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EVALUATION OF THE ANTI-*ALTERNARIA SOLANI* ACTIVITY OF *ALLIUM HIRTIFOLIUM* BOISS.

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Abstract

The inhibitory effect of methanolic extract of 100 plant species from 45 plant families were investigated on mycelial growth of *Alternaria solani*, the cause of early blight of tomatoes, based on paper disc diffusion method. The study was conducted in a completely randomized design with 4 replications. The results showed that methanolic extracts of 21 species exhibited measurable inhibitory effect on mycelial growth of *A. solani.* The stongest inhibitory effect was obtained for the extracts of *Allium hirtifolium* and *Teucrium chamaedrys* with radius inhibitory zones of 19.08 mm ± 0.48 and 15.58 mm ± 0.48, respectively. Furthermore, an experiment was performed to investigate synergistic effect of *A. hirtifolium*, *Ferula assa-foetida* and *Artemisia sieberi* separately and in combinations with each other based on agar dilution method on the growth of *A. solani*. Results indicated that a mixture of *Allium hirtifolium* and *F. assa-foetida* extracts caused complete mycelial growth inhibition of *A. solani*. The IC50 and IC95 values for *A. hirtifolium* bulbs were equal to 78 and 946 ppm, respectively. Moreover, methanolic extract of *A. hirtifolium* bulb significantly showed the highest inhibitory effect on mycelial growth of *A. solani* when compared with other plant parts. The results of inhibitory effect of fractions obtained by thin layer chromatography of *A. hirtifolium* bulbs showed that one band with Rf= 0.41 had an inhibitory effect against *A. solani*.

Introduction

Early blight is one of the main diseases of tomatoes, potatoes, and eggplants all over the world. The disease is caused by the species of *Alternaria* and the most destructive and important species is *A. solani* (Eliss and Martin). In humid areas with high humidity and frequent rains followed by hot and dry weather, the disease inflicts considerable reductions in yield (Yazici *et al*., 2011). In Iran, the amount of tomato infection in Jiroft and Kahnouj has been reported between 60 and 90% (Shahbazi *et al*., 2011). There are some methods such as fungicides application, resistant varieties, biological elements and crop rotation to control this disease (Neeraj & Verma, 2010).

Increasing concerns for public health and environment have caused growers to seek for new strategies to control the disease considering decreasing the use of pesticides. To achieve this goal, using plant extracts and natural compounds is of great importance since these products have no bad effects on health or cause any pollution. They are even cheaper than chemicals and have minimum or in fact, no harmful side effects on hosts as well (Neeraj & Verma, 2010).

Several useful studies were conducted to find out the effects of plant extracts on the control of early blight disease. Suleiman (2010) studied antifungal activity of crude extracts of neem (*Azadirachta indica* Juss) and Pawpaw (*Carica papaya* L.) in *In vitro* conditions on *A. solani* separated from rotting yam tubers whose pathogenesis was proved and evaluated. They found that the mentioned extracts consisted of antifungal compounds postponed mycelial growth of the fungus. Moreover, antifungal activities of crude methanolic extracts of *Tinospora cordifolia*, *Acorus calamus*, and C*elestrus piniculatus* were studied on the growth of *A. solani* at various concentrations. The results obtained from this test indicated that crude methanolic extracts of *C. paniculatus* at the concentration of 5000 µg/ml showed greater inhibitory effect on *A. solani* (Singh *et al*., 2010). Satya *et al*., (2005) reported that the leaf extracts of zimmu (*Allium cepa*×*A. sativum*) were effective in preventing the growth of agronomically important fungal and bacterial pathogens like *A. solani.* Sharma *et al.,* (2007) reported that the extract of neem leave had the highest inhibitory effect nearly 43.3% and 26.7% at the concentrations of 0.1% and 0.01%, respectively on radial growth of *A. solani*. Pawar & Thaker (2007) indicated that *Melaleuca alternifolia* oils could reduce the outbreak and severity of foliar diseases derived from *A. solani* on potatoes.

The objectives of the present study, as part of larger screening program, were to identify plant sources with anti-*Alternaria* activity, to determine which plant parts or concentrations have higher activity and to find the active fractions in active plant species.

