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Evaluation Of Allelopathic Potential Of *Cissus sicyoides* Against the Growth Of *Echinochloa Crus-Galli* And Some Tested Plants

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Abstract

Many plant species in nature exert significant allelopathic potential as part of the defense mechanism system, many among their secondary metabolites (allelochemicals), including mineral constituents, which are responsible for the suppression of weeds and enhancing crop yield when directly incorporated into paddy fields**.** *Cissus sicyoides* is considered a high-potential allelopathic plant because of its invasion in nature and detected allelochemicals from the plant parts in some studies. The objective of this research was to exploit the allelopathic properties of *C.sicyoides* against paddy weeds and some indicator plants under laboratory bioassays and greenhouse conditions. The results demonstrated that *C. sicyoides* had significant inhibition on *E. crus-galli*, tested plants, and other paddy weeds. In the laboratory conditions, the extracts from *C.sicyoides* leaves inhibited the growth of *Echinochloa crus-galli* by 54.3%. The powders from *C.sicyoides* leaves inhibited the emergence of paddy weeds by approximately 100.0%. In the greenhouse conditions, the powders from *C.sicyoides* leaves by adding after 3 and 13 days inhibited the growth of *E. crus-galli* and the emergence of paddy weeds by 64.4%. Remarkably, negligible harmful effects on rice growth were observed. The findings of the study may provide useful information for the exploitation of this plant species to effectively control weeds in the rice fields for sustainable agriculture production.

Keywords: Allelopathy, paddy weed, Cissus sicyoides, inhibition, Echinochloa crus-galli, paddy weed, emergence.

1. INTRODUCTION

Allelopathy is defined as the interaction(s) between plant species including positive and negative influences by releasing plant-produced secondary metabolites, referred to as allelochemicals[1]. Kong et al[2] and Xuan et al[3] stated that released allelochemicals as "phytotoxins" from allelopathic plant species can reduce significantly the emergence of weeds in crop systems. Almost allelochemicals are secondary metabolites, which can affect primary metabolites and plant growth regulators in flowering plants[4]. High allelopathic plants are considered to have characteristics including (1) strong growth in nature, (2) invasive plants, (3) weed inhibition under the canopy, and (4) release of allelochemicals from plant parts. Allelopathy is defined as the interaction(s) between plant species, including positive and negative influences, by releasing plant-produced secondary metabolites, also known as phytotoxins[5].

Weed infestation is a big challenge and significantly reduces the yields of crops. To control them, some methods are being applied such as hand-weeding, water management, land preparation and sowing techniques. However, all these methods are dependent on weather conditions, time-consuming and require intensive labor, which does not conform to the current trends of urbanization in this country[6]. Synthetic herbicides and insecticide utilization can minimize the time spent on weed, pests, insects and disease control and stabilize the crop yield. However, the overuse of synthetic chemicals is a serious problem in Vietnam, causing environmental pollution, weed-resistant herbicides, unsafe agricultural products, and human health concerns. Biological control is somewhat less known and has been carried out sporadically in this country. Hence, reducing the dependency on synthetic herbicides and agrochemicals in agricultural production in Vietnam is an important task to develop environmentally friendly and maintain sustainable agricultural production. Biological management by using allelopathy may enhance crop yield without environmental cost, which is one of the most important considerations for this country[7-8].

To date, herbicide is widely used in the world because of its convenience and high effect on weed management. However, the environment as well as human and animal health are seriously affected by the frequent application of chemical herbicides. Moreover, resistance to weeds against herbicides could be increased, which is also a serious problem threatening worldwide sustainable development in agriculture[9-10]. Numerous studies on allelopathic potential from plants and crops such as rice, wheat, clovers, kava and sorghum, etc. were successfully exploited for weed management during the last four decades in cropping systems[11-14].

C. sicyoides is traditionally known as a medicinal plant[12-13]. In nature, *C. sicyoides* is observed as a strong invasion plant, which sometimes becomes a serious problem for commercial plants such as flowers, fruits, etc. However, there is sporadical research on the allelopathic potential of this plant. Therefore, in this study, *C.sicyoides* was selected and to assess the allelopathic potential in laboratory bioassay and greenhouse conditions.

2. MATERIALS AND METHODS

2.1. Plant collection and Aqueous extract

C.sicyoides plants were collected from Co Nhue Commune, Tu Liem district, Hanoi, Vietnam. Leaves, stems and roots of *C.sicyoides* were separately cut into small pieces and dried in an oven at 60^{o}C until the moisture level was not exceeded by 5%. After that, the samples were powdered less than 4 mm using a kitchen grinder.

The powdered leaves, stems and roots of *C. sicyoides* (5.0g) were separately extracted in 100 ml distilled water for 24h at 30^{o}C. The extracts were subsequently filtered by filter papers. After that, the extracts were dissolved in distilled water at the concentration of 50 g/L, 25 g/L, 12/5 g/L and 6,25 g/L respectively and kept in the refrigerator for the next experiments.

2.2. Tested plants

The tested plants or indicator plants included: Seeds of lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus* L.), barnyardgrass (*Echinochloa crus-galli* L.), and Khang dan rice variety (*Oryza sativa* L.) were collected from Department of Genetic Engineering, Agricultural Genetics Institute in 2021. The germination of indicator plants was tested before conducting the experiment and showed >95%.

2.3. Laboratory bioassays

Experiment 1: Petri dishes (9 cm diameter) containing filter paper were added by 2 ml of distilled water.

