Allelopathic Potential of Four Emergent Macrophytes on the Growth of Terrestrial Plant Species

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Abstract

Four emergent macrophytes (semi-aquatic plants), Centrostachys aquatica, Polygomum pulchrum, Ischaenum hirtum and Hymenachne acutigluma, grow abundantly worldwide in natural wetlands and fresh waterways. To discover novel bio-resources for weed management, we have assessed the allelopathic potential of the aqueous methanol extracts of these species on the growth of two terrestrial weeds: barnyard grass (Echinochloa crus-galli (L). Beauv.) and rye grass (Lolium multiforum Lam), and three model test plants: alfalfa (Medicago sativa L.), cress (Lepidium sativum L.), lettuce (Lactuca sativa L.). Among the aqueous methanol extracts of the four emergent macrophytes, the C. aquatica aqueous methanol extract showed the greatest inhibitory activity, completely inhibiting the shoot and root growth of rye grass (0.1 and 0.3 g dry weight equivalent extract, respectively) and barnyard grass (1g dry weight equivalent extract). The inhibitory activity of the H. acutigluma aqueous methanol extract on shoot growth of test plant was greater than P. pulchrum and I. hirtum while aqueous methanol extract of the P. pulchrum inhibited root growth greater than H. acutigluma and I. hirtum. The inhibitory efficacy of these emergent macrophytes was dependent on their potential activity, the test plant species and concentration of the extracts. The present results that all plants may contain allelopathically active substances and that C. quatica may contain the greatest herbicidal substance(s).

Keywords: *Allelopathy, Barnyard grass, Centrostachys aquatica, Growth inhibitor, Hymenachne acutigluma, Ischaenum hirtum, Natural bio-resource, Polygomum pulchrum, Rye grass, Weed management*

1. Introduction

The use of pesticides, of which herbicides account for 49% of total use, has led to increased agricultural production [25]. However, their use has also seen the emergence of several negative effects, such as the development of herbicide-resistant weeds [5, 45]. They have become deleterious to the environment, and have induced human health problems [19]. In order to reduce these harmful effects, it is necessary to diversify weed management options

[19]. One of the new feasible options for reducing herbicide dependency would be the utilization of allelopathy as a tool in weed management [32]. Allelopathy is any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influences the growth and development of agricultural and biological systems [1]. Bhowmik and Inderjit [36] noted that resistant weed biotypes were more affected by allelopathic compounds than susceptible biotypes. To date, many species, including macrophytes, have shown strong allelopathic activity [31, 11, 14, 46].

Four emergent macrophytes, *Centrostachys aquatica* (R.Br.). Wall ex Moq Tand (Amaranthaceae), *Polygonum pulchrum* Blume (Polygonaceae), *Hymenachne acutigluma* Steud. (Poaceae), and *Ischaemum hirtum* Hack. (Poaceae), grow abundantly in natural wetlands, irrigation channels, and fresh waterways, worldwide [2, 8, 20, 39, 41, 44]. Numerous kinds of secondary compounds in *Polygonum* spp. have antifungal and antibacterial activities [4, 21] and allelopathic activity [16, 23, 28]. Chou [6] suggested that living plants or residues of *P. aviculare* is probably useful in the control of bermuda grass and some other weeds. Several species of Amaranthaceae family had a strong allelopathic influence on the germination and growth of several plants. For instance, *Aramathus retroflexus* reduced the growth of *Nicotiana tabacum*; *A. spinosus* diminished the growth and establishment of congress weed (*Parthenium hysterophorus*) [33]. Many allelochemicals obtained from Poaceae species [3] were reported to have strong activity, such as sorgoleone from *Sorghum bicolor* (L.) [26] and momilacton B from rice [17].

Screening plant species with strong allelopathic potential is a fundamental study for chemical analyses [39] and for weed control program [43] because it provides important basic information on their growth inhibitory effects as well as their potential for weed control [12]. In addition, laboratory bioassays are an important part of allelopathic research because they allow researchers to study large amounts of plant material in a short space of time. Bioassays, by design, also allow researchers to eliminate interference factors other than the one under study [24].

In the present research, allelopathic activity of four emergent macrophytes, *C. aquatica*, *P. pulchrum*, *H. acutigluma* and *I. hirtum* was determined against terrestrial plants, barnyard grass, ryegrass, alfalfa, cress and lettuce, and their inhibitory activity was evaluated. Effects of main effects and interaction among plant extract, test plants, and concentration of extract on the growth of plants were also discussed.