Materials and Methods

Plant materials and fungal isolate: One hundred plant species from 45 plant families (Table 1.) were collected from their natural inhabitant in different Provinces; Kermanshah and Hamadan in West, Ardabil in North and Fars South-West of Iran from 2010 to 2012. Plants were randomly collected to increase the chance of finding plants with bioactive extracts. The plants were identified by staff at the Herbarium at Razi University, College of Agriculture and scientific names were checked in the International Plant Names Index (http://www.ipni.org/ipni/plantnamesearchpage.do). Each sample was cleaned, shade dried and ground to a fine powder with a coffee grinder before methanol extraction. The isolate of *A. solani* was kindly provided by Dr. Youbert Ghosta from Department of Plant Protection at Urmia University.

Preparation of plant crude extract: Methanolic extracts were also obtained as described by Bahraminejad *et al.,* (2008). Briefly, 5 g ground sample was extracted with 100 ml methanol for 24 h by shaking on an orbital shaker at 300 rpm. Then 30ml distilled water was added to 70 ml of the methanolic extract and lipids were removed with 100 ml n-hexane mixed at 250 rpm for 2 h. Methanolic phase was concentrated using a rotary evaporator. Finally, the residues were dissolved in 45% methanol in distilled water and a sample of extract at a concentration of 100 mg/ml for bioassay was provided.

Table 1. *In vitro* screening for anti-*Alternaria solani* activity (mean ± standard error) of plant extracts at a concentration of 5 mg/paper disc. Each mean was calculated from four replicates.

Plant	Family	Location	Plant part used	Radius zone of inhibition (mm)
Allium colchicifolium Boiss.	Alliaceae	Kermanshah	Leaf	NI
Allium hirtifolium Boiss.	Alliaceae	Tuiserkan	Bulb	19.08 ± 0.5 [a*]
Rhus coriaria L.	Anacardiaceae	Sarpolezahab	Total	8.58 ± 0.1 [gh]
Carum copticum Benth. & Hook.f.	Apiaceae	Kermanshah	Seed	NI[*]
Cuminum cyminum L.	Apiaceae	Kermanshah	Seed	NI
Dorema aucheri Boiss.	Apiaceae	Sarpolezahab	Total	NI
Falcaria vulgaris Bernh.	Apiaceae	Kermanshah	Leaf	NI
Ferula assa-foetida L.	Apiaceae	Fars-Lar	*Gum*	14.67 ± 0.3 [c]
Ferulago angulata Boiss.	Apiaceae	Sarpolezahab	Shoot	8.33 ± 0.2 [ghi]
Foeniculum vulgare Mill.	Apiaceae	Kermanshah	Leaf	NI
Oliveria decumbens Vent.	Apiaceae	Sarpolezahab	Total	NI
