INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13S–019/2013/15–6–1359–1362
http://www.fspublishers.org

Full Length Article

Allelopathic Activity of Some Herb Plant Species

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Abstract

Some plant species have been used traditionally as herb because of their bioactive properties. These properties may allow for some of those herb plants to have allelopthic activity. Therefore, allelopathic activity of sixteen herbs was determined by sandwich method. All plants except for rosemary inhibited significantly lettuce root growth. Among them horseradish had the greatest inhibitory activity and completely inhibited the lettuce root growth. When lettuce seeds were grown on agar medium with living horseradish plants by plant-box method, the growth of lettuce roots was inhibited. The inhibition of lettuce roots was significantly correlated with the distance between horseradish and lettuce. The inhibition was the maximum at the nearest position from horseradish and the least at the far position from horseradish. Thus, some allelopathic substances may be secreted from horseradish, diffused in the medium and inhibited the lettuce root growth, although allelopathic substances have not yet determined. This research suggests that most herb plants have allelopathic activity and maximum activity was recorded by horseradish. Living horseradish may also secret unknown allelopathic substances into the medium. Thus, horseradish may be one of the promising candidates for the sources of novel biologically active compounds as well as candidates for intercropping crops and/or soil additive materials to control weeds in variety of agricultural settings.

Keywords: Allelopathy; Biological activity; Growth inhibitor; Herb; Horseradish

Introduction

The negative impacts of commercial herbicide use on the environment make it desirable to diversify weed management options (Putnam, 1988; Weston, 1996; Narwal, 1999). Some plants provided excellent weed control in intercropping or as soil additives (Weston, 1996; Narwal, 1999; Semidey, 1999; Caamal-Maldonado *et al.,* 2001). Plants produce hundreds of secondary metabolites, and some of these compounds show allelopathic activity such as growth inhibitory effects on other plants (Putnam, 1988; Gross and Parthier, 1994; Inderjit, 1996; Duke *et al.,* 2000; Macías *et al.,* 2007). Thus, allelopathy is one strategy to reduced commercial herbicide dependency in practical weed control programs (Farooq *et al*., 2011). In addition, natural compounds are considered to be more environmentally benign than most synthetic herbicides (Duke *et al.,* 2000; Macías *et al.,* 2007). However, only a small percentage of the plant species have been phytochemically analyzed despite the fact that plants provide a rich source of biologically active compounds (Hostettmann and Wolfender, 1997; Ambrosio *et al.,* 2006).

Some plants have been used traditionally as herb to treat certain human diseases and other symptoms because of their bioactive properties (Diamond *et al.,* 2000; Ling *et al.,* 2009; Ogbuewu *et al., 2*011). Those plants probably contain some bioactive compounds. Thus, herbs may be not only candidates for the sources of novel biologically active compounds, but also candidates for intercropping crops and/or soil additive materials to control weeds in the sustainable agriculture. This study was, therefore, conducted to determination the allelopathic activity of sixteen herb plants and the investigation of possible secretion of allelopahic substance from the most active herb plants.

Materials and Methods

Plant Materials

Leaves of sixteen herb plants (Table 1) were collected in the research farm of Prefectural University of Hiroshima in Shobara, Japan. The leaves were dried at 40°C for 24 h in oven. Lettuce (*Lactuca sativa* L.) was chosen as test plant for the bioassays.

Bioassay by Sandwich Method

Allelopathic activity of 16 herb plants was determined by sandwich method developed by Fujii *et al.* (2004). Every 50 mg of dried herb leaves was placed into a well of six-well multi-dish plastic plates (BD Biosciences, NJ, USA). The first layer of agar (5 mL, 0.5% w/v, Nacalai, Kyoto, Japan) was then added into the wells and the plant leaves rose to the surface of agar. After gelatinization of the agar, the second layer of agar (5 mL) was added onto the plant leaves. After gelatinization of the second agar layer, five seeds of lettuce were arranged on the agar surface. Then, the six-well multi-dish plastic plate was coved with a plastic film and kept in darkness at 25°C. Control lettuce seeds were sown onto the second layer of agar without plant leaves between agar layers and treated exactly same as described above. After incubation for three days, root length of lettuce was measured and the percentage length of the roots was determined by reference to control lettuce roots as the formula: [(root length of lettuce incubated with herb)/control root length of lettuce] x 100. The bioassay was repeated four times. Significant difference between treatment and control plants was examined by Tukey's-test.

