rice[1]

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ABSTRACT

Secondary metabolites from weeds have potential as a natural herbicide and are safe for the main crop. This study aimed to screening secondary metabolites in *Mikania micrantha* extract and their effect on the agronomic, physiological and histological characteristics of barnyardgrass and lowland rice. A non-factorial randomized block design was used, with extract concentrations of *Mikania micrantha* (0; 20; 40; 60; 80; 100 %) and 2,4-D dimethyl amine herbicide as comparison. The *M. micrantha* extract presented flavonoids, tannins, alkaloids and saponins. The concentration of 20-100 % inhibited the barnyardgrass growth, but was considered safe for the lowland rice, and increased the root length and volume, while the concentration of 60 % increased the number of leaves and stomatal density in the lowland rice. The concentration of 20-100 % significantly inhibited the root volume, biomass, SPAD total chlorophyll and growth reduction of barnyardgrass, and it was classified as an inhibitor. The barnyardgrass tocixity also increased as the concentration grew from 60 to 100 %. The *M. micrantha* extract reduced the barnyardgrass growth by 63.5 %, being close to the percentage presented by the 2,4-D dimethyl amine (65.6 %).

KEYWORDS: *Echinochloa crus-galli* (L.) Beauv, *Oryza sativa* L., secondary metabolites.

RESUMO

Efeitos alelopáticos de *Mikania micrantha* Kunth em capim-arroz e arroz de planície

Metabólitos secundários de ervas daninhas têm potencial como herbicida natural e são seguros para a cultura principal. Objetivou-se identificar os metabólitos secundários em extrato de *Mikania micrantha* e o seu efeito nas características agronômicas, fisiológicas e histológicas de capim-arroz e arroz de planície. Utilizou-se delineamento não-factorial de blocos casualizados, com concentrações de extrato de *Mikania micrantha* (0; 20; 40; 60; 80; 100 %) e o herbicida 2,4-D de dimetilamina como comparação. O extracto de *M. micrantha* apresentou flavonoides, taninos, alcaloides e saponinas. A concentração de 20-100 % inibiu o crescimento do capim-arroz, mas foi considerada segura para o arroz de planície, e aumentou o comprimento e volume das raízes, enquanto a concentração de 60 % aumentou o número de folhas e a densidade estomatal no arroz de planície. A concentração de 20-100 % inibiu significativamente o volume das raízes, biomassa, clorofila total SPAD e a redução do crescimento do capim-arroz, sendo classificada como um inibidor. A tocixidade do capim-arroz também aumentou à medida que a concentração elevou-se de 60 para 100 %. O extrato de *M. micrantha* reduziu o crescimento do capim-arroz em 63,5 %, porcentagem próxima à apresentada pela 2,4-D dimetilamina (65,6 %).

PALAVRAS-CHAVE: *Echinochloa crus-galli* (L.) Beauv, *Oryza sativa* L., metabólitos secundários.

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, the rice crop yield will increase by 42 % to fulfill the 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 weeds reached 32 % and was significantly greater than the 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

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[*Echinochloa crus-galli* (L.) Beauv], and Ottis & Talbert (2007) reported 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 inhibits the number of tillers and reduces the number of grains, maturity and 1,000-grain weight of lowland rice.

One of the weed managements for *E. crus-galli* in lowland rice fields is 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 increase of the number of tillers and the total dry weight of lowland rice by 3.54 and 58.54 %, respectively, if 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 weeds, 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 proved 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 aimed to screening secondary metabolites in *M. micrantha* extract and determine their effect on the characteristics of *E. crus-galli* and lowland rice.

MATERIAL AND METHODS

This study was conducted at a farmland in Padang Bulan Selayang I, Medan Selayang sub-district, Medan city, Indonesia, from June to October 2021. The barnyardgrass seeds were taken at lowland rice fields in Padang Bulan, Medan (98º38.601'E and 3º33.560'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 was taken from the bucket and put into a 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 placed in the middle. Subsequently, the barnyardgrass seedlings were transplanted after 3-4 leaves into a bucket of five seedlings per bucket, in a position surrounding the lowland rice (Figure 1).

