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Seasonal Variation in Allelopathic Potential of *Artemisia princeps* var. *orientalis* on Plants and Microbes

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We investigated seasonal variations in allelopathic potential of *Artemisia princeps* var. *orientalis*. Aqueous and methanol extracts and volatile substances were prepared in the laboratory from samples collected monthly (April through October). Their impacts were then assessed on the germination and seedling growth of *Lactuca sativa* and *Achyranthes japonica*. The allelopathic potential varied with the time of sample collection and the concentration tested. For example, germination of *L. sativa* was not inhibited by the aqueous extract but seedling growth (shoots and roots) was, with its seasonal effect being significant. For *A. japonica*, seed germination was not inhibited at lower concentrations (except for August samples). However, at higher concentrations and in certain months (especially July), germination was more negatively affected. The degree of seedling growth inhibition also differed by month and by extract concentration, with roots being impacted more than shoots. Volatile substances also had a time-dependent influence on the germination and seedling elongation of *A. japonica*. In a separate experiment, the ethyl-acetate and water fractions of a crude methanol extract were prepared monthly from *A. princeps* var. *orientalis*. Here, we examined their antimicrobial activities against three gram-positive bacteria (*Bacillus cereus, Bacillus subtilis,* and *Staphylococcus aureus*), two gram-negative bacteria (*Escherichia coli* and *Pseudomonas fluorescens*), and one lactic acid bacterium, *Lactobacillus plantarum*. The ethyl-acetate fraction that was sampled in September was remarkably potent against *B. cereus* and *B. subtilis*, whereas the water fraction collected in August and September showed great antimicrobial activity against the gram-positive and -negative bacteria. In contrast, *L. plantarum* was not inhibited by the water fraction, regardless of the sampling month. Likewise, the ethyl-acetate and water fractions collected in April and October had the lowest levels of antimicrobial activity.

Keywords: allelopathic potential, *Artemisia princeps* var. *orientalis*, seasonal variation

Artemisia is one of the largest genera in the family Compositae. Some species are aromatic, and are used as herbs, spices, and folk medicines (Wagner, 1977). *Artemisia princeps* var. *orientalis* is a common weed but an important Korean medicinal plant used as a styptic. The water-soluble and volatile chemicals of this species have an allelopathic effect on some plants and microbes, either inhibiting or stimulating their processes, depending on the concentration and receptor species (Yun, 1991). Yun and Choi (2002) have reported that aqueous extracts from this species also influence mycorrhizal colonization and mycorrhizal plant growth.

The allelopathic potential of some genera may vary according to their developmental stage. For example, Wardle et al. (1993) have shown that the aqueous extract and leachates collected at the rosette stage in nodding thistle (*Carduus nutans*) are the most allelopathic for neighboring seed germination. Kim (1996) has also reported seasonal variations in the concentration and composition of monoterpenes from *A. princeps* var. *orientalis* sampled in May and June. Likewise, seeds of *Artemisia annua* that originate from a wild population in Japan exhibit seasonal and positional variations in their levels of artemisinin, artemisinic acid, arteannuin B, and artemisitene (Kawamoto et al., 1999). Agerbirk et al. (2001) have demonstrated seasonal fluctuations (August to November) in leaf glucosinolates and degrees of insect resistance by *Barbarea vulgaris* ssp. *arcuata* (Brassicaceae).

No research has been reported on seasonal changes in the allelopathic potential of *A. princeps* var. *orientalis*. Therefore, the objective of the current study was to analyze the effect of its extracts and volatile substances on seed germination, seedling growth, and antimicrobial activity.

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MATERIALS AND METHODS

Plants

Foliage of *A. princeps* var. *orientalis* was collected monthly from April to October (2001, 2002) in the field near Sunchon National University, Suncheon of Korea. For our test plants, seed of *Achyranthes japonica* was collected the year before, while seed for *Lactuca sativa* was purchased.

Microorganisms

Our test microorganisms included three gram-positive bacteria, *Bacillus cereus* ATCC 27348, *Bacillus subtilis* ATCC 9327 and *Staphylococcus aureus* ATCC 13301; two gram-negative bacteria, *Escherichia coli* ATCC 15489 and *Pseudomonas fluorescens* ATCC 11250; and one lactic acid bacterium, *Lactobacillus plantarum* ATCC 8014. The gram-positive and negative bacteria were cultured on nutrient broth agar, while *Lactobacillus* was reared on an MRS broth agar.

