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Allelopathic Potential of Essential Oils from *Carum copticum* L., *Cuminum cyminum* L., *Rosmarinus officinalis* L. and *Zataria multiflora* Boiss.

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ABSTRACT

Essential oils (EOs) obtained from dried fruits of *Carum copticum* L. and *Cuminum cyminum* L., and aerial parts of *Rosmarinus officinalis* L. and *Zataria multiflora* Boiss. were evaluated *in vitro* to examine their potential allelopathic effects. The inhibitory effects of EOs at concentrations of 0, 100, 200, 300 and 400 µl. L^{-1} on seed germination and seedling growth of common bermudagrass (*Cynodon dactylon* L.), tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) from the Poaceae family were tested. Germination rate and seedling length were significantly ($P \leq 0.05$) reduced by all EOs, however, the highest effect was observed at 300 and 400 µl. L^{-1}. Moreover, the allelopathic impact of *Z. multiflora* EO on seed germination and seedling growth was most effective (100% germination and growth inhibition) when compared with other tested EOs.

Keywords: allelochemical, growth inhibition, Poaceae, seed germination, seedling growth

INTRODUCTION

Inhibition of growth of plants by other plants in their vicinity has been known for a long time. The chemical interaction between plants, which can cause enhancement or inhibition of growth, has been termed 'allelopathy' and has been extensively studied (Rice 1984; Macias *et al*. 2007; Azirak and Karaman 2008). Allelopathy is generally known as a significant ecological factor in determining the structure and composition of plant communities (Scrivanti *et al*. 2003). The production and accumulation of secondary metabolites, which inhibit and/or stimulate germination of seeds and development of other plants, are important mechanism in the interactions between plants. Aromatic plants, known to be rich in active principles, can play an important role in plant-plant interactions and constitute a primary source of potential allelochemicals (Vokou 1992; Macias *et al*. 2007). Evidence for allelopathic interactions in nature from plants containing volatile allelochemicals has been frequently described (Bonner 1950; Asplund 1986; Ramezani *et al*. 2008; Zhao *et al*. 2009). Most germination and growth inhibitors produced by perennial angiosperms identified by Rice (1984) were phenolic compounds or derivatives of cinnamic acid. Other authors have also found EOs, coumarins, flavonoids, alkaloids, cyanoglycosides, proteins, and amino acids among the inhibitory compounds (Putnam 1985; Macias *et al*. 2007).

The biological activity of EOs depends on the synergistic or antagonistic effects of constituent types present in different concentrations (Burt 2004; Macias *et al*. 2007), therefore, EOs present in aromatic plants cause a number of positive or negative effects (Vokou 1999). Germination inhibition by EOs, when applied to dry seeds such as wheat (*Triticum aestivum* L.), black mustard (*Brassica nigra* (L.) Koch), palmer amarant *(Amaranthus palmeri* S. Watson), and *Arabidopsis thaliana* has been reported by several researchers (Zhang *et al*. 1995; Dudai *et al*. 1999; Zhao *et al*. 2009). The allelopathic competence of aerial parts and roots of many plants and trees is also well documented in both laboratory and greenhouse experiments. Tworkoski (2002) determined the phytotoxicity effects of some EOs e.g. red thyme (*Thymus vulgaris*), summer savory (*Satureja hortensis*) as post emergence growth inhibitors on several weeds. Singh *et al*. (2005) reported the phytotoxic activity of volatile oils from *Eucalyptus citriodora* on *Parthenium hysterophorus* and Ramezani *et al*. (2008) showed the allelopathic effects of four EOs against three weed species at pre-emergence.

Investigation on allelopathic and phytotoxicity effects of EOs provides important basic information on their growth inhibitory effects as well as their potential for weed control.

The aim of this study was to investigate the allelopathic effects of four important Iranian essential oil-bearing plants against three genera within the *Poaceae* family.

Table 1 Selected essential oil plants and main components for determining their allelopathic effects.