The *Mikania micrantha* shoot was taken from a experimental field in the Universitas Sumatera Utara, Medan (98º39.214'E and 3º33.467'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.

The screening of *M. micrantha* secondary metabolites was conducted using a qualitative method

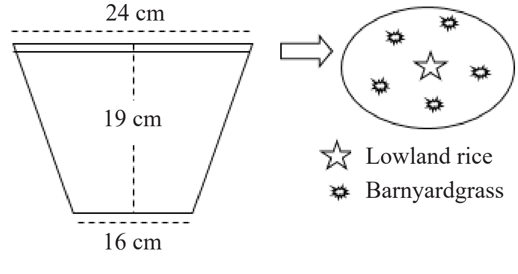

Figure 1. Position for planting seedlings of lowland rice and barnyardgrass in the bucket.

by obtaining 10 mL of the extract (Figure 2) and analyzing the contents of alkaloids, tannins, saponins, flavonoids, steroids and triterpenoids. 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 was 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 colloidal black, when the sample was positive.

The screening of saponin was conducted by soaking the sample with ethanol and putting it in a test tube. Distilled water and 96 % alcohol reagent were further added, 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 the extract were inserted into a test tube, then 2 drops of Liebermann-Burchard reagent were 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 for one minute, when the positive sample changed to colloidal black.

This study used a non-factorial randomized block design, with concentration factors of *M. micrantha* extract such as NH_0 = 0 % (untreated); NH_1 = 20 % (157.8 g L^{-1}); NH_2 = 40 % (315.6 g L^{-1}); NH_3 = 60 % (473.4 g L^{-1}); NH_4 = 80 % (631.2 g L^{-1}); NH_5 = 100 % (789 g L^{-1}); NH_6 = 2,4-D dimethyl amine herbicide (720 g a.i. ha^{-1}) as a comparison, with three replications. The application of natural herbicides from *M. micrantha* extract and comparison herbicide were conducted when the barnyardgrass had 5-6 leaves. Furthermore, a manual spraying was conducted at 9:40-10:53 a.m., when the temperature, humidity, air and spray pressure were 29.3 ºC, 88 %, 1,008 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 were sown evenly. Subsequently, they were sprayed with deltamethrin insecticide (25 g L^{-1}) to control pests and propine fungicide (70 %) to control diseases, based on the recommended dose.

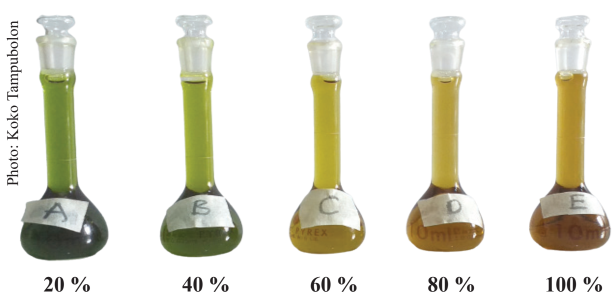

Figure 2. Samples of *Mikania micrantha* weed ethanol extract in qualitative secondary metabolite screening tests.