Determining Germination and Seedling Growth

Aqueous extracts of *A. princeps* var. *orientalis* were prepared according to the methods of Yun (1991). Briefly, fresh foliage was cut into 1-cm pieces and 1,000 mL of distilled water was added to 200 g of the tissue, all at 20°C. After 24 h, the extract was filtered through a sieve. This aqueous extract was considered a 100% stock solution, so it was diluted with distilled water to 50% and 10% test concentrations. Distilled water also served as our control. To test the effect of extract concentration on germination and seedling development, one layer of filter paper was placed in a glass Petri dish and wetted with one of the extracts. Fifty seeds were then evenly arranged in each dish. The dishes were placed in a growth chamber maintained at 25°C during the day (14 h) and 18°C at night (10 h). After 4 to 6 days, germination percentages and seedling elongation were recorded.

To assess the time-dependent effect of volatile substances on germination and radicle elongation, 50 seeds of the test plants (*Achyranthes* and *Lactuca*) were placed in a 1.8-L glass chamber on filter paper that was layered on moist, absorbent cotton. Different quantities of sliced *Artemisia* leaves (fresh weights of 5, 10, 15, 20, 25, or 30 g) were collected each month and placed in a glass beaker within the chamber. The glass chambers were then covered with vinyl wrap and placed in a growth chamber that was maintained at 25°C during the day (14 h) and 18°C at night (10 h). Ratios of germination and elongation were calculated according to the method of Kil and Yun (1992).

Antimicrobial Assay

Approximately 100 g of monthly-collected, air-dried *Artemisia* foliage was added to 500 mL methanol and homogenized for 20 min. After being held at room temperature for 30 min, the solution was filtered through Whatman No. 2 paper. Methanol extract was extracted three times with 500 mL hexane, then concentrated to produce the hexane-extracted fraction. The remaining water extract was successively fractionated with diethyl ether and ethyl acetate (i.e., the ether- and ethyl acetate-extracted fractions); the residue was considered the water fraction. Each fraction was concentrated in vacuo to 30 mL. To determine antimicrobial activity, each bacterial strain was grown in a nutrient broth at 30°C for 18 to 24 h prior to testing, then subcultured three times for 18 to 24 h. The turbidity of the bacterial cell suspension was adjusted with the same sterile broth to 0.3 optical density (OD) units at 660 nm. For the disk-plate test, 0.1 mL of the bacterial cell suspension was poured uniformly on the nutrient broth agar in each plate. Paper disks containing the 200-μL extracts were then carefully placed on the seeded Petri dishes. The diameter of the inhibition zone was measured (in mm) after the plates were incubated at 37°C or 30°C for 24 to 48 h, depending on the strain (Bauer et al., 1966).

RESULTS AND DISCUSSION

Seasonal Variations in Allelopathic Potential of *A. princeps* var. *orientalis* on Plants

The genus *Artemisia* possesses allelopathic potential. For example, Funke (1943) has suggested that the alkaloid absinthium of *Artemisia absinthium* is the main cause for the lack of growth in neighboring plants. Volatile materials produced by the leaves of *Artemisia californica* inhibit root growth in *Cucumis* and *Avena* seedlings (Muller et al., 1964), while the aqueous leaf extracts of *Artemisia tridentata*, *Artemisia cana*, *Artemisia nova*, and *Artemisia tripartita* stop the germination of some species (Reid, 1964). Water-soluble and volatile chemicals from *A. princeps* var. *orientalis* exhibit phytotoxic and antimicrobial activity, with intensity depending on their concentrations and receptor species (Yun, 1991).

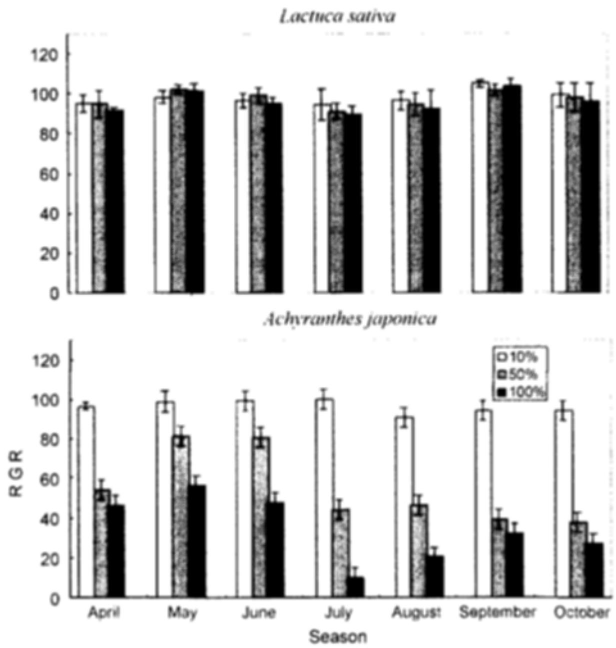

Figure 1. Relative germination ratio (RGR ± SD) of two test species sown in Petri dishes, as affected by different concentrations of aqueous extracts collected monthly from *A. princeps* var. *orientalis*.

