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# 学位論文全文に代わる要約
# Dissertation Abstract

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学位論文題目：
Title of Dissertation Allelopathic potential and allelopathic substances in six Vietnamese macrophyte species
（6種類のベトナム産水生植物のアレロパシー活性とアレロパシー物質）

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**Introduction**

Weeds have always represented one of the main limiting factors in crop production (Soloneski and Larramendy 2011). The application of herbicides, which account for 49% of the total use of pesticides, has led to improved agricultural production (Pretty 2008). Unfortunately, they result in weed resistance, serious environmental risks, and substantial health dangers to the population. Many efforts have been deployed in designing alternative weed management strategies. More recently, the role of allelopathy in controlling the spreads of weeds, have being been interested (Peng *et al.* 2004, Upadhyaya and Blackshaw 2007, Rea *et al*. 2008). Allelopathy, the phenomenon by which living or dead plants, or parts thereof, of one species affect other adjacent species through the release of secondary compounds into the environment (Rice 1984), has been documented in a wide range of plant species, including crop plants (Kruse *et al.* 2000), weeds, forest trees (Kohli *et al.* 2001), and aquatic plants (Gross 2003).

Many researchers have reported allelopathic macrophytes as a potential source to control aquatic weeds (Elakovich 1989, Quayyum *et al.* 1999, Kathiresan 2006, Zu *et al.* 2010). However, only several researchers have focused on studying effect of macrophytes on terrestrial weed (Zu *et al.* 2010).

Duckweed (*Lemna minor* L.,) and water lettuce (*Pistia stratiotes* L., family Araceae) is free-floating freshwater macrophytes and widely distributed in tropical and sub-tropical regions (Neuenschwander *et al.* 2009, Skillicorn *et al.* 1993). Duckweed had an antibacterial effect (GülÇin *et al.* 2010) and is inhibitory to algae (Ping *et al.* 2001). Water lettuce limits the growth of submerged hydrophytes and phytoplankton (Chokder, 1968). Alilotta *et al.* (1991) found six allelochemicals in water lettuce to be inhibitory to algae and microalgae.

*Centrostachys aquatica* (R. Br.) Wall ex Mogtand (family Amaranthaceae), *Polygomum pulchrum* Blume (family Polygonaceae), *Ischaenum hirtum* Hack (family Poaceae) and *Hymenachne acutigluma* (Steud.) Gilliland (family Poaceae) are emergent macrophytes and grow abundantly in natural wetlands and waterways worldwide (Mani 2011, Srivastava and Nair 2010, Germishuizen and Meyer 2003, Armold and De Wet 1993). Several species of these families had a strong allelopathic influence on the germination and growth of several plants (Dhole *et al.* 2011, de Souza *et al.* 2011, D'Abrosca *et al.* 2006, Alsaadawi *et al.* 1982). Many allelopathic compounds have been isolated form these family (de Souza *et al.* 2011, Tullanithi *et al*. 2010, Rasmussen *et al*. 1992, Kato-Noguchi 2011).

The primary objective of this research was to evaluate the allelopathic potential of the aqueous methanol extracts of two floating macrophytes (duckweed and water lettuce) and four emergent macrophytes (*Centrostachys aquatica* (R. Br.) Wall ex Mogtand, *Polygomum pulchrum* Blume, *Ischaenum hirtum* Hack, *Hymenachne acutigluma* (Steud.) Gilliland) on the germination and growth of several terrestrial test plants, including Italian ryegrass and barnyard grass. Additionally, a series of experiments focused on the isolation,

identification and molecular biological activities of allelochemicals to elucidate allelopathy in duckweed (floating macrophytes) and *Censtrotachys aquatica* (emergent macrophytes).

