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Allelopathic Potential of *Allamanda cathartica* L. under Different Screening Conditions

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Abstract

Allamanda cathartica is an ornamental plant and is widely distributed in many areas of the world. This study aims to determine the allelopathic effects of this plant (leaf and stem dried powders) under bioassays and greenhouse conditions against the growth of *Echinochloa crus-galli* and some indicator plants. At the applied dose (50g/L) of the leaf powders, the root length of *E. crus-galli* and *Bidens pilosa* was significantly reduced by over 90%. Interestingly, rice growth was negligibly inhibited and the elongation of shoot and root was stimulated. In a greenhouse trial, the average inhibition of *E. crus-galli* growth was reduced by 54.9% at a dose of 50g/m^2 of the leaf powders. Moreover, the biomass of natural paddy weeds was significantly reduced by 46.4%. However, rice growth parameter was increased by about 20% at a dose of 12.5g/m^2. The allelopathic property of the leaf powders showed higher than its stems. Consequently, our results indicated that *A. cathartica* possesses a strong allelopathic property and may be served this plant as a natural herbicide source to control paddy weeds and increase rice growth for sustainable agricultural production.

Keywords: Allelopathic potential, *Allamanda cathartica* bioassays, greenhouse condition, inhibition, stimulation

Introduction

In plant ecosystems, allelopathy is narrated as a natural phenomenon where one plant can influence the germination, growth and development of a nearby plant by releasing certain allelochemicals via a chemical pathway. The allelopathy topic has received much attention from worldwide scientists in recent years since allelopathy was recognized to possibly alternate the synthetic herbicides for weed control [1]. Many plant species in plant ecosystems, including leguminous, medicinal, and invasive plants exert significant allelopathic potential as part of plant defense mechanism systems, many among their secondary metabolites (allelochemicals), including mineral constituents, which are responsible for weed suppressing and enhancing rice yield when directly incorporate into paddy fields [2]. To the best of our knowledge, over 2000 plant species (39 families) have exerted the weed-suppressing effects [3], in which several hundreds of higher plants showed great allelopathic potential, for instance, stylo (*Stylosanthes guianensis*), passion fruit (*Passiflora edulis*), dodder (*Cuscuta hygrophilae*), alfalfa (*Medicago sativa*); buckwheat (*Fagopyrum esculentum*), fragrant thoroughwort (*Eupatorium canabium*), galactia (*Galactia pendula*), billy goat weed (*Ageratum conyzoides*); leucaena (*Leucaena glauca*), neem (*Azadirachta indica*) [2, 4]. Therefore, searching for plants with strong allelopathic potential for decreasing weed emergence and lessening the dependency on synthetic herbicides may be one of the most feasible solutions to make an eco-friendly environment, safer agriculture products and human health.

The genus of *Allamanda* presents about 15 different species and is widely distributed worldwide. *A.cathartica* is an ornamental plant native to Central America and Brazil. *A.cathartica* is an evergreen vine, robust shrubs reaching 6 m in height and growing all year around. The typical leaves are elliptical to obovate, opposite, or in whorls, trumpet-shape with yellow flowers (Fig. 1)

Fig. 1. Climbing *A. cathartica*

A. cathartica is primarily used as a medicinal plant for human health, an ornamental plant, either for grounds or for walls and fences. To the best of our knowledge, over 153 phytochemical compounds were detected and isolated from the

different parts of *A.cathartica*. Most of them belong to fatty acids, volatile compounds, phenolics, flavonoids, alkaloids, steroids, terpenes, lactones, ketones, etc [5]. Moreover, multiple biological activities were reported, including analgesic, anti-inflammatory, antidepressant, antidiabetic, antiviral, antimicrobial, antimalarial, antioxidant, nematicide, etc [4]. However, very little information is available on the allelopathic properties of this plant. The objective of this study focused on evaluating the allelopathic activity of this plant on bioassays and greenhouse conditions.

