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Allelopathic Potential of Fennel (*Foeniculum vulgare* Mill.)

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ABSTRACT

The ethanolic extract obtained from dried fruits of fennel (*Foeniculum vulgare* Mill.) was evaluated *in vitro* to examine its potential allelopathic effects. The inhibitory effect of the extract at 0, 2.5, 5 and 10% (i.e., g amounts of original extract in 100 ml distilled water) on germination and seedling growth of four weeds, perennial ryegrass (*Lolium perenne*), wild barley (*Hordium spontaneum*), oat (*Avena ludoviciana*) and dandelion (*Taraxicum officinalis*), were tested. All concentrations suppressed the germination of perennial ryegrass and dandelion, while oat showed significantly reduced ($P \leq 0.05$) germination and seedling growth at 2.5%. The germination rate and percentage and seedling growth of wild barley were significantly ($P \leq 0.05$) decreased at 2.5 and 5%. At 10%, none of the tested weeds germinated.

Keywords: allelochemical, inhibitory effect, phenolic compound, seed germination, seedling growth

INTRODUCTION

Allelopathy is an important mechanism of plant interference mediated by the addition of plant-produced phytotoxins to the plant environment and is a competitive strategy of plants (Oussama 2003). Allelochemicals are produced by plants as end products, by-products and metabolites and exist in the stems, leaves, roots, flowers, inflorescence, fruits and seeds of plants (Sisodia and Siddiqui 2010). The release of these chemical compounds into the environment acts on other organisms such as plants – including weeds – animals and microorganisms to either inhibit or stimulate activity (Fujii *et al*. 2003). There is increasing evidence that these plant chemicals can suppress germination and growth of different weed species (Singh *et al*. 2003; Turk and Tawaha 2003; Sampietro and Vattuone 2006; Saharkhiz *et al*. 2010). Worldwide, enormous amounts of chemical herbicides are used to manage these weeds. However, synthetic herbicides are often toxic and cause environmental problems (Khanh *et al*. 2004; Sodaeizadeh *et al*. 2009). Moreover, overuse of artificial herbicides has led to the development of weed biotypes with herbicide resistance (Sodaeizadeh *et al*. 2009). In agriculture, there is a worldwide effort to reduce the amount of chemicals used in crop production through modern biological and ecological methods. One of the possible solutions is the use of allelopathy to explore the negative chemical interaction between plants (Azizi and Fujii 2006). The importance of allelopathy in natural control of weeds and crop productivity is now highly recognized (Khan *et al*. 2009). In recent years, exploration of medicinal plants for their allelopathic potential is of prime interest (Anjum *et al*. 2010). Medicinal plants may contain bioactive compounds such as ferulic, coumaric, vanilic, caffeic and chlorogenic acid that possess inhibitory activity (Modallal and Al-Charchafchi 2006). Allelopathic experiments on medicinal plants were conducted by different scientists (Anjum *et al*. 2010). Nazir *et al*. (2006) evaluated the allelopathic effects of aqueous extracts of *Rheum emodi*, *Saussaurea lappa* and *Potentilla fulgens* on some traditional food crops; germination of all crops was significantly reduced by *S. lappa* and *P. fulgens* extracts. Fujii *et al*. (2003) used 239 medicinal plants to evaluate the allelopathic activity on lettuce. They concluded that 223 species were inhibitory.

Fennel (*Foeniculum vulgare* Mill.; family Apiaceae) is an important medicinal plant with a long story of herbal uses (Anwar *et al*. 2009). Roots, young shoots, leaves, flowering stems, mature inflorescences and fully ripened and dried seeds are commonly used as homemade remedies (Barros *et al*. 2009). It can be utilized as carminative, diuretic, expectorant and stimulant, antispasmodic and stomachic. It is also used in gasteroenteritis, hernia, indigestion and abdominal pain (Ahmad *et al*. 2004). Also, Kwon *et al*. (2002) reported that the $CHCl_3$-soluble fraction from the methanolic extract of fennel stem contains dillapional (a phenyl propanoid derivative) and scopoletin (a coumarin derivative) which are responsible for antimicrobial activity. Investigation on allelopathic effects of a plant provides important basic information on their growth inhibitory effects as well as their potential for weed control (Macias *et al*. 2007; Saharkhiz *et al*. 2009). The water and ethanol extracts of fennel seeds showed strong antioxidant activity. For example, 100 μg of water and ethanol extracts exhibited 99.1 and 77.5% inhibition of peroxidation in the linoleic acid system, respectively, and greater than the same dose of α-tocopherol (36.1%) (Oktay *et al*. 2003). Although the results of research studies indicate that fennel seed is a potential source of natural antioxidants, the activity of such extracts still need to be demonstrated in biological systems.