The measured agronomic characters of the lowland rice included phytotoxicity at 2, 7 and 14 days after spraying (DAS), number of leaves at 7-14 DAS, root length and volume, and biomass (roots and shoots) at 42 DAS. The 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 were carried out visually and scored based on leaf color, as it follows: score 0 = green; score 1 = yellowish-green; score 2 = overall yellow; score 3 = yellow-brown spots; score 4 = brown and shrink (Alridiwirsah et al. 2020b). The score was calculated and converted to percentage using the equation: Phytotoxicity = {[(∑ leaves x score 4) + (∑ leaves x 3) + (∑ leaves x 2) + (∑ leaves x 1)]/total number of leaves} × 100 %. The fresh weight was measured by cleaning the roots from the soil, then drying for two hours and weighing with an analytical scale. Meanwhile, the dry weight measurement was carried out in an oven at 65 ºC, for 72 hours, and the weed growth reduction was measured based on the total dry weight, using the equation: Growth reduction = 100 - [total dry weight (sprayed)/total dry weight (control)] × 100 %. The ARI measurement was based on the shoot dry weight, using the formula by Williamson & Richardson (1988): ARI = [shoot dry weight (control)/shoot dry weight (sprayed)] - 1. When the ARI value is greater than 0, the allelopathic effect is stimulatory, and if the ARI value < 0, the allelopathy effect is inhibitory (Sunmonu & Van Staden 2014).

The measured histological characters of lowland rice and barnyardgrass included upper and lower epidermis, mesophyll and stomata density. The upper and lower epidermis and the mesophyll tissue were measured by taking the second leaf at 7 DAS with a transverse incision, using the paraffin method (Johansen 1940). The 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 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 10 x 10. The physiological characters of lowland rice and barnyardgrass included the total chlorophyll at 2, 7 and 14 DAS. Subsequently, the 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 Anova, followed by the Duncan's multiple range test at $p < 0.05$, using the 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 (Table 1). The secondary metabolites from the extract had alkaloids, flavonoids, saponins and tannins, but steroids and triterpenoids compounds were not detected. 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.

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

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

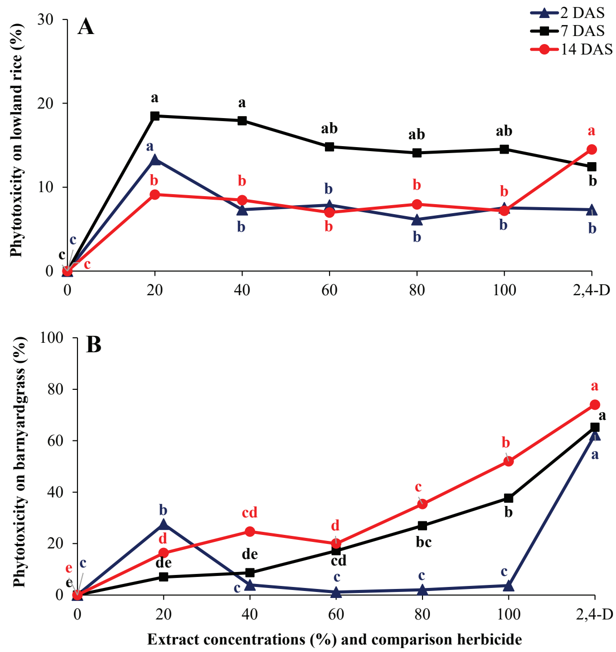

Figure 3. Effect of extract concentrations of *Mikania micrantha* and comparison herbicide (2,4-D dimethyl amine) on the phytotoxicity of lowland rice (A) and barnyardgrass (B). Different letters on the graph indicate a significant effect by the Duncan's multiple range test at $p < 0.05$. DAS: days after spraying.

Table 1. Screening of *Mikania micrantha* secondary metabolites for different extract concentrations.

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

+: detected; -: not detected.

Table 2. Effect of extract concentrations of *Mikania micrantha* and comparison herbicide (2,4-D dimethyl amine) on the number of leaves in lowland rice at 7-14 days after spraying (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

Different lowercase letters in the column are significant by the Duncan's multiple range test at $p < 0.05$ ± standard error.

The *M. micrantha* extracts at any concentration and the 2,4-D dimethyl amine herbicide significantly affected the root volume of barnyardgrass, but were not significant on the root length and volume of the lowland rice and root length of the barnyardgrass (Figure 4). Furthermore, the exposure to *M. micrantha* extracts caused a decrease in the root volume of barnyardgrass from 22.2 to 42.1 % and the 2,4-D dimethyl amine herbicide by 55.2 %, with the highest decrease found at the concentration of 100 %. This indicated that the 100 % exposure to *M. micrantha* extracts inhibited the growth and development of the barnyardgrass roots.