Prangos ferulacea Lindl.	Apiaceae	Ardabil	Shoot	9.75 ± 0.2 [f]
Aristolochia bottae Jaub. & Spach	Aristolochiaceae	Tuiserkan	Total	6.83± 0.2 [l]
Acroptilon repens (L.) DC.	Asteraceae	Kermanshah	Shoot	WI
Achillea filipendulina Lam.	Asteraceae	Kermanshah	Shoot	12.09 ± 0.4[d]
Artemisia sieberi Besser	Asteraceae	Ardabil	Shoot	11.75 ± 0.3 [d]
Calendulla officinalis L.	Asteraceae	Kermanshah	Shoot	8.50 ± 0.3 [g]
Centaurea behen L.	Asteraceae	Harsin	Shoot	NI
Centaura depressa M.Bieb	Asteraceae	Kermanshah	Shoot	NI
Centaurea sp.	Asteraceae	Sarpolezahab	Total	NI
Cynara scolymus L.	Asteraceae	Kermanshah	Fruit	11.83 ± 0.2 [d]
Gundelia tournefortii L.	Asteraceae	Sarpolezahab	Total	WI
Onopordon sp.	Asteraceae	Kerend	Total	NI
Xanthium strumarium L.	Asteraceae	Kermanshah	Total	NI
Anchusa italica Retz.	Boraginaceae	Kermanshah-Sahne	Shoot	NI
Borago officinalis L.	Boraginaceae	Super market	Shoot	NI
Onosma sp.	Boraginaceae	Kermanshah-Sahne	Total	NI
Brassica rapa L.	Brassicaceae	Super market	Corm	NI
Conringia orientalis L.	Brassicaceae	Kermanshah	Total	NI
Descurainia sophia (L.) Prantl	Brassicaceae	Kermanshah	Shoot	NI
Goldbachia laevigata DC.	Brassicaceae	Kermanshah	Shoot	NI
Raphanus sativus L.	Brassicaceae	Super market	Root	NI
Cannabis sativa L.	Cannabinaceae	Kermanshah	Total	NI
Vaccaria pyramidata Medik	Caryophyllaceae	Sarpolezahab	Total	NI
Convolvulus arvensis L.	Convolvulaceae	Super market	Leaf	NI
Citrullus colocynthis (L.) Schrad. S	Cucurbitaceae	Sarpolezahab	Shoot	NI
Cucumis melo L.	Cucurbitaceae	Sarpolezahab	Shoot	NI
Cucumis schema	Cucurbitaceae	Sarpolezahab	Shoot	NI
Chrozophora tinctoria A. Juss.	Euphorbiaceae	Kermanshah	Total	NI
Euphorbia rigida Loisel.	Euphorbiaceae	Kermanshah	Shoot	8.16 ± 0.2 [ghij]
Alhagi camelorum Fisch.	Fabaceae	Sarpolezahab	Total	NI
Glycyrrhiza glabra L.	Fabaceae	Kermanshah	Shoot	NI
Melilotus officinalis Lam.	Fabaceae	Kermanshah	Total	NI
Prosopis stephaniana (Willd.)	Fabaceae	Sarpolezahab	Fruit	NI
Robinia pseudoacacia L.	Fabaceae	Kermanshah	Shoot	NI
Sophora alopecuroides L.	Fabaceae	Kermanshah	Shoot	NI
Quercus persica Jaub. & Spach	Fagaceae	Kermanshah	Fruit	WI
Muscari armeniacum Leichtlin ex Baker	Hyacinthaceae	Tuiserkan	Total	7.58 ± 0.1 [jk]
Hypericum scabrum L.	Hypericaceae	Tuiserkan	Total	7.91 ± 0.8 [hijk]
Juglans regia L.	Juglandaceae	Tuiserkan	Peel of fruit	NI

