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Allelopathic Potential of *Ephedra*

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ABSTRACT

The ethanolic extract obtained from dried *Ephedra pachyclada* Boiss. Flowering aerial parts was evaluated *in vitro* to examine its potential allelopathic effects. The alkaloids ephedrine and pseudoephedrine are the active constituents of the plant. The inhibitory effect of the extract at 0, 2.5, 5, 10 and 20% (i.e., g amounts of original extract in 100 ml of distilled water) on germination and seedling growth of perennial ryegrass (*Lolium perenne*) and bread wheat (*Triticum aestivum*) as representative monocotyledonous plants and soybean (*Glycine max*) and mung bean (*Vigna radiata*) as representative dicotyledonous plants were tested. All concentrations of the stock *Ephedra* extract significantly reduced ($P \leq 0.05$) germination rate and percentage and seedling growth of the four examined plants. At 20% of the original extract, the germination of perennial ryegrass, soybean and mung bean was completely suppressed. In this study, the allelopathic potential of *E. pachyclada* extract on seed germination of the two dicotyledonous plants belonging to the *Poaceae* family was higher than the two monocotyledonous plants belonging to the *Fabaceae* family.

Keywords: allelochemical, alkaloid compound, ephedrine, inhibitory effect

INTRODUCTION

The genus *Ephedra* (family Ephedraceae; gymnosperm), comprises about 50 species native to arid and semi-arid regions of Asia, Europe, northern Africa, western North America and South America (Price 1996; Motomura *et al*. 2007) but with restricted distribution (Singh *et al*. 1999). Several species of *Ephedra* have traditionally been used for a variety of medicinal purposes. *Ephedra* has been used as a herbal remedy in traditional medicine for the treatment of asthma and hay fever, as well as for common colds (Abourashed *et al*. 2003). The alkaloids ephedrine and pseudoephedrine are the active constituents of the plant. Ephedrine constitutes 40–90% of the alkaloid content, the remainder consisting of pseudoephedrine and the methylated forms of each compound. Some species of *Ephedra* have a total alkaloid content of 1–3% by dry weight (Abourashed *et al*. 2003; Bent *et al*. 2003; Bohn *et al*. 2003; Barker *et al*. 2007; Gholami 2010).

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 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, animals and microorganisms to either inhibit or stimulate their growth (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 interactions 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 that possess inhibitory activity (Modallal and Al-Charchafchi 2006). 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. Investigation on the 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; Nourimand *et al*. 2011).

In this study, the allelopathic potential of *E. pachyclada* extract on germination and seedling growth of two monocotyledonous plants belonging to the *Poaceae* family and two dicotyledonous plants belonging to the *Fabaceae* family has been considered.

MATERIALS AND METHODS

Plant material

Ephedra pachyclada plants were collected from near Shiraz, Iran. The seeds, namely of perennial ryegrass (*Lolium perenne*), bread wheat (*Triticum aestivum*), soybean (*Glycine max*) and mung bean (*Vigna radiata*), were prepared by the College of Agriculture, Shiraz University.