Materials and Methods

The aboveground parts of *A.cathartica* were collected from Dan Phuong district, Hanoi city (21°05'32.8"N 105°39'53.3"E). The leaves and stems were separated, then cleaned with tap water, then chopped into pieces of 2, and dried at 45oC until water content was reduced to 12-15%. The materials were then ground into fine powders for bioassays and greenhouse experiments. The seeds of *E.crus-galli* (barnyardgrass), *B.pilosa* (beggarstick), *Brassica junea* (mustard) and rice (Khang dan variety) were provided by Department of Genetic Engineering, Agricultural Genetics Insitute and used as the indicator plants [6]. Before conducting experiments, the germination (%) of all seeds of indicator plants was randomly checked and was shown to be over 90% [6].

Bioassays

The bioassay experiments were performed at the laboratory of the Genetic Engineering Department, AGI, Hanoi, Vietnam in 2021. The powders (leaves and stems) of *A. cathartica* were separately diluted to 5% agar and distilled water at different concentrations (50 g/L, 25 g/L, 12.5 g/L và 6.2 g/L) following the previous method [8]. Each diluted solution was poured into the plastic pot (120 mL). The pots were then sterilized at 120°C for 15 min and kept cool at room temperature. Twenty seeds of each indicator plant were evenly grown on the agar surface of the pot. The control treatment was used only with distilled water. The pots were placed in the growth chamber at 25o C, 4000 Lux, light time: 9:00-17:00, and the humidity was adjusted by 75%. After 7 days of sowing, germination rate, root and stem length were recorded. All treatments were done with at least three replicates and compared with the controlled treatment. The inhibition (%) was calculated following the formula:

Inhibition (%)=[(1-sample/control) x 100]

Greenhouse trial

The experiments were conducted at the greenhouse of Agricultural Genetics Insititute in 2021. Five hundred grams of commercially heat-treated soil without any weed seeds or microorganisms (pH 4.5-5.8, EC 1.0 ± 0.2, N: 1,100 ± 100 mg kg^{-1}, P_2O_5 400± 100mg kg^{-1}) were filled up to a pot (total vol: 740 ml, 12cm in height) and was moistened with 200ml of distilled water. Simultaneously, 20 healthy seeds of *E. crus-galli* and *Oryza sativa* (Khang dan 18 variety) were sown on the surface of the soil pot. After 2 days, the dry powders of *A. cathartica* (leaves and stems for each treatment) were diluted at different doses (control: 0 g; 12.5 g; 25 g; 50 g/ m^2), and evenly applied by scattering on the surface of the pots, while the control treatment was used only distilled water. Each treatment was performed at least thrice. All pots

were provided water about 2 cm above the surface of soil. The greenhouse temperature was about 25-30℃. After 3 weeks, plant number of indicator plants, shoot and root length were determined [7]. In another trial, paddy soils were collected to a depth of 5cm from the AGI Experiment station where previously grown Khang dan rice variety, then was air dried and mixed until treatments were done. The experimental protocol was done following the method described [10].

Statistical Analyses

The laboratory bioassays were performed with six replications and repeated three times. The greenhouse trials were done in a completely randomized design with three replications. The statistical analyses were performed using Excel version 2016, Minitab 1.8 and IRRISTAT ver 2010. The means were separated based on the least significant differences (LSD) and coefficient of variations (CV) at the 0.05 probability level.

Results and Discussion

Allelopathic potential of leaves and stems *A. cathartica* on the growth and *B. junea* and *B. pilosa* in bioassays

As shown in Table 1, both leaf and stem powders of *A. cathartica* showed strong allelopathic effects against the growth of *B.junea*. Specifically, at the applied dose (50g/L), root length was the most inhibited by over 90%, followed by germination (81.4%) and shoot length (66.7%). Our results agreed with some authors'studies who reported that the roots of indicator plants were most inhibited [7,8]. The inhibitory levels were gradually reduced depending on the dose concentrations. Consequently, the average inhibition (AI) showed that 79.5% (50g/L); 42.8% (25g/L); and 19.2% (12.5g/L), respectively. Similarly, the stem powders of *A. cathartica* significantly reduced the growth of roots by over 76.0% (50g/L), 57.1% (25g/L) and 38.1% (12.5g/L). Overall, the leaves of *A. cathartica* was higher allelopathic properties than its stems. Therefore, allelopathic potential of leaf powders was further evaluated, but the growth suppression levels of indicator plants were proportional to the applied dose (Table 1, Fig 1).