2. Materials and Methods

2.1.Aqueous Methanol Extraction

The whole plants of *C. aquatica*, *P. pulchrum*, *H. acutigluma*, and *I. hirtum*, at the vegetative stage, were collected in riparian zones and fresh waterways of Cần Thơ City, South Vietnam (9°27′ N, 106°E), in August, 2011, then washed with tap water and dried in the sun. Dry materials were then packed and protected from air humidity by a silica gel-desiccant, then stored at 3°C in a fridge until use.

Fifty grams of the dried plant materials was separately soaked in 500 mL of 70% (v/v) aqueous methanol for 48 h, and filtered through filter paper No. 1 (Toyo Ltd., Tokyo, Japan). The residue was re-extracted with methanol for 24 h and filtered. The two filtrates of each species were combined and evaporated at 40°C to produce an aqueous methanol extract.

2.2. Bioassay

Cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), and alfalfa (*Medicago sativa* L.) were chosen as test plant due to their known seedling growth behavior and due to their sensitivity to allelochemicals [43, 35]. Barnyard grass (*Echinochloa crus-galli* (L). Beauv.) and rye grass (*Loliummultiflorum* Lam) were chosen for the bioassay because they are common weeds and also have already evolved resistance to herbicides. In some regions, barnyard grass was resistant to propanil, thiobencard, quinclorac and butachlor [5] while rye grass was resistant to glyphosate [45].

The aqueous methanol extracts of *C. aquatica*, *P. pulchrum*, *H. acutigluma*, or *I. hirtum* was added to filter paper in 2.8-cm diameter Petri dishes to obtain final concentrations of 0.01, 0.03, 0.1, 0.3, and 1.0 g dry weight equivalent extract mL^{-1} (g DW eq. extract mL^{-1}). Petri dishes were maintained in a draft chamber for 40 min to evaporate the methanol. After soaking seeds in distilled water 48 h, barnyard grass and rye grass seeds were germinated in the dark at 25°C for 30 and 48 h, respectively. Cress, lettuce, and alfalfa seeds were incubated in the dark at 25°C for 16 h. The seedlings with roots in 1 mm length were chosen for growth bioassay. The experiment was conducted in a completely randomized design, consisted of three factors with 120 treatments (4 aqueous methanol extracts × 6 concentrations × 5 test plant species), 10 seedlings per Petri dish for each treatment, and repeated three times. Root and shoot length was measured 48 h after incubation in the dark at 25°C. The percentage of shoot or root growth in each treatment was calculated and compared to that of the control which had been treated with distilled water without aqueous methanol extract: Shoot (or root) growth (%) = the shoot (or root) length of treatment containing aqueous methanol extract / shoot (or root) length of the control × 100.

2.3. Statistical Analysis

Statistical analysis was performed by three-way ANOVA for assessing the effect of three factors: extract, test plant and concentration of extracts, and the interactions among themselves. Duncan's multiple range test ($P \leq 0.05$) was applied to test for significant differences between means. Data were analysed by using PASWSTAT version 18.0 (SPSS, Inc., Chicago, USA). The concentration at which the growth of a test plants was reduced by 50% (IC_{50}) was established on the basis of curve fitting to a logistic equation, using GraphPad Prism ver. 5.0. (GraphPad, Inc., San Diego, CA, U.S.A.).

3. Results

Three-way ANOVA analysis indicated that all three main factors: extracts, concentrations of these extracts and test plants, had significant effects ($P \leq 0.001$). Among the extracts, *C. aquatica* extract had the highest inhibitory activity on the shoot growth of test plants, followed by *H. acutigluma*, *P. pulchrum* and *I. hirtum* extract (Figure 1). The inhibitory activity of the *C. aquatica* extract on root growth of test plants was highest. The *P. pulchrum* extract demonstrated significantly greater inhibitory activity on root growth than *H. acutigluma* and *I. hirtum* extract. The shoot growth of cress was most sensitive to those extracts than other test plants while the root growth of lettuce was most sensitive to the

extracts (Figure 2). The shoot and root growth of barnyard grass was least sensitive to those extracts.