Botanical name	Extraction part	Main components	Concentration (%)
Zataria multiflora	Aerial parts	Carvacrol, linalool	10-75
Carum copticum *	Fruits	Thymol, *p*-cymene	40-24
Cuminum cyminum	Fruits	α-Pinene, limonene, linalool	29, 21, 10
Rosmarinus officinalis	Aerial parts	α-Pinene 1,8-cineole, linalool	15, 7, 14

Main compounds of Iranian plant material were reported by Mohagheghzadeh *et al*. 2000, Saharkhiz *et al*. 2005, Gachkar *et al*. 2007. * Syn. *Trachyspermum ammi*

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MATERIAL AND METHODS

Plant material

The four plants and plant parts used for extraction of EOs and their main oil components are shown in **Table 1**. To test the allelopathic potential of these four EOs, common bermudagrass (*Cynodon dactylon* L.), tall fescue (*Festuca arundinacea* Schreb.), and perennial ryegrass (*Lolium perenne* L.) were selected as plants to be exposed to selected EOs.

Essential oil isolation

Dried fruits of *Carum copticum* L. and *Cuminum cyminum* L., and flowering tops of *Rosmarinus officinalis* L. and *Zataria multiflora* Boiss. were hydrodistillated for 2.5 hr, using an all-glass Clevenger-type apparatus, according to the method outlined by the British Pharmacopoeia (1988). Sample oils were dried over anhydrous sodium sulphate and stored in sealed vials at 4°C before allelopathic assessments. The plant species from which the oils were obtained, had been collected from Shiraz suburbs and were identified and authenticated by A.R. Khosravi, a plant taxonomist, at Shiraz University, Herbarium, Shiraz, Iran. Voucher specimens (no. 24985, 24986, 24987, 24988) have been deposited in the herbarium.

Bioassay of inhibition induced by EOs

In order to detect the allelopathic effects of the studied EOs, four concentrations (100, 200, 300, and 400 μl.L $^{-1}$) and a control (0 μl.L $^{-1}$) were used. Whatman no. 1 filter paper was sterilized in oven at 72°C for 48 hr and placed in 9 cm diameter Petri dishes. Four replications of 100 seeds were used for each treatment. The seeds of the experimental plants were soaked in distilled water for 3 days then placed in Petri dishes and supplemented with 3 ml of the EO solutions. To prevent evaporation, Petri dishes were sealed with parafilm and placed in a phytotron at 25 ± 2°C with a 16 hr photoperiod. They were monitored daily and moistened with EO solutions as needed. The number of germinated seeds was recorded daily in all experiments. Final germination percentages were calculated for each trial. After 20 days, all germinated and non-germinated seeds were counted. The seeds showing radicle emergence were recorded as germinated. Total lengths of seedlings (root plus shoot) were also measured in all treatments.

Statistical analysis

The design of the experiment was a completely randomized factorial arrangement. Data were analyzed using MSTAT-C and mean comparisons were made following Tukey's test at $P \leq 0.05$.

RESULTS

The allelopathic effects of the EOs from *C. copticum*, *R. officinalis*, *Z. multiflora*, and *C. cyminum* on the germination and seedling length of examined plants were determined. The EOs resulted in decreasing seed germination and seedling length in all studied plants. The effects of concentration treatments on each tested plant seed and seedling lengths were analyzed statistically and are shown in **Figs. 1-6**.

As shown in **Figs. 1**, **3**, **5**, EOs strongly inhibited germination after 20 days ($P \leq 0.05$) whereas control treatments showed maximum germination rates. On the other hand, different EOs and concentrations exhibited different effects in controlling germination and seedling length (**Figs. 2, 4,**

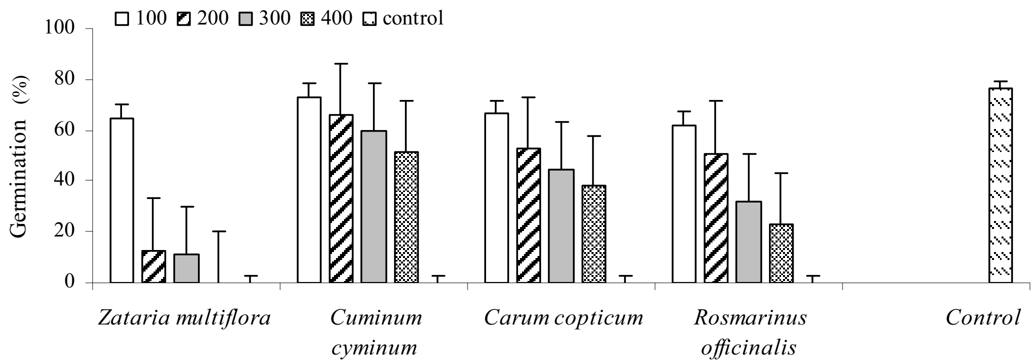

Fig. 1 Effect of different concentrations (μl.L $^{-1}$) of tested EOs on perennial ryegrass seed germination ($P \leq 0.05$).

