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Assessment of the Allelopathic Potential of Cucumber Plants

Ho Le THI and Hisashi KATO-NOGUCHI

Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761–0795, Japan

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The allelopathic potential of cucumber (*Cucumis sativus* L.) plants after their crop harvest was investigated. Aqueous methanol extracts inhibited the growth of shoots of cress (*Lepidium sativum* L.), alfalfa (*Medicago sativa* L.) and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv). A potent growth inhibitory substance in the extract was isolated by chromatographic separation of silica gel and Sephadex columns, Sep-Pack cartridge and HPLC. At concentration of 3 μg mL^{-1}, this substance inhibited cress shoot growth by 23% of shoot growth of control plants. These results suggest that cucumber plants may contain at least one growth inhibitory substance, which may be released into the environment, either as exudates from living plant tissues or leachates from residues of the plants, and may act as allelochemicals to neighboring plants.

Keywords : allelopathy, *Cucumis sativus*, cucumber, growth inhibition, weed management

INTRODUCTION

The negative impacts of commercial herbicide use on the environmental contamination make necessary to diversify weed management options (Putnam, 1988; Weston, 1996; Einhellig, 1996). Controlling weeds through allelopathy is one of the strategies to reduce commercial herbicide dependency (Rice, 1984; Putnam, 1988; Duke et al., 2000). It has been observed that many 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 a hundred of secondary compounds, and some of these compounds are phytotoxic and have potential as herbicides or templates for new herbicide classes (Putnam, 1988; Gross and Parthier, 1994; Inderjit, 1996; Duke et al., 2000). However, only a fraction of these compounds have been evaluated their herbisscidal or allelopathic activity (Dodge, 1987; Einhellig and Leather, 1988).

Cucumber (*Cucumis sativus* L.) is one of the major crops in Vietnam. After the crop harvesting, their plants (stems, leaves and roots) are mostly discarded as waste. It is therefore of interest to assess the allelopathic potential of cucumber plant waste for possible weed control purpose. In this study, the allelopathic potential of Vietnam local cucumber variety was determined and allelopathic substance in aqueous methanol extract of the cucumber plants was isolated.

MATERIALS AND METHODS

Plant materials

Cucumber (*C. sativus* L. cv. Phung Tuong) plants (including stems, leaves and roots, exclud-

Corresponding author : Hisashi Kato-Noguchi, fax : +81–87–891–3086,
e-mail : hisashi@ag.kagawa-u.ac.jp

ing flowers and fruit) were obtained from the field in Cuu Long Delta, Vietnam after the final crop harvest and dried at 50ºC 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 two days. After filtration using filter paper (No. 2; Toyo, Tokyo, Japan), the residue was extract again with 1 L of cold methanol for two days and filtered, and the two filtrates were combined.

An aliquot of the extract (final assay concentration was 0.1 or 0.3 g dry weight equivalent extract mL^{-1}) was evaporated to dryness, dissolved in a small volume of methanol, added to a sheet of filter paper (No. 2; Toyo Ltd.) in a 2.8-cm Petri dish and dried. 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, and 10 seeds of cress (*Lepidium sativum* L.), alfalfa (*Medicago sativa* L.) or barnyardgrass (*Echinochloa crus-galli* (L.) Beauv) were sown on it. After 48 h of incubation in the darkness at 25ºC, the length of their shoots was measured. Control seedlings were sown on the filter paper moistened with the aqueous solution of Tween 20.

Purification of inhibitor

Dried cucumber plants were extracted as described above and extract was concentrated at 40ºC *in vacuo* to produce an aqueous residue. The residue was adjusted to pH 7.0 with 1 M phosphate buffer and partitioned four times against an equal volume of ethyl acetate. The ethyl acetate phase was evaporated to dryness after drying over anhydrous Na_2SO_4. The crude material was then chromatographed on a column of silica gel (60 g, silicagel 60, 70–230 mesh; Merck, Darmstadt, Germany), 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 C_{18} 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, and detected at 220 nm.

RESULTS AND DISCUSSION

Aqueous methanol extract of cucumber plants inhibited shoot growth of cress, alfalfa and barnyardgrass and increasing the concentration of the extract increased the inhibition (Fig. 1). At the concentration of the extract obtained from 0.3 g dry weight cucumber plants, the shoot length of cress, alfalfa and barnyardgrass was 6.0, 6.5 and 8.3% of that of control plant shoots, respectively. These results suggest that this cucumber plants may possess allelopathic activity.