Bioassay by Plant-box Method

Allelopathic activity of horseradish was determined by plant-box methods as described by Fujii *et al.* (2007). Horseradish grown in sand culture for two months was washed with distilled water. One horseradish plant was then placed into the root zone-separating tube in a plant-box (Mggenta Corporation, Chicago, USA, 60×60 mm (width) x100 mm (height). The tube containing horseradish was fixed at the corner of the box and agar (0.5% w/v) was then poured into the box and the zone-separating tube in the 65 mm height. Horseradish roots were held with agar in the tube. After gelatinization of agar, lettuce seeds were arranged on agar surface. Lettuce seeds in the plant-box were incubated in darkness at 20°C. The distilled water was added on the agar surface to keep moisture every day. Control lettuce seeds were sown onto the agar in plant-box as describe above but the tube did not contain horseradish. After incubation for five days, root length of lettuce was measured and the percentage length of the roots was determined by reference to control lettuce roots as the formula described above. The bioassay was repeated four times.

Results

Inhibitory Activity of Herb Leaves

All plants except for rosemary inhibited lettuce root growth significantly (Fig. 1; Table 1). Leaves of fennel, horseradish, lavender and mallow inhibited the root growth less than 30% of control root growth. Chamomile, Jerusalem artichoke, autumn sage, lamb's-ear and myoga inhibited the root growth less than 40% of control root growth, and artichoke, meadow sage, thyme, wild strawberry and Japanese pepper inhibited less than 60% of control.

Inhibitory Activity of Horseradish Plants

Inhibitory activity of living horseradish plants was determined by plant-box method (Fujii *et al.,* 2007). Horseradish plants inhibited the growth of lettuce roots, and the inhibition was the greatest at the nearest distance position from horseradish and the least at the far distance position from horseradish (Fig. 2). The inhibitory activity of horseradish was significantly correlated with the distance from horseradish ($r = 0.84$, $P<0.01$).

Discussion

Inhibitory activities of leaves of 16 herb plants differed among plant species even in the same family (Table 1; Fig. 1). However, horseradish had the greatest inhibitory activity of all and inhibited the root growth completely. This result indicates that all herb plants except for rosemary possess allelopathic activity and some allelopathic substances may diffuse from those leaves into agar medium and inhibit the growth of lettuce roots. Thus, most herb plants may be candidates for soil additive materials to control weeds.

Table 1: Plant materials

Common name	Scientific name	Family
Fennel	*Foeniculum vulgare* Mill.	Apiaceae
Artichoke	*Cynara scolymus* L.	Asteraceae
Chamomile	*Matricaria recutita* L.	Asteraceae
Jerusalem artichoke	*Helianthus tuberosum* L.	Asteraceae
Horseradish	*Armoracia rusticana* G. Gaertn., B. Mey. and Scherb.	Brassicaceae
Autumn sage	*Salvia greggii* A.Gray	Lamiaceae
Lamb's-ear	*Stachys byzantina* K.Koch	Lamiaceae
Lavender	*Lavandula angustifolia* Mill.	Lamiaceae
Lemon balm	*Melissa officinalis* L.	Lamiaceae
Meadow sage	*Salvia pratensis* L.	Lamiaceae
Thyme	*Thymus vulgaris* L.	Lamiaceae
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae
Mallow	*Malva sylvestris* L.	Malvaceae
Wild strawberry	*Fragaria vesca* L.	Rosaceae
Japanese pepper	*Zanthoxylum piperitum* (L.) DC.	Rutaceae
Myoga	*Zingiber mioga* (Thunb.) Roscoe	Zingiberaceae