Table 1. (Cont'd.)

Plant	Family	Location	Plant part used	Radius zone of inhibition (mm)
Teucrium chamaedrys L.	Lamiaceae	Ardabil	Shoot	15.59 ± 0.1^{b}
Lavandula officinalis Chaix	Lamiaceae	Kermanshah	Shoot	NI
Melissa officinalis L.	Lamiaceae	Super market	Shoot	WI
Mentha piperata Stokes	Lamiaceae	Kermanshah	Shoot	NI
Mentha pulegium L.	Lamiaceae	Sarpolezahab	Shoot	NI
Rosmarinus officinalis L.	Lamiaceae	Kermanshah	Total	NI
Thymus sp.	Lamiaceae	Ardabil	Shoot	NI
Thymus vulgaris L.	Lamiaceae	Super market	Shoot	7.33 ± 0.9^{kl}
Salvia sclarea L.	Lamiaceae	Kermanshah	Flower	WI
Satureja hortensis L.	Lamiaceae	Kermanshah	Shoot	WI
Cinnamomum zeylanicum Blume	Lauraceae	Super market	Bark	NI
Laurus nobilis L.	Lauraceae	Kermanshah	Shoot	NI
Gagea sp.	Liliaceae	Tuiserkan	Total	NI
Linum usitatissimum L.	Linaceae	Kermanshah	Seed	NI
Lawsonia inermis L.	Lythraceae	Super market	Shoot	NI
Punica granatum L.	Lythraceae	Sarpolezahab	Leaf	WI*
Abelmoschus esculentus Moench	Malvaceae	Sarpolezahab	Leaf	NI
Abutilon theophrasti Medik.	Malvaceae	Kermanshah	Total	NI
Melia azedarach L.	Meliaceae	Kermanshah	Shoot	7.74 ± 0.4^{ijk}
Melia indica Brand.	Meliaceae	Kermanshah	Leaf	NI
Morus alba L.	Moraceae	Kermanshah	Leaf	NI
Eucalyptus sp.	Myrtaceae	Super market	Leaf	NI
Syzygium aromaticum(L.) Merr. & L.M.Perry	Myrtaceae	Super market	Flower	10.08 ± 0.3^{ef}
Jasminum officinale L.	Oleaceae	Kermanshah	Shoot	NI
Orobanche alba Rchb.	Orobanchaceae	Tuiserkan	Shoot	NI
Papaver dubium L.	Papaveraceae	Tuiserkan	Total	WI
Pinus eldarica Medw.	Pinaceae	Kermanshah	Leaf	NI
Avena sativa L.	Poaceae	Kermanshah	Shoot	NI
Echinochloa crus-galli L.	Poaceae	Kermanshah	Total	NI
Polygonum aviculare L.	Polygonaceae	Kermanshah	Shoot	NI
Portulaca oleraceae L.	Portulacaceae	Kermanshah	Total	NI
Anagallis arvensis L.	Primulaceae	Sarpolezahab	Total	10.59 ± 0.1^{e}
Consolida sp.	Rananculaceae	Super market	Leaf	NI
Ziziphus jujuba Lam.	Rhamnaceae	Super market	Fruit	WI
Ziziphus spina-christi Willd.	Rhamnaceae	Super market	Leaf	NI
Mespilus germanica L.	Rosaceae	Kermanshah	Leaf	NI
Cruciata coronata (Sibth. & Sm.) Ehrend.	Rubiaceae	Tuiserkan	Total	WI
Verbascum sp.	Scrophulariaceae	Tuiserkan	Total	8.83 ± 0.1^{g}
Veronica anagallis-aquatica L.	Scrophulariaceae	Sarpolezahab	Total	NI
Scrophularia striata Boiss.	Scrophulariaceae	Sarpolezahab	Shoot	NI
Capsicum annuum L.	Solanaceae	Super market	Fruit	NI
Nicotiana persica Lindl.	Solanaceae	Kermanshah	Shoot	NI
Nicotiana tabacum L.	Solanaceae	Kermanshah	Shoot	WI
Physalis alkekengi L.	Solanaceae	Sarpolezahab	Leaf	12.25 ± 0.1^{d}
Solanum nigrum L.	Solanaceae	Kermanshah	Fruit	7.42 ± 0.1^{kl}
Celtis caucasica Willd.	Ulmaceae	Kermanshah	Shoot	NI
Urtica dioica L.	Urticaceae	Tuiserkan	Shoot	WI
Viscum album L.	Viscaceae	Kerend	Shoot	NI
Curcuma longa L.	Zingiberaceae	Super market	Rhizome	NI
Peganum harmala L.	Zygophyllaceae	Ardabil	Shoot	NI

***,** Means followed by the same letters are not significantly different at $p \leq 0.05$

Bioassay: To determine the effect of plant extracts on the mycelial growth of *A. solani*, a disc of 6 mm was cultured using a cork borer from the margin of the growing pathogenic colony and placed in the center of Petri dish containing PDA medium. The plates were incubated in the 25°C germinator. In this study, the paper disc method was used to test for any inhibitory effect of crude plant extracts. Positive control discs loaded by mancozeb at a concentration of 1 mg/disc were tested against *A. solani* in order to determine the effectiveness of the crude extracts. When the diameter of the colony was 3 cm long, the sterile filter paper disc of 6 mm containing 50 µl of the extract suspension (concentration of 100 mg/ml) equal to 5 mg was placed on the agar surface at 1 cm away from the growing mycelial and 45% methanol was used as a negative control. Loaded paper discs were allowed to be dried well. Inoculated plates were incubated at 25°C and radius zone of inhibition (distance between the centre of the paper disc and margin of inhibited colony from three different directions) was recorded in millimeters as described by Bahraminejad *et al.,* (2008).