Seeds of all indicator plants were rinsed three times with distilled water. After that, they were soaked in 0.1% sodium hypochlorite for 30 min. Finally, they were rinsed three times again with distilled water. Twenty seeds of each indicator plants were separately placed in a Petri dish and treated by 10 mL solution of extracts from *C.sicyoides* leaves, stems, and roots at concentrations of 50 g/L, 25 g/L, 12.5 g/L and 6.25 g/L with at least three replications. The control was applied with distilled water only. The designed experiment was completely random in the growth chamber with the condition for a 14h photoperiod (328 – 450 lux) at 26ºC, with a moisture of 75%. After 7 days, the rates of gemination and survival, shoot height, root length and dry weight of indicator plants were recorded over the controls[14].

Experiment 2: Following the method of Khanh et al. (2018), paddy soils (pH: 6.3, total C: 2.22%, total N: 0.18%, CEC: 8.8 meq per 100 g soil; CaO: 91, MgO: 13, K2O: 17, K2O5: 18, SiO2: 25 mg per 100 g soil) were collected randomly from the experimental farm of Agricultural Genetics Institute, Hanoi, Vietnam, where early-matured rice had been grown. The soils were collected to a depth of 10 cm in the summer of 2017. After that, the soils were dried and mixed for further use. Paddy soils (400 g) and water (200 mL) were put in plastic beakers (9 cm diameter, 500 mL capacity). After 2 days, powders from *C.sicyoides* leaves, stems, and roots were added to the soil surface with the dose of 1.0, 1.5, 2.0 tons/ha. The control was applied with water only. The designed experiments were completely random with three replications. All pots were put in the growth chamber with the condition for a 14h photoperiod (328 – 450 lux) at 26ºC, with moisture of 75%. After 21 days, the types and the number of dry weight of natural weeds were evaluated over the control.

2.4. Greenhouse trials

Experiment 1: Clean soils (3 kg) and water (1 L) were added to the plastic pots (25 cm diameter, 7 L capacity) in the greenhouse. Thirty seeds of rice and barnyardgrass were separately placed on the surface of the soil. Two treatments with three replications were carried out as follows: Treatment 1: the extracts from *C. sicyoides* leaves (25 g/L) were sprayed on the soil surface after 3 days with a concentration of 80 mL. Treatment 2: the extracts from *C. sicyoides* (25 g/L) were sprayed on the soil surface with a concentration of 80 mL. The treatments were divided into two times (after 3 and 16 days). The control was applied with only water. The designed experiments were completely random. After 21 days, germination rate, survival rate, shoot height, root length and dry weight of rice and barnyardgrass were evaluated over the control.

Experiment 2: Paddy soils (3 kg) and water (1 L) were put in plastic pots (25 cm diameter, 7 L capacity). Two treatments with three replications were carried out as below: Treatment 1: After 3 days, powders from *C.sicyoides* were added to the soil surface with a dose of 1 ton/ha. Treatment 2: After 3 and 16 days, powders from *C. sicyoides* were added to the soil surface with a total dose of 1 ton/ha. The control was applied with only water. The designed experiments were completely random. After 21 days, the types and the number, of dry weight of natural weeds were evaluated over the control.

Experiment 3: Clean soils (3 kg) and water (1 L) were added to the plastic pots (25 cm diameter, 7 L capacity) in the greenhouse. Thirty seeds of rice and barnyardgrass were separately placed on the surface of the soil. Two treatments with three replications were carried out as below: Treatment 1: After 3 days, powders from *C. sicyoides* were added to the soil surface with a dose of 1 ton/ha. Treatment 2: After 3 and 16 days, powders from *C. sicyoides* were added to the soil surface with a total dose of 1 ton/ha, divided into two times. The control was applied with only water. The designed experiments were completely random. After 21 days, germination rate, survival rate, shoot height, root length and dry weight of rice and barnyardgrass were evaluated over the control.

2.5. Statistical analysis

The data was analyzed using Excel 2010 and one-way ANOVA. The percentage of inhibition/stimulation was calculated following the formula below:

Percentage of inhibition/stimulation (%) = [1 – (treatment/control)] x 100

3. RESULTS

3.1. Effects of extracts from *Cissus sicyoides* roots, stems, and leaves on germination of indicator plants in laboratory condition

As shown in Table 1, the results showed that the germination rate (GR) of lettuce was inhibited. The treatments including extracts from stems (25 and 6.25 g/L) and from leaves (50 g/L) induced a high rate of inhibition on germination of lettuce by 42.3%, 45.8%, and 50.9% respectively. However, the extracts from *C.sicyoides* had an insignificant effect on the survival rates of lettuce in comparison to the control. For shoot height (SH), stimulation was recorded for lettuce under the effect of extracts from *C.sicyoides*, except for the extracts from stems (50 and 25 g/L). However, the treatments including extracts from roots, stems (6.25 g/L), and from leaves (6.25, 12.5, 25 g/L) showed high rates of stimulation on shoot height of lettuce by over 30%, especially the extracts from leaves (42.4%) at the concentration of 12.5 g/L. The shoot height (SH) of lettuce was reduced (15.2%) by only the effect of extracts from stems (50 g/L). All the treatments inhibited significantly the root elongation of lettuce from 5.9% to over 80.0%. Remarkably, the extracts from stems (50 g/L, 25 g/L) had inhibition rates of 82.4% and 72.5% respectively, followed by the extracts from roots and leaves (50 g/L) with rates of 60.8%. In general, the extracts at low concentrations (6.25, 12.5, 25 g/L) had negligible effects on the dry weight of lettuce. Contrarily, at the concentration of 50 g/L, dry weights of lettuce were decreased by 51.7%, 54.4%, and 69.1% under the effects of extracts from leaves, roots, and stems, respectively. In summary, the extracts from *C.sicyoides* stems had the highest average inhibition on the germination of lettuce in laboratory conditions.