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Short Communication

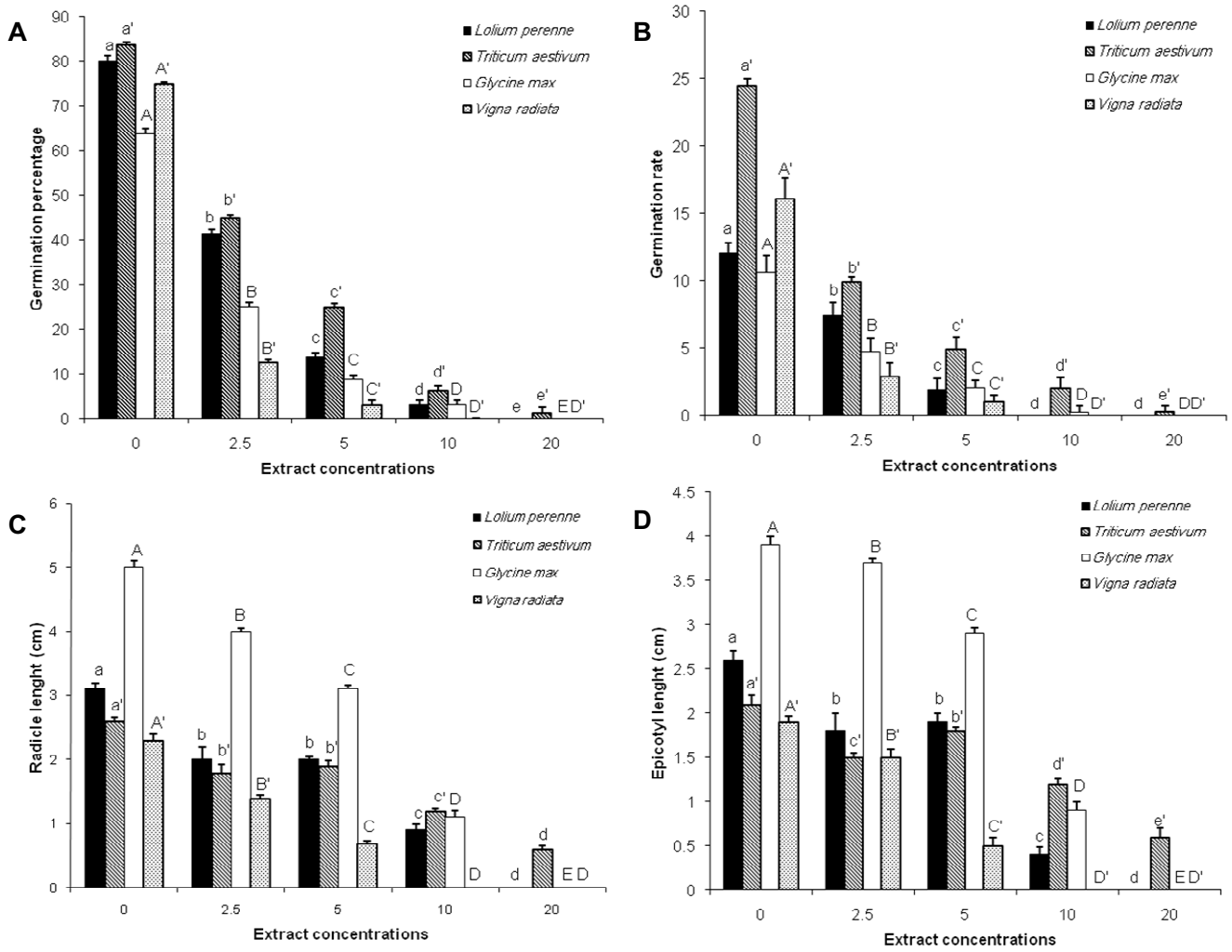

Fig. 1 Effect of different concentrations of stock *Ephedra* extract on seed germination percentage (A), seed germination rate (B), radicle length (C) and epicotyl length (D) of four examined plants. Different lower-case and capital letters indicate significant differences (LSD test at $P \leq 0.05$).

Extraction from *Ephedra* plants

The aerial parts of flowering plants of *E. pachyclada* were dried at room temperature and then powdered in a knife mill. Ground sample (7.5 g) was mixed with petroleum benzene for 3 h in closed glass at 25°C to remove lipids. The solvent was removed and the remaining material was mixed with 100 ml of 96% ethanol for 24 h at 4°C. The extract was separated from solids 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 *E. pachyclada* plant extract, dilutions were made of the original extract to 2.5, 5, 10 and 20% of the stock extract. Twenty seeds of each of four plants were surface sterilized with a solution of water and bleach (95: 5) and were placed on sterilized filter paper in 6-cm diameter Petri dishes. 3 ml of each solution was added to each Petri dish; distilled water served as the control. Petri dishes were placed in the light at 25°C for 12 days. They were monitored daily and the evaporated contents were compensated with distilled water. The number of germinated and non-germinated seeds were counted and final radicle and epicotyl length were measured at the end of the 12th day. Seeds from which a radical emerged 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 *E. pachyclada* plant 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 plants.

Different concentrations of the stock *Ephedra* extract exhibited different effects on the germination rate and percentage and seedling growth of the four examinated plants (**Figs. 1A-D**). Germination percentage was 75-80% and germination rate was 12-24.5 germinated seeds/day in the control group of the four tested plants. At 20% of the original extract, the germination of perennial ryegrass, soybean and mung bean were completely suppressed.