Table 1. Allelopathic effect of *A. cathartica* leaf and stem powders on the growth of *B.junea*

Dose (g/L)		Inhibition (%)			
		Germination	Shoot length (cm)	Root length (cm)	AI (%)
Leaf	50	18.3^{d} (81.4)	0.8^{b}(66.7)	0.2^{d}(90.5)	79.5
	25	62.2^{c}(36.7)	1.8^{a}(25.0)	$0,7^{c}$(66.7)	42.8
	12.5	86.7^{abc}(11,8)	2.1^{a}(12.5)	1.4^{b}(33.3)	19.2
Stem	50	62.3^{c} (36.6)	1.6^{ab}(33.3)	0.5^{cd}(76.2)	48.7
	25	69.3^{bc} (29.5)	1.5^{ab}(37.5)	0.9^{c}(57.1)	41.4
	12.5	92.5^{a}(5.9)	1.9^{a}(20.8)	1.3^{b}(38.1)	21.6
Control		98.3^{a} (0.0)	2.4^{a} (0.0)	2.1^{a} (0.0)	-
LSD $_{(0.05)}$		17.2	0.6	0.2	-
$CV_{(\%)}$		14.1	19.1	13.8	-

AI: Average inhibition; CV%: Coefficient variation. The means with the same letters in a column are not significantly different at P<0.05. The value in parentheses indicated the inhibition percentage over the control treatment.

Figure 1. Effect of leaf and stem of *A.cathartica* extract on growth of *B.junea*. A: Leaf; B: stem

The growth of *B. Pilosa* (germination rate, shoot and root length) was remarkably reduced. Among the growth factors, the root length was the most suppressed by 92.0% at the applied dose (50g/L). Generally, the average inhibition (AI) ranged from 32.8% to about 74.0%, based on the dose applied (Table 2, Fig 2).

Table 2. Allelopathic effect of *A. cathartica* leaves powders on the growth of *B. Pilosa*

Dose (g/L)	Inhibition (%)			
	Germination	Shoot length (cm)	Root length (cm)	AI
50	15.0^{c} (8.4)	1.2^{b} (52.0)	0.2^{b} (92.0)	73.8
25	65.6^{b} (27.5)	25^{a} (14.2)	$0,7^{b}$ (72.0)	39.7
12.5	65.9^{b}(27.2)	2.2^{a}(12.0)	1.0^{ab}(60.0)	32.8
Control	90.5^{a}(0.0)	25.0^{a}(0.0)	$2,5^{a}$(0.0)	-
$LSD_{(0,05)}$	7.4	1.2	1.1	-
$CV_{(\%)}$	15.3	31.4	53.5	-

AI: Average inhibition; CV%: Coefficient variation. The means with the same letters in a column are not significantly different at P<0.05. The value in parentheses indicated the inhibition (%) over the control treatment.

Figure 2. Allelopathic effects of *A. Cathartica* leaf powders at different concentrations on the growth of *B. pilosa*.

Allelopathic effect of leaf and stem powders of A. cathartica on the growth of O.sativa (Khang dan 18 variety)

In this experiment, we further evaluated the allelopathic effect of leaves and stem powders of *A. cathartica* against the Khang dan 18 (a mega rice variety) to find out whether *A. cathartica* causes any allelopathic effect on the growth of rice variety, a major food crop. At the highest dose (50g/L) of leaves powder, shoot and root length and germination rate were reduced by 60.1%, 15.7% and 44.8%, respectively. However, the stems of *A. cathartica* disclosed less inhibition against the growth of rice than its leaves. Only root length was inhibited by 23.9%, while the shoot length was stimulated by over 8.1%. At the lower doses, it showed either negligible

inhibition or stimulation of rice growth parameters (Table 3). Specifically, shoot length was invigorated by arranging from 2.6% to 12.4%, respectively (Fig 3).