Table 1. Effects of extracts from *Cissus sicyoides* leaves, stems, and roots on germination and growth of lettuce in laboratory conditions.

Treatment (g/L)		GR (%)	SR (%)	SH (cm)	RL (cm)	DW (mg)	AI
Roots	**50**	78.3^{cd} (-20.3)	93.6^{a} (-6.4)	3.4^{e} (+3.0)	2.0^{f} (-60.8)	6.8^{e} (-54.4)	-27.8
	25	80.0^{cd} (-18.6)	95.7^{a} (-4.3)	4.0^{c} (+21.2)	3.5^{d} (-31.4)	12.9^{b} (-13.4)	-9.3
	12.5	75.0^{d} (-23.7)	95.6^{a} (-4.4)	4.0^{c} (+21.2)	3.9^{c} (-23.5)	12.2^{bc} (-18.1)	-9.7
	6.25	83.3^{bcd} (-15.3)	95.9^{a} (-4.1)	4.3^{abc} (+30.3)	4.8^{ab} (-5.9)	10.8^{d} (-27.5)	-4.5
AI		-19.5	-4.8	+18.9	-30.4	-28.4	-12.8
Stems	**50**	90.0^{ab} (-8.4)	100.0^{a} (0.0)	2.8^{f} (-15.2)	0.9^{h} (-82.4)	4.6^{f} (-69.1)	-35.0
	25	56.7^{e} (-42.3)	88.4^{a} (-11.6)	3.2^{ef} (-3.0)	1.4^{g} (-72.5)	11.8^{cd} (-20.8)	-30.0
	12.5	78.3^{cd} (-20.3)	95.8^{a} (-4.2)	3.9^{cd} (+18.2)	3.2^{d} (-37.3)	14.6^{a} (-2.0)	-9.1
	6.25	53.3^{e} (-45.8)	93.5^{a} (-6.5)	4.3^{abc} (+30.3)	2.4^{e} (-52.9)	14.8^{a} (-0.7)	-15.1
AI		-29.2	-5.6	+7,6	-61.3	-23.2	-22.3
Leaves	**50**	48.3^{e} (-50.9)	93.3^{a} (-6.7)	4.1^{bc} (+24.2)	2.0^{f} (-60.8)	7.2^{e} (-51.7)	-29.2
	25	86.7^{bc} (-11.8)	94.0^{a} (-6.0)	4.6^{ab} (+39.4)	3.4^{d} (-33.3)	12.2^{bc} (-18.1)	-6.0
	12.5	81.7^{bcd} (-16.9)	100.0^{a} (0.0)	4.7^{a} (+42.4)	4.5^{b} (-11.8)	14.3^{a} (-4.0)	+1.9
	6.25	86.7^{bc} (-11.8)	100.0^{a} (0.0)	4.4^{abc} (+33.3)	4.8^{ab} (-5.9)	14.1^{a} (-5.4)	+2.0
AI		-22.9	-3.2	+34.8	-28.0	-19.8	-7.8
Control		98.3^{a} (0.0)	100.0^{a} (0.0)	3.3^{e} (0.0)	5.1^{a} (0.0)	14.9^{a} (0.0)	
LSD 5%		5.4	7.3	3.0	2.1	0.6	
CV%		4.2	4.6	4.7	3.8	2.9	

Means within a column followed by the different letters were significantly different at $p < 0.05$. Numbers in parentheses are the rates in comparison with control; "+" symbol shows the promotion percentage over control, "−" symbol shows the inhibition percentage over control. AI: Average Inhibition. GR: germination rate, SR: survival rate, SH: shoot height, RL: root length, DW: dry weight.

For radish, the results showed that the germination of radish was reduced significantly by the effects of some treatments. Concretely, the extracts from roots (50 g/L) and leaves of *C.sicyoides* (25 g/L) suppressed the germination of radish by 12.1%. The extracts from roots (12.5 g/L and 6.25 g/L) and leaves (12.5 g/L) reduced the germination of radish by over than 15.0%. On the other hand, the survival rates of radish had negligible reduction with the effect of the treatments in comparison to the control except for the extracts from leaves (50 g/L) with approximately 20.0% inhibition (Table 2).

For shoot length (SH), almost all the treatments had stimulation effects on the length of radish shoots, especially the extracts from roots and shoots of *C.sicyoides* at 6.25 g/L (29.3%), leaves 6.25 g/L (31.4%), leaves 12.5 g/L (37.9%), the highest stimulation rate was 42.7% under the effects of extracts from leaves 25 g/L. However, the extracts from stems (50 g/L) inhibited the SH of radish by 16.9%. The root length (RL) of the radish was decreased by the effects of almost the treatments except for the extracts from roots and leaves (6.25 g/L) with insignificant differences in comparison to the control.