In this study the allelopathic potential of fennel seed extract on germination and seedling growth of four weeds belonging to the *Poaceae* and *Asteraceae* families has been considered.

MATERIALS AND METHODS

Plant material

Fennel seeds were brought from medicinal herb centers at Shiraz, Iran. The seeds of weeds, namely wild barley (*Hordium spontaneum*), perennial ryegrass (*Lolium perenne*), oat (*Avena ludoviciana*) and dandelion (*Taraxicum officinalis*), were prepared by the College of Agriculture, Shiraz University.

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Extraction from fennel seeds

Fennel seeds were powdered in a knife mill. Ground sample (20 g) was mixed with 200 ml of 96% ethanol using a shaking water bath for 24 h at room temperature. The extract was separated from solid by filtering through Whatman No. 1 filter paper. The remaining residue was re-extracted twice and the extracts were pooled. The solvent was removed under vacuum at 40°C using a rotary vacuum evaporator (Laborota 4000-Heidolph Germany).

Bioassay

In order to detect the allelopathic effect of the fennel seed extract, dilutions were made of the original extract to 2.5, 5 and 10% of the stock extract. Twenty seeds of each weed were surface sterilized with a water-bleach solution (95: 5) and were placed on sterilized filter paper in 6-cm diameter Petri dishes. Each solution (3 ml) was added to each Petri dish; distilled water served as the control. Petri dishes were placed in the light at 25°C for 14 days. They were monitored daily and the evaporated contents were compensated with distilled water. The numbers of germinated and non-germinated seeds were counted and final radicle and epicotyl length were measured at the end of 14th day. The seeds showing radical emergence were considered to be germinated.

Statistical analysis

The experimental design was a complete randomized design with four replications for each treatment. Data were analyzed using SPSS v. 17.0 and mean comparisons were made following the LSD test at $P \leq 0.05$.

RESULTS

The allelopathic effect of fennel seed extract on the germination and seedling length of examined plants was determined. The extract caused a significant ($P \leq 0.05$) decrease or inhibited seed germination and seedling length in all four studied weeds.

Different concentrations of seed extract exhibited different effects on the germination of weeds' seeds (**Figs. 1, 2**). Germination percentage was 66-100% and germination rate was 10-58 germinated seeds/day, in the control group of four tested weeds. All of the concentrations of fennel seed extract inhibited the germination of perennial ryegrass and dandelion. At 2.5%, the germination of wild barley and oat seeds was significantly lowered. At 5% only wild barley seeds could germinate, but significantly ($P \leq 0.05$) less than the control.

The length of the radicle and epicotyl of wild barley and oat was significantly reduced at 2.5% (**Figs. 3, 4**). At 5%, only the growth of wild barley radicles was possible but epicotyl emergence was inhibited. At 10% the germination of all four weeds was completely suppressed.

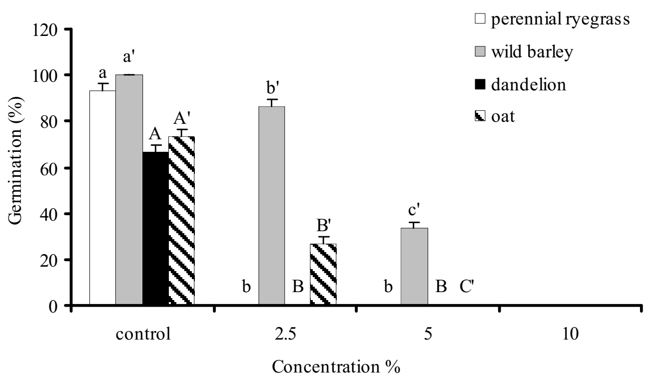

Fig. 1 Effect of different concentrations of the stock fennel extract on perennial ryegrass, wild barley, dandelion and oat seed germination. Different small and capital letters show significant differences ($P \leq 0.05$).

