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学位論文要旨
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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Due to a numerous increase of herbicide-resistant weeds and environmental concern regarding to the use of synthetic herbicides, considerable effort has been deployed in designing alternative weed management strategies. On the other hand, there is an interest in utilization of aquatic weeds as bio-resources in benefit ways. One of the new feasible options for reducing synthetic herbicide dependency and utilizing weeds as bio-resources would be the utilization of allelopathy in aquatic weeds for weed management. Allelopathy is any processes involving secondary metabolites produced by plants, algae, bacteria, and fungi that influent the growth and development of agricultural and biological systems. To date, many weed species including macrophytes have shown strong allelopathic activity. This study was undertaken to (1) explore the allelopathic potential of six macrophyte species on the growth of terrestrial plants and (2) to isolate and identify allelopathic substances.

The allelopathic potential of two floating macrophytes, duckweed (*Lemna minor* L.) and water lettuce (*Pistia stratiotes* L.) was examined on the seedling growth of eight test terrestrial plants, alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), barnyard grass (*Echinochloa crus-galli* (L). Beauv.), crabgrass (*Digitaria sanguinalis* L.), jungle rice (*Echinochloa colonum* (L.) Link.), Italian ryegrass (*Lolium multiforum* Lam) and timothy (*Phleum pratense* L.), and on the germination of cress and barnyard grass. Aqueous methanol extracts of the two macrophytes inhibited root growth of all test terrestrial plants at ≥ 0.01 g dry weight equivalent extract mL^{-1} (g DW eq. extract mL^{-1}) and shoot growth at ≥ 0.03 g DW eq. extract mL^{-1}. Although the inhibitory effects of the two aqueous methanol extracts on test terrestrial plants varied and were non-selective, an irreversible dose-threshold (1 g DW eq. extract mL^{-1}) was necessary to completely inhibit the seedling growth and germination of all test plants. The results suggest that the two floating marcrophytes may contain growth inhibitory substances which are inhibitory to the growth of test terrestrial plants.

For isolation of allelopathic active substances, the aqueous methanol extract of duckweed was partitioned into water and ethyl acetate fractions. Subsequently, ethyl acetate fraction was purified by the columns of silica gel, Diaion HP20, Sephadex LH-20, Diaion HP20SS and C_{18} Sep-Pak cartridges. Three growth inhibitory substances were finally isolated by reverse phase HPLC with 45% aqueous methanol, at retention time of 95- 98, 147- 150, and 175- 180 min. One of these growth inhibitory substances was identified as 3-hydroxy-β-ionone by spectra data. Although this substance was found in several plant species, its presence in duckweed was first reported. The concentrations of 3-hydroxy-β-ionone inhibited significantly seedling growth of cress and Italian ryegrass were at concentrations ≥ 0.1 and ≥ 5 μM, respectively. The concentration of 3-hydroxy-β-ionone required to inhibit 50% of the growth (IC_{50}) of cress shoots and roots was 0.1 μM, and the concentration of the substance required to inhibit 50% of the growth of shoots and roots of Italian ryegrass was 3.4 and 2.4 μM, respectively. The presence of (3R)-(-)-3-hydroxy-β-ionone in duckweed and growth inhibitory activity of this substance suggest that it may contribute the allelopathic potential of duckweed.

Four emergent macrophytes (semi-aquatic plants), *Centrostachys aquatica* (R.Br.). Wall ex Moq Tand, *Polygonum pulchrum* Blume, *Hymenachne acutigluma* Steud., and *Ischaemum hirtum* Hack., grow abundantly worldwide in natural wetlands and fresh waterways. To discover novel bio-resources for weed management, the allelopathic potential of the aqueous methanol extracts of these species on the growth of two terrestrial weeds: barnyard grass and rye grass, and three test plants: alfalfa, cress, lettuce were determined. 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 inhibitory activity of the *H. acutigluma* aqueous methanol extract on shoot growth of test plants 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 allelopathic activity, the test plant species, and concentration of the extracts. Extract of *C. aquatica* caused necrotic symptoms on roots of Italian ryegrass and barnyard grass, implying an irreversible death of roots. These results indicate that all plants may contain allelopathically active substances and that *C. quatica* may contain the greatest herbicidal substance(s).

The aqueous methanol extract of *C. aquatica* was then purified for isolatation of allelopathic substances. A growth inhibitory substance was finally isolated and identified as (-)- loliolide by its spectral data. It is the first report of this substance existing in *Censtrotachys* genus. (-)- Loliolide significantly inhibited the growth of cress and barnyard grass seedlings at concentrations ≥ 0.03 μM. The concentration of (-)- loliolide required to inhibit 50% of the growth 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. The variation of IC_{50} value among the test plants suggest some selectivity of (-)-loliolide effects. The presence of (-)- loliolide in *C. aquatica* and growth inhibitory activity of this substance suggest that (-)- loliolide may contribute the allelopathic potential of *C. aquatica.*

With capability of 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), duckweek and *C. auquatica* are potentially useful bio-resources for weed management in sustainable agriculture.