Table 3. Allelopathic effect of *A. cathartica* leaves and stems powders on the growth of *O.sativa*

Dose (g/L)		Inhibition (%)			
		Germination	Shoot length	Root length	AI
Leaf	50	54.3^{c} (44.8)	5.3^{c} (15.7)	2.8^{c} (60.1)	40.2
	25	96.7^{ab} (1.6)	6.4^{b} (+ 2.6)	5.5^{b} (22.5)	7.2
	12.5	96.7^{ab} (1.6)	6.7^{ab} (+ 8.0)	7.7^{a} (+ 8.5)	+ 5.0
Stem	50	98.3^{ab}(1.7)	7.5^{ab} (+ 8.1)	5.4^{b}(23.9)	10.1
	25	91.7^{b}(8.3)	7.8^{a} (+ 12.0)	7.8^{a} (+ 9.9)	+ 4.5
	12.5	100^{a}(0)	7.2^{ab} (+ 4.0)	8.0^{a} (+ 12.7)	+ 5.6
Control		100^{a}(0.0)	7.0^{ab}(0.0)	7.1^{a}(0.0)	-
$LSD_{(0.05)}$		3.3	1.1	1.2	-
$CV_{(\%)}$		2.6	10.9	9.0	-

AI: Average inhibition; CV%: Coefficient variation. The means with the same letters in a column are not significantly different at P<0.05. The value in parentheses indicated the inhibition (%) over the control treatment.

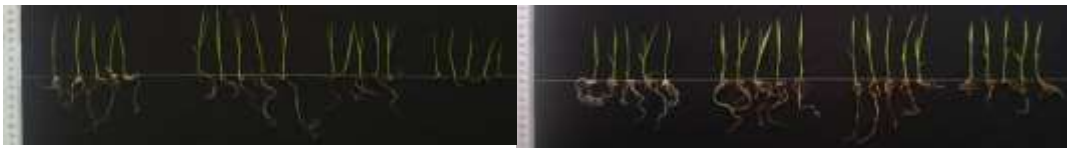

Figure 3. Allelopathic effects of *A. Cathartica* at different concentrations on the stem length and root length of *O. sativa* (Khang Dan 18 variety).A: Leaf; B: Stem

Greenhouse trial

Allelopathic effect of *A. cathartica* leaves powders on the growth of *E. crus-galli*

Barnyardgrass is one of the most noxious weeds and has been considered as the world's worst rice weed [9]. Therefore, we further evaluated the allelopathic effects of this plant against the growth of *E. crus-galli* under greenhouse conditions. The results showed that the leaf powders significantly reduced the growth of *E. crus-galli*. At the applied dose of 50g/m^2, The root length was the most inhibited by 75.0%, followed by 46.5% (the plant number), and shoot length was the least (43.2%), respectively. At the lower doses (25.0 and 12.5g/m^2), root length of *E. crus-galli* was minimized from 68.8 to 43.8%. The highest average inhibition was 54.9% at the applied dose 50.0g/m^2, followed by 51.7% (25.0g/m^2) and 27.9% (12.5g/m^2), respectively (Table 4).

Table 4. Allelopathic effect of *A. cathartica* leaf extract on the growth of *E. crus-galli*

Dose (g/m^2)	Inhibition (%)			
	Germination (%)	Shoot length (cm)	Root length (cm)	AI
50	48.5^{b}(46.5)	2.5^{b}(43.2)	0.8^{c}(75.0)	54.9
25	57.9^{b}(36.2)	2.2^{b}(50.0)	1.0^{bc}(68.8)	51.7
12.5	60.7^{b}(33.1)	4.1^{a}(6.8)	1.8^{b}(43.8)	27.9
Control	90.7^{a}(0.0)	4.4^{a}(0.0)	3.2^{a}(0.0)	-
$LSD_{(0,05)}$	10.2	1.2	0.7	-
$CV_{(\%)}$	8.4	19.3	20.3	-

The means with the same letters in a column are not significantly different at P<0.05. The value in parentheses indicates inhibition % over the control. The symbol "+" presents the stimulation % over the control.