**Materials and methods**

Whole plant of the two floating macrophytes (duckweed and water lettuce) and four emergent macrophytes (*Centeostachys aquatica, Polygomum pulchrum, Ischaenum hirtum, Hymenachne acutigluma*) were collected then washed with tap water and individually dried in the sun. Fifty gram of dried each macrophyte was soaked in 500 mL of 70% (v/v) aqueous methanol for 48 h, and filtered. The residue was re-extracted with methanol for 24 h and filtered. The two filtrates were combined and evaporated at 40°C to produce an aqueous methanol extract. Aqueous methanol extracts were diluted with methanol to obtain final concentrations of 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, and 1.0 g dry weight equivalent extract $mL^{-1}$ (g DW eq. extract $mL^{-1}$).

A germination test was conducted on two species, cress (*Lepidium sativum* L.) and barnyard grass (*Echinochloa crus-galli* (L). Beauv. Eigth test species, cress, lettuce (*Lactuca sativa* L.), timothy (*Phleum pratense* L.), alfalfa (*Medicago sativa* L.), barnyard grass, crab grass (*Digitaria sanguinalis* L.), junglerice (*Echinochloa colonum* (L.) Link.), and Italian ryegrass (*Lolium multiflorum* L.) were selected for growth bioassay.

One hundred μL of aqueous methanol extract of each macrophyte spcies of each concentration were added to filter paper in a 2.8-cm diameter Petri dish and kept in a draft chamber for 30 min to evaporate the methanol. For germination bioassay, ten seeds of each test species were placed on filter paper containing extracts in each Petri dish, moistened with 0.6 mL of a 0.05% (v/v) aqueous solution of polyoxyethylenesorbitan monolaurate. Then the Petri dishes were incubated in the dark at 25°C. The germination bioassay was laid out using a completely randomized design with eight concentrations (50 seeds for each concentration) and repeated three times. Germination was considered to have occurred only after the radicle had protruded by at least 1 mm (based on Gallardo *et al*. 2002). Germination was determined by counting the number of germinated seeds at 12-h intervals over a 48-h period. The percentage of germination in each treatment was calculated and compared to that of the control which had been treated with distilled water without residue. The percentage germination was calculated using the following equation: Germination (%) = n/N×100, where n is the number of seeds germinated and N is the number of seeds sown. For growth bioassay, ten germinated seeds were then placed on filter paper in a Petri dish with different concentration of extract or without extrac (control). The bioassay was designed in a completely randomized design using eight concentrations (20 plants for each concentration) with three replications. Root and shoot elongation was measured 48 h after incubation in the dark at 25°C. The percentage of shoot or root growth was calculated using the following equation: Shoot (or root) growth (%) = Le/Lc × 100, where Lc is the shoot (or root) length of the control and Le is the shoot (or root) length of the sample extract.

For isolation of active compounds, dried plant of duckweed (4 kg) and C. *aquatica* (300 g) were extracted as described above to produce aqueous methanol extracts. The aqueous methanol extracts were adjusted to pH 7.0 with 1 M phosphate buffer and partitioned by a liquid partitioning (water: ethyl acetate, 1:3 v/v). After drying with sodium sulfate, the ethyl acetate phase was evaporated to dryness and chromatographed on columns of silica gel, Diaion HP20, Sephadex LH-20, Diaion HP20ss, $C_{18}$ cartridge and finally purified by a reserve phase HPLC. The active substance was characterized by high-resolution ESI mass, $^{1}$H-NMR spectra and optical rotation. Growth bioassay of active substances isolated from duckweed or *C. aquatica* was determined by test plants as described above.

**Result**

**Chapter 2: Allelopathic potential of two aquatic plants, duckweed (*Lemna minor* L.) and water lettuce (*Pistia stratiotes* L.), on terrestrial plant species**