The *M. micrantha* extracts at any concentration and the 2,4-D dimethyl amine herbicide significantly inhibited the barnyardgrass biomass, but were not significant for the lowland rice biomass (Figure 5). Furthermore, the exposure to the extracts caused a decrease in the root fresh and dry weight of the barnyardgrass from 58.9 to 71.6 % and 63.4 to 73.2 %, respectively, with the highest decrease at the concentration of 20 %. This was also observed in the treatment with the 2,4-D dimethyl amine herbicide, which decreased the root fresh and dry weight of barnyardgrass by 75.4 and 75.9 %, respectively. There was a decrease in the shoot fresh and dry weight of barnyardgrass due to the exposure to the *M. micrantha* extracts, which ranged 41.6-62.6 and 27.0-60.9 %, respectively, with the highest decrease at the concentration of 60 %. This was similar to the treatment with the 2,4-D dimethyl amine herbicide, which decreased the shoot fresh and dry weight of barnyardgrass by 57.6 % and 55.3 %, respectively.

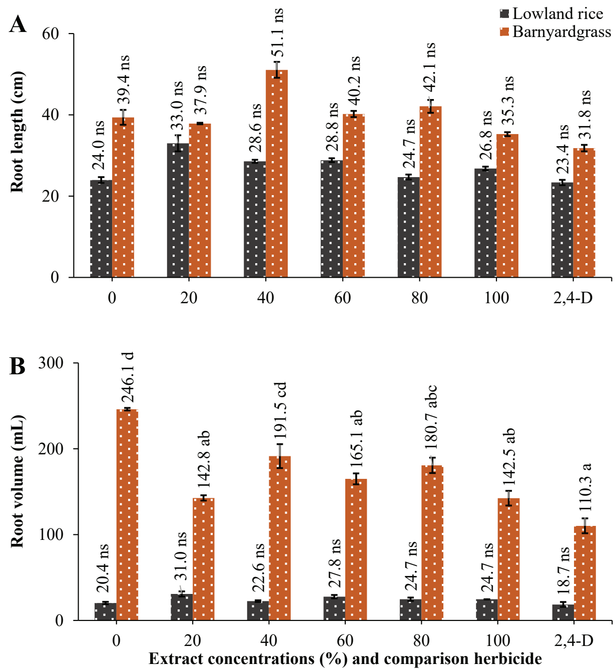

Figure 4. Effect of extract concentrations of *Mikania micrantha* and comparison herbicide (2,4-D dimethyl amine) on root length (A) and root volume of lowland rice and barnyardgrass (B). Different letters for each species (lowland rice and barnyardgrass) on the graph indicate a significant effect by the Duncan's multiple range test at $p < 0.05$. ns: not significant. The vertical lines on the graph indicate the standard error.

The *M. micrantha* extracts at any concentration and the 2,4-D dimethyl amine herbicide significantly affected the leaf stomata density of the lowland rice and barnyardgrass, but were not significant for the upper and lower epidermis and mesophyll tissue (Table 3; Figure 6). The extract concentration of 60 % and the 2,4-D dimethyl amine herbicide increased the highest stomatal density of lowland rice by 96.9 and 91.1 %, respectively, if compared to the control. However, the extract concentration of 100 % significantly increased the stomatal density of the barnyardgrass by 81.7 %, when compared to the control.