Table 2. Effects of extracts from *C. sicyoides* leaves, stems, and roots on germination and growth of radish in laboratory conditions

Treatment (g/L)		GR (%)	SR (%)	SH (cm)	RL (cm)	DW (mg)	AI
Roots	50	85.0^{bc} (-12.1)	98.1^{a} (-1.9)	6.4^{cd} (+3.6)	6.6^{f} (-46.3)	61.6^{cdef} (+18.5)	-7.6
	25	88.3^{abc} (-8.7)	98.0^{a} (-2.0)	5.7^{de} (-7.4)	7.8^{de} (-36.6)	52.7^{efgh} (+1.3)	-10.7
	12.5	81.7^{c} (-15.5)	97.9^{a} (-2.1)	5.6^{de} (-8.9)	7.3^{e} (-40.7)	45.1^{h} (-13.3)	-16.1
	6.25	81.7^{c} (-15.5)	97.9^{a} (-2.1)	4.8^{e} (-22.3)	6.6^{f} (-46.3)	54.1^{efgh} (+4.0)	-16.4
AI		-13.0	-2.0	-8.8	-42.5	+2.6	-12.7
Stems	50	93.3^{ab} (-3.5)	96.5^{a} (-3.5)	8.7^{a} (+40.5)	5.8^{gh} (-52.8)	63.4^{cde} (+21.9)	+0.5
	25	93.3^{ab} (-3.5)	92.9^{ab} (-7.1)	8.5^{ab} (+38)	6.3^{fg} (-48.8)	88.3^{a} (+69.8)	+9.7
	12.5	86.7^{abc} (-10.3)	92.9^{ab} (-7.1)	9.3^{a} (+49.6)	8.0^{d} (-35.0)	77.6^{ab} (+49.2)	+9.3
	6.25	93.3^{ab} (-3.5)	94.6^{a} (-5.4)	9.0^{a} (+46.0)	10.0^{b} (-18.7)	73.8^{bc} (+41.9)	+12.1
AI		-5.2	-5.8	+43.5	-38.8	+45.7	+7.9
Leaves	50	95.0^{ab} (-1.8)	80.8^{b} (-19.2)	7.4^{bc} (+20.0)	3.6^{j} (-70.7)	46.7^{gh} (-10.2)	-16.4
	25	85.0^{bc} (-12.1)	100.0^{a} (0.0)	8.5^{ab} (+37.5)	4.5^{i} (-63.4)	69.7^{bcd} (+34.0)	-0.8
	12.5	81.7^{c} (-15.5)	100.0^{a} (0.0)	8.6^{a} (+39.3)	6.0^{fg} (-51.2)	49.4^{fgh} (-5.0)	-6.5
	6.25	91.7^{abc} (-5.2)	100.0^{a} (0.0)	6.4^{cd} (+4.0)	5.3^{h} (-56.9)	59.7^{defg} (+14.8)	-8.7
AI		-8.7	-4.8	+25.2	-60.6	+8.4	-8.1
Control		96.7^{a} (0.0)	100.0^{a} (0.0)	6.2^{d} (0.0)	12.3^{a} (0.0)	52.0^{efgh} (0.0)	
LSD 0.05		6.3	6.9	6.3	3.5	7.4	
CV%		4.2	4.3	5.1	2.9	7.2	

Means within a column followed by the different letters were significantly different at p < 0.05. Numbers in parentheses are the rates in comparison with control; "+" symbol shows the promotion percentage over control, "−" symbol shows the inhibition percentage over control. AI: Average Inhibition. GR: germination rate, SR: survival rate, SH: shoot height, RL: root length, DW: dry weight.

The RL of radish was reduced by the effects of extracts from roots 25 g/L (72.4%). The extracts from roots, stems, and leaves (50 g/L) inhibited the RL of radish by 61.2%, 81.9%, and 61.9% respectively. For dry weight (DW), almost all the treatments stimulated the DW of radish over the control. For example, the DW of radish was stimulated by the effects of extracts from leaves 25 g/L (34.0%), stems 12.5 g/L (49.2%), especially the extracts from stems 25 g/L (69.8%) in compared to the control. The effects of the extracts from *C.sicyoides* on barnyardgrass are shown in Table 3. The results showed that the GR of barnyardgrass was extremely inhibited by the extracts from stems (50 g/L) by 20.7% and from leaves (25, 50 g/L) by 32.8% and 50.1% respectively over the control. The SR of barnyardgrass was not much affected by all the treatments except the extracts from leaves (25 g/L) with a rate of 15.1%. For the SH, almost all the treatments induced inhibition effects on barnyardgrass except the extracts from stems (12.5 g/L). The extracts from leaves (50 g/L) had the maximum inhibition rate of 56.1% on the SH of barnyardgrass. Similar to the SH, the RL of barnyardgrass was reduced by the effect of all the treatments. The extracts from leaves (12.5, 25, and 50 g/L) had the most serious inhibition on the RL of barnyardgrass with rates of 63.8%, 58.4%, and 68.1% respectively. The DW of barnyardgrass was affected negatively by all the treatments over the control, especially the extracts from leaves (25 g/L) with a rate of 85.4%.

Table 3. Effects of extracts from *Cissus sicyoides* leaves, stems, and roots on germination and growth of barnyardgrass in laboratory conditions

