*Environmental and Experimental Biology (2014) 12: 83–87* *Original Paper*

# Evaluation of allelopathic effects of methanolic extracts from *Salicornia herbacea* seed and leaves on germination and seedling growth *in vitro* of two medicinal plants and two weeds

**Javad Sharifi Rad[1], Majid Sharifi Rad[2]*, Jaime A. Teixeira da Silva[3]**

[1]Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol 61615-585, Iran
[2]Department of Range and Watershed Management, Faculty of Natural Resources, University of Zabol, Zabol, Iran
[3]P. O. Box 7, Ikenobe 3011-2, Miki-cho, Kita-gun, Kagawa-ken, 761-0799, Japan

*Corresponding author, E-mail: majid.sharifirad@gmail.com

**Abstract**

In this study, methanolic extracts obtained from dried seeds and leaves of *Salicornia herbacea*, a medicinal plant, were evaluated at 2.5, 5 and 10% (v/v) *in vitro* for their potential allelopathic effects on the germination and seedling parameters of medicinal plants *Hyssopus officinalis* and *Nigella sativa*, and weeds *Taraxacum officinale* and *Amaranthus retroflexus*. Root length of *T. officinale* and *A. retroflexus* decreased significantly after exposure to 10% leaf extract, while all concentrations decreased the germination percentage of both weeds. *Amaranthus retroflexus* shoot length decreased significantly only after exposure to 10% leaf extract. In *N. sativa*, increasing concentration of leaf extracts increased shoot length and in *H. officinalis* maximum shoot length occurred at 5% concentration, but a further increase in concentration of leaf extracts had no negative impact on root length or seed germination. A very similar trend was observed for seed extracts. The negative effects on both weeds may be due to active compounds and/or allelochemicals in *S. herbacea*, whose seed and leaf extracts could be used as effective herbicides when applied at 5 or 10%, although the allelochemical(s) responsible for this allelopathic activity have yet to be identified.

**Key words:** allelopathic, germination, growth, methanolic extract, *Salicornia herbacea*.

## Introduction

*Salicornia herbacea* L. (Chenopodiaceae) is a halophyte that grows in salt marshes, salt lakes and tidal floodways (Anwar et al. 2002; Shepherd et al. 2005). *Salicornia* species are native to North America and are widely distributed in Europe, South Africa, South Asia and in Central and South Iran. *S. herbacea* has several active constituents including tungtungmadic acid (3-caffeoyl-4-dihydrocaffeoyl quinic acid), a chlorogenic acid derivative which has higher anti-oxidative activity in the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging test and in iron-induced liver microsomal lipid peroxidation assay (Chung et al. 2005). Chlorogenic acid, an ester of caffeic acid with quinic acid, is a recognized antioxidant found in several plants (Medina et al. 2007). In addition, other active compounds, including β–sitosterol, stigmasterol, uracil, quercetin 3-*O*-β-D-glucopyranoside, and isorhamnetin 3-*O*-β-D-glucopyranoside, have been isolated from *S. herbacea* by repeated column chromatography (Lee et al. 2004; Park, Kim 2004). Oh et al. (2007) found that the methanolic extract of viscozyme-treated *S. herbacea* fresh shoots exhibited the strongest radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl, superoxide and hydroxyl radicals. Oh et al. (2007) isolated and identified five phenolic compounds (ferulic acid, procatechuic acid, isorhamnetin, caffeic acid, and quercetin) by antioxidant assay-guided fractionation and purification. *S. herbacea* contains large amounts of salt and minerals, especially calcium, magnesium and iodine (Min et al. 2002).

Allelochemical substances, which mainly inhibit the growth or germination of a plant by another through the release of toxic metabolites into the environment (Oussama 2003), play a significant role in the ecology and evolution of plants (Deef, Abd El-Fattah 2008). Plant-derived allelochemicals have been suggested as useful herbicides in weed control (Babu, Kandasamy 1997).