The inhibitory effect of the aqueous methanol extracts obtained from duckweed and water lettuce on the

percent germination of cress was significant at all concentrations 24 h after incubation (Fig 2.1). At concentrations ≤ 0.03 g DW eq. extract $mL^{-1}$ of duckweed or at a concentration ≤ 0.1 g DW eq. extract $mL^{-1}$ of water lettuce, the inhibitory effect decreased over time and did not suppress germination 48 h after incubation. The aqueous methanol extract of duckweed or water lettuce significantly inhibited percent germination of barnyard grass at ≥ 0.003 g or ≥ 0.1 g DW eq. extract $mL^{-1}$ 36 h after incubation, respectively (Fig 2.2). Increasing the extract concentration resulted in stronger inhibition. Applying 0.1 g DW eq. extract $mL^{-1}$ of duckweed or 0.3 g DW eq. extract $mL^{-1}$ of water lettuce completely inhibited the percent germination of cress. Likewise, both aqueous methanol extracts of the two floating macrophytes completely suppressed the percent germination of barnyard grass at 1 g DW eq. extract $mL^{-1}$.

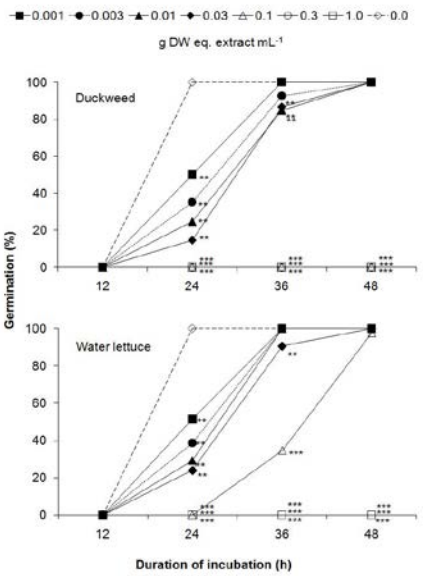


Fig 2.1 Effects of duckweed and water lettuce aqueous methanol extracts on the percent germination of cress in different periods and concentrations

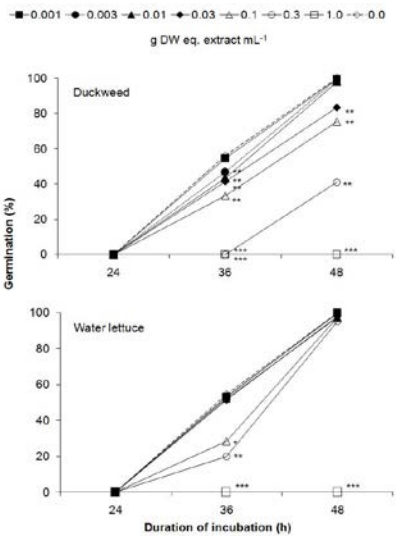


Fig 2.2 Effects of duckweed and water lettuce aqueous methanol extracts on the percent germination of barnyard grass in different periods and concentrations.

The growth response of each test species to the inhibitory activity of duckweed and water lettuce aqueous methanol extract varied due to a concentration-test species interaction (Fig 2.4) and was diverse, ranging from stimulation to inhibition (Fig 2.5). By increasing the concentration, the inhibitory activity increased. The root and shoot growth of cress, alfalfa, lettuce and timothy was completely inhibited by duckweed aqueous methanol extract at 0.1 g DW eq. extract $mL^{-1}$. Complete inhibition of the shoot and root growth of barnyard grass, junglerice, crab grass and rye grass seedlings was also observed at 1 g DW eq. extract $mL^{-1}$. Shoot growth of cress, lettuce, and timothy was completely inhibited at 0.3 g DW eq. extract $mL^{-1}$.

The shoots of barnyard grass, crab grass, junglerice and rye grass could not develop at 1 g DW eq. extract $mL^{-1}$. At 1 g DW eq. extract $mL^{-1}$, both aqueous methanol extracts caused necrotic symptoms on roots of test species, implying an irreversible death of roots .