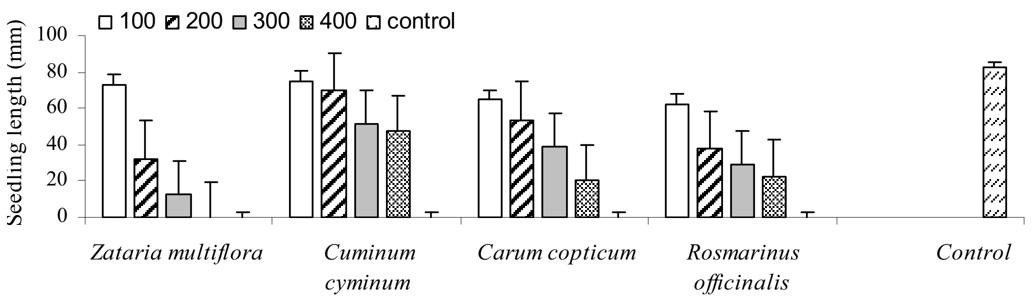

Fig. 2 Effect of different concentrations (μl.L $^{-1}$) of tested EOs on perennial ryegrass seedling length ($P \leq 0.05$).

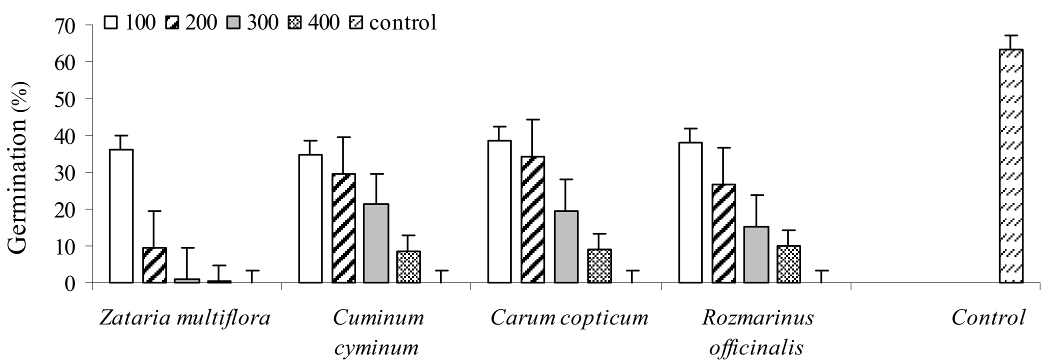

Fig. 3 Effect of different concentrations (μl.L $^{-1}$) of tested EOs on tall fescue seed germination ($P \leq 0.05$).

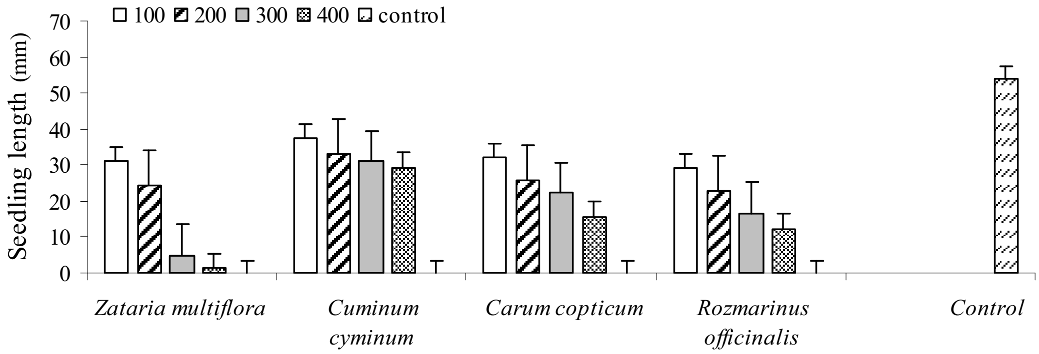

Fig. 4 Effect of different concentrations (ppm) of tested EOs on tall fescue seedling length ($P \leq 0.05$).