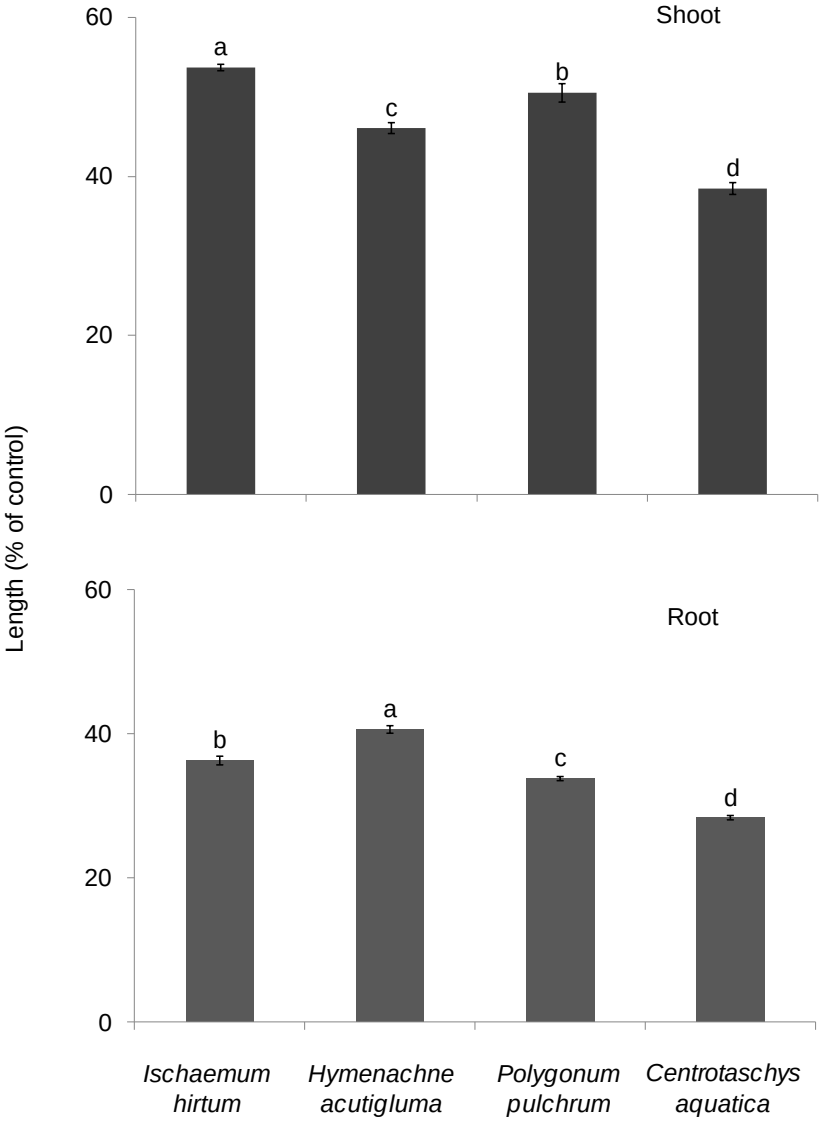

Figure 1. Comparison of marginal means of extract factor (aqueous methanol extracts of *I. hirtum, H. acutigluma*, *P. pulchrum* and *C. aquatica*) on the growth of plants, averaging over levels of factors of test plant and concentration. Main effect (extract effect) of variables were computed multiple comparisons in three ways ANOVA model by using General linear model and Post hoc test (PASWSTAT version 18.0). Means above each bar followed by the same letter are not significantly different as determined by Duncan's multiple range test at $P \leq 0.05$, replicates: 3.