Treatment (g/L)		GR (%)	SR (%)	SH (cm)	RL (cm)	DW (mg)	AI
Roots	50	86.7^{bc} (-10.3)	94.1^{ab} (-4.2)	42.9^{d} (-43.0)	16.3^{fg} (-56.3)	7.5^{f} (-54.3)	-33.6
	25	98.3^{a} (+1.7)	94.8^{ab} (-3.5)	47.3^{cd} (-37.1)	27.1^{bc} (-27.3)	12.8^{cd} (-22.0)	-17.6
	12.5	95.0^{ab} (-1.8)	92.9^{ab} (-5.4)	46.8^{cd} (-37.8)	25.1^{bc} (-32.7)	13.7^{bc} (-16.5)	-18.8
	6.25	95.0^{ab} (-1.8)	96.4^{a} (-1.8)	51.0^{cd} (-32.2)	21.0^{de} (-43.7)	16.0^{a} (-2.4)	-16.4
AI		-3.1	-3.7	-37.5	-40.0	-23.8	-21.6
Stems	50	76.7^{c} (-20.7)	95.7^{ab} (-2.5)	45.6^{cd} (-39.4)	20.8^{de} (-44.2)	7.9^{f} (-51.8)	-31.7
	25	93.3^{ab} (-3.5)	92.8^{ab} (-5.5)	53.5^{c} (-28.9)	23.5^{cd} (-37.0)	11.9^{de} (-27.4)	-20.5
	12.5	95.0^{ab} (-1.8)	96.3^{a} (-1.9)	68.3^{ab} (-9.2)	16.9^{f} (-54.7)	14.1^{bc} (-14.0)	-16.3
	6.25	91.7^{ab} (-5.2)	98.1^{a} (-0.1)	65.3^{b} (-13.2)	27.5^{b} (-26.3)	14.7^{b} (-10.4)	-11.0
AI		-7.8	-2.5	-22.7	-40.6	-25.9	-19.9
Leaves	50	48.3^{e} (-50.1)	86.4^{ab} (-12.0)	33.0^{e} (-56.1)	11.9^{h} (-68.1)	2.4^{g} (-85.4)	-54.3
	25	65.0^{d} (-32.8)	83.4^{b} (-15.1)	49.1^{cd} (-34.7)	15.5^{fgh} (-58.4)	10.6^{e} (-35.4)	-35.3
	12.5	86.7^{bc} (-10.3)	86.5^{ab} (-11.9)	52.2^{cd} (-30.6)	13.5^{gh} (-63.8)	14.8^{b} (-9.8)	-25.3
	6.25	88.3^{ab} (-8.7)	94.3^{ab} (-4.0)	53.6^{c} (-28.7)	18.8^{ef} (-49.6)	12.6^{cd} (-23.2)	-22.8
AI		-25.5	-10.8	-37.5	-60.0	-38.5	-34.4
Control		96.7^{ab} (0)	98.2^{a} (0)	75.2^{a} (0)	37.3^{a} (0)	16.4^{a} (0)	
LSD 0.05		6.6	6.9	5.3	2.1	1.0	
CV%		4.6	4.5	6.0	5.9	5.0	

Means within a column followed by the different letters were significantly different at $p < 0.05$. Numbers in parentheses are the rates in comparison with control; "+" symbol presented the promotion percentage over control, "−" symbol showed the inhibition percentage over control. AI: Average Inhibition. GR: germination rate, SR: survival rate, SH: shoot height, RL: root length, DW: dry weight.

The data in Table 4 showed that the GR of rice was not different between the treatments and the control except for the extracts from leaves (25 g/L) with a slight inhibition of 11.7%. For the SR, only the extracts from leaves (50 g/L) induced an inhibition rate of 18.8% over the control. All the remaining treatments had insignificant effects on the SR of rice. The SH of rice was inhibited by the effects of the extracts from leaves, stems, and roots at high concentrations. Concretely, the inhibition rates on rice shoots of extracts from leaves and stems at 50 g/L and from roots at 25 g/L were 31.9%, 34.0%, and 36.2% respectively. For the RL, the extracts from stems and leaves at the concentration of 50 g/L inhibited rice roots by 21.3% and 40.3% over the control.

Table 4. Effects of extracts from *Cissus sicyoides* leaves, stems, and roots on germination and growth of rice in laboratory condition

Treatment (g/L)		GR (%)	SR (%)	SH (cm)	RL (cm)	DW (mg)	AI
Roots	**50**	96.7^{ab} (-3.3)	96.7^{a} (-3.3)	3.0^{g} (-36.2)	62.2^{fg} (-10.5)	26.8^{c} (-31.1)	-16.9
	25	93.3^{ab} (-6.7)	98.2^{a} (-1 8)	3.6^{ef} (-23.4)	76.9^{de} (+10.6)	56.0^{a} (+44.0)	+4.5
	12.5	91.7^{ab} (-8.3)	98.2^{a} (-1.8)	4.1^{cde} (-12.8)	83.0^{bcde} (+19.4)	54.1^{a} (+39.1)	+7.1
	6.25	93.3^{ab} (-6.7)	100^{a} (0)	4.7^{ab} (0)	95.5^{ab} (+37.4)	51.7^{a} (+32.9)	+12.7
AI		-6.3	-1.7	-18.1	+14.2	+21.2	+1.9
Stems	**50**	98.3^{ab} (-1.7)	96.7^{a} (-3.3)	3.1^{fg} (-34.0)	54.7^{gh} (-21.3)	20.1^{d} (-48.3)	-21.7
	25	96.7^{ab} (-3.3)	93.1^{a} (-6.9)	3.8^{de} (-19.1)	85.3^{abcd} (+22.7)	27.5^{c} (-29.3)	-7.2
	12.5	95.0^{ab} (-5.0)	93.2^{a} (-6.8)	4.5^{bc} (-4.3)	80.1^{cde} (+15.3)	43.4^{b} (+11.6)	+2.2
	6.25	98.7^{ab} (-1.3)	93.2^{a} (-6.8)	5.2^{a} (+10.6)	99.1^{a} (+42.6)	40.6^{b} (+4.4)	+9.9
AI		-2.8	-6.0	-11.7	+14.8	-15.4	-4.2
Leaves	**50**	93.3^{ab} (-6.7)	81.2^{b} (-18.8)	3.2^{fg} (-31.9)	41.5^{h} (-40.3)	18.5^{d} (-52.4)	-30.0
	25	88.3^{b} (-11.7)	100^{a} (0)	4.4^{bcd} (-6.4)	70.4^{ef} (+1.3)	20.1^{d} (-48.3)	-13.0
	12.5	98.3^{ab} (-1.7)	100^{a} (0)	4.5^{bc} (-4.3)	77.5^{de} (+11.5)	43.5^{b} (+11.8)	+3.5
	6.25	98.3^{ab} (-1.7)	100^{a} (0)	5.3^{a} (+12.8)	93.2^{abc} (+34.1)	51.6^{a} (+32.6)	+15.6
AI		-5.5	-4.7	-7.5	+1.7	-14.1	-6.0
Control		100^{a} (0)	100^{a} (0)	4.7^{ab} (0)	69.5^{ef} (0)	38.9^{b} (0)	
LSD 0.05		6.4	5.2	3.6	7.9	3.7	
CV%		4.0	3.2	5.1	6.2	5.8	