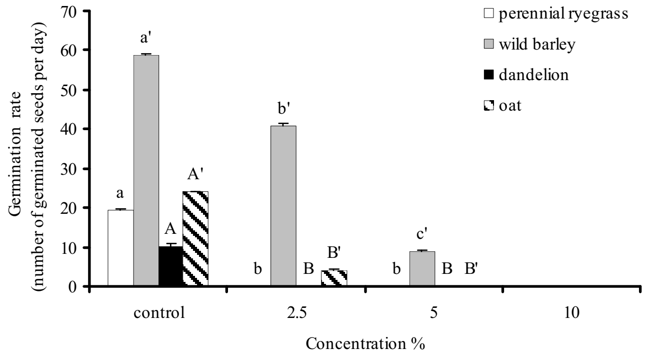

Fig. 2 Effect of different concentrations of the stock fennel extract on perennial ryegrass, wild barley, dandelion and oat seed germination rate. Different small and capital letters show significant differences ($P \leq 0.05$).

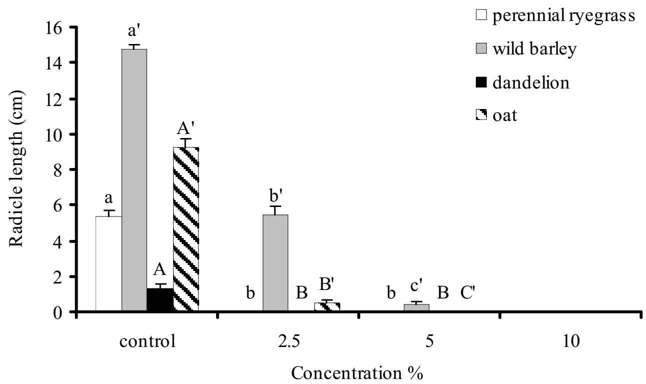

Fig. 3 Effect of different concentrations of the stock fennel extract on perennial ryegrass, wild barley, dandelion and oat radicle length. Different small and capital letters show significant differences ($P \leq 0.05$).

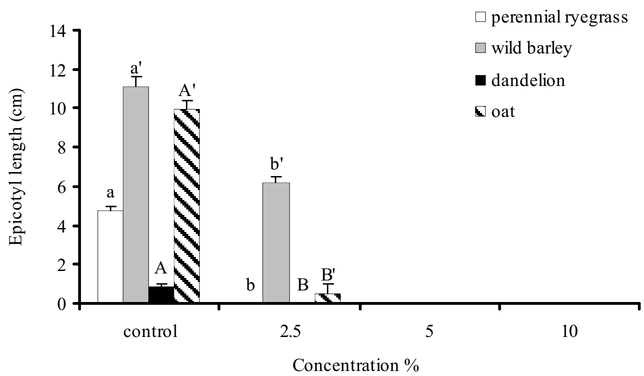

Fig. 4 Effect of different concentrations of the stock fennel extract on perennial ryegrass, wild barley, dandelion and oat epicotyl length. Different small and capital letters show significant differences ($P \leq 0.05$).

DISCUSSION

In this study, basic research on the allelopathic potential of fennel (*Foeniculum vulgare*) seed at several concentrations showed that this medicinal plant exhibited a significant inhibitory effect on the seed germination and seedling lengths of all four examined weeds.

