

Allelopathy and the allelopathic activity of a phenylpropanol from cucumber plants

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Abstract The growth inhibitory effect of cucumber (*Cucumis sativus* L.) plants after crop harvested was investigated. Aqueous methanol extracts of the cucumber plants inhibited the growth of roots and shoots of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), ryegrass (*Lolium multiflorum* L.), timothy (*Pheleum pratense* L.), crabgrass (*Digitaria sanguinalis* L.), *Echinochloa crus-galli* (L.) Beauv and *Echinochloa colonum* (L.) Link, and increasing the extract concentration increased the inhibition. These results suggest that cucumber plants may possess allelopathic activity. The aqueous methanol extract of cucumber plants was divided into ethyl acetate and aqueous fractions, and the growth inhibitory activity of ethyl acetate fraction was greater than that of aqueous fraction. Thus, ethyl acetate fraction was further purified and a main

allopathically active substance in the fraction was isolated and determined as (*S*)-2-benzoyloxy-3-phenyl-1-propanol by spectral data. This substance inhibited root and shoot growth of cress seedlings at concentrations greater than 10 μ M, and the concentration required for 50% inhibition of root and shoot growth was 21 and 23 μ M, respectively. These results suggest that (*S*)-2-benzoyloxy-3-phenyl-1-propanol may contribute to the growth inhibitory effect of cucumber plants and may play an important role in cucumber allelopathy. Thus, cucumber plants may be potentially useful for weed management in a field setting.

Keywords Allelopathy · *Cucumis sativus* · Cucumber · Growth inhibition · Weed management

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Introduction

Cucumber is one of the important crop plants in the gourd family Cucurbitaceae and currently widely cultivated. After crop harvesting, however, cucumber plants (stems, leaves, and roots) are mostly dumped as waste at large expense. The manipulation of wastes is now becoming a serious environmental issue.

It has been observed that some plant species can provide excellent weed suppression after incorporation of their residues into soil (Narwal 1999; Semidey 1999; Caamal-Maldonado et al. 2001). Plants

produce hundreds of secondary compounds, and some of these compounds play an important role in the defense mechanism in the plant rhizosphere as allelopathic substances (Putnam 1988; Gross and Parthier 1994; Inderjit 1996; Duke et al. 2000). The plant rhizosphere is a densely populated zone in which plant roots must compete with invading root systems of neighboring plants for light, water, and minerals. Allelopathic substances have an ability to inhibit the growth of competitive neighbors to improve the survival of the host plants to survive (McCully 1999; Hawes et al. 2000; Bais et al. 2004). However, only a fraction of these root-derived substances have been evaluated their allelopathic activity (Dodge 1987; Einhellig and Leather 1988).

Cucumber plants have also been reported to possess allelopathic activity (Putnam and Duke 1974), and extract of cucumber plants after crop harvested are known to inhibit the germination and growth of *Echinochloa crus-galli* under laboratory and greenhouse conditions (Thi et al. 2008). These findings suggest that cucumber plants may have substances with allelopathic activity, and may be potentially useful for weed management in some agricultural field settings. However, the chemical basis of cucumber allelopathy is not fully understood. In this study, the allelopathic activity of cucumber plants was determined and a growth inhibitor causing the allelopathic effect was isolated and characterized.

Materials and methods

Plant material

Cucumber (*Cucumis sativus* L. cv. Phung Tuong) plants (including stems, leaves, and roots) were obtained from the field in Cuu Long Delta, Vietnam after the final crop harvesting and dried at 50°C for 3 days.

Extraction and bioassay

Dried cucumber plants (100 g dry weight) were extracted with 1 l of 70% of aqueous cold methanol for 2 days. After filtration using filter paper (No. 2; Toyo, Tokyo, Japan), the residue was extracted again with 1 l of cold methanol for 2 days and filtered, and the two filtrates were combined.

An aliquot of the extract (final assay concentration was 0.03, 0.1, or 0.3 g dry weight cucumber equivalent extract ml⁻¹) was evaporated to dryness, dissolved in a 0.2 ml of methanol, and added to a sheet of filter paper (No. 2; Toyo Ltd.) in a 3-cm Petri dish. Methanol was evaporated in a draft chamber. Then, the filter paper in the Petri dishes was moistened with 0.8 ml of a 0.05% (v/v) aqueous solution of Tween 20. After germination in the darkness at 25°C for 24–120 h, 10 seeds of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), ryegrass (*Lolium multiflorum* L.), timothy (*Phleum pratense* L.), crabgrass (*Digitaria sanguinalis* L.), *Echinochloa crus-galli* (L.) Beauv or *Echinochloa colonum* (L.) Link were then placed into the Petri dishes. The length of their shoots and roots was measured after 48 h of incubation in the darkness at 25°C. Methanol (0.2 ml) was added to the filter paper in the Petri dish and evaporated as described above. Control seedlings were then placed into filter paper moistened with the aqueous solution of Tween 20 without the methanol extract.