This study aimed to assess the allelopathic effects of methanolic extract obtained from *S. herbacea* seeds and leaves on germination and seedling growth *in vitro* of two medicinal plants (*Hyssopus officinalis* L. and *Nigella sativa* L.) and two common weeds (*Taraxacum officinale* F.H. Wigg and *Amaranthus retroflexus* L.). Hyssop (*Hyssopus officinalis*) is an endemic Iranian perennial with a long history of medicinal use. Black cumin (*Nigella sativa*) is naturally distributed throughout various parts of Iran and is extensively cultivated for its medicinal value. Dandelion (*Taraxacum officinale*) is a perennial herb with deep roots. It is the most significant and abundant weed in turfgrass fields and a risk to agriculture lands in Iran since seed are dispersed by wind. Redroot pigweed (*Amaranthus retrofiexus*) is one of the most important weeds of common bean and has spread to many agricultural areas in Iran.

## Materials and methods

### *Plant material*

The seeds and fresh leaves of mature (one year old) *S. herbacea* were harvested in the area around Maharlo salt lake (located 27 km southeast of Shiraz, Iran) in Iran between July and August of 2012. The seeds of both medicinal plants and weeds were obtained from a commercial seed company (Pakan Bazr, Esfahan, Iran).

### *Plant extracts*

Mature seeds and fresh leaves were powdered separately in a knife mill. Each ground sample (20 g) was mixed with 200 mL of 85% methanol using a shaking water bath for 24 h at room temperature. The extract was separated from the solid concentrate 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 *S. herbacea* seed and leaf extracts, dilutions of the original extract were made using distilled water to 2.5, 5 and 10%. Twenty seeds of similar size from each test species were surface sterilized with a water-bleach solution (95:5) and then placed on sterilized double-layered filter paper in Petri dishes 6 cm in diameter. Extract (5 mL) from each concentration (2.5, 5 and 10%) of seed and leaf extract were added separately to each Petri dish and distilled water was used as the control. All Petri dishes were maintained under laboratory conditions (light (350 μmol $m^{-2}$ $s^{-1}$) at 25 °C) for 14 days, monitored daily and the evaporated contents were compensated with fresh 2.5, 5 and 10% solution. The numbers of germinated and non-germinated seeds were counted and final root and shoot lengths were measured at the end of the 14th day.

### *Statistical analysis*

In this study, the experimental design was a complete randomized design with four replications for each treatment. Significant differences in mean germination percentage, as well as root and shoot length in different treatments was tested using one-way analysis of variance (ANOVA) and the least significant difference (LSD) test. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS software version 11.5, IBM SPSS, New York, USA) at $P \leq 0.05$.

## Results

All concentrations (2.5, 5 and 10%) of the leaf extract decreased the germination percentage of both weeds (Fig. 1C). Root length of *T. officinale* and *A. retroflexus* decreased significantly after exposure to 10% (Fig. 1A). *A. retroflexus* shoot length decreased significantly only after exposure to 10% leaf extract (Fig. 1B). In *N. sativa*, as the leaf extract concentration increased, shoot length increased. In *H. officinalis*, maximum shoot length was observed in the presence of 5% leaf extract (Fig. 1B), and no negative impact on root length or seed germination was observed (Fig. 1A and 1C, respectively). An almost identical trend was observed for the seed extract (Fig. 2), which suggested that the allelopathic component(s) of leaves and seeds is likely to be very similar.