Allelopathic screening of 81 medicinal plant species collected from Pakistan demonstrated that *F. vulgare* is one of the top 10 medicinal plants with the highest inhibitory effect against the germination of lettuce (*Lactuca sativa* L.) seeds (Gilani *et al.* 2010). In addition, the essential oils of *F. vulgare* showed an inhibitory effect against common weeds including *Alcea pallida* Waldst. and Kit., *Amaranthus retroflexus* L., *Centaurea salsotitialis* L., *Raphanus raphanistrum* L., *Rumex nepalensis* Spreng., *Sinapis arvensis* L. and *Sonchus oleraceus* L. (Azirak and Karaman 2008). Aqueous extracts from the shoots and roots of switch grass (*Panicum* *virgatum* L.) inhibited the germination and growth of perennial ryegrass. Black mustard (*Brassica nigra* L.) extracts reduced wild barley (*Hordeum spontaneum*) hypocotyl length, hypocotyl weight, radicle weight, seed germination, and radicle length (Tawaha and Turk 2003). The phytotoxic effect of barley extracts (*Hordeum vulgare* L.) on wild oat (*Avena ludoviciana*) was reported by Kolahi *et al*. (2008). In another experiment, *Taraxacum officinale* responded by delayed germination in response to *Rumex obtusifolius* extract applied as a spray (Zaller 2006). The inhibitory effect of the extract on seed germination and seedling length of tested weeds may be related to the presence of allelochemicals, including tannins, wax, flavonoids and phenolic acids. Furthermore, toxicity might be due to a synergistic effect rather than the effect of any one compound or class of secondary metabolite (Saharkhiz *et al*. 2009). According to the findings of Oktay *et al*. (2003) both aqueous and ethanolic fennel extracts contain a high phenolic content. Reversed-phase high performance liquid chromatography (HPLC) illustrated that the major phenolic compounds of the methanolic extract contained caffeoylquinic, chlorogenic, dicaffeoylquinic, rosmarinic acids, eriocitrin, rutin, and miquelianin (Krizman *et al*. 2007). Many studies have revealed that phenolics can seriously interfere with metabolic processes during germination, seedling, and later growth stages (Demos *et al*. 1975; Rasmussen and Einhellig 1977; Einhellig and Rasmussen 1978; Lynch 1980; Williams and Hoagland 1982; Blum *et al*. 1984; Nandakumar and Rangaswany 1985; An *et al*. 2000). Phenolic acids may interfere with IAA (indole-3-acetic acid) metabolism, mitochondrial metabolism and respiration, photosynthesis, synthesis of proteins, and ion uptake and transport (Williams and Hoagland 1982, Rice 1984, Einhellig 1986, Al-Charchafchi *et al*. 1987, Hussain and Khan 1988). The accumulation of phenolics played a protective role in strengthening plant cell walls during growth by polymerization into lignins (Diaz 1997). Rohn *et al*. (2002) demonstrated that phenolic substances that are able to form quinones (i.e., caffeic acid, chlorogenic acid, gallic acid, etc.) might react with amino acid side chains and free thiol groups on the enzymes. The inhibitory effect of chlorogenic and caffeic acids on germination of *Artemisia herba alba* seeds (Al-Charchafchi and Al-Quadan 2006) was strongly correlated to the inhibition of the very sensitive enzyme activities of the oxidative pentose phosphate pathway (Al-Quadan *et al*. 2008). In general, phenolics have the property of altering mitochondria and chloroplasts membranes, hindering the energy transfer necessary for ion transport (Moreland and Novitzky 1987). Ferulic acid and coumarin seemed to act alike as mitosis-disrupting herbicides on microtubules, which are responsible for setting the plane for cell division and cellulose deposition (Cornman 1946, Vaughn and Lehnen 1991). The inhibition of germination and growth by coumarin has been explained in terms of the inhibitor, the thiol group, responsible for oxidative phosphorylation, membrane permeability and cellulose biosynthesis (Mino *et al*. 1975). Rosmarinic acid has inhibitory activity against plant α-amylase during seed germination and seedling growth (Kusano *et al*. 1998; Nagpal *et al*. 2008). The lower water availability for seed germination due to binding water by compounds present in an extract might play an effective role in reducing seed germination (Bogatek *et al*. 2006).

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