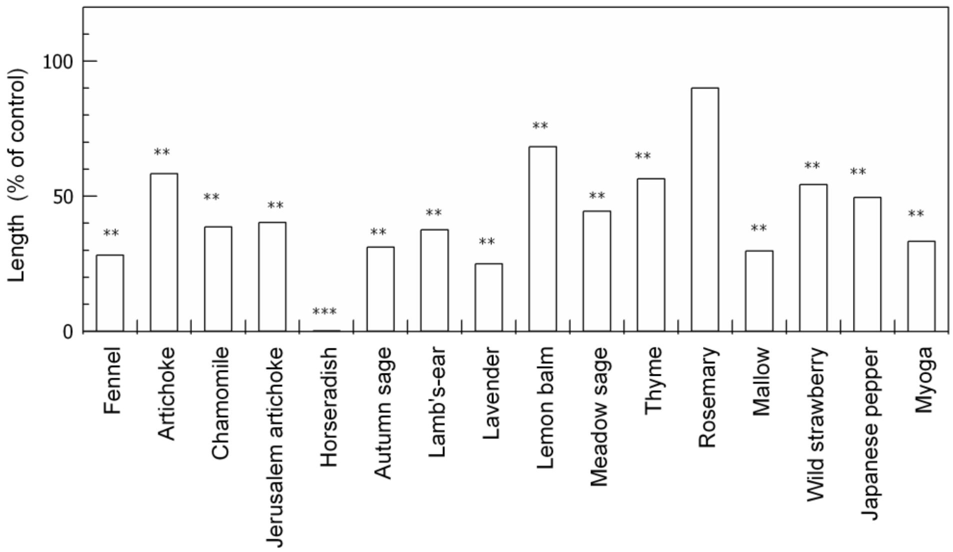

Fig. 1: Effects of leaves of sixteen herb plants on lettuce root growth. Inhibitory activity of 50 mg of dry leaves was determined by sandwich method. The bioassay was repeated four times. Asterisk indicates significant difference between control and treatment: **, $P < 0.01$, ***, $P < 0.001$

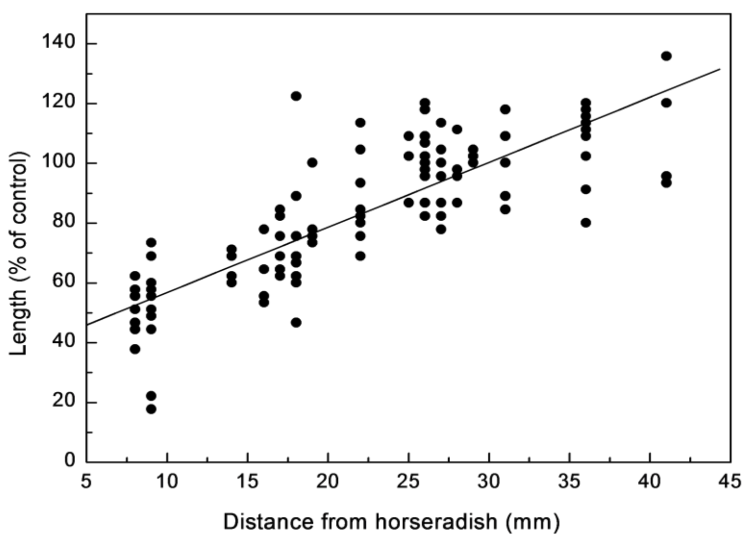

Fig. 2: Effect of living horseradish on lettuce root growth. Inhibitory activity of living horseradish was determined by plant-box method. Horseradish plant was transplanted onto agar medium and lettuce seeds was then placed on the medium at diffrect distanse from horseradish and grown at 20°C with a 12-h photoperiod for five days. Each solid circle indicates that root length and disctanse from horseradish of lettuce seedling

When lettuce seeds were grown on agar medium together with living horseradish plants, the growth of lettuce roots was inhibited (Fig. 2). Lettuce may grow with horseradish without competition for nutrients, because nutrients are considered to be unnecessary during the germination stage of seeds where most nutrients are withdrawn from seed reserves (Fuerst and Putnam, 1983). These results suggest that the inhibitory effect of horseradish on lettuce may not be due to competitive interference for nutrients, but rather due to an allelopathic effect. It is suggested that, although mechanisms of the exudation are not well understood, plants are able to secrete a wide variety of compounds from root cells by plasmalemma-derived exudation, endoplasmic-derived exudation and proton-pumping mechanisms (Hawes *et al.,* 2000; Bais *et al.*, 2004). Through the exudation of compounds, plants are able to inhibit the growth of competing plant species (McCully, 1999; Hawes *et al.*, 2000; Bais *et al.,* 2004). Therefore, horseradish plants may secrete some allelopathic substances into the agar medium. The concentrations of those allelopathic substances are probably high at the nearest distance position from horseradish and low at the far distance position because of diffusion of the substances from horseradish. The results indicate that horseradish has strong allelopathic activity (Fig. 1) and may secrete some allelopathic substances into the neighboring environments (Fig. 2). Thus, horseradish may be candidate for the sources of novel biologically active compounds and work as intercropping crop to control weeds. Allyl isothiocyanate was fond in horseradish as a bioactive compound (Cole, 1976). However, there has been no information in literatures on alleopahtic substanses secreted from horseradish plants. The identification of those allelopathic substances should be investigated further.

In conclusion, allelopathic activity of sixteen herbs was determined by sandwich method and horseradish had the greatest inhibitory activity. Allelopathic potential of living horseradish plants was also determined by plant-box method and the horseradish inhibited the growth of lettuce. The present research suggests that horseradish contains potent allelopathic substances and horseradish plants secrete allelopathic substances into the medium. Thus, horseradish may be one of the promising candidates for the sources of novel biologically active compounds as well as candidates for intercropping crops and/or soil additive materials to control weeds in variety of agricultural settings.

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(Received 18 March 2013; Accepted 08 July 2013)