## Discussion

Allelochemicals are produced by plants as products, by-products and primary and secondary metabolites and they

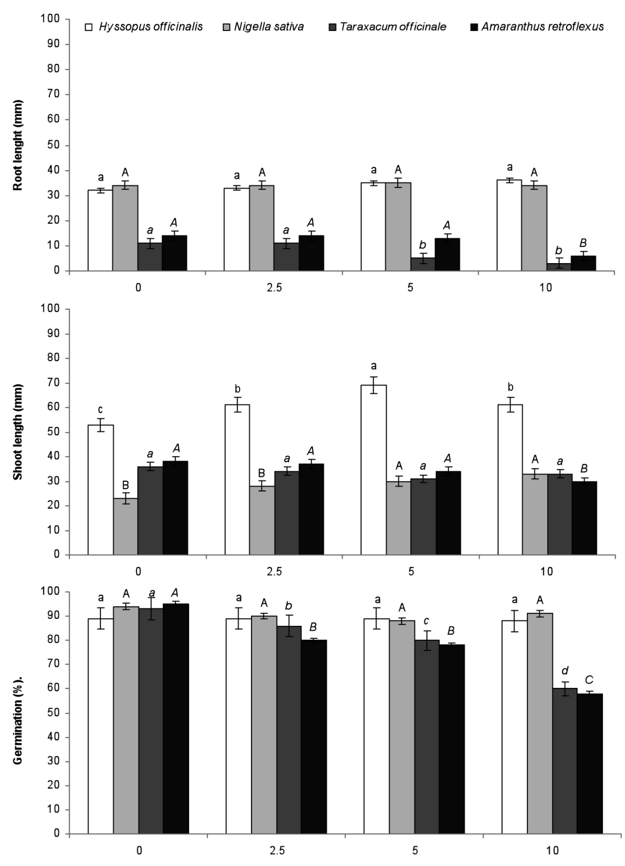


**Fig. 1.** Effect of four concentrations of *Salicornia herbacea* leaf extracts on root length (A), shoot length (B) and seed germination (C) of the four examined plants. Different letters show significant differences (for each parameter, between different extract concentrations and within a single plant species) between means at $P \leq 0.05$ (LSD test).

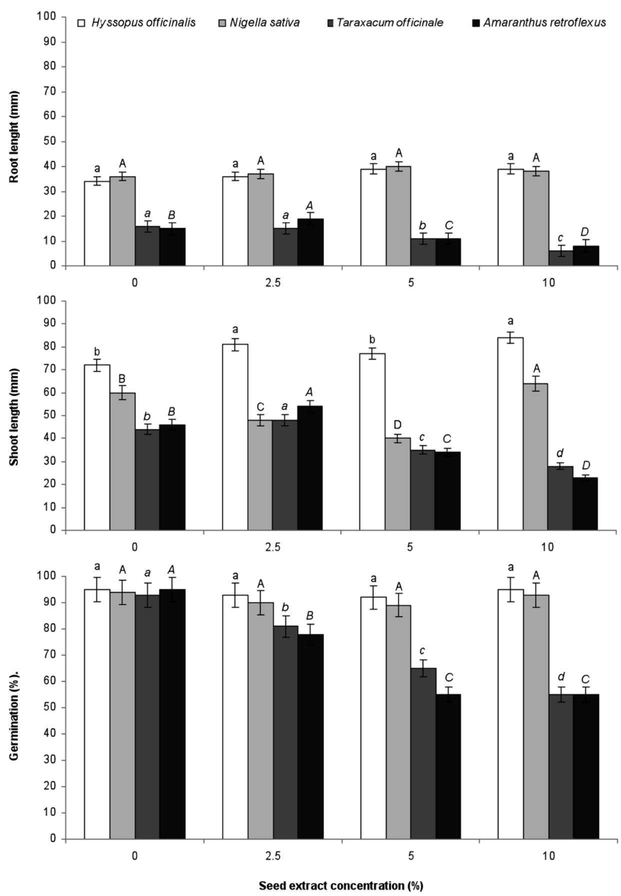


**Fig. 2.** Effect of four concentrations of *Salicornia herbacea* seed extracts on root length (A), shoot length (B) and seed germination (C) of the four examined plants. Different letters show significant differences (for each parameter, between different extract concentrations and within a single plant species) between means at $P \leq 0.05$ (LSD test).

exist in the stems, leaf, roots, flowers, inflorescences, fruits and seeds of plants (Sisodia, 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 such plant chemicals can suppress the germination and growth of different weed species (Singh et al. 2003; Turk, Tawaha 2003; Sampietro, Vattuone 2006; Mohsenzadeh et al. 2011; Nourimand et al. 2011; Mohsenzadeh et al. 2012). In particular, some medicinal plants have shown allelopathic activity, particularly towards seed germination: *Ephedra pachyclada* (Mohsenzadeh et al. 2011), *Trachyspermum copticum* (Mohsenzadeh et al. 2012), and *Foeniculum vulgare* (Nourimand et al. 2011).