Separation of extract

Dried cucumber plants were extracted 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, partitioned four times against an equal volume of ethyl acetate, and separated ethyl acetate and aqueous phase. The biological activities of the ethyl acetate and aqueous fractions were determined using the cress bioassay as described above.

Purification of active substance in ethyl acetate fraction

The ethyl acetate fraction was evaporated to dryness and chromatographed on a column of silica gel (60 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). The biological activity of the fractions was determined using a cress bioassay as described above. After evaporation of the active fraction, the residue was purified by a column of Sephadex LH-20 (50 g, Amersham Pharmacia Biotech, Buckinghamshire, UK), and eluted with 20, 40, 60, and 80% (v/v)

aqueous methanol (50 ml per step) and methanol (100 ml). After evaporation of the active fraction, the residue was dissolved 20% (v/v) aqueous methanol (2 ml) and loaded onto reverse-phase C₁₈ Sep-Pak cartridges (Waters). The cartridge was eluted with 20, 40, 60, 80% (v/v) aqueous methanol and methanol (15 ml per step). After evaporation of the active fraction, the residue was finally purified by reverse-phase HPLC (10 mm id × 50 cm, ODS AQ-325; YMC Ltd, Kyoto, Japan) eluted at a flow rate of 2 ml min⁻¹ with 50% aqueous methanol, detected at 220 nm. Inhibitory activity was only found in a peak fraction eluted between 74 and 77 min, yielding an active substance (2.1 mg) as colourless oil. The active substance was characterized by ¹H-NMR and nuclear Overhauser effect spectra.

Results and discussion

Allelopathic activity of cucumber extract

Aqueous methanol extract of cucumber plants inhibited root and shoot growth of all the species used

including weed species (crabgrass, *E. crus-galli*, and *E. colonum*), and increasing the extract concentration increased the inhibition (Fig. 1). The extract obtained from 0.3 g dry weight cucumber plants inhibited the root growth of cress, lettuce, alfalfa, ryegrass, timothy, crabgrass, *E. crus-galli*, and *E. colonum* by 97.7, 90.4, 95.5, 100, 100, 98.8, 96.3, and 90.7%, respectively, and inhibited the shoot growth of cress, lettuce, alfalfa, ryegrass, timothy, crabgrass, *E. crus-galli*, and *E. colonum* by 95.3, 80, 93.2, 100, 87, 88, 58, and 52%, respectively. Thus, the extract of cucumber plants contains a growth inhibitory substance and cucumber plants appear to possess an allelopathic activity.

The aqueous methanol extract of cucumber plants was divided into ethyl acetate and aqueous fractions as described in the section of “Separation of extract” in “Materials and methods”, and their biological activities were determined. Both fractions suppressed root and shoot growth of cress seedlings. However, inhibitory activity of the ethyl acetate fraction was greater than that of the aqueous fraction (Fig. 2). Thus, isolation of allelopathic active substances proceeded using the ethyl acetate fraction.

Fig. 1 Effects of aqueous methanol extract of cucumber plants on root and shoot growth of cress, lettuce, alfalfa, ryegrass, timothy, crabgrass, *E. crus-galli*, and *E. colonum*. Concentrations of tested samples corresponded to the extract obtained from 0.03, 0.1, and 0.3 g dry weight cucumber plants. Means ± SE from three independent experiments with 10 plants for each determination are shown. Asterisk indicates significant difference between control and treatment: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$