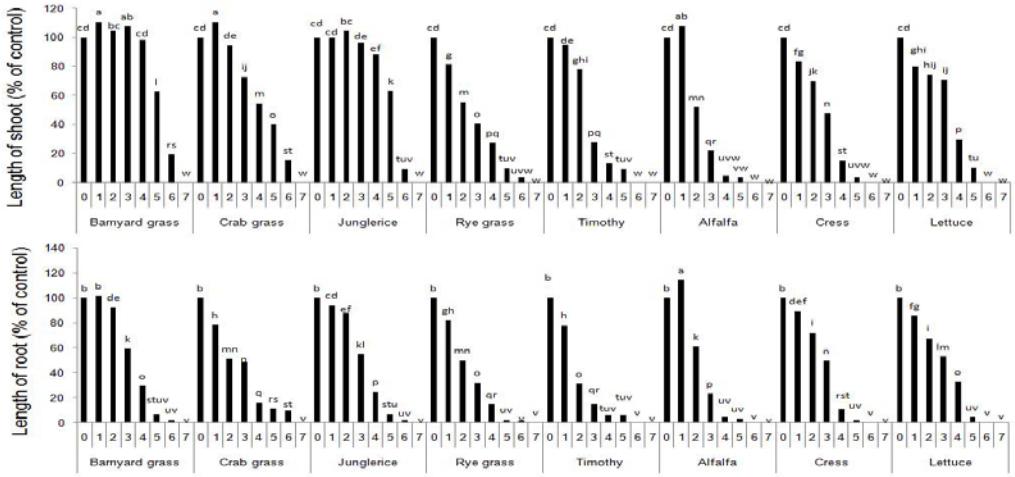


Fig 2.4 Comparison of the response of eight test species to duckweed aqueous methanol extract at different concentrations: (1) 0.001, (2) 0.003, (3) 0.01, (4) 0.03, (5) 0.01, (6) 0.1, (7) 1 g DW eq. extract $mL^{-1}$ and (0) without extract.

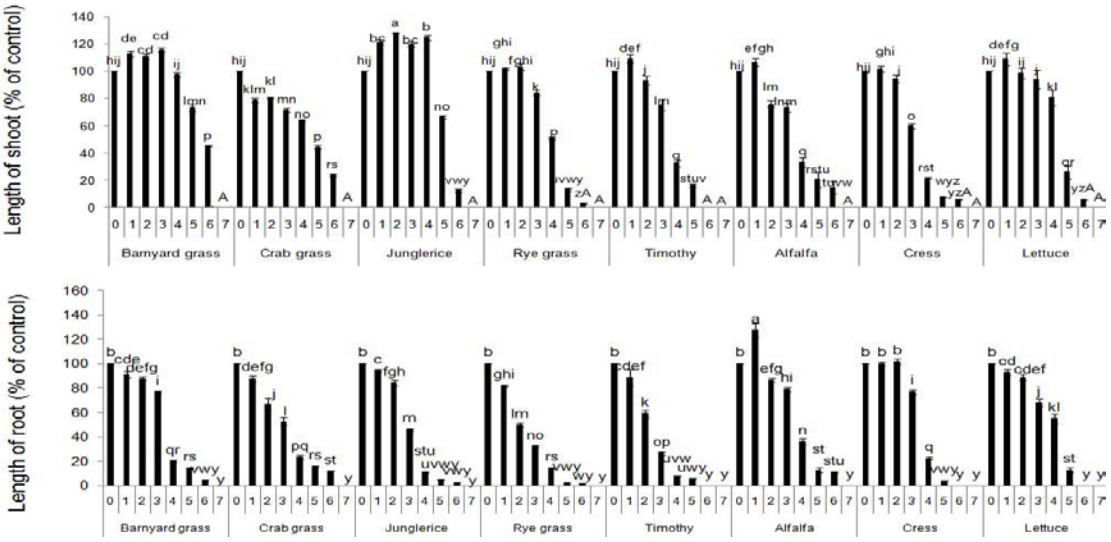


Fig 2.5 Comparison of the response of eight test species to water lettuce aqueous methanol extract at different concentrations: (1) 0.001, (2) 0.003, (3) 0.01, (4) 0.03, (5) 0.01, (6) 0.1, (7) 1 g DW eq. extract $mL^{-1}$ and (0) without extract.