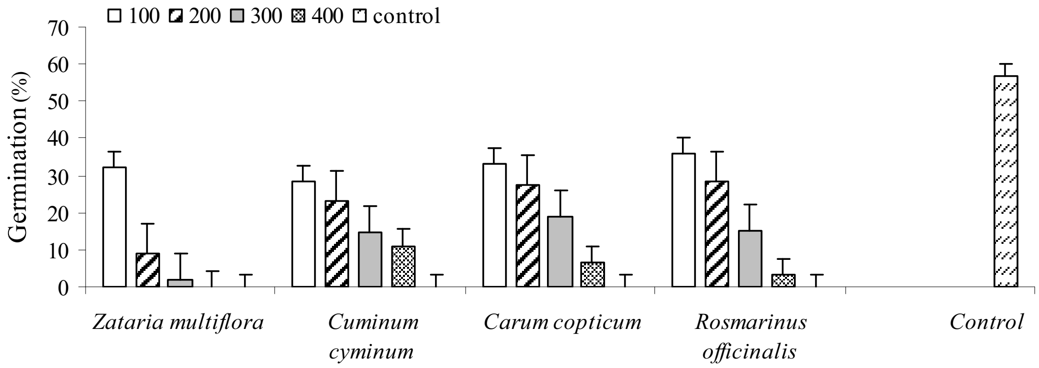

Fig. 5 Effect of different concentrations (µl.L^{-1}) of tested EOs on Bermudagrass seed germination ($P \leq 0.05$).

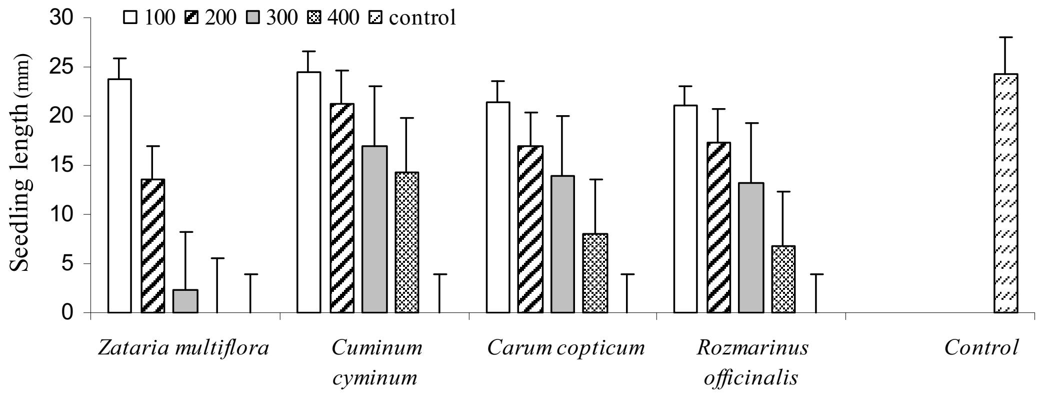

Fig. 6 Effect of different concentrations (µl.L^{-1}) of tested EOs on Bermudagrass seedling length ($P \leq 0.05$).

6) of tested plants. While germination was 57-76% in the control group, *Z. multiflora* EO showed the greatest inhibition effect on seed germination and seedling length in all tested plants. Germination was significantly ($P \leq 0.05$) suppressed to 0-0.25% by application of *Z. multiflora* EO at 400 µl.L^{-1} and reached between 1-11% at 300 µl.L^{-1}. The results also demonstrate that, with the exception of bermudagrass seedling length, 100 µl.L^{-1} of *Z. multiflora* EO significantly ($P \leq 0.05$) reduced seed germination and seedling length of all tested plants. However, the maximum inhibitory effect among all tested EOs was that of *Z. multiflora* at 300, 400 µl.L^{-1}, especially on perennial ryegrass and bermudagrass seeds and seedlings (**Figs. 1, 2, 5, 6**).

R. officinalis EO showed varying inhibition effects on the seeds and seedlings. All concentrations of *R. officinalis* EO significantly ($P \leq 0.05$) inhibited seed germination in tested plants (**Figs. 1, 3, 5**) and decreased seedling length of perennial ryegrass and tall fescue (**Figs. 2, 4**). However, *R. officinalis* EO exhibited no significant allelopathic effect at 100 µl.L^{-1} on bermudagrass seedling length compared with the control ($P \leq 0.05$).