Allelopathic effect of *A. cathartica* leaf powders on the growth of *O. sativa*

As presented in Table 6, leaf powders of *A. cathartica* exhibited negligible suppression at the highest dose ($50g/m^2$). Especially, the shoot length of rice was stimulated at all doses from 1.4 to 4.3%. The root length was increased by 54.5% at the dose of $12.5g/m^2$, and rice growth was stimulated by about 20.0%, respectively (Table 5).

Table 5. Allelopathic effect of *A. cathartica* leaf powders on the growth of *O. sativa* under greenhouse conditions

Dose (g/m^2)	Inhibition (%)			
	Plant number	Shoot length (cm)	Root length (cm)	AI
50	92.7^{a}(5.7)	14.1^{a} (+ 1.4)	3.6^{b}(18.2)	7.5
25	96.5^{a}(1.8)	14.5^{a} (+ 4.3)	3.5^{b}(20.5)	6.0
12.5	100^{a} (+ 1.7)	14.4^{a} (+ 3.6)	6.8^{a}(+ 54.5)	+ 19.9
Control	98.3^{a}(0.0)	13.9^{a}(0.0)	4.4^{b}(0.0)	-
$LSD_{(0,05)}$	8.9	1.4	1.3	-
$CV_{(\%)}$	4.9	5.1	14.8	-

The means with the same letters in a column are not significantly different at P<0.05. The value in parentheses indicates inhibition % over the control. The symbol "+" presents the stimulation % over the control.

As the results are shown in Table 6, both leaves and stems of *A. cathartica* significantly reduced the growth of natural paddy weeds including cotyledon and dicotyledon weeds and total biomass of dry weight. At the dose applied 2.0 ton/ha of the leaf powders, the cotyledon weeds were inhibited by 58.8%, and total biomass dry weight was 46.9%. The average inhibition disclosed that the allelopathic effect of the leaves was higher than its stems which were similar to the results attained in bioassay experiments.

Table 6. Allelopathic effects of leaf and stem powders of *A. cathartica* against the growth of natural paddy weeds

Dose (ton/ha)		Natural weeds biomass inhibition (%)			
		Cotyledon	Dicotyledon	Total biomass dry weight (g)	AI
Leaf	1.0	9.7^{bc}(48.1)	6.7^{cd}(44.2)	16.3^{cd}(46.9)	46.4
	2.0	7.7^{c} (58.8)	8.7^{bc}(27.5)	16.3^{cd}(46.9)	44.4
Stem	1.0	11.3^{bc}(39.6)	7.3^{cd}(39.2)	18.7^{bc}(39.1)	39.3
	2.0	12.0^{b}(35.8)	11.0^{ab}(8.3)	23.0^{b}(25.1)	23.1
Control		18.7^{a}	12.0^{a}	30.7^{a}	-
$LSD_{(0.05)}$		2.1	2.5	3.2	-
$CV_{(\%)}$		9.6	13.0	14.3	-

AI: Average inhibition; CV%: Coefficient variation. The value in parentheses indicated the inhibition (%) over the control treatment. The means with the same letters in a column are not significantly different at P<0.05.

Conclusions

In summary, *A. cathartica* showed a significant allelopathic effect on the growth of b *E. crus-galli* and indicator plants in bioassays and greenhouse conditions. At the applied dose (50g/L) of the leaf powders, the root length of *E. crus-galli* and *B. pilosa* were significantly reduced by over 90%. Interestingly, rice growth was negligibly inhibited and the elongation of shoot and root was stimulated at the lower doses. Our findings have provided useful information for further isolating and identifying allelochemicals in this plant and using this plant as a natural herbicide to control weeds for a sustainable agriculture system.

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