At 2.5%, the germination of mung bean was significantly lowered and at 10 and 20% none of the mung bean seeds germinated. The length of the epicotyl of perennial ryegrass and mung bean were significantly reduced at 10% (**Figs. 1C, 1D**). At 20%, only the growth of wheat radicle and epicotyl was possible.

DISCUSSION

In this study, basic research on the allelopathic potential of *Ephedra pachyclada* Boiss. at several concentrations showed that this medicinal plant exhibited a significant inhibitory effect on the seed germination rate and percentage

and seedling lengths of all four examined plants encompassing mono- and dicotyledonous species. The inhibitory effect of the plant extract on germination and growth of other plants may be related to the presence of allelochemicals. 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). 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).

The alkaloids ephedrine and pseudoephedrine are the active constituents of *Ephedra* (Abourashed *et al*. 2003). Nasr and Shariati (2005) reported that different concentrations of ephedrine reduced the seed germination of *Astragalus cycluphyllus*. Ephedrine exhibits optical isomerism and has two chiral centres, giving rise to four stereoisomers. By convention, the enantiomers with opposite stereochemistry around the chiral centres are designated as ephedrine, while pseudoephedrine has same stereochemistry around the chiral carbons (Griffith and Johnson 1995; Ma *et al*. 2007).

A reduction in seed germination of perennial ryegrass was consistent with different concentrations of the stock *Ephedra* extract (**Fig. 1A**) but at the highest concentration (20% of the stock *Ephedra* extract), its seed germination was completely suppressed. However, in our previous work on fennel allelopathy with the same design, the germination of perennial ryegrass was suddenly reduced at the first concentration and seed germination was completely inhibited (Nourimand *et al*. 2011).

Seed germination rate and percentage of wheat were highest among the four tested plants and the germination and seedling growth were not completely inhibited even with 20% of the stock *Ephedra* extract compared to other tested plants. In this study, the allelopathic potential of *E. pachyclada* extract on seed germination of two monocotyledonous plants belonging to the *Poaceae* family were lower than the two dicotyledonous plants belonging to the *Fabaceae* family. Some plants produce natural herbicides that can be used as safe chemical substitutes for weed control. This study showed that the *E. pachyclada* extract might be used at an optimal concentration as part of a natural herbicide on dicotyledons plants. There are over 70,000 plant species thought to be of medicinal value in the world (Li *et al*. 2010). Most of the medicinal plants in the world like *Ephedra* species are still harvested in the wild and have not been developed as crops.

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REFERENCES

Abourashed E, El-Alfy A, Khan I, Walker L (2003) *Ephedra* in perspective: A current review. *Phytotherapy Research* **17 (7)**, 703-712

Anjum A, Hussain U, Yousaf Z, Khan F, Umer A (2010) Evaluation of allelopathic action of some selected medicinal plant on lettuce seeds by using sandwich method. *Journal of Medicinal Plants Research* **4**, 536-541

Azizi M, Fujii Y (2006) Allelopathic effect of some medicinal plant substances on seed germination of *Amaranthus retroflexus* and *Portulaca oleraceae*. *Acta Horticulturae* **699**, 61-68

Barker W, Antia U (2007) A study of the use of *Ephedra* in the manufacture of methamphetamine. *Forensic Science International* **166 (2)**, 102-109

Bent S, Tiedt T, Odden M, Shlipak M (2003) The relative safety of ephedra compared with other herbal products. *Annals of Internal Medicine* **138 (6)**, 468-471

Bogatek R, Gniazdowska A, Zakrzewska W, Oracz K, Gawronski SW (2006) Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. *Biologia Plantarum* **50**, 156-158

Bohn A, Khodaee M, Schwenk T (2003) Ephedrine and other stimulants as ergogenic aids. *Current Sports Medicine Reports* **2 (4)**, 220-225

Fujii Y, Parvez SS, Parvez MM, Ohmae Y, Iida O (2003) Screening of 239 medicinal plant species for allelopathic activity using sandwich method. *Weed Biology and Management* **3**, 233-241

Gholami M (2010) Screening allelopathy activity, antifungal, antibacterial and total phenol content of extract from *Ephedra* sp. under various ecophysiological environments. MS thesis, Shiraz University, Iran, 125 pp

Griffith RK, Johnson EA (1995) Adrenergic drugs. In: Foye WO, Lemke TL, Williams DA (Eds) *Principles of Medicinal Chemistry*, Williams and Wilkins, Baltimore, pp 345-365