The *M. micrantha* extracts at any concentration and the 2,4-D dimethyl amine herbicide significantly inhibited the total chlorophyll of the barnyardgrass at 2, 7 and 14 DAS, as well as the total chlorophyll in the lowland rice at 2 and 14 DAS, but were not significant at 7 DAS (Figure 7). There was a decrease in the SPAD total chlorophyll content of the lowland rice due to exposure to the *M. micrantha* extracts ranging from 13.0 to 28.0 %, when compared to the

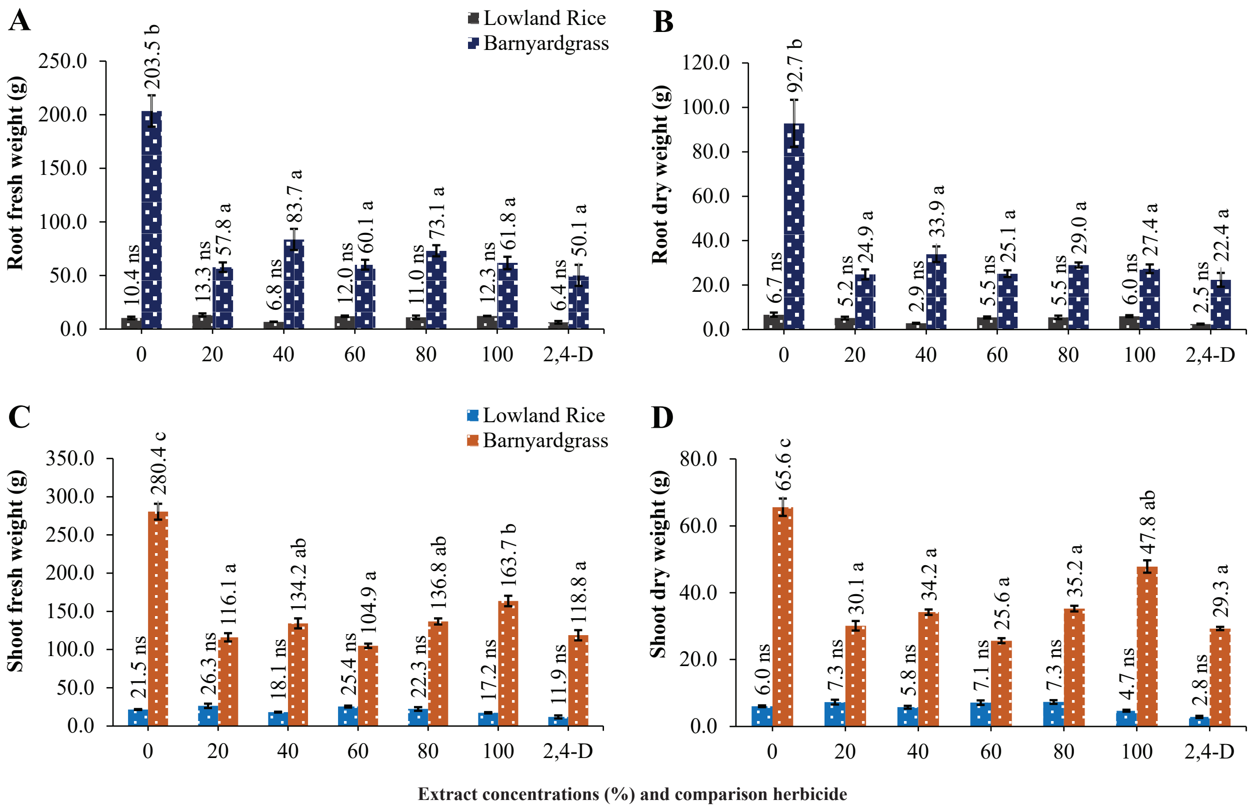

Figure 5. Effect of extract concentrations of *Mikania micrantha* and comparison herbicide (2,4-D dimethyl amine) on root fresh weight (A), root dry weight (B), shoot fresh weight (C) and shoot dry weight (D) in lowland rice and barnyardgrass. Different letters for each species (lowland rice and barnyardgrass) on the graph indicate a significant effect by the Duncan's multiple range test at $p < 0.05$. ns: not significant. The vertical lines on the graph indicate the standard error.