Mohsenzadeh et al. (2011) found that all concentrations (2.5, 5, 10 and 20%) of methanolic extract of flowering aerial parts of *E. pachyclada* extract significantly reduced germination and seedling growth of four evaluated plants: ryegrass (*Lolium perenne*), bread wheat (*Triticum aestivum*), soybean (*Glycine max*), and mung bean (*Vigna radiata*). At 20% of the original extract, the germination of perennial ryegrass, soybean and mung bean was completely suppressed. Further, Mohsenzadeh et al. (2012) tested the inhibitory effect of the methanolic seed extract of *T. copticum* at 2.5 and 5% on germination and seedling growth of corn (*Zea mays*), cowpea (*Vigna unguiculata*), redroot amaranth (*Amaranthus retroflexus*) and dandelion (*Taraxicum officinalis*). The effects on cowpea and corn as cultivated crops were found to differ from the weeds (redroot amaranth and dandelion). At 2.5 and 5% seed extract, seed germination and seedling length of both weeds were completely inhibited, but in corn and cowpea there was no change or only a slight decrease. Nourimand et al. (2011) tested methanolic extract obtained from dried fruits of fennel (*Foeniculum vulgare*) *in vitro* to examine its potential allelopathic effects. Inhibitory effect of methanolic extract obtained from dried fruits of fennel (*F. vulgare)* at 2.5, 5 and 10% on germination and seedling growth of four weeds, perennial ryegrass (*Lolium perenne*), wild barley (*Hordeum spontaneum*), oat (*Avena ludoviciana*) and dandelion was tested. All concentrations suppressed the germination of perennial ryegrass and dandelion, while oat showed significantly reduced germination and seedling growth at 2.5%. The germination rate and percentage and seedling growth of wild barley were significantly decreased at 2.5 and 5%. At 10%, none of the examined weeds germinated. Worldwide, enormous amounts of chemical herbicides are used to manage these weeds. However, synthetic herbicides are often toxic and cause environmental problems (Khan 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 has been 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, i.e. chemical interaction between plants (Azizi, Fujii 2006). The importance of allelopathy in the natural control of weeds and crop productivity is now highly recognized (Khan et al. 2004).

Medicinal plants have been increasingly explored for their herbicidal potential (Anjum et al. 2010) as they may contain bioactive compounds such as ferulic, coumaric, vanillic, caffeic and chlorogenic acid that possess inhibitory activity (Modallal, Al-Charchafchi 2006). Nazir et al. (2007) evaluated the allelopathic effects of the 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* leaf and rhizome aqueous extracts at 2%. Fujii et al. (2003) tested 239 medicinal plants for their allelopathic activity on lettuce. They concluded that 223 species had inhibitory effects on germination and seedling growth.

Islam and Kato-Noguchi (2007) investigated the allelopathic potential of a medicinal plant *Leucas aspera*. Aqueous methanol extract of this plant at four concentrations (3, 10, 30 and 100 mg dry mass equivalent $mL^{-1}$ of extract) was tested on seven plant species: alfalfa (*Medicago sativa*), barnyard grass (*Echinochloa crusgalli*), water cress (*Lepidum sativum*), lettuce (*Lactuca sativa*), timothy (*Phleum pratense*), jungle rice (*Echinochloa colonum*) and Italian ryegrass (*Lolium multiflorum*). Extract from *L. aspera* significantly inhibited the growth of seedlings of all test plant species and at all concentrations. Root growth was more sensitive than shoot growth in response to *L. aspera* extract but this inhibitory activity was dependent on concentration. That study indicated that *L. aspera* may contain growth inhibitory substances (i.e., allelochemicals), allowing it to exert wide allelopathic activity.