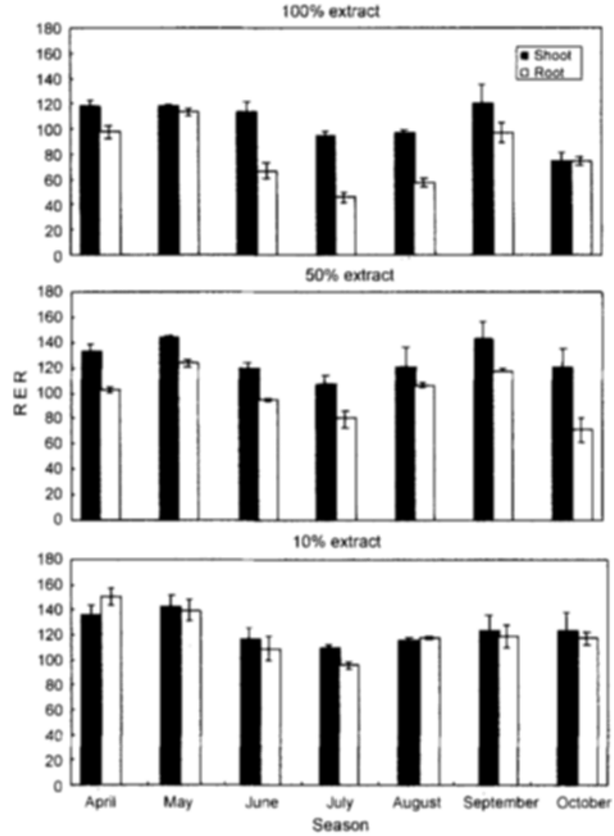

Figure 2. Relative elongation ratio (RER ± SD) of *L. sativa* sown in Petri dishes, as affected by different concentrations of aqueous extracts collected monthly from *A. princeps* var. *orientalis*.

In this study, we tested how extracts from *A. princeps* var. *orientalis* collected monthly influenced seed germination and seedling elongation in two target species. Whereas different concentrations of aqueous extracts did not affect germination by *L. sativa*, concentrations of 50% to 100% did prevent seeds of *A. japonica* from germinating (Fig. 1). Furthermore, the elongation of *L. sativa* and *A. japonica* seedlings was not inhibited by the 10 and 50% extracts, regardless of the month samples were collected (Figs. 2 and 3) These results agree with those from other research, in which germination and growth were higher for seedlings treated with low concentrations, compared with the control (Rice, 1984). Furthermore, root elongation for our two species was reduced only by the 100% extract concentration.

Germination rates varied for *A. japonica* according to the month in which *A. princeps* var. *orientalis* was sampled. However, no significant difference in performance was observed with *L. sativa*. In contrast, seedling elongation was significantly influenced by collection time for both species tested (Figs. 2 and 3). Richardson and Williamson (1988) have also demonstrated seasonal variations in allelopathic effects of shrubs of sand pine dcrub on pines and grasses of the sandhills. We tested the effect of volatile substances from *A. princeps* var. *orientalis* on *A. japonica*. There, concentrations of 20, 25, and 30 g/1.8 L (prepared each month) effectively inhibit germination. In our analysis, April was the only month without such an effect, while samples collected in August, July, and September (in that order) were the most detrimental (Fig. 4). Ahn et al. (2003) have reported that yields of lacquer and aromatic essential oils from *Dendropanax morbifera* are highest in August. In the current study, radicle elongation of *A. japonica* was affected by the timing of collection for volatile substances, being more inhibited later in the growing season (Fig. 5). This was in contrast to results by Wardle et al. (1993), who showed that it was the younger (rosette) plants of nodding thistle that were most phytotoxic (especially against radicle growth).

