Aquatic Botany 117 (2014) 56–61

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Growth limiting effects on various terrestrial plant species by an allelopathic substance, loliolide, from water hyacinth

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ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 19 April 2014
Accepted 2 May 2014
Available online 14 May 2014

Keywords:
Allelopathy
Bioactive compound
Eichhornia crassipes
Growth inhibitor
Weed control

ABSTRACT

Water hyacinth (*Eichhornia crassipes*) is one of the most noxious invasive aquatic plants in many countries due to its rapid growth and reproduction rate. Most practical methods to control water hyacinth are mechanical removal of the plants, and chemical and biological controls with large expense for those treatments. For recovery of control costs, beneficial use options for water hyacinth after mechanical removal would be helpful. Therefore, we investigated possible allelopathic effects of extracts and isolated allelopathic substances in water hyacinth. An aqueous methanol extract of water hyacinth inhibited the growth of roots and shoots of cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), timothy (*Phleum pratense*) and ryegrass (*Lolium multiflorum*). Increasing extract concentration increased the inhibition. These results suggest that water hyacinth may cause allelopathic effects and contain allelopathically active substances. The extract was then purified by several choromatographic runs with monitoring the inhibitory activity during all purification steps, and a main allelopathically active substance was isolated. The chemical structure of the substance was determined by spectral data as loliolide. Loliolide inhibited the growth of cress and ryegrass at concentrations greater than 3 and 10 μM. The concentrations required for 50% inhibition of the root and shoot growth ranged from 12.7–27.5 to 27.5–49.7 μM for cress and ryegrass, respectively. These results suggest that loliolide may be an allelopantic substance and contribute to the growth inhibitory effect of water hyacinth. A water extract of water hyacinth also inhibited all test plant species extract-concentration dependently and contained loliolide. In addition, powder of water hyacinth inhibited germination of barnyard grass in the greenhouse condition with powder-concentration dependently. Therefore, water hyacinth may potentially be useful as soil additive materials to control weeds in the sustainable agriculture.

1. Introduction

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach) is a floating aquatic plant belongs to Pontederiaceae family and originated from South America. The plants reproduce both sexually and asexually and form large, dense and interlocking mats on freshwater systems. Water hyacinth has become one of the most noxious invasive aquatic plants in over 50 countries on five continents from tropical to temperate zones due to its rapid rate of growth and reproduction. The spreading of the water hyacinth is also enhanced by water stream and wind as well as human activity. Most practical methods to control water hyacinth are mechanical removal of the plants, and chemical and biological controls with large expense (Gunnarsson and Petersen, 2007; Villamagna and Murphy, 2010).

Plants produce hundreds of secondary metabolites, and some of these compounds show allelopathic property such as growth inhibitory effects against other plants (Putnam, 1988; Gross and Paritheir, 1994; Inderjit, 1996; Duke et al., 2000; Macías et al., 2007). Some plants provided excellent weed control ability in intercropping and/or as soil additives due to their allelopathic property (Weston, 1996; Semidey, 1999; Caamal-Maldonado et al., 2001). Therefore, allelopathy of plants is potentially useful for weed management options in several agriculture settings to reduce commercial herbicide dependency (Putnam, 1988; Weston, 1996; Narwal, 1999). In addition, allelopathic substances have potential as either herbicides or templates for new synthetic herbicide classes (Putnam, 1988; Gross and Paritheir, 1994; Seigler, 1996; Duke et al., 2000; Macías et al., 2007).

It was reported that acetone and methanol extracts of water hyacinth and their crude fractions separated by thin layer chromatography had growth inhibitory activity against algae and microbes (Jin et al., 2003; Shanab et al., 2010). Isocyanoethy acetate,

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http://dx.doi.org/10.1016/j.aquabot.2014.05.001
0304-3770/

2-2-dimethyl cyclopentane and propane amide were isolated from the acetone extracts of water hyacinth as growth inhibitors against algae (Jin et al., 2003). Therefore, after mechanical removal of water hyacinth from the natural water systems, the plants might be one of the candidates for soil additive materials to control weeds because of its inhibitory activity. However, allelopathic activity of water hyacinth on terrestrial plants has remained obscure (Ahmed et al., 1982). The objective of this study was the investigation of possible allelopathic property against terrestrial plants and allelopahic substances of water hyacinth.

2. Materials and methods

2.1. Plant materials

Whole plants of water hyacinth (*E. crassipes* (Mart.) Solms-Laubach) were collected from freshwater ponds in Cần Tho City, South of Vietnam in September, 2011 (the late wet season), 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. Seeds of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa.* L.), timothy (*Phleum pratense* L.) and ryegrass (*Lolium multiflorum* L.) were chosen as test plants for bioassay owing to their known seedling growth behavior. Barnyard grass (*Echinochloa crus-galli* [L.] Beauv.) was selected for green house experiments.