C. copticum and *C. cyminum* EOs at 400 µl.L^{-1} showed considerable inhibition effects on the germination as well as seedling length of the tested plants compared with the control. However, no significant difference was observed between control and 100 µl.L^{-1} of *C. copticum* EO on bermudagrass seedling growth (**Fig. 6**) and perennial ryegrass seed germination (**Fig. 1**). Significant difference ($P \leq 0.05$) was also observed between 300 and 400 µl.L^{-1} of *C. cyminum* EO on the seed germination of tall fescue (**Fig. 3**) and perennial ryegrass but no significant difference was with the control, 100 or 200 µl.L^{-1} on the perennial ryegrass seed germination and/or bermudagrass seedling length (**Figs. 1, 6**).

DISCUSSION

EOs are generally used in the cosmetic, medicinal, and food industries, and are thought to be safe compounds for humans, animals, and the environment. However, in recent years, numerous reports have appeared regarding the potential use of EOs for naturally controlling weeds, pests, nematodes, fungi, bacteria, and other harmful organisms in organic farming systems.

In the present study, which is a basic work regarding the allelopathic potential of some important Iranian aromatic plants, all tested EOs, particularly of *Z. multiflora*, exhibited a significant inhibitory effect on the seed germination and seedling lengths of examined plants and showed allelopathic effects. The concentration of the EOs was an important factor which changed the degree of allelopathic effects. With an increase in EO concentration, germination capacity and seedling lengths markedly decreased.

A number of reports on EOs' allelopathic effects confirm results obtained in this study. Azirak and Karaman (2008) investigated the allelopathic effects of 10 EO-bearing plants and reported that the EOs of *Carum carvi*, *Mentha spicata*, *Origanum onites*, and *Thymbra spicata* showed high inhibitory activities (allelopathic effect) on the germination of seven weeds *in vitro*. They also observed that the inhibitory effects of the EOs varied according to the kind of EO and concentration used. Ramezani *et al*. (2008) detected the allelopathic effects of rosemary, eucalyptus, Lawson cypress, and white cedar oils against seed germination of three weed species including knapweed, purslane, and amaranth and concluded that 300 and 400 $\mu l.L^{-1}$ of the tested EOs showed the maximum inhibition effect on germination. They also observed differences in the sensitivity of the weed species to the EOs and demonstrated that the inhibitory activity against weed seed germination varied with species from which the EO was extracted. This is in agreement with the results obtained in the present study. Inhibition of germination has been reported for several monoterpenes (e.g. linalool, camphor, pinenes, cineoles, carvacrol, and thymol) in pure form or as EO mixtures (Macias *et al*. 2007; Azirak and Karaman 2008). Furthermore, toxicity may increase due to a synergistic effect rather than being single (Burt 2004; Macias *et al*. 2007). EOs of the Iranian plants in this study have been reported to contain abundant amounts of monoterpenes and phenolic components (Mohagheghzadeh *et al*. 2000; Saharkhiz *et al*. 2005; Gachkar *et al*. 2007), which show strong allelopathic effects (Vyvyan 2002; Macias *et al*. 2007). Therefore, it can be concluded that the inhibitory effects of the EOs on seed germination and seedling length of the tested plants could be attributed to these components. The mode of action of EOs against germination and growth of plants in not quite clear. However, some studies have indicated that volatile monoterpenes are potent inhibitors of cell mitosis (Romagni *et al*. 2000; Macias *et al*. 2007). Vaughn (1991) reported that EOs from cinnamon (*Cinamommum zeylanicum* Blume) and red thyme (*Thymus vulgaris* L.) reduced sprout growth in potato, possibly by killing meristematic cells. Tworkosk (2002) suggested that EOs altered membrane permeability based on the electrical conductivity measured by electrolyte leakage in leaf disks of dandelion. On the other hand, some components like 1,8-cineol cause a variety of effects e.g. swollen root tips, inhibition of mitochondrial respiration, mitosis blockage, and inhibition of DNA synthesis (Macias *et al*. 2007). However, this aspect needs further study.

CONCLUSION

The results obtained in this study suggest the use of *Zataria multiflora*, *Rosmarinus officinalis*, *Carum copticum*, and *Cuminum cyminum* EOs at 300 and/or 400 $\mu l.L^{-1}$ to control seed germination and seedling growth of the examined plants belonging to the Poaceae family. The findings of this work may be extended to the noxious weeds in this family. The results reported here can be considered the first steps towards a possible practical application of EOs as potentially natural allelochemicals. Further studies on the phytotoxicity and especially on formulation of EOs, are still required before this technique could be used for weed control in agriculture.

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