Patel and Pandya (2013) assayed the allelopathic or phytotoxic effect of four medicinal plants (*Boerhaavia diffusa, Aerva lanata, Acalypha indica*, and *Synedrella nodiflora*) on radish (*Raphanus sativus*). Seed germination was significantly inhibited by stem and root aqueous extract of *B. diffusa*, radical growth was noticeably affected by *A. indica* leaf extract, while *S. nodiflora* extract affected plumule dry and fresh mass.

Miri et al. (2013) evaluated the potential *in vitro* allelopathic effects of the ethanolic extract from seeds of *Cardaria draba* (syn. *Lepidium draba*). Effect of extract from *C. draba* at 2.5, 5 and 10% (w/v) on germination and seedling growth of two cultivated crops, common bean (*Phaseolous vulgaris*) and wheat (*Hordeum vulgare*) and two weeds, dandelion (*Taraxicum officinalis*) and redroot amaranth (*Amaranthus retroflexus*) was tested. The seed extract affected all four test plants equally, with 2.5, 5 and 10% decreasing seed germination and seedling growth (shoot and root length). This allelopathic effect may be related to the presence of allelochemicals, including glucoerucin (4-methylation-butyl-glucosinolate), glucosinolate, glucoraphanin (4-methylsulfinyl-butyl-glucosinolate) and sinalbin (*p*-hydroxy-benzyl-glucosinolate).

Mahmoodzadeh and Mahmoodzadeh (2013) used aqueous extracts obtained from the shoots and roots of soybean (*Glycine max*), also a medicinal plant, to determine its allelopathic potential against the germination and seedling growth of two weed species, Johnson grass (*Sorghum halepens*) and rye (*Secale cereale*). The *G. max* shoot extract (100, 75, and 50%) decreased seed germination of both weeds. The shoot extract caused the greatest reduction in the germination of *S. halepense*. Similarly, in our study, seed and leaf extracts of *S. herbacea* had stronger allelopathic effects on weeds than on medicinal plants.

Allelopathic effects are desirable for researchers seeking means of natural control of weeds without any or with very little effect on other plants such as crops in rangelands and fields. Medicinal plants have a wealth of primary and secondary metabolites that contribute to their medicinal properties. Some of these, including *Aloe vera* (Alipoor et al. 2012) and *Tecomella undulata* (Mohsenzadeh et al. 2012b), may also inhibit the growth of other plants, i.e., display allelopathic properties, serving thus multiple purposes for society and agriculture (Teixeira da Silva et al. 2013).

In conclusion, the results of this study indicate that *S. herbacea* seed and leaf extracts had negative effects on two weeds (*Taraxicum officinalis* and *Amaranthus retroflexus*) but no effect on two medicinal plants (*Hyssopus officinalis* and *Nigella sativa*). *Salicornia herbacea* can thus be used as a herbicide against weeds when applied at 5 or 10% (seed or leaf extract), although pot, greenhouse and field trials would be necessary to confirm this claim.

**Acknowledgements**

The authors express their sincere appreciation to Professor A.K. Uniyal, College of Agriculture Science and Technology, Dehradun, UA, India, for his suggestions in improving this article.

**Conflicts of interest**

The authors declare no conflicts of interest, financial or other.

**References**

Alipoor M., Mohsenzadeh S., Teixeira da Silva J.A., Niakousari M. 2012. Allelopathic potential of *Aloe vera*. *Med. Aromatic Plant Sci. Biotechnol.* 6: 78–80.

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. *J. Med. Plants Res.* 4: 536–541.

Anwar F., Bhanger M.I., Nasir M.K., Ismail S. 2002. Analytical characterization of *Salicornia bigelovii* seed oil cultivated in Pakistan. *J. Agric. Food Chem.* 50: 4210–4214.