The inhibitory activity of different parts of *A. hirtifolium* against *A. solani:* Persian shallot (*Allium hirtifolium)* was selected among 100 tested plant species and the methanolic extracts of the plant parts such as leaves, stems, inflorescence, and bulb were separately prepared. Inhibitory effects of the stock suspension of the mentioned parts were studied on the mycelial growth of *A. solani* at 100 mg/ml concentration and the quantity of 50 µl equal to 5 mg per paper disc was loaded on each paper disc.

The inhibitory effect of different concentration of *A. hirtifolium* bulb: In order to determine the growth inhibition of shallot bulb, its methanolic extract was considered at 7 serial concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.62 ppm) with four replicates on the mycelial growth of *A. solani* on the basis of agar dilution method. To provide the concentrations, required quantities of the extract were first solved in the solvent (45% methanol) and added to 100 ml of the medium when the temperature reached about 45°C. After the solidifying the medium, discs of 6 mm diameter were taken from the side of the growing colony and placed in the center of each Petri dish containing the medium. For the test control, a disc was loaded by 1ml of methanol (45%) as negative control. The plates were incubated in the 25°C ± 1 germinator. Inhibitory effect was measured in two perpendicular directions using a ruler and its average was calculated, once the medium surface in the control plates thoroughly occupied by the fungus. The percentage of inhibition was calculated for each concentration with the following formula:

$$IP= ((C-T)/C) \times 100$$

IP: Inhibitory Percentage; C: The average diameter of fungal colony in the control

T: The average diameter of fungal colony in the desired treatment (Hadian *et al*., 2006).

The 50 and 95% growth inhibition (IC_{50} and IC_{95}) were determined from a probit analysis on the logarithm of the concentrations which was analyzed using SPSS 17.0.

The study of the synergistic effect of the plant extracts against *A. solani*: To examine the possibility of the cumulative or synergistic effect of the plant extracts, the inhibition of methanolic extracts was separately and in combinations with each other investigated against *A. solani* using agar dilution method. There were four replicates for this test and 0.8 ml of 45% methanol was applied as a negative control.

Thin layer chromatography: Considering the determination of the most effective components of the most effective species (shallot bulb) in accordance to thin layer chromatography, aluminum-backed plates (0.20 mm, silica 60, Merck KGA) were used. The TLC plates were developed with a solvent system of isopropanol: ammonia: water (8: 1: 1) as stated by Satya *et al.,* (2005). The developed chromatograms were examined under UV light (254/365 nm). Observed fractions with similar relative to the front, Rf, were pooled, and materials were recovered from the silica gel. The Rf of the components in each band was calculated via the following formula (Bahraminejad *et al*., 2008).

Rf= (A/B) ×100, Rf = relative to front in each band; A= The distance passed by the considered material; B= the distance passed by the solvent.

Eluted fractions were separately tested for anti-*Alternaria solani* activity at a concentration of 2mg per paper disc using the paper disc diffusion method.

The present study was carried out on the basis of completely randomized design (CRD) and each treatment was replicated four times. The results were analyzed using MSTAT-C software program. The means were compared according to Duncan's new multiple ranges test (α= 0.05).

Results

The results of *In vitro* antifungal activity of 100 plant extracts are summarized in Table 1 and indicated as radius inhibition zone. These results showed highly significant differences among different plant extracts. According to Table 1, among 100 tested plant species of 45 plant families, methanolic extract of 21 species (>21%) from 13 families has shown a measurable inhibitory effect on *A. solani*. Methanolic extract of *A. hirtifolium* had the maximum activity (19.08± 0.5 mm) on mycelial growth of the tested fungus, followed by *Teucrium chamaedrys*, *Ferula assa-foetida, Physalis alkekengi*, *Achillea filipendulina, Cynara ascolymus, Artemisia sieberi, Anagallis arvensis, syzygium aromaticum* with more than 10 mm radius inhibition zones. The observed inhibitory effect of *A. hirtifolium* at 5 mg per paper disc crude extract was higher than the inhibition obtained from mancozeb as a common fungicide at 1 mg per paper disc (17.00 ± 0.43 mm).