**Chapter 3: Isolation and identification of an allelopathic substance from duckweed**

The active substance isolated from aqueous methanol extract of duckweed has a molecular formula of $C_{13}H_{20}O_2$ and was identified as (3R)-(-)-3-hydroxy-β-ionone (Fig 3.10)

(3R)-(-)-3-Hydroxy-β-ionone isolated from duckweed inhibited the shoot and root growth of cress seedlings at concentrations ≥ 0.1μM and the root and shoot growth of Italian ryegrass seedlings at concentrations ≥5 μM (Fig 3.11; F 3.12). Shoot and root growth of cress was equally inhibited by 3-hydroxy-β-ionone, with $IC_{50}$ of 1.00 and 1.01 μM, respectively. In contrast, Italian ryegrass (monocotyledon species) was less sensitive to (3R)-(-)-3-hydroxy-β-ionone than cress (dicotyledon species), suggesting some selectivity.

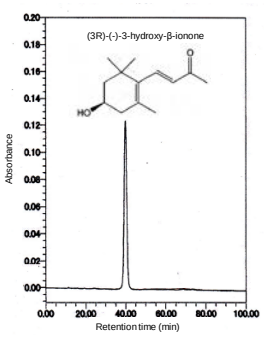


Fig 3.10 Chemical structure of (3R)-(-)-3-hydroxy-β-ionone

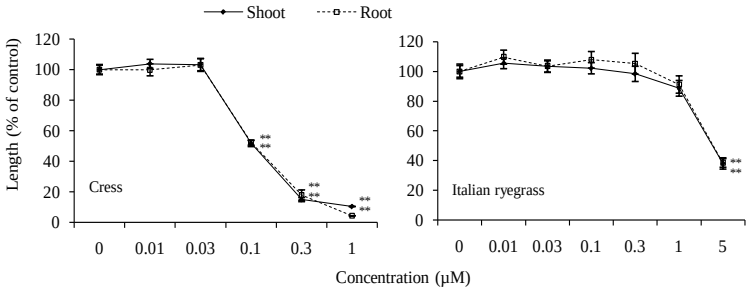


Fig 3.11 Effects of 3-hydroxy-β-ionone on the root and shoot growth of Italian ryegrass and cress seedlings.

**Chapter 4: The allelopathic potential of four emergent macrophytes on the growth of terrestrial plant species**

Table 4.1 Comparison the inhibitory activity of aqueous methanol extracts obtained from the four semi-aquatic species on the shoot growth of five test species under interaction among aqueous methanol extracts of semi-aquatic species, concentration of these extracts and test species.

| Concentration ($g\ mL^{-1}$) | Extract | Shoot growth (% of control) | | | | |
|---|---|---|---|---|---|---|
| | | Test species | | | | |
| | | 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 |
| 0.1 | *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 |
| 0.3 | *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 | *I. hirtum* | .00 G | 12.78 A…E | 0.00 G | 0.00 G | 0.00 G |
| | *H. acutigluma* | .00 G | 8.91 C…G | 0.00 G | 0.00 G | 0.00 G |
| | *P. pulchrum* | .00 G | 6.10 DEFG | 0.00 G | 0.00 G | 0.00 G |
| | *C. aquatica* | .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 in this table followed by the same letter are not significantly different as determined by Duncan's multiple range test at $P \leq 0.05$, replicates: 3, $g\ mL^{-1}$ is gram dry weigh equivalent extract.

At each concentration, each test plant responded differently to the inhibitory activity of each extract of emergent macrophytes (Table 4.1 and 4.2). 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 4.2 Comparison the inhibitory activity of aqueous methanol extracts obtained from the four semi-aquatic species on the root growth of five test species under interaction among aqueous methanol extracts of semi-aquatic species, concentration of these extracts and test species.

| Concentration ($g\,mL^{-1}$) | Extracts | Root growth (% of control) | | | | |
|---|---|---|---|---|---|---|
| | | Test species | | | | |
| | | 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 in this table followed by the same letter are not significantly different as determined by Duncan's multiple range test at $P \leq 0.05$, replicates: 3. $g\,mL^{-1}$ is gram dry weigh equivalent extract.