The aqueous methanol extract of cucumber plants was subjected to a chromatography of silica gel, and the biological activity of the eluted fractions was evaluated by the cress bioassay. Growth inhibitory activity was detected in fraction obtained by elution with 70 and 80% ethyl acetate in *n-hexane.* These active fractions were combined and further purified by a column of Sephadex LH-20. Growth inhibitory activity was detected in fraction obtained by elution with 40% aqueous methanol. The active residue was passed through a C_{18} Sep-Pak cartridge and the activity was detected in fraction eluted with 40% aqueous methanol. The active residue was finally purified by reverse-phase HPLC and growth inhibitory activity was found in a peak fraction eluted between 72–76 min (Fig. 2), yielding an active substance (0.3 mg). This substance inhibited the growth of

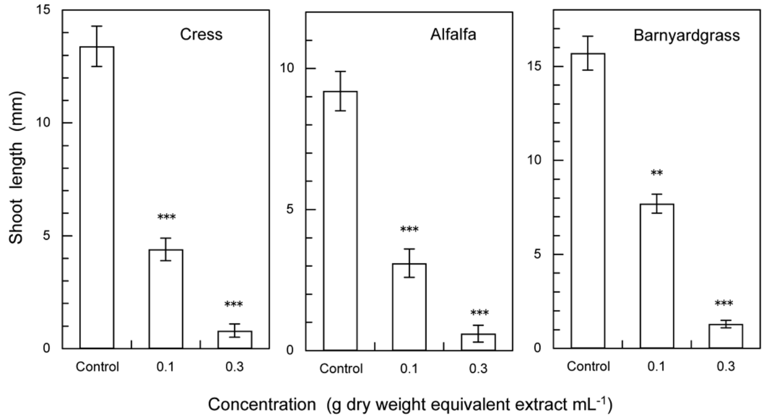

Fig. 1 Effect of aqueous methanol extract of cucumber plants on the shoot growth of cress, alfalfa and barnyardgrass seedlings. Concentrations of tested samples corresponded to the extract obtained from 0.1 and 0.3 g dry weight cucumber. Means ± SE from 3 independent experiments with 10 plants for each determination are shown. ** Significant different at $P < 0.01$, *** Significant different at $P < 0.001$ level as compared with control seedlings.

cress shoots. At concentration of 1 and 3 μg mL^{-1} of the substance, the length of cress shoots became 34 and 23% of that of control plant shoots, respectively (Fig. 3). For identification and characterization of the growth inhibiting substance, large-scale purification of the inhibitory substance in cucumber plants is now underway.

Many secondary plant metabolites are considered to be associated with the allelopathic effects of plants (Rice, 1984; Putnam and Tang, 1986; Inderjit and Nishimura, 1999). Under certain conditions, these compounds are released into the environment, either as exudates from living tissues or leachates from residues in sufficient quantities to affect the neighboring or successional plants

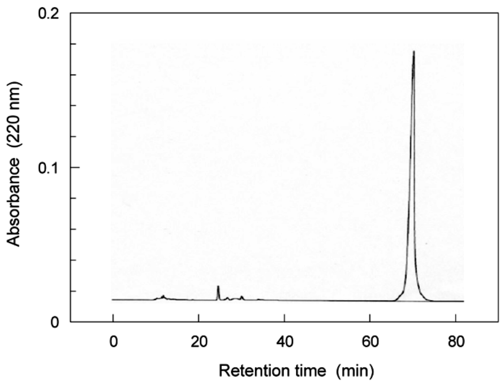

Fig. 2 HPLC chromatogram of active substance purified from cucumber extract.

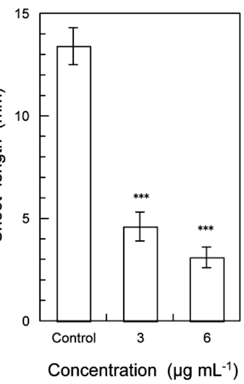

Fig. 3 Effect of a growth inhibitor purified from cucumber on the growth of cress shoots. Means ± SE from 3 independent experiments with 10 plants for each determination are shown. *** Significant different at $P < 0.001$ level as compared with control seedlings.

(Rice, 1984; Putnam, 1988; Einhellig, 1999).

In the present research, methanol extract of cucumber plants was found to have allelopathic potential and a growth inhibitory substance was isolated from the extract by chromatographic separations, although the active component was not characterized. These results suggest that cucumber plants probably contain a growth inhibitory substance that may be released into the environment, either as exudates from living tissues or leachates from residues after decomposition of the cucumber plants, and act as allelochemical to other plants. Therefore, it is possible that cucumber plant waste after crop harvest could be use as a weed-suppressive residue in a field setting.

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