Khanh MA, Marwat KB, Hassan Z (2004) Allelopathic potential of some multipurpose trees species (MPTS) on the wheat and some of its associate's weeds. *International Journal of Biology and Biotechnology* **1**, 275-278

Li S, Yuan W, Yang P, Antoun MD, Balick MJ, Cragg GM (2010) Pharmaceutical crops: an overview. *Pharmaceutical Crops* **1**, 1-17

Ma G, Bavadekar SA, Davis YM, Lalchandani SG, Nagmani R, Schaneberg BT, Khan IA, Feller DR (2007) Pharmacological effects of ephedrine alkaloids on human α_1- and α_2-adrenergic receptor subtypes. *The Journal of Pharmacology and Experimental Therapeutics* **322**, 214-221

Macias FA, Molinillo J, Varela RM, Galindo CG (2007) Allelopathy – a natural alternative for weed control. *Pest Management Science* **63**, 327-348

Modallal N, Al-Charchafchi FMR (2006) Allelopathic effect of *Artemisia harba alba* on germination and seedling growth of *Anabasis setifera*. *Pakistan Journal of Biological Sciences* **9**, 1795-1798

Motomura H, Noshiro S, Mikage M (2007) Variable wood formation and adaptation to the alpine environment of *Ephedra pachyclada* (Gnetales: Ephedraceae) in the Mustang District, Western Nepal. *Annals of Botany* **100**, 315-324

Nasr M, Shariati M (2005) The use of allelochemicals to delay germination of *Astragalus cycluphyllus* seeds. *Journal of Agronomy* **4 (2)**, 147-150

Nazir T, Uniyal AK, Todaria NP (2006) Allelopathic behavior of three medicinal plant species on traditional agriculture crops of Garhwal Himalaya, India. *Agroforestry Systems* **3**, 183-187

Nourimand M, Mohsenzadeh S, Teixeira da Silva JA, Saharkhiz MJ (2011) Allelopathic potential of fennel (*Foeniculum vulgare* Mill.). *Medicinal and Aromatic Plant Science and Biotechnology* **5 (1)**, 54-57

Oussama O (2003) Allelopathy in two durum wheat (*Triticum durum* L.) varieties. *Agriculture, Ecosystems and Environment* **96**, 161-163

Price RA (1996) Systematics of the Gnetales: A review of morphological and molecular evidence. *International Journal of Plant Science* **157**, S40-49

Saharkhiz MJ, Ashiri F, Salehi MR, Ghaemaghami J, Mohammadi SH (2009) Allelopathic potential of essential oils from *Carum copticum* L., *Cuminum cyminum* L., *Rosmarinus officinalis* L. and *Zataria multiflora* Boiss. *Medicinal and Aromatic Plant Science and Biotechnology* **3**, 32-35

Saharkhiz MJ, Smaeili S, Merikhi M (2010) Essential oil analysis and phytotoxic activity of two ecotypes of *Zataria multiflora* Boiss growing in Iran. *Natural Product Research* **24**, 1598-1609

Sampietro DA, Vattuone MA (2006) Sugarcane straw and its phytochemicals as growth regulators of weed and crop plants. *Plant Growth Regulation* **48**, 21-27

Singh HP, Batish DR, Kaur S, Kohli RK (2003) Phytotoxic interference of *Ageratum conyzoides* with wheat (*Triticum aestivum*). *Journal of Agronomy and Crop Science* **189**, 341-346

Singh HP, Kohli RK, Batish DR, Kaushal PS (1999) Allelopathy of gymnospermous trees. *Journal of Forestry Research* **4**, 245-254

Sisodia S, Siddiqui B (2010) Allelopathic effect by aqueous extracts of different parts of *Croton bonplandianum* Baill. on some crop and weed plants. *Journal of Agricultural Extension and Rural Development* **2**, 22-28

Sodaeizadeh H, Rafieiolhossaini M, Havlík J, Van Damme P (2009) Allelopathic activity of different plant parts of *Peganum harmala* L. and identification of their growth inhibitors substances. *Plant Growth Regulation* **59 (3)**, 227-236

Turk MA, Tawaha AM (2003) Weed control in cereal in Jordan. *Crop Protection* **22**, 239-246