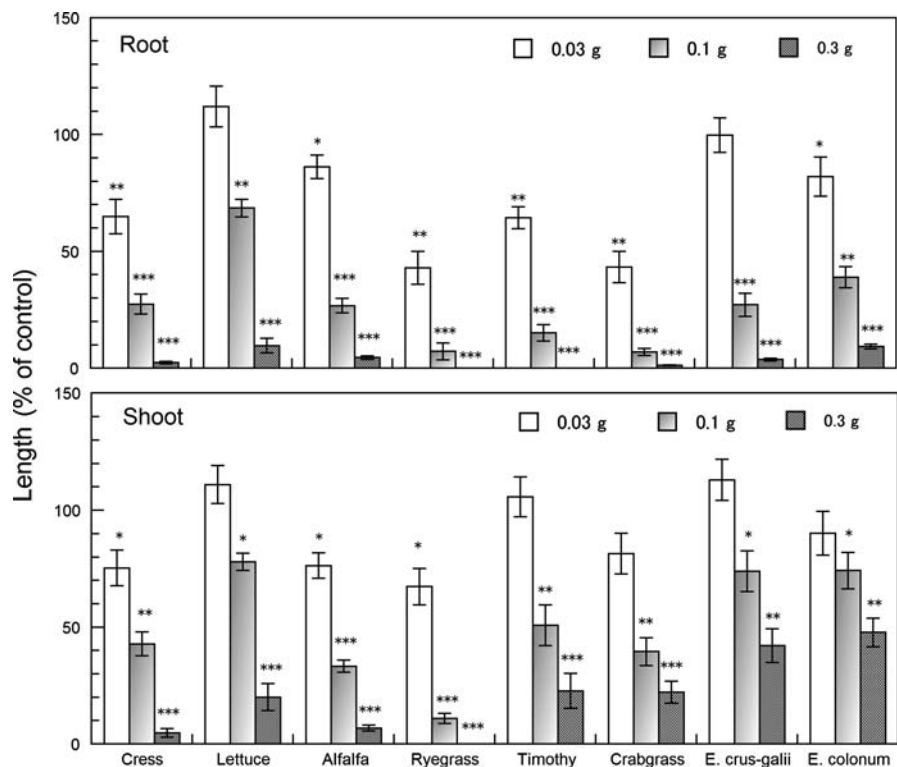
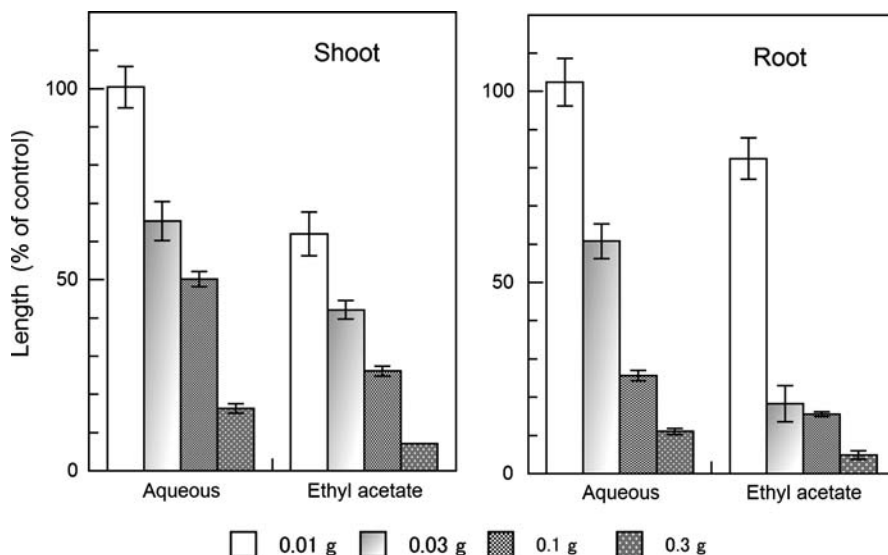


Fig. 2 Effects of ethyl acetate and aqueous fraction isolated from an aqueous methanol extract of cucumber plants on root and shoot growth of cress. Concentrations of tested samples corresponded to the extract obtained from 0.01, 0.03, 0.1, and 0.3 g dry weight cucumber plants. Other detail as for Fig. 1