Highly significant differences were found among the extracts obtained from different parts of shallot in terms of inhibitory effect on mycelial growth of *A. solani*. The methanolic extract of shallot bulb with 23.67 ± 0.4 mm inhibitory zone significantly showed the greatest inhibitory effect on *A. solani*. Besides, no significant differences were found among leaf, stem and inflorescence for their inhibitory effects (Fig. 1).

Different concentrations of methanolic extract of shallot bulb were tested against mycelial growth of *A. solani* using agar dilution method. The results revealed that there are highly significant differences among the inhibitory percentages of miscellaneous concentrations of shallot bulb extract on mycelial growth of *A. solani*. Although, the fungal mycelial growth has considerably reduced (90.83%) at concentration of 1000 ppm, no concentration has caused the complete inhibition among the tested range (Fig. 2). The IC50 and IC95 values for *A. hirtifolium* bulbs were equal to 78 and 946 ppm, respectively.

Results of the synergistic effect of the extracts through evaluating the inhibitory percentage of methanolic extracts of *A. hirtifolium* (bulb), *Ferula assa-foetida* (Gum) and *Artemisia sieberi* (shoot) plants have demonstrated that the mixture of *A. hirtifolium* and *F. assa-foetida* extracts (each at 500 ppm concentration) caused a complete inhibition of *A. solani*, whereas none of the mentioned plant extracts was able to inhibit the mycelial growth of *A. solani* completely when they were applied at the doubled concentration (Fig. 3). Moreover, the results showed that there are significant differences among tested treatments for the percentage of inhibition, so that a mixture of *A. hirtifolium* and *F. assa-foetida* significantly controlled the growth of fungus when compared to the other treatments.

Comparing the effectiveness of 4 diverse solvent systems has exhibited that the solvent system of isopropanol: ammonia: water (8:1:1) establishing distinctive bands under UV is the most appropriate liquid phase for making the components of shallot bulb extract isolated apart. The results have demonstrated the existence of six bands, four inter-band and the remnant residue of the extract on the beginning line on the silica gel plates. The bands were numbered from 1 to 6 and inter band areas. Rf quantities for each band were calculated (Table 2). The results of studying inhibitory effect of shallot bulb showed that only band 2 had distinguishable inhibitory effect on *A. solani* at concentration of 2 mg per paper disc. No inhibitory effects observed for the other bands (Fig. 4).

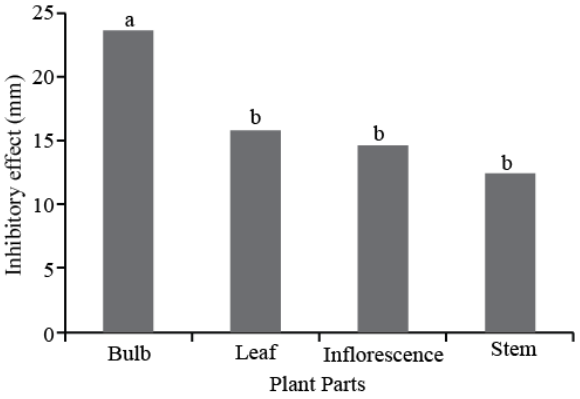

Fig. 1. Means of inhibitory effect of various parts of *Allium hirtifolium* including bulb, leaf, inflorescence and stem against mycelial growth of *Alternaria solani.*

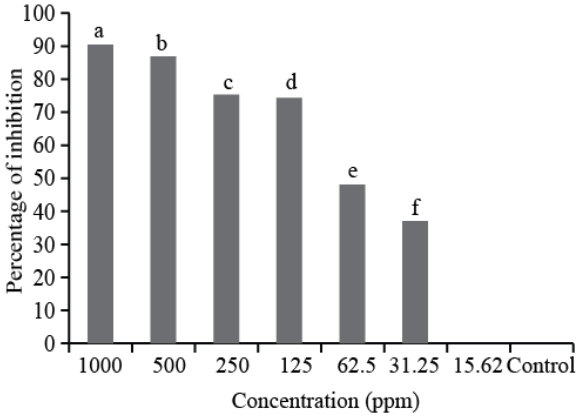

Fig. 2. The mean percentage of inhibition of methanolic extract of *Allium hirtifolium* bulb on mycelial growth of *Alternaria solani* at various concentrations through agar dilution method.