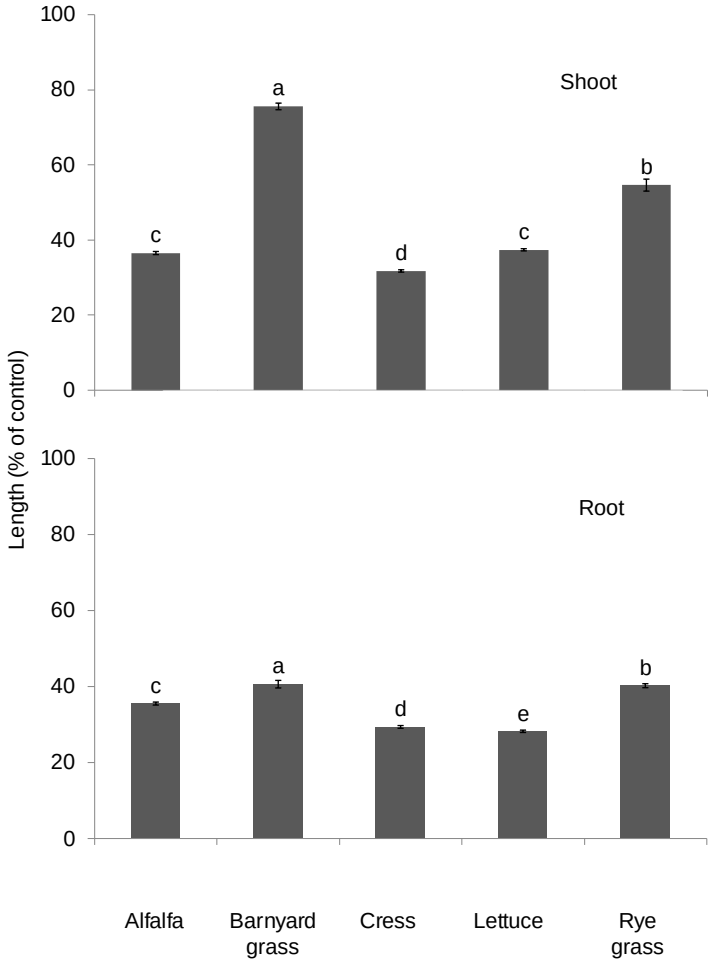

Figure 2. Comparison of marginal means of test plant factor (alfalfa, barnyard grass, cress, lettuce, ryegrass) on the growth of plants, averaging over levels of extract and concentration. Main effect (effect of test plants) of variables were computed multiple comparisons in three ways ANOVA model by using General linear model and Post hoc test (PASWSTAT version 18.0). Means above each bar followed by the same letter are not significantly different as determined by Duncan's multiple range test at $P \leq 0.05$, replicates: 3

The interaction between extracts×concentration and test plants (Figure 3) indicates that the *C. aquatica* extract inhibited the shoot and root growth of rye grass and barnyard grass more than other extracts. Shoot growth of barnyard grass was inhibited by the *H. acutigluma* extract more than *P. pulchrum* and *I. hirtum* extracts, while root growth of this test species was inhibited by the *P. pulchrum* extract more than *H. acutigluma* and *hirtum* extracts. The *C. aquatic* and *P. pulchrum* extracts inhibited similarly the root growth of rye grass and cress, the latter being a test plant that is very sensitive to allelochemicals [43].

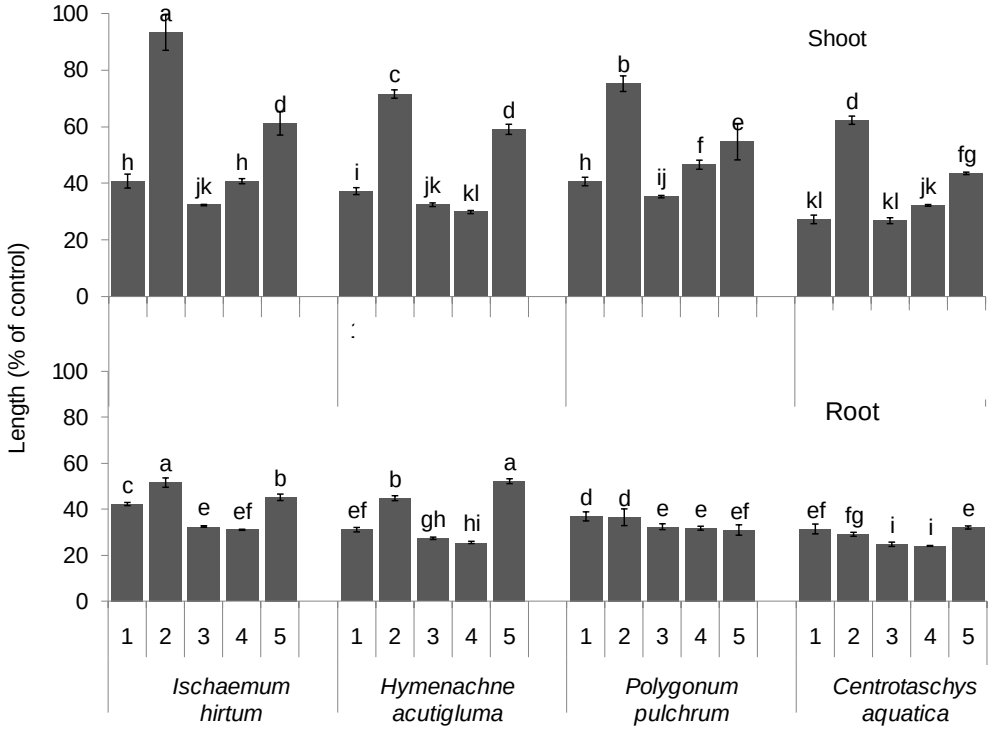

Fig 3. Effect of extract factor (aqueous methanol extracts of *I. hirtum, H. acutigluma*, *P. pulchrum* and *C. aquatica*) on the growth of plant at various test plants ((1) alfalfa, (2) barnyard grass, (3) cress, (4) lettuce, (5) ryegrass), averaging over levels of concentration factor. The interaction between variables of extracts and test plants were computed multiple comparisons in three ways ANOVA model by using General linear model and Post hoc test (PASWSTAT version 18.0). Means above each bar followed by the same letter are not significantly different as determined by Duncan's multiple range test at $P \leq 0.05$.