2.2. Aqueous methanol extraction and bioassay

Whole plants of water hyacinth (100 g dry weight) were cut into small pieces and soaked with 1 L of 70% (v/v) aqueous methanol for two days. After filtration using filter paper (No. 2; Toyo, Tokyo, Japan), the residue was soaked again with 1 L of methanol for one day and filtered. Then, the two filtrates were combined. An aliquot of the extract (final assay concentration was 0.01, 0.03, 0.1 and 0.3 g dry weight water hyacinth equivalent extract mL^{-1}) was evaporated to dryness at 40 °C in vacuo, dissolved in 0.2 mL of methanol and added to a sheet of filter paper (No. 2; Toyo, Tokyo) in a 3-cm Petri dish. Methanol was evaporated in a fume hood at room temperature. Then, the filter paper in the Petri dishes was moistened with 0.8 mL of a 0.05% (v/v) aqueous solution of polyoxyethylene sorbitan monolaurate (Tween 20; Nacalai, Japan). Ten seedlings of cress, lettuce, timothy or ryegrass were placed on the filter paper in Petri dishes after germination in the darkness at 25 °C for 16–72 h. The length of roots and shoots of these seedlings was measured after 48 h of incubation in the darkness at 25 °C, and the percentage length of the seedlings was determined by reference to the elongation of control seedlings. For control treatment, methanol (0.2 mL) was added to the filter paper in the Petri dish and evaporated as described above. Control seedlings of test plants were then placed on the filter paper moistened with the aqueous solution of Tween 20. The bioassay was repeated four times using a completely randomized design with 10 plants for each determination. Significant differences between treated and control plants were examined by Welch's *t*-test for each test plant species.

2.3. Purification of active substance

Whole plants of water hyacinth (1 kg dry weight) were soaked with 70% (v/v) aqueous methanol and methanol as described above and the extract was concentrated at 40 °C in vacuo to produce an aqueous residue. The aqueous residue was adjusted to pH 7.0 with 1 M phosphate buffer, and partitioned three times against an equal volume of ethyl acetate. The ethyl acetate fraction was evaporated to dryness and separated on a column of silica gel (100 g, silica gel 60, 70–230 mesh; Merck), eluted stepwise with *n*-hexane containing increasing amounts of ethyl acetate (10% per step, v/v; 100 mL per step) and methanol (200 mL). The biological activity of the fractions was determined using a cress bioassay as described above (four independent bioassays with 10 plants for each fraction), and activity was found in a fraction obtained by elution with 80% ethyl acetate in *n*-hexane fraction. The active fraction was evaporated and purified using a column of Sephadex LH-20 (100 g, Amersham Pharmacia Biotech, Buckinghamshire, UK), and eluted with 20, 40, 60 and 80% (v/v) aqueous methanol (100 mL per step) and methanol (200 mL). The active fraction was eluted with 40% aqueous methanol and evaporated to dryness. The residue was dissolved in 20% (v/v) aqueous methanol and loaded onto reverse-phase C_{18} Sep-Pak cartridges (Waters). The cartridge was eluted with 20, 40, 60, 80% (v/v) aqueous methanol (15 mL per step) and methanol (30 mL per step). The active fraction was eluted by 40% aqueous methanol and evaporated to dryness. The residue was finally purified by reverse-phase HPLC (10 mm i.d. × 50 cm, ODS AQ-325; YMC Ltd., Kyoto, Japan) with a flow rate of 1.5 mL/min with 50% aqueous methanol, and detected at 220 nm. Inhibitory activity was found in peak fractions eluted between 66 and 74 min. The active substance was characterized by high-resolution ESI mass, ^{1}H-NMR (400 MHz, CD_3OD, TMS as internal standard) and optical rotation.

2.4. Bioassay of the active substance

Active substance was dissolved in a 0.2 mL of methanol, added to a sheet of filter paper (No. 2) in a 3-cm Petri dish, and the methanol was evaporated in a fume hood at room temperature. The filter paper in the Petri dish was moistened with 0.8 mL of 0.05% (v/v) aqueous Tween 20. Ten cress or ryegrass seedlings were placed on the filter paper in Petri dishes after germination as described above. The length of roots and shoots of the seedlings was measured after 48 h of incubation in the darkness at 25 °C, and the percentage length of the seedlings was determined by reference to the elongation of control seedlings as described above. The bioassay was repeated four times using a completely randomized design with 10 plants for each determination.

2.5. Water extraction and bioassay

Whole plants of water hyacinth (100 g dry weight) were cut into small pieces and soaked with 1 L of distil water for two days and filtrated using filter paper (No. 2). Biological activity of the water extract was determined by the bioassay of cress, lettuce, timothy and ryegrass seedlings as described by above. All experiments were carried out under sterile conditions. The bioassay was repeated four times using a completely randomized design with 10 plants for each determination. Significant differences between treated and control plants were examined by Welch's *t*-test for each test plant species.

2.6. Water hyacinth powder and bioassay

Sandy loam soil (pH 6.5) was dried and filled into pots (30 cm i.d. × 20 cm, 8 kg soil per pot). Dried water hyacinth was ground into powder by mechanical blender and spread over soil surface of the pots at 0 (control), 0.7, 2.1, 7, 21 or 70 g powder per pot. After watering, these pots were kept in a greenhouse for 9 days. Then, 100 seeds of barnyard grass were sown onto the soil in the pots. Water was supplied adequately. Germination rate and was determined at day 14 after sowing, and compared to those of control barnyard grass. The bioassay was repeated four times using a completely randomized design with 100 seeds for each determination.