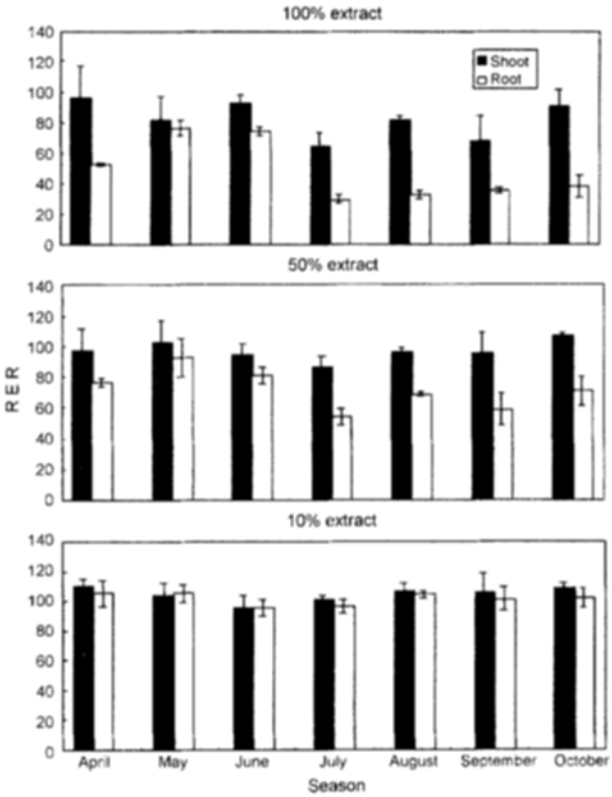

Figure 3. Relative elongation ratio (RER ± SD) of *A. japonica sativa* sown in Petri dishes, as affected by different concentrations of aqueous extracts collected monthly from *A. princeps* var. *orientalis*.

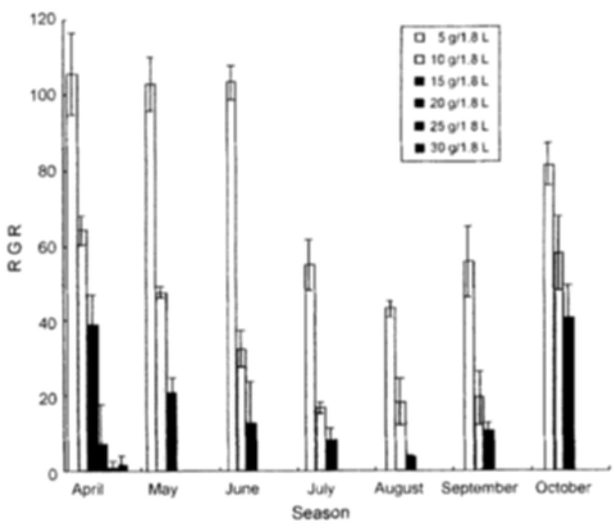

Figure 4. Relative germination ratio (RGR ± SD) of *A. japonica* sown in a glass chamber, as affected by different concentrations of volatile substances collected monthly from *A. princeps* var. *orientalis*.

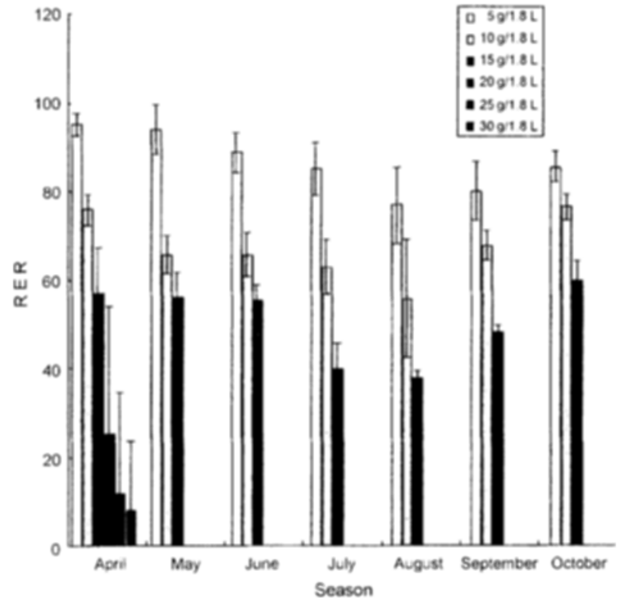

Figure 5. Relative elongation ratio (RER ± SD) of *A. japonica* sown in a glass chamber, as affected by different concentrations of volatile substances collected monthly from *A. princeps* var. *orientalis*.