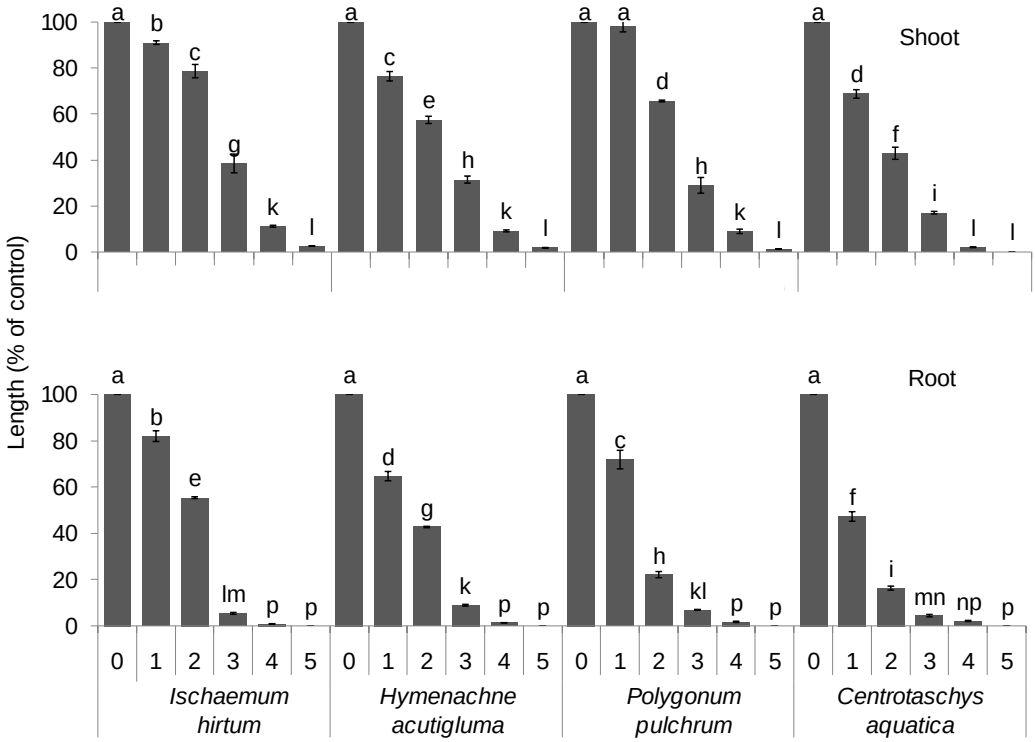

Figure 4. Effect of extract factor (aqueous methanol extracts of *I. hirtum, H. acutigluma*, *P. pulchrum* and *C. aquatica*) on the growth of plant at every levels of concentrations (1) 0.01, (2) 0.03, (3) 0.1, (4) 0.3, (5) 1.0 gram dry weight equivalent extract, averaging over different test plants. The interaction between variables of extracts and concentrations were computed multiple comparisons in three ways ANOVA model by using General linear model and Post hoc test (PASWSTAT version 18.0). Means above each bar followed by the same letter are not significantly different as determined by Duncan's multiple range test at $P \leq 0.05$, replicates: 3.

The effect of the concentration can be observed in Figure 4: an increase in the concentration of extracts led to a significant reduction in the shoot and root growth of test plants. The interaction between the test plant×extract and concentration factor also showed that shoot or root growth under the effect of concentration factor at high levels (0.3 and 1 g DW eq. extract) was not an effective parameter for comparing the inhibitory efficacy of extracts. When compared at the same concentration, the *C. aquatica* extract significantly inhibited the shoot growth of test plants, except for two concentrations (≥0.3 and ≥1 g DW eq. extract) at which all extracts almost completely inhibited the root and shoot growth of test plants.