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 (Fig 4.5 and 4.6). 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 1 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.

**Chapter 5: Isolation and identification of an allelopathic substance from emergent macrophyte: *Centrostachys aquatica***

The active substance has a molecular formula of $C_{11}H_{20}O_3$ and was identified as (-)(-) loliolide (Fig 5.4) by comparing this data with that in the literature (Hodges and Porte 1964). (-)- loliolide significantly inhibited the growth of cress and barnyard grass seedlings at low concentrations (≥ 0.03 µM). The concentration of (-)-loliolide required to inhibit 50% of the growth ($IC_{50}$) was 45.6 and 0.73 µM for barnyard grass shoots and roots, 0.18 and 0.15 µM for cress shoots and roots, and 34.9 µM for Itatian ryegrass roots.

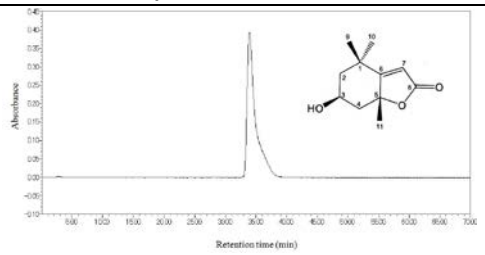


Fig 5.7 Chemical structure of (-) - loliolide and HPLC chromatogram

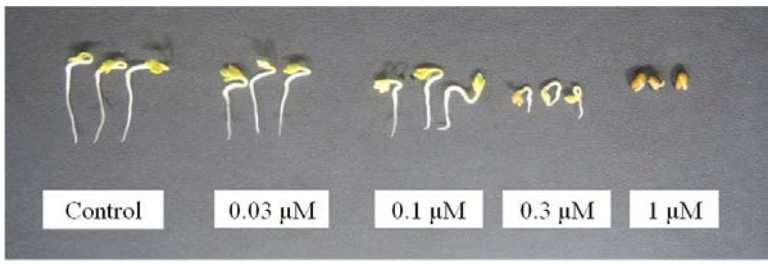


Fig 5.11 Effect of (-)-loliolide on the growth of cress seedlings in various concentrations.

**Chapter 6: General discussion**

The growth of test terrestrial plants in this study was significantly inhibited by aqueous methanol extracts of the two floating (duckweed and water lettuce) (chapter 2) and four emergent macrophytes (*C. aquatica, P. pulchrum, I. hirtum, Hacutigluma* ) (chapter 4), providing strong evident that these macrophytes possess allelopathic potential.

The results in chapter 2 showed that inhibition of duckweed and water lettuce aqueous methanol extracts on germination of different test plants is various. This may be due to the differences in the amount and the composition of the compounds in plants that rendered the difference in potential phytotoxicity (An *et al*. 2002). By increasing concentration of aqueous methanol extracts of duckweed and water lettuce resulted in increasing the inhibitory activity of the extracts. Dose threshold of aqueous methanol extract of the two floating macrophyes to completely inhibit the growth and germination of test plants is at 1g DW eq. extract $mL^{-1}$. The concentration dependence in allelopathy have been observed and documented in literature (Lovett *et al*. 1989, An *et al*. 2005, Batlang and Shushu 2007, Ashrafi *et al*. 2009). The application of allelopathic plant extracts can effectively control weeds (Farooq *et al*. 2011) and the results in chapter 2 offer a promise for future research and applications of these two aquatic species as natural alternatives for pre- and post-emergent bioherbicides.