Azizi M., Fujii Y. 2006. Allelopathic effect of some medicinal plant substances on seed germination of *Amaranthus retroflexus* and *Portulaca oleraceae. Acta Hortic.* 699: 61–68.

Babu R.C., Kandasamy O.S. 1997. Allelopathic effect of *Eucalyptus globulus* Labill on *Cyperus rotundus* L. and *Cynodon dactylon* L. *J. Agron. Crop Sci.* 179: 123–126.

Chung Y.C., Chun H.K., Yang J.Y., Kim J.Y., Han E.H., Kho Y.H., Jeong H.G. 2005. Tungtungmadic acid, a novel antioxidant, from *Salicornia herbacea. Arch. Pharmacal. Res.* 28: 1122–1126.

Coban D., Kamaci H.O., Suzer C., Saka S., Firat K. 2009. Allometric growth in hatchery-reared gilthead sea bream. *N. Am. J. Aquacult.* 71: 189–196.

Deef H.E., Abd El-Fattah R.I. 2008. Allelopathic effects of water extract of *Artemisia princeps* var. *orientalis* on wheat under two type of soils. *Acad. J. Plant Sci.* 1: 12–17.

Fujii Y., Parvez S.S., Parvez M.M., Ohmae Y., Iida O. 2003. Screening of 239 medicinal plant species for allelopathic activity using sandwich method. *Weed Biol. Manage.* 3: 233–241.

Islam A.K.M.M., Kato-Noguchi H. 2012. Allelopathic potentiality of medicinal plant *Leucas aspera. Int. J. Agr. Sustain.* 4: 1–7.

Khan M.A., Marwat K.B., Hassan Z. 2004. Allelopathic potential of some multipurpose trees species (MPTS) on the wheat and some of its associate's weeds. *Int. J. Biol. Biotechnol.* 1: 275–278.

Kong C.S., Kim Y.A., Kim M.M., Seo Y. 2008. Flavonoid glycosides isolated from *Salicornia herbacea* inhibit matrix metalloproteinase in HT1080 cells. *Toxicol. In Vitro* 22: 1742–1748.

Kumar S.R., Ramanathan G., Subhakaran M., Inbaneson J. 2009. Antimicrobial compounds from marine halophytes for silkworm disease treatment. *Int. J. Med. Med. Sci.* 1: 184–191.

Lee C.H., Kim I.H., Kim Y.E., Oh S.W., Lee H.J. 2004. Determination of betaine from *Salicornia herbacea* L. *J. Korean Food Nutr.* 33: 1584–1587.

Mahmoodzadeh H., Mahmoodzadeh M. 2013. Allelopathic potential of soybean (*Glycine max* L.) on the germination and root growth of weed species. *Life Sci J.* 10: 63–93.

Manikandan T., Neelakandan T., Usha Rani G. 2009. Antibacterial activity of *Salicornia brachiata*, a halophyte. *J. Phytol.* 1: 441–443.

Medina I., Gallardo J.M., Gonzalez M.J., Lois S., Hedges N. 2007. Effect of molecular structure of phenolic families as hydroxycinnamic acids and catechins on their antioxidant effectiveness in minced fish muscle. *J. Agric. Food Chem.* 55: 3889–3895.

Min J.G., Lee D.S., Kim T.J., Park J.H., Cho T.Y., Park D.I. 2002. Chemical composition of *Salicornia herbacea* L. *J. Food Sci. Nutr.* 7: 105–107.

Miri A., Rad J.S., Rad M.S., Teixeira da Silva J.A. 2013. Allelopathic activity of medical plant, *Cardaria draba* (*Lepidium draba* L.). *Ann. Biol. Res.* 4:76–79.

Modallal N., Al-Charchafchi F.M.R. 2006. Allelopathic effect of *Artemisia harba alba* on germination and seedling growth of *Anabasis setifera. Pak. J. Biol. Sci*. 9: 1795–1798.