At each concentration, each test plant responded differently to the inhibitory activity of each extract (Table 1 and 2). At the lowest concentration (0.01 g DW eq. extract), the *C. aquatica* extract stimulated the shoot growth of barnyard grass. Similarly, the extracts of *I. hirtum* and *P. pulchrum* stimulated the shoot growth of barnyard grass and rye grass at this

concentration. On the other hand, the shoot growth of barnyard grass and rye grass was not affected by *H. acutigluma* extract, while the shoot growth of all other test plants was inhibited by these extracts at 0.01 g DW eq. extract. At 0.1 g DW eq. extract, the *I. hirtum* extract still stimulated the shoot growth of barnyard grass, whereas the *C. aquatica* extract completely inhibited the shoot growth of rye grass. Lettuce shoots could not grow when exposed to the *H. acutigluma* and *I. hirtum* extracts at ≥ 0.1 g DW eq. extract, or the *C. aquatica* and *P. pulchrum* extracts at ≥ 0.3 g DW eq. extract. Likewise, the *C. aquatica* extract inhibited the shoot growth of all species at 1 g DW eq. extract. The extracts of *H. acutigluma*, *I. hirtum* and *P. pulchrum* also completely inhibited the seedling growth of all test species at 1 g DW eq. extract, except for barnyard grass seedlings.

Table 1. Effect of Aqueous Methanol Extracts Obtained from Four Semi-aquatic Species on the Shoot Growth of Five Test Plants at Different Concentrations

Conc. (g mL^{-1})	Extract	Shoot growth (% of control)				
		Test plants				
		Alfalfa	Barnyard grass	Cress	Lettuce	Rye grass
0.01	*I. hirtum*	65.88 kl	118.85 c	66.67 kl	88.24 h	116.09 cd
	H. acutigluma	79.66 i	104.87 fg	43.51 opq	50.52 mno	103.91 fg
	P. pulchrum	74.39 ijk	129.60 b	70.01 jk	102.73 fg	113.89 cde
	C. aquatica	47.12 nop	111.75 cdef	42.19 opq	54.34 mn	88.59 h
0.03	*I. hirtum*	58.66 lm	141.34 a	22.53 u…z	56.60 m	114.51 cde
	H. acutigluma	27.32 s…w	107.08 efg	21.02 v…A	29.01 stuv	103.07 fg
	P. pulchrum	40.69 pqr	108.21 defg	33.51 rst	58.36 lm	87.86 h
	C. aquatica	10.32 B…F	102.37 g	9.68 B…G	19.64 wyzA	72.79 ijk
0.10	*I. hirtum*	20.54 v…A	139.72 a	5.10 DEFG	0.00 G	27.23 s…w
	H. acutigluma	14.58 z…D	77.70 ij	17.70 y…C	0.00 G	47.58 nop
	P. pulchrum	20.19 v…A	71.33 ijk	8.58 C…G	18.59 w…B	26.27 t…y
	C. aquatica	3.62 EFG	52.76 mn	9.37 C…G	19.64 wyzA	0.00 G
0.30	*I. hirtum*	.00 G	46.67 nop	0.00 G	0.00 G	9.29 C…G
	H. acutigluma	2.24 FG	30.70 stu	12.75 A…E	0.00 G	0.00 G
	P. pulchrum	8.89 C…G	35.90 qrs	0.00 G	0.00 G	0.00 G
	C. aquatica	2.96 FG	7.03 DEFG	0.00 G	0.00 G	0.00 G
1.00	*I. hirtum*	0.00 G	12.78 A…E	0.00 G	0.00 G	0.00 G
	H. acutigluma	0.00 G	8.91 C…G	0.00 G	0.00 G	0.00 G
	P. pulchrum	0.00 G	6.10 DEFG	0.00 G	0.00 G	0.00 G
	C. aquatica	0.00 G	0.00 G	0.00 G	0.00 G	0.00 G
0.0	Control	100.00 g	100.00 g	100.00 g	100.00 g	100.00 g

Means followed by the same letter are not significantly different as determined by Duncan's multiple range test at $P \leq 0.05$, replicates: 3, Conc. is concentration; g mL^{-1} is gram dry weigh equivalent extract per milliliter.

Table 2. Effect of Aqueous Methanol Extracts Obtained from Four Semi-aquatic Species on the Root Growth of Five Test Plants at Different Concentrations