Seasonal Variation in Allelopathic Potential of *A. princeps* var. *orientalis* on Microbes

The antimicrobial activities of our ethyl-acetate and water fractions differed by collection month in their effects on bacterial growth. The water fraction did not form a clear zone on *L. plantarum* regardless of the month when samples were collected. In contrast, extracts were more highly inhibitory toward gram-positive bacteria than for gram-negative bacteria, especially with the samples from July, August, and September (Fig. 6). These results agree with those from research by Ueda et al. (1982), who found that many spices were more inhibitory to the gram-positive bacteria.

In conclusion, seasonal variations were found in the allelopathic potential of *A. princeps* var. *orientalis* on plants and bacteria. This effect was weaker in April (i.e., the sprouting stage) and in October (fading stage) but much more pronounced in August and in July (thriving stage). These findings concur with those from an investigation by Kawamoto et al. (1999), in which the contents of artemisinin and related sesquiterpenes in *A. annua* were lower in May and October and higher in August and September. Research will continue on the monthly fluctuations in chemical composition of *A. princeps* var. *orientalis*.

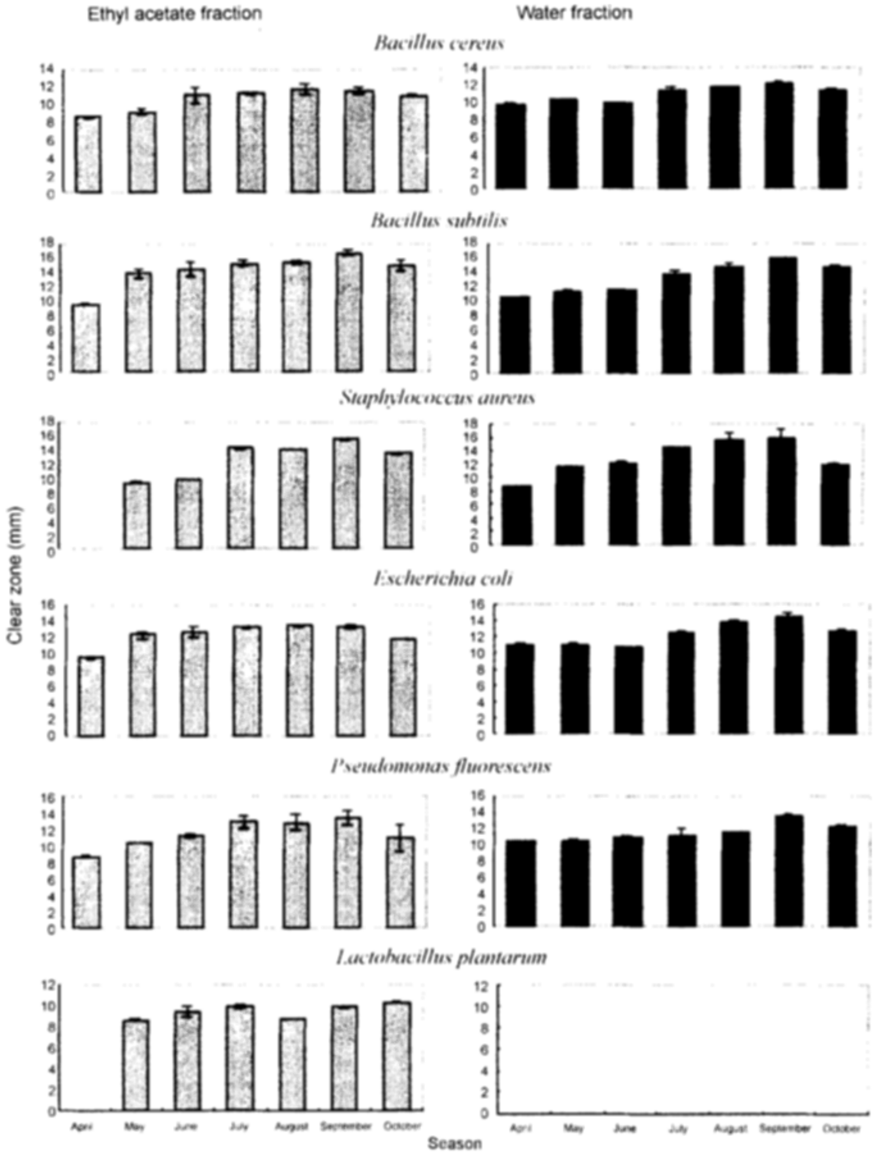

Figure 6. Antimicrobial activities of ethyl acetate and water fractions of methanol extracts collected monthly from *A. princeps* var. *orientalis*.

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