Identification of allelochemical and biological activity

The ethyl acetate fraction was purified by columns of silica gel and Sephadex LH-20, C₁₈ Sep-Pak cartridges, and HPLC, and an active substance was isolated as colourless oil. The ¹H NMR (CD₃OD, 400 MHz) spectrum of the substance showed; δ 2.85 (1H, dd, *J* = 13.7, 8.3 Hz), 3.02 (1H, dd, *J* = 13.7, 6.3 Hz), 3.64 (2H, d, *J* = 5.4 Hz), 4.33 (1H, m), 7.16 (1H, tt, *J* = 8.8, 1.8 Hz), 7.26 (4H, m), 7.41 (2H, dd, *J* = 7.4, 7.4 Hz), 7.49 (1H, tt, *J* = 7.4, 1.5 Hz), and 7.72 ppm (2H, dd, *J* = 7.4, 1.5 Hz). The specific rotation of the substance was $[\alpha]_D^{24} -65.8^\circ$ (*c* = 0.087, MeOH). From the comparison of these data with those reported in the literature (Yaoita and Kikuchi 1997), the substance was identified as (*S*)-2-benzoyloxy-3-phenyl-1-propanol (Fig. 3). This substance was first isolated from *Petasites japonicus* as a new phenylpropanoid by Yaoita and Kikuchi (1997). However, it has not been reported so far that (*S*)-2-benzoyloxy-3-phenyl-1-propanol has growth inhibitory activity.

The biological activity of (*S*)-2-benzoyloxy-3-phenyl-1-propanol was determined with cress seedlings (Fig. 4). At concentration greater than 10 μM, the substance inhibited the growth of the cress roots and shoots. When percentage length of cress plants was plotted against logarithm of the concentrations, concentration-response curves were linear between 20 and 80% inhibition. The concentration required for 50% inhibition of the cress roots and shoots in the

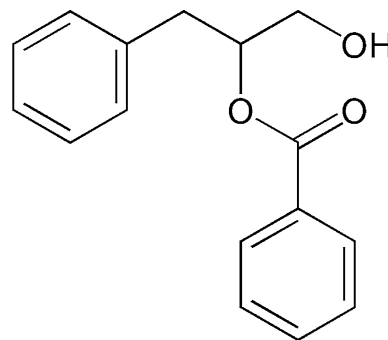


Fig. 3 Chemical structure of (*S*)-2-benzoyloxy-3-phenyl-1-propanol

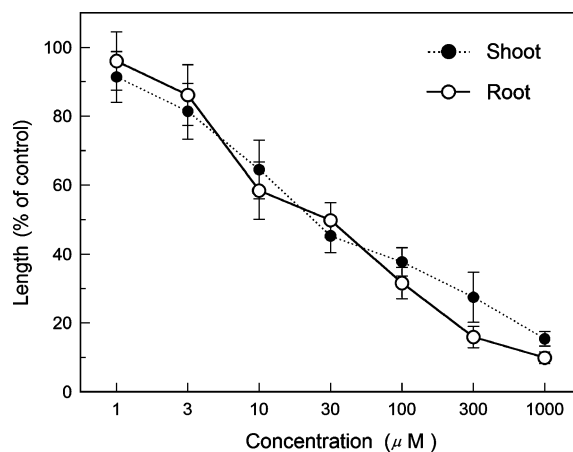


Fig. 4 Effects of (*S*)-2-benzoyloxy-3-phenyl-1-propanol on the root and shoot growth of cress seedlings. Other details as for Fig. 1

assay (defined as I_{50}), as determined by a logistic regression analysis, was 21 and 23 μM , respectively.

Allelopathy of cucumber

An aqueous methanol extract of cucumber plants inhibited the growth of all species tested (Fig. 1), which indicates that cucumber plants may possess allelopathic activity. A growth inhibitory substance was isolated from the aqueous methanol extract and its chemical structure was determined as (*S*)-2-benzoyloxy-3-phenyl-1-propanol. This substance inhibited the growth of cress at concentrations greater than 10 μM for roots and shoots (Fig. 2). Under the certain conditions allelopathic compounds are released into the plant rhizosphere, either as exudates from living tissues or by decomposition of plant residues in sufficient quantities to inhibit the growth of neighboring plants (Rice 1984; Putnam 1988; Seigler 1996; Einhellig 1999). Therefore, a growth inhibitory substance, (*S*)-2-benzoyloxy-3-phenyl-1-propanol may provide the competitive advantage for root establishment of cucumber plants in the rhizosphere as an allelopathic substance which is involved in the defense mechanism through the inhibition of the growth of neighboring plant species that can be competitive with cucumber plants.

Many attempts have been made to exploit allelopathy of plants for weed control in a variety of agricultural settings (Inderjit 1996; Seigler 1996; Duke et al. 2000). Synthetic chemical herbicides may continue to be a key component in many integrated weed management systems, but controlling weeds through allelopathy is one strategy to reduce herbicide dependency (Putnam 1988; Einhellig 1996; Weston 1996; Duke et al. 2000).

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