Conc. (g mL^{-1})	Extracts	Root growth (% of control)				
		Test plants				
		Alfalfa	Barnyard grass	Cress	Lettuce	Rye grass
0.01	*I. hirtum*	82.69 def	120.22 a	65.33 h	55.26 jk	86.24 cd
	H. acutigluma	58.54 ij	89.59 c	42.44 mn	32.35 op	100.55 b
	P. pulchrum	73.09 g	102.10 b	65.52 h	46.67 lm	71.71 g
	C. aquatica	50.86 kl	47.31 lm	38.16 n	37.09 no	62.96 hi
0.03	*I. hirtum*	57.61 ij	80.54 ef	27.70 pq	31.66 p	79.47 f
	H. acutigluma	17.69 stu	79.81 f	8.72 v…C	21.61 rs	85.77 cde
	P. pulchrum	31.04 pq	12.41 uvwy	25.32 qr	30.39 pq	11.68 vwyz
	C. aquatica	18.64 st	18.75 st	6.55 y…D	7.82 w…C	29.99 pq
0.10	*I. hirtum*	13.87 tuvw	4.78 ABCD	2.53 CD	0.00 D	6.02 z…D
	H. acutigluma	9.59 v…A	.00 D	4.48 ABCD	0.00 D	27.42 pq
	P. pulchrum	8.73 v…C	5.11 ABCD	7.91 w…C	14.05 tuv	2.98 BCD
	C. aquatica	10.11 v…A	7.71 w…C	4.17 ABCD	0.00 D	0.00 D
0.30	*I. hirtum*	0.00 D	4.55 ABCD	0.00 D	0.00 D	0.00 D
	H. acutigluma	1.43 D	0.00 D	5.69 z…D	0.00 D	0.00 D
	P. pulchrum	8.96 v…B	0.00 D	0.00 D	0.00 D	0.00 D
	C. aquatica	9.48 v…A	1.41 D	0.00 D	0.00 D	0.00 D
1.00	*I. hirtum*	0.00 D	0.00 D	0.00 D	0.00 D	0.00 D
	H. acutigluma	0.00 D	0.00 D	0.00 D	0.00 D	0.00 D
	P. pulchrum	0.00 D	0.00 D	0.00 D	0.00 D	0.00 D
	C. aquatica	0.00 D	0.00 D	0.00 D	0.00 D	0.00 D
0.00	Control	100.00 b	100.00 b	100.00 b	100.00 b	100.00 b

Means followed by the same letter are not significantly different as determined by Duncan's multiple range test at $P \leq 0.05$, replicates: 3, Conc. is concentration; g mL^{-1} is gram dry weigh equivalent extract per milliliter.

At 0.01 g DW eq. extract, *P. pulchrum* and *H. acutigluma* extracts had no effect on the root growth of barnyard grass and rye grass, respectively. The *I. hirtum* extract stimulated the root growth of barnyard grass when grown at 0.01 g DW eq. extract. Conversely, the root growth of the other test plants was inhibited by the four extracts at the same concentration (0.01 g DW eq. extract). Necrotic symptoms on the roots of test plants were observed at concentration ≥0.1 g DW eq. extract (Figure 5). Dark brown roots implied the reversible death of roots in treatments in which roots were completely inhibited by extracts. The *I. hirtum* extract completely inhibited the root growth of lettuce (0.1 g DW eq. extract), alfalfa, cress, rye grass (0.3 g DW eq. extract) and barnyard grass (1 g DW eq. extract) while the *H. acutigluma* extract completely inhibited barnyard grass and lettuce at 0.1g DW eq. extract, rye grass at 0.3 g DW eq. extract and other test plants at 1 g DW eq. extract. Similarly, the extract of *P. pulchrum* completely inhibited barnyard grass and cress at l g DW eq. extract, rye grass at 0.3 g DW eq. extract and alfalfa at 1 g DW eq. extract. The *C. quatica* extract completely inhibited the root growth of lettuce, rye grass at 0.1 g DW eq. extract, cress at 0.3 g DW eq. extract, and barnyard grass as well as alfalfa at 1 g DW eq. extract.

Root growth of test plants was much more sensitive to extracts than shoot growth, except for the root growth of alfalfa which was inhibited by *C. aquatica* extract and the root growth of cress which was inhibited by *I. hirtum*, *H. acutigluma*, and *C. aquatica* extracts (Table 3).

Table 3. IC_{50} Values of Aqueous Methanol Extracts for Shoots and Roots of the Test Plants

Extracts	Test species	IC_{50} (g DW eq. extract)	
		Shoot	Root
I. hirtum	Alfalfa	0.0385	0.0364
	Barnyard grass	0.2434	0.0373
	Cress	0.0148	0.0158
	Lettuce	0.0339	0.0138
	Rye grass	0.0728	0.0539
H. acutigluma	Alfalfa	0.0182	0.0119
	Barnyard grass	0.1671	0.0400
	Cress	0.0055	0.0083
	Lettuce	0.0117	0.0049
	Rye grass	0.0967	0.0649
P. pulchrum	Alfalfa	0.0235	0.0174
	Barnyard grass	0.1106	0.0240
	Cress	0.0187	0.0152
	Lettuce	0.0369	0.0108
	Rye grass	0.0616	0.0140
C. aquatica	Alfalfa	0.0092	0.0094
	Barnyard grass	0.0996	0.0090
	Cress	0.0080	0.0078
	Lettuce	0.0107	0.0074
	Rye grass	0.0389	0.0158