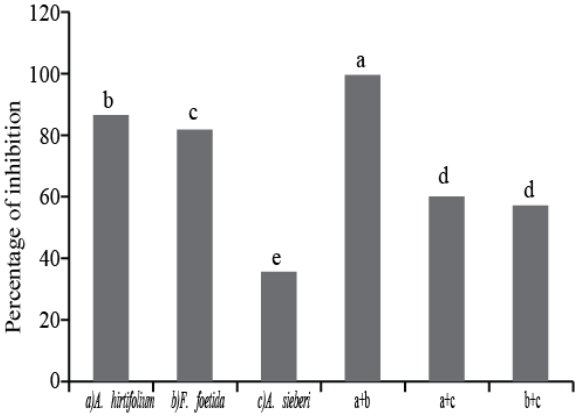

Fig. 3. Mean inhibitory effect of the methanolic extracts obtained from *A. hirtifolium*, *F. foetida, A. sieberi,* separately at a concentration of 1000 ppm and a combination (500 ppm of each) of the mentioned extracts on mycelial growth of *Alternaria solani.*

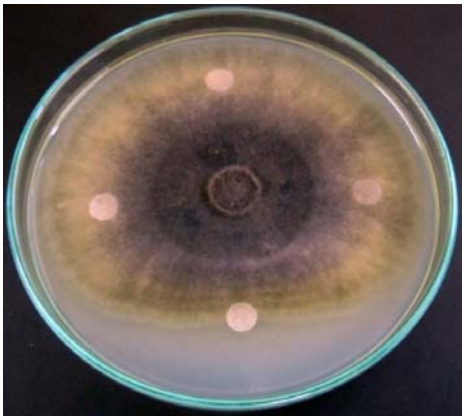

Fig. 4. Inhibitory effect of the extracted fractions of methanolic *Allium hirtifolium* bulb extract resulted from TLC on mycelial growth of *Alternaria solani* at concentration of 2 mg/ paper disc.

Table 2. Anti-*Alternaria solani* effect of the fractions obtained from the methanolic extract of *Allium hirtifolium* bulb resulted from TLC fractionation at concentration of 2 mg/paper disc using paper disc diffusion method.

Number	RF	Color	Inhibitory effect
-	0.00	-	NI[a]
-	0.09	-	NI
1	0.23	Blue	NI
-	0.32	-	NI
2	0.41	Strong blue	++[b]
-	0.46	-	NI
3	0.51	Pale blue	NI
-	0.68	-	NI
4	0.84	White	NI
5	0.89	Red	NI
6	0.96	White	NI

a, not inhibited; b, inhibited

Discussion

This study showed that the methanolic extract of Persian shallot bulbs followed by the shoot extracts of *Teucrium chamaedrys* were the strongest extracts among 100 tested plant species. The Persian shallot is an annual herbaceous plant belongs to Alliaceae grows wildly in the Zagros mountains, Iran. There are more than 500 species in the genus of *Allium* (Fateh *et al*., 2010). The most important species in this genus are onion (*A. cepa*), garlic (*A. sativum*) and shallot (*A. hirtifolium*). The antibacterial activity of *In vitro* and *In vivo* grown garlic (*A. sativum*) was investigated and documented earlier (Fatima *et al*., 2011). Moreover, it has been reported that the extract of garlic at high concentration could completely suppressed the mycelial growth of *Magnaporthe oryzae*, the cause of rice blast (Hajano *et al*., 2012). In *Allium* plants, different biological active compounds such as, alliin, allicin, allicipin, saponins, steroids, flavones, fistulosin and polyphenol carboxylic acids, ajoene were reported (Singh *et al.,* 1990; Carotenuto *et al.,* 1999; Phay *et al*., 1999; Barile *et al.,* 2007; Huma *et al*., 2009; Pârvu *et al.,* 2010). Bagiu *et al*., (2012) showed that allicin and S-methyl cystein in *A. ursinum* were responsible to observed antimicrobial activity. Besides, it was reported that the antifungal activity of flower extract of this plant species was stronger than leaf extract. This was because of higher allicin content in flower part of the plant (Pârvu *et al*., 2011). Pârvu *et al*., (2009) stated that aliin is an important antifungal active compound in hydro alcoholic extract of *A. obliquum*. The quality and quantity of the antimicrobial substances in *Allium* sp. depend on plant part used, plant species, harvest time and geographical conditions. Therefore, the antifungal activity observed in this study might be due to presence of allicin, aliin, saponin, S-methyl cystein or a combination of all of them in *A. hirtifolium.*