Means within a column followed by the different letters, were significantly different at $p < 0.05$. Numbers in parentheses are the rates in comparison with control; "+" symbol shows the promotion percentage over control, "−" symbol shows inhibition percentage over control. AI: Average Inhibition. GR: germination rate, SR: survival rate, SH: shoot height, RL: root length, DW: dry weight.

However, the treatments at the low concentration induced stimulation effects on root elongation of rice, for example, the extracts from roots 6.25 g/L (37.4%), leaves 6.25 g/L (34.1%), especially the extracts from roots of 6.25 g/L with the maximum stimulation rate of more than 42.0%. For the DW, rice was inhibited by the treatments at high concentrations and stimulated by the treatments at low concentrations. For instance, the extracts from leaves 25 g/L and 50 g/L had inhibition rates of 48.3% and 52.4% respectively. On the contrary, the extracts from leaves 12.5 g/L and 6.25 g/L had stimulation rates of 11.8% and 32.6% respectively. Similar to the extracts from leaves, the extracts from roots, and stems 50 g/L had inhibition rates of 31.1 % and 48.3% respectively. The extracts from roots 25 g/L and 12.5 g/L had stimulation rates of 44.0% and 39.1%.

3.2. Effects of powder from roots, stems, and leaves from *Cissus sicyoides* on germination and growth of natural weeds in laboratory and greenhouse conditions.

Besides the indicator plants, the natural weeds in soil collected from the paddy field were calculated under the effects of powder from *C. sicyoides* roots, stems, and leaves in laboratory conditions. The results showed that the emergence of natural weeds significantly inhibited the both germination and dry weight of weeds. The germination of monocotyledons was highly reduced by the effects of powder from *C. sicyoides*, especially the powder from leaves with the dose of 2 tons/ha had an inhibition rate of 91.5% over the control. The number of dicotyledons was not different between the treatments and the control. The dry weight of monocotyledons, dicotyledons and total weeds was seriously decreased by the effects of all the treatments in comparison to the control.

The effects of powders from *C. sicyoides* on the germination and growth of natural weeds in the greenhouse are shown in Table 6. It can be easily observed that the number of weeds was reduced under the effects of all the treatments over the control. The treatment performed (after 3 and 13 days), which had a higher inhibition effect on monocotyledons than the treatment of adding all the powders after 3 days. On the contrary, the treatments of adding all the powders after 3 days had higher inhibition on dicotyledons than the remaining treatment. As a result, these two ways of application had a similar inhibition on the emergence of natural weeds.

For dry weight, the application after 3 and 13 days had higher inhibition on natural weeds than the application after 3 days. Especially, the treatments after 3 and 13 days had inhibition rates of 73.4% on monocotyledons and 82.8% on dicotyledons. The results showed that the divided application 2 times had higher inhibition on natural weeds than adding the powders only 1 time.

The effects of extracts and powders from *C. sicyoides* on rice variety Khang dan 18 are shown in Table 7. The results showed that the GR of rice was not affected by all the treatments in comparison to the control. For SR, the extracts from *C. sicyoides* had higher negative effects on rice than the powders. For example, the application of extracts after 3 days induced inhibition rates of 11.4% on the GR of rice. The treatment of adding powders after 3 and 13 days had negligible effects on rice compared to the control. For SH and RL, rice was insignificantly affected by the extracts and powders from *C. sicyoides*. Similarly, the DW was not different between treatments and the control except for the application of the extracts after 3 days with inhibition rates of 17.8%.

The effects of extracts and powders from *C. sicyoides* on barnyardgrass in the greenhouse condition are shown in Table 8. The application of powders had better potential for weed suppression than the extracts. For instance, the application of the powders after 3 days had an inhibition rate of 15.2% and after 3 and 13 days had an inhibition rate of 22.1% on the GR of barnyardgrass. While the extracts did not affect GR of barnyardgrass. For SR, there weres no differences between all the treatments and the control. The SH of barnyardgrass was inhibited by the application extracts and powders after 3 and 13 days with rates of 11.7% and 13.3% respectively. The remaining treatments had insignificant effects on barnyardgrass. The treated roots of barnyardgrass by the extracts from *C. sicyoides* were similar to the control roots. However, the treated roots by the powders after 3 days were shorter (20.4%) than the control and after 3 and 13 days were shorter (25.9%) than the control. For the DW, the application of the powders after 3 and 13 days had an inhibition rate of 17.9% on barnyardgrass. The application of the extracts after 3 days had an inhibition rate of 19.6% and after 3 and 13 days had an inhibition rate of 24.1% over the control.