Note: IC_{50} values were determined by a logistic regression analysis after bioassays

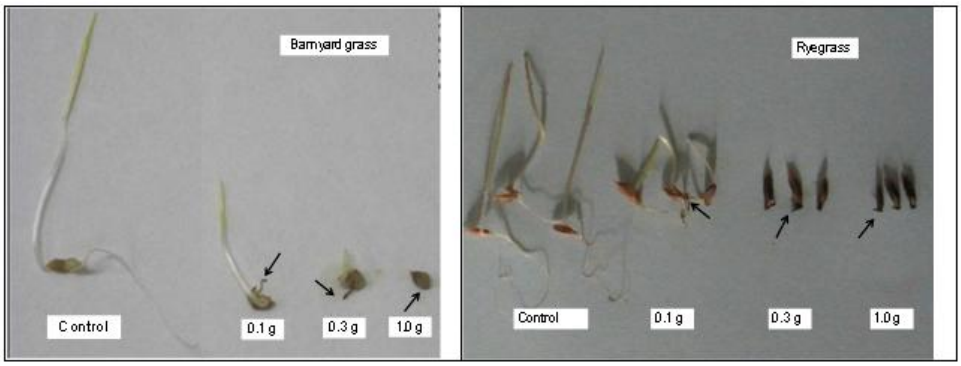

Figure 5. Necrotic symptoms on roots of barnyard grass and ryegrass cause by *Centrostachys aquatica* aqueous methanol extract at concentration ≥0.1 g dry weight equivalent extract.

4. Discussion

Morphological changes of test plants, shortening shoot and root length, and death of roots, applied four emergent macrophytes extracts in the present study indicates that those four plants may contain plant growth inhibitors (Table 1, 2, Figure 5). Dayan and Duke [11] demonstrated that morphological changes of seedling growth provide important information in evaluating the effect of a phytotoxin and may offer some mechanism options. One of the reasons for shortening shoot and root length, or death of roots is due to increase of production of reactive oxygen species (ROS) [19]. Overproduction of ROS causes oxidative stress, leads to membrane lipid damage, and results in cell death. Excessive ROS also affects mitotic phase, lead to decrease cell division and subsequently reduce seedling growth [19].

The concentration-response relationship in growth of some test plant species in this study is hormesis of which phenomenon is characterized by low-dose stimulation and high-dose inhibition. Hormesis phenomenon is well recognized in allelopathy of terrestrial plants (9, 15, 27, 29, 30, 34, 37, 38, 42).

The distinction between inhibitory activities of extracts obtained from monocotyledon (*H. acutigluma and I. hirtum*) and that of extracts obtained from dicotyledon (*C. aquatica and P. pulchrum*) on the growth of test plants is not clear in this study (Figure 1). Our result also showed that the inhibitory effects of the extracts on seedling growth depended on test plant species, test plant organs, and extract concentration (Table 1, 2, 3). The variations in the inhibitory effects in this study may be due to the facts that the sensitivity of test plant species to active components is diverse, and types and amount of active components are different in the extracts [28]. It was reported that that types of active components depend on species [21, 46] and active components activity of types of secondary metabolites affect variously on the seedling growth [18].

Utilization of the allelopathy in weed management has been promoted [6, 32]. Allelopathic potential of some emergent and floating macrophytes (aquatic plants) have been suggested as a feasible bio-resource for future studies on weed control [16, 46]. The inhibitory activity of extracts of *C. aquatica, P. pulchrum H. acutigluma and I. hirtum* on seedling growth of test plants in this study added fundamental information of allelopathic potential of emergent macrophytes for future researches on weed management. Aqueous methanol extracts of *C. aquatica, H. acutigluma and P. pulchrum* have great inhibitory activity on the growth of barnyard grass and ryegrass which have developed high resistance to herbicides. Chou [7] emphasized that although many biological active compounds have been found, researchers still need to isolate and identify for discovering new compounds from plants. When these allelochemicals are eventually identified, the suppressive function of common herbicides on weeds may be improved [43]. Therefore, further research will be focus on identifying the growth inhibitory allelochemicals of *C. aquatica, H. acutigluma and P. pulchrum* that might supply fundamental information for development of bio-herbicides.

Acknowledgements

The first author is grateful to the Ministry of Agriculture and Rural Development of Vietnam for a PhD grant under AST scholarship project; specific thanks to Dr. Jaime A. Teixeira da Silva for critically reviewing and improving English of the paper; and great appreciation to Mr. Ho Van Tru, Tri Viet Corp. for supplying emergent macrophyte materials used in this study.

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