Table 3. Effect of the extract concentrations of *Mikania micrantha* and comparison herbicide (2,4-D dimethyl amine) on the histological characteristics of lowland rice and barnyardgrass.

Extract concentrations	Histological characteristics			Stomatal density (n μm^{-1})
	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

Different lowercase letters for each column and species indicate a significant effect by the Duncan's multiple range test at $p < 0.05$ ± standard error. ns: not significant.

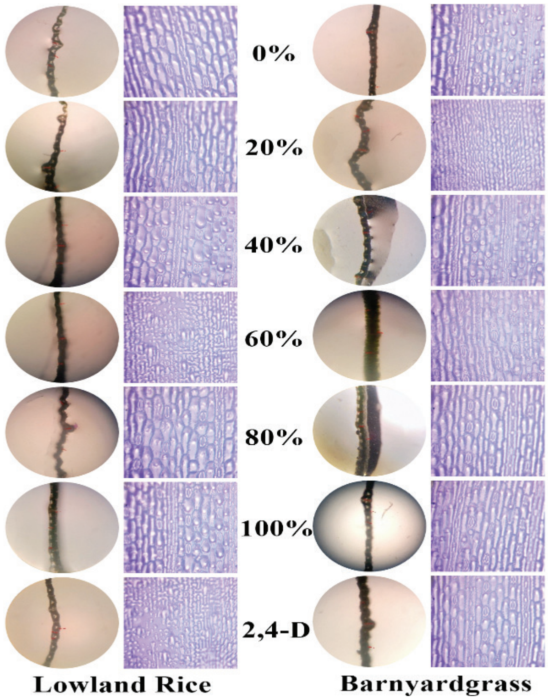

Figure 6. Histological tissue of lowland rice and barnyardgrass due to exposure to *Mikania micrantha* extracts and 2,4-D dimethyl amine herbicide.

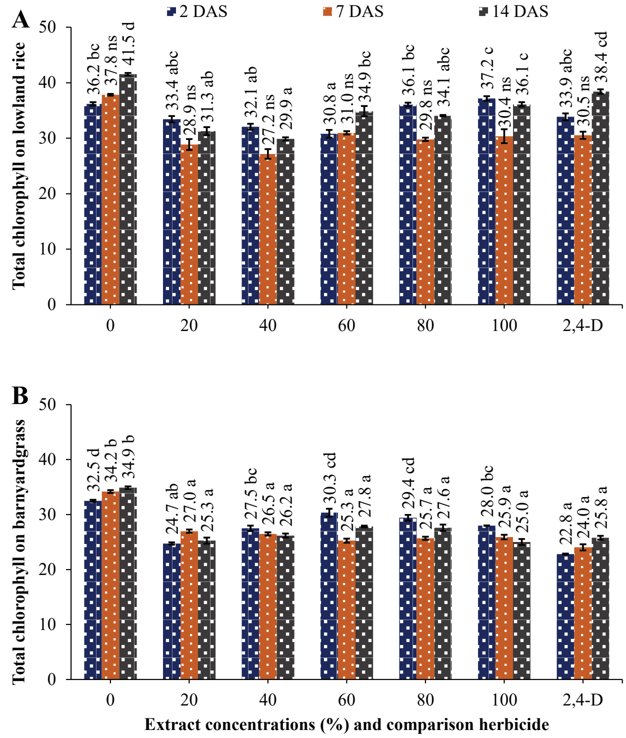

Figure 7. Effect of extract concentrations of *Mikania micrantha* and comparison herbicide (2,4-D dimethyl amine) on SPAD total chlorophyll in lowland rice (A) and barnyardgrass (B). Different letters for each species (lowland rice and barnyardgrass) on the graph indicate a significant effect by the Duncan's multiple range test at $p < 0.05$. ns: not significant. The vertical lines on the graph indicate the standard error. DAS: days after spraying.