Table 5. Effects of powders from *Cissus sicyoides* leaves, stems, roots on the emergence of natural weeds in laboratory condition

Treatment (tons/ha)		**Number of weeds**			**Dry weight of weeds (mg)**			**AI**
		Monocotyledons	**Dicotyledons**	**Total**	**Monocotyledons**	**Dicotyledons**	**Total**	
Roots	**1.0**	5.7b (-63.7)	1.0a (-41.2)	6.7b (-61.3)	3.7b (-82.0)	0.3b (-81.3)	4.0b (-81.9)	-68.6
	1.5	5.0b (-68.2)	0.3a (-82.4)	5.3bc (-69.4)	3.5b (-82.9)	0.0b (-100)	3.5b (-84.2)	-81.2
	2.0	4.0bc (-74.5)	0.0a (-100)	4.0bc (-76.9)	1.6bc (-92.2)	0.0b (-100)	1.6bc (-92.8)	-89.4
AI		-68.8	-74.5	-69.2	-85.7	-93.8	-86.3	-79.7
Stems	**1.0**	4.0bc (-74.5)	0.3a (-82.4)	3.3bc (-80.9)	1.8bc (-91.2)	0.1b (-93.8)	1.8bc (-91.9)	-85.8
	1.5	3.0bc (-80.9)	0.3a (-82.4)	3.3bc (-80.9)	3.5b (-82.9)	0.1b (-93.8)	3.6b (-83.7)	-84.1
	2.0	3.3bc (-79.0)	1.3a (-23.5)	4.7bc (-72.8)	2.9bc (-85.9)	0.5b (-68.8)	3.4b (-84.6)	-69.1
AI		-78.1	-62.8	-78.2	-86.7	-85.5	-86.7	-79.7
Leaves	**1.0**	2.7bc (-82.8)	0.3a (-82.4)	3.0bc (-82.7)	1.7bc (-91.7)	0.1b (-93.8)	1.8bc (-91.9)	-87.6
	1.5	2.3bc (-85.2)	0.0a (-100)	2.3bc (-86.7)	0.5c (-97.6)	0.0b (-100)	0.5c (97.7)	-94.5
	2.0	1.33c (-91.5)	0.0a (-100)	1.0c (-94.2)	0.4c (-98.0)	0.0b (-100)	0.4c (-98.2)	-97.0
AI		-86.5	-94.1	-87.9	-95.8	-97.9	-95.9	-93.0
Control		15.7a (0)	1.7a (0)	17.3a (0)	20.5a (0)	1.6a (0)	22.1a (0)	
LSD 0.05		0.6	-	1.4	0.4	-	0.5	
CV%		13.6	-	16.6	6.6	-	6.8	

Means within a column followed by the different letters were significantly different at $p < 0.05$. Numbers in parentheses are the rates in comparison with control; "+" symbol shows the promotion percentage over *control, "−" symbol shows the inhibition percentage over control. AI: Average Inhibition. GR: germination rate, SR: survival rate, SH: shoot height, RL: root length, DW: dry weight.*

Table 6. Effects of powders from *C.sicyoides* leaves on the emergence of natural weeds in the greenhouse conditions

Treatment	Number of weeds			Dry weight (mg)			AI
	Monocotyledons	Dicotyledons	Total	Monocotyledons	Dicotyledons	Total	
After 3 days	10.7^{b} (-34.4)	12.3^{c} (-52.1)	23^{b} (-63.5)	28.3^{b} (-55.2)	5.0^{b} (-75.4)	33.4^{b} (-60.0)	-56.8
After 3 and 13 days	8.7^{c} (-46.6)	15.3^{b} (-40.5)	23.3^{b} (-73.4)	16.8^{c} (-73.4)	3.5^{b} (-82.8)	20.3^{c} (-75.7)	-65.4
AI	-40.5	-46.3	-68.5	-64.3	-79.1	-67.9	-61.1
Control	16.3^{a} (0)	25.7^{a} (0)	42^{a} (0)	63.1^{a} (0)	20.3^{a} (0)	83.4^{a} (0)	
LSD 0.05	1.2	1.6	2.4	2.8	1.5	3.0	
CV%	4.9	4.6	4.1	3.9	7.6	2.7	

Means within a column followed by different letters were significantly different at $p < 0.05$. Numbers in parentheses are the rates in comparison with control; "+" symbol shows the promotion percentage over control, "−" symbol shows the inhibition percentage over control. AI: Average Inhibition. GR: germination rate, SR: survival rate, SH: shoot height, RL: root length, DW: dry weight.

Table 7. Effects of extracts and powders from *C. sicyoides* leaves on germination and growth of rice in greenhouse

Treatment		GR (%)	SR (%)	SH (cm)	RL (cm)	DW (mg)	AI
Extracts	**After 3 days**	86.7^{a} (-6.0)	87.6^{b} (-11.4)	17.9^{a} (-2.7)	6.3^{a} (-3.1)	74.8^{b} (-17.8)	-8.2
	After 3 and 13 days	96.7^{a} (+4.9)	90.7^{b} (-8.3)	16.9^{a} (-8.2)	6.3^{a} (-3.1)	83.7^{ab} (-8.0)	-4.5
Powders	**After 3 days**	91.1^{a} (-1.2)	98.9^{a} (0)	18.3^{a} (-0.5)	6.9^{a} (+6.2)	89.9^{ab} (-1.2)	+0.7
	After 3 and 13 days	81.1^{a} (-12.0)	98.6^{a} (-0.3)	19.5^{a} (+6.0)	6.8^{a} (+4.6)	93.4^{a} (+2.6)	+0.2
AI		-3.6	-5.0	-1.4	+1.2	-6.1	-3.0
Control		92.2^{a} (0)	98.9^{a} (0)	18.4^{a} (0)	6.5^{a} (0)	91.0^{a} (0)	
LSD 0.05		13.6	3.6	-	-	10.6	
CV%		8.2	2.1	-	-	6.7	

Means within a column followed by the different letters were significantly different at $p < 0.05$. Numbers in parentheses are the rates in comparison with control; "+" symbol shows the promotion percentage over control, "−" symbol shows the inhibition percentage over control. AI: Average Inhibition. GR: germination rate, SR: survival rate, SH: shoot height, RL: root length, DW: dry weight.