Mohsenzadeh S., Gholami M., Teixeira da Silva J.A. 2011. Allelopathic potential of *Ephedra. Med. Aromatic Plant Sci. Biotechnol.* 5: 160–162.

Mohsenzadeh S., Tayyebi N.S., Teixeira da Silva J.A. 2012. Allelopathic potential of *Tecomella undulate. Med. Aromatic Plant Sci. Biotechnol.* 6: 75–77.

Mohsenzadeh S., Zaboli J., Teixeira da Silva J. A. 2012. Allelopathic potential of ajwain (*Trachyspermum copticum*). *Med. Aromatic Plant Sci. Biotechnol.* 6: 72–74.

Nazir T., Uniyal A.K., Todaria N.P. 2007. Allelopathic behavior of three medicinal plant species on traditional agriculture crops of Garhwal Himalaya, India. *Agroforest Syst.* 3: 183–187.

Nourimand M., Mohsenzadeh S., Teixeira da Silva J.A., Saharkhiz M.J. 2011. Allelopathic potential of fennel (*Foeniculum vulgare* Mill.). *Med. Aromatic Plant Sci. Biotechnol.* 5: 54–57.

Oh J.H., Kim E.O., Lee S.K., Woo M.H., Choi S.W. 2007. Antioxidant activities of the ethanol extract of hamcho (*Salicornia herbacea* L.) cake prepared by enzymatic treatment. *Food Sci. Biotechnol.* 16: 90–98.

Oussama O. 2003. Allelopathy in two durum wheat (*Triticum durum* L.) varieties. *Agr. Ecosyst. Environ.* 96: 161–163.

Park S.H., Kim K.S. 2004. Isolation and identification of antioxidant flavonoids from *Salicornia herbacea* L. *J. Korean Soc. Appl. Biol. Chem.* 47: 120–123.

Patel D.A., Pandya N.R. 2013. Allelopathic effect of few medicinal plants on *Raphanus sativus* L. *Int. J. Res. Plant Sci.* 3: 88–93.

Rhee M.H., Park H., Cho J.Y. 2009. *Salicornia herbacea*: Botanical, chemical and pharmacological review of halophyte marsh plant. *J. Med. Plants Res.* 3: 548–555.

Sampietro D.A., Vattuone M.A. 2006. Sugarcane straw and its phytochemicals as growth regulators of weed and crop plants. *Plant Growth Regul.* 48: 21–27.

Sarker S.K., Park S.R., Kim G.M., Yang C.J. 2010. Hamcho (*Salicornia herbacea*) with probiotics as alternative to antibiotic for broiler production. *J Med. Plants Res.* 4: 415–420.

Shepherd K.A., Macfarlane T.D., Colmer T.D. 2005. Morphology, anatomy and histochemistry of Salicornioideae (Chenopodiaceae) fruits and seeds. *Ann. Bot.* 95: 917–933.

Singh H. P., Batish D. R., Kaur S., Kohli R. K. 2003. Phytotoxic interference of *Ageratum conyzoides* with wheat (*Triticum aestivum*). *J. Agron. Crop Sci*. 189: 341–346.

Sisodia S., Siddiqui B. 2010. Allelopathic effect by aqueous extracts of different parts of *Croton bonplandianum* Baill. on some crop and weed plants. *J. Agr. Ext. Rural Dev.* 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 Regul.* 59: 227–236.

Teixeira da Silva J.A., Bich T.T.N., Mohsenzadeh S. 2013. Allelopathic potential of medicinal plants: focus on aloe, ajwain and rohida tree, In: Govil J.N., Tiwai L. (eds) *Recent Progress of Medicinal Plants. Phytoconstituents and Biochemical Processes*. St. Press, LLC Houston, USA, Vol. 35, pp. 373–390.

Turk M.A., Tawaha A.M. 2003. Weed control in cereal in Jordan. *Crop Prot.* 22: 239–246.

*Received 13 February 2014; received in revised form 3 April 2014; accepted 15 May 2014*