control, and the highest decrease was observed for the concentration of 40 % at 14 DAS. The 2,4-D dimethyl amine herbicide also slightly decreased the SPAD total chlorophyll content of the lowland rice by 7.5 %. On the other hand, the extract concentrations of *M. micrantha* had no effect in decreasing the SPAD total chlorophyll content for the barnyardgrass at 2 DAS, but the chlorophyll decreased from 7 to 14 DAS. The highest decrease in total chlorophyll (28.4 %) was observed at the concentration of 100 % of *M. micrantha* extract, when compared to the control at 14 DAS. Furthermore, the 2,4-D dimethyl amine herbicide also decreased the SPAD total chlorophyll content for the barnyardgrass by 26.1 % at 14 DAS.

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

In general, the effect of the *M. micrantha* concentration 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 resulted in the highest stomatal density of the lowland rice (Tables 2 and 3). Furthermore, the length and volume of the lowland rice root at all the extract concentrations (20-100 %) were higher than for the control (Figure 4). These parameters are 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 in the shoot, because the roots are

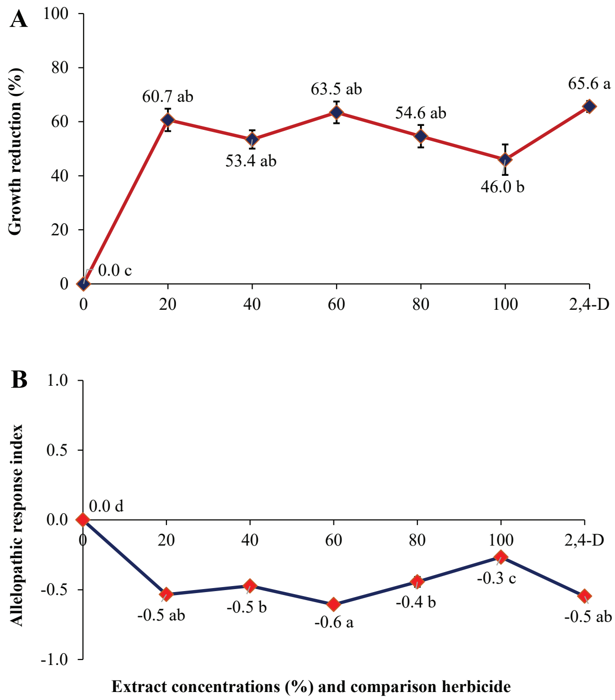

Figure 8. Effect of extract concentrations of *Mikania micrantha* and comparison herbicide (2,4-D dimethyl amine) on the growth reduction (A) and allelopathic response index (B) in barnyardgrass. Different letters on the graph indicate a significant effect by the Duncan's multiple range test at $p < 0.05$. The vertical lines on the graph indicate the standard error.

more sensitive to phytotoxic compounds. Similarly, Li et al. (2010) also emphasized that roots are the first organs directly related to allelochemistry, and that the absorption of water and ions is disrupted, while the accumulation of abscisic acid increases, thus affecting the stomata opening width and the photosynthesis process.

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

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

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

CONCLUSIONS

1. The *Mikania micrantha* extract contains flavonoids, tannins, alkaloids and saponins, and concentrations of 20-100 % showed to be safer for the growth of lowland rice than the use of the 2,4-D dimethyl amine herbicide;
2. The concentration of 60 % resulted in the highest number of leaves and stomatal density for the lowland rice, while the concentration of 20-100 % significantly inhibited the barnyardgrass growth in the range of 46.0-63.5 %, with a negative allelopathic response index. Specifically, it reduced the SPAD total chlorophyll, root volume, fresh and dry weight of roots and shoots;
3. There was an increase in the weed toxicity of the barnyardgrass along the concentration increase from 60 to 100 %;
4. The ability of the *Mikania micrantha* extract to reduce the barnyardgrass growth was similar to that of the 2,4-D dimethyl amine herbicide.

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