In chapter 3, a growth inhibitory substance was isolated from the aqueous methanol extract of duckweed and identified by spectral data as (3R)-(-)-3-hydroxy-β-ionone (3-hydroxy-5,7-megastigmadien-9-one). (3R)-(-)-3-hydroxy-β-ionone has previously been isolated from *Bunias orientalis* (Dietz and Winterhalter 1996),

burley tobacco (*Nicotiana tabacum* L.) (Fujimori *et al*. 1974), quince fruit (*Cydonia oblonga* Mill.) (Güldner and Winterhalter 1991), grape vine (*Vitis vinifera* L.) (Mathieu *et al.* 2005), moss (*Rhynchostegium pallidifolium* (Mitt.) A. Jaeger) (Kato-Noguchi *et al.* 2010a) and rice (Kato-Noguchi 2011). To the best of our knowledge, this is the first report of the existence of this substance in *Lemna* genus. (3R)-(-)-3-hydroxy-β-ionone inhibited seedling growth of cress (dicotyledonous species) and Italian ryegrass (monocotyledonous species) at the concentrations greater than 0.1 and 5 µM, respectively. Kato-Noguchi *et al.* (2011) noticed that the efficacy of (3R)-(-)-3-hydroxy-β-ionone purified from the rice cultivar Kartikshail was greater on cress roots and shoots than on barnyardgrass (*Echinochloa crus-galli)* roots and shoots (another monocotyledoneous species). The substance inhibited 50% of cress roots and shoots growth with 0.1 µM, and 50% of Italian ryegrass root and shoot growth at 2.4 and 3.4 µM, respectively. These results suggested that (3R)-(-)-3-hydroxy-β-ionone has some selective activity. The presence of (3R)-(-)-3-hydroxy-β-ionone in duckweed and growth inhibitory activity of this substance suggest that it may contribute to the allelopathic potential of duckweed.

In chapter 4, we compared the allelopathic potential of the four emergent macrophytes on the growth of terrestrial plants. According to Hong *et al*. (2003), it is necessary to find stronger allelopathic plants for weed control. 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 DW eq. extract $mL^{-1}$, respectively) and barnyard grass (1 g DW eq. extract $mL^{-1}$). The present results indicate that all plants may contain allelopathic active substances and that *C. quatica* may contain the greatest herbicidal substance(s). It was reported that types and amount of active components are different between source plant species (Back and Kim 1988, Smolarz 2002). Wu *et al.* (2001) noticed in a review that test species and biotypes within species differ significantly in their susceptibility to an allelopathic source. Necrotic symptom on several test plant species was observed in our study. (Chou and Patrick, 1996) also observed necrotic root tips and shorter roots of lettuce seedlings due to damage of meristematic tissue when lettuce seedling exposed in aqueous extracts of decomposing corn residues. Golisz et al. (2008) in their review noted that some alellochemicals can act as herbicides and some of them can cause root cell death indirectly by facilitating the production of reactive oxygen species.

(-)-Loliolide was identified as the active ingredient in the *C. aquatica* extract by its spectral data and by comparison with the reported data. (-)- loliolide inhibited 50% of the seedling growth of cress, Italian ryegrass and barnyard grass at various concentration, suggesting some selectivity. Allelochemicals have the selective activity (Patrick *et al.* 2002, Li and Hu 2005) because allelochemicals may have selective action or because the test plants may have different capacities for phytotoxin detoxification (An et al. 2000, Gatti *et al.* 2010).

The inhibitory efficacy of these macrophytes was dependent on their potential activity, the test plant species, and concentration of the extracts. To the best of our knowledge, it has no been reported the existence of 3-hydroxy-β-ionone in duckweed and of (-)- loliolide in *C. aquatica.* Thus, our results are the first isolation and identification the two substances from these macrophytes. With the rapid growth, large biomass productivity, high water content in plant materials, and the inhibitory activity of the six macrophytes as well as the existence of the growth inhibitors in duckweek (3-hydroxy-β-ionone) and *C. auquatica* (loliolide), it is necessary to study these macrophytes as bio-resource of growth inhibitors for application in sustainable agriculture.

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