Table 8. Effects of extracts and powders from *C.sicyoides* leaves on germination and growth of barnnyardgrass in greenhouse

Treatment		GR (%)	SR (%)	SH (cm)	RL (cm)	DW (mg)	AI
Extracts	**After 3 days**	83.3^{ab} (-12.9)	98.7^{a} (+3.5)	17.7^{ab} (-5.9)	4.7^{ab} (-13.0)	39.0^{b} (-19.6)	-9.6
	After 3, 13 days	82.2^{ab} (-14.0)	98.8^{a} (+3.6)	16.6^{b} (-11.7)	4.8^{ab} (-11.1)	36.8^{b} (-24.1)	-11.5
Powders	**After 3 days**	81.1^{b} (-15.2)	98.6^{a} (+3.4)	17.4^{ab} (-7.4)	4.3^{b} (-20.4)	40.2^{ab} (-17.1)	-11.3
	After 3, 13 days	74.5^{b} (-22.1)	97.1^{a} (+1.8)	16.3^{b} (-13.3)	4.0^{b} (-25.9)	39.8^{b} (-17.9)	-15.5
AI		-16.1	+3.1	-9.6	-17.6	-197	-12.0
Control		95.6^{a} (0)	95.4^{a} (0)	18.8^{a} (0)	5.4^{a} (0)	48.5^{a} (0)	
LSD 0.05		9.5	-	1.5	0.5	5.7	
CV%		6.3	-	7.7	4.7	6.3	

Means within a column followed by different letters were significantly different at $p < 0.05$. Numbers in parentheses are the rates in comparison with control; "+" symbol shows the promotion percentage over control, "−" symbol shows the inhibition percentage over control. AI: Average Inhibition. GR: germination rate, SR: survival rate, SH: shoot height, RL: root length, DW: dry weight.

4. DISCUSSION

Laboratory bioassay is considered as one of the most popular methods in the identification of plant allelopathy because of its essential in initial research on the early stage of plant growth duration. Additionally, this is a simple convenient method to save time and be able to be conducted at any time annually. Moreover, the plant allelopathic potential can be evaluated more accurately by controlling factors including temperature, nutrition, and light[15,6]. Plant powders and aqueous extracts were used in numerous studies on allelopathy[16-19]. In this research, the powders and extracts from *C. sicyoides* roots, stems, and leaves were separately evaluated for the allelopathic potential against weeds and some indicator plants. The indicator plants including lettuce and radish were used because they are sensitive to allelochemicals at low concentrations in laboratory conditions[20]. In addition, barnyardgrass and paddy weeds were also essential for the research on allelopathy because barnyardgrass directly competes with rice in the paddy fields[21].

In this study, our findings showed that the extracts from *C. sicyoides* inhibited on germination rates of lettuce and radish. This phenomenon could be occurred by the effect of phenolic acids including caffeic, p-coumaric, ferulic, gallic, 4-hydroxybenzoic, resorcylic, salicylic, and vanillic acids[20]. However, the percentage of inhibition depends on the concentration as well as the interaction between phenolic acids[21]. Almost the extracts stimulated the shoot height of the lettuce and radish, otherwise, they inhibited the root length, and consequently, the lettuce and radish were leading to dead. Barnyardgrass is also a common weed in the rice field. In this study, Barnyardgrass shoots, roots and dry weights were inhibited by all the extracts from *C. sicyoides*. The effects of *C. sicyoides* on rice were also evaluated, the results showed that rice growth was negligibly affected except for the extracts at high concentrations (50g/L). Some phenolic compounds and steroids extracted from *C. sicyoides* were reported and referred to as allelochemicals[22-24]. Additionally, phenolic acids are the most common allelochemical because of their abundance in a wide range of soil[25-26]). Li et al[27] and Anh et al[26] reported that the phenolics are related to the allelopathic mechanisms and considered as potential weed suppressors through various biological pathways including (1) changes in membrane permeability and inhibition of plant nutrient uptake; (2) inhibition of cell division, elongation, and submicroscopic structure; (3) plant photosynthesis and respiration; (4) various enzyme function and activities; (5) synthesis of plant endogenous hormones; (6) protein synthesis. In this study, the powders and extracts from *C. sicyoides* were shown a significant inhibition against the growth of paddy weeds and tested plants under laboratory and greenhouse conditions. However, it should be continuously carried out research in paddy field conditions as well as evaluated for allelochemicals in further research.

5. CONCLUSIONS

In summary, *C. sicyoides* had high allelopathic potential against paddy weeds and indicator plants. The leaves of *C. sicyoides* showed the highest allelopathic potential against barnyardgrass and other paddy weeds. The powders from *C. sicyoides* leaves by adding in twice times (after 3 and 13 days) showed higher allelopathic potential than one time. On the contrary, a slight stimulation of rice growth was recorded under the effects of powders from *C. sicyoides* leaves.

Conflict of Interest

There is no conflict of interest.

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