Teucrium chamaedrys as the one of the most active plant species against *A. solani* is a perennial plant species belong to genus *Teucrium* and family Lamiaceae. Usually, different plant species in this genus have antimicrobial activity but the antibacterial activity of these plants was greater than their antifungal properties (Mosadegh *et al.,* 2002; Stanković *et al.,* 2012). Bel Hadj Salaha *et al*., (2007) found that the methanolic extract of *T. sauvagei* could inhibit the *In vitro* growth of dermatophytes. The methanolic extract of *T. montanum* was shown to be active against *Fusarium oxysporum* (Vukovic *et al*., 2008). The aerial part of a plant species of *T. polium* was analyzed and it has been found that it contains terpenoids, tannins, saponins, estroles, flavonoids and leucoantocyanins (Mosadegh *et al.*, 2002). Moreover, a relationship was found between the phenolic compounds and biological activities (Stanković *et al.,* 2012).

The antifungal effect of some plant extracts and essential oils of different plant species grown in some other parts of the world were previously documented (Satya *et al*., 2005; Pawar & Thaker 2007; Sharma *et al.,* 2007; Neeraj & Sharma, 2010). Satya *et al*., (2005) showed that the leaf extract of zimmu (*Allium cepa* × *A. sativum*) was active in inhibiting the growth of *A. solani*. Furthermore, foliar damage causded by *A. solani* decreased to 80-93% when tomato is intercropped with marigold and the fruit yield increases to 70-75% in comparison to non-intercropped tomato (Riaz *et al*., 2008). Neeraj & Verma (2010) reported that garlic bulb considerably inhibited the growth of *A. solani* on tomatoes. Aslam *et al*., (2010) showed that *Dodonaea viscosa* have maximum inhibitory effect on *A. solani* when the grown mycellia were exposed to diffusate of 5 medicinal plant species of Pakistan. They concluded that *D. viscosa* could be used to manage the fungal disease caused by *A. solani*. Besides, in an experiment carried out by Sitara *et al.,* (2011), the anti- *Alternaria alternata* activity of *Aloe vera* gel was prone.

Replacing one extract with a combination of the extracts could enhance their efficiency in some cases (Neeraj & Verma, 2010). In this study, the combination of *A. hirtifolium* and *Ferula assa-foetida* extracts (each at 500 ppm concentration) caused the complete mycelial growth inhibition of *A. solani*; whereas none of the mentioned plant extracts was capable of the complete mycelial growth of *A. solani* at the doubled (1000 ppm). Therefore, it can be concluded that it is possible to combine the extracts of the active plant species to improve the control of *A. solani* growth.

It could be concluded that plant crude extracts could be of greatest importance to control fungal pathogens. The extracts of *A. hirtifolium, T. chamaedrys* and a combination of *A. hirtifolium* and *F. assa-foetida* could be three promising candidates for further *In vivo* biological control of disease caused by *A. solani*. Moreover, they could form the basis for further investigation of fractionation for determining the inhibitory compounds in the introduced active plant species. The dermination of quantity and quality of bioactive compounds in different plant parts at different plant growth stages and finally greenhouse and field analyses which are required to be compared with synthetic fungicides for the management of early blight of tomatoes will be the future research. Besides, these results and the encouraging percentage of plants with antifungal activity (more than 21% in this research) indicated that the flora in Iran can be regarded as rich sources of plants with antifungal activity. Therefore, screening more plant species will be continued. As one fraction of *A. hirtifolium* extract showed inhibitory effect, therefore we should analyze this fraction to find out the compound/s responsible to the observed activity. Authors are now trying to address the above mentioned subjects. Furthermore, we have to develop natural fungicides which are as effective as and safer than synthetic fungicides for human and environment.

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