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In vitro and In vivo antifungal activities of Iranian plant species against Pythium aphanidermatum

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ABSTRACT

Crude aqueous and methanolic extracts of 97 plant species belonging to 35 families collected from the west of Iran were screened for antifungal activity against economically important phytopathogenic fungi, Pythium aphanidermatum. Bioassay of the extracts was conducted by paper disc diffusion method on agar plate cultures with four replications. Seventeen of 97 (17.5%) plant species showed inhibitory activity against the tested fungi. Centaurea sp., Papaver dubium, C.behen, C.depressa, Hypericum perforatum, C.iberica, Juglans regia, Vaccaria pyramidata, Mespilus germanica, Verbascum sp., Avena sativa, Alhagi camelrum, H. scabrum, Glycyrrhiza glabra, Haplophylum perforatum, Xanthium strumarium and Portulaca oleraceae were the active plant species against P.aphanidermatum. The efficacy of the selected plant crude extracts against P. aphanidermatum was also evaluated in greenhouse condition. All tested plant extracts were superior in reducing the disease severity as compared to the control. Among the different treatments, seed treatments of G. Glabra and P.oleraceae extracts reduced disease severity from 70% for infected control to 43%. This disease severity was reduced to 46% for C.behen. Therefore, weeding and returning these plant species to the soil to retain the soil healthy is a way to reduce phytopathogenic fungi and success in a long-term crop production program. According to these results, we conclude that the flora in the west of Iran can be regarded as a rich source of plants with antifungal activity. Therefore, further screening of other plant species, identifying active fractions or metabolites and application of active extracts under field condition are warranted.

Keywords: Antifungal activity, Crude extract, Iranian plants, Paper disc, *Pythium aphanidermatum.*

INTRODUCTION

Crop losses due to plant diseases are estimated to be about 14% worldwide [1] and 20% for major foods and cash crops [23]. Nowadays, synthetic pesticides are known to be the most effective method of the pest and disease control. However, they are not considered as a long-term solution due to the concerns associated with pesticides application such as problems of public health, environmental pollution, reduction in crop quality, toxic effect on non-target organisms and causing resistance in pest and disease agents, [17, 24,25]. Thus, it is believed that pesticides should be optimized under integrated pest management programs [15]. IPM for conserving agro-ecosystem is include the use of pest-resistance cultivars, holding pests at tolerable levels, and making use of natural products [25].

In recent years, natural plant products as environmentaly safe option have recieved attention for controlling phytopathogenic diseases. Therefore, considerable research to search for biocides that are environmentally safe and easily biodegradable have been carried out during last two decades [2, 32]. Investigation of plants containing natural antimicrobial metabolites for plant protection has been identified as a desirable method of disease control [19,25]. Given the effect of the plant species origin and genetic diversity on chemical composition, studies screening for novel antifungal compounds in plants from different part of the world are needed. So, in this research, we screened plants from west of Iran.

Iran is divided to 31 provinces including Kermanshah and Hamadan, with a vast range of climatic conditions, located in the west of the country. Plant diversity is very rich in these two provinces; therefore it is expected to find significant and distinct variation in secondary metobolites with antifungal activity. Iranian plants have been screened previously for antimicrobial activity [13,14,26 ,28], but with a focus on activity against agents of diseases in human. There have been no comprehensive screening studies for activity of Iranian plants against the phytopathogenic fungi. Author screened 63 plant species collected from western Iran for inhibitory activity against *Rhizoctonia solani*, *Fusarium oxysporum* and *Cochliobolus sativus* [3]. He found that the extracts of *Glycyrrhiza glabra*, *Rosmarinus officinalis*, *Avena sativa*, *Vaccaria pyramidata*, *Centaurea behen*, *Anagalis arvensis* and *Tribulus terrestris* have broad-spectrum of antifungal activity.

The destructive phytopathogenic fungi, *Pythium aphanidermatum* were considered to test the antifungal activity of plant species. This fungus is a phytopathogenic fungus at farm level. The disease caused by this fungus is a major problem for a wide range of horticultural crops [8]. No single method is available to provide adequate control of the disease [2]. However, nowadays seed treatment with chemical fungicides especially systemic fungicides is the effective method for the control of these kinds of diseases. Evaluation of plant species against root rot agent, *P.aphanidermatum* has been earlier investigated under laboratory and greenhouse conditions in different parts of the world. An aqueous extract of fenugreek was reported to inhibit mycellial growth of *P.aphanidermarum* [16]. It was also reported that this fungus is the most resistant species among five tested phytopathogenic fungi when exposed to the extract of fenugreek. Suleiman and Emua (2009) stated that ginger and aloe could completely inhibit the mycelial growth of *P. aphanidermatum* under laboratory condition [31]. Both extracts were shown to be effective against the fungus for short period under field conditions. It has been reported that the aqueous extract of *Zygophyllum fabago* inhibits the growth of *P.aphanidermatum*[11]. Ethanolic extracts of *Allium sativum*, *Azadirachta indica*, *Curcuma longa* and *Zingiber officinalis* were shown to inhibit mycellial growth of *P.aphanidermatum*, respectively, in *in vitro* condition [29]. The extracts of *A.sativum*, *A. indica*, *Z. officinalis* and *Datura stramonium* were also introduced respectively to reduce the disease severity caused by fungus significantly. The leaf extract of zimmu was also reported to be effective against *P.aphanidermatum* in *in vitro* and *in vivo* experiments [21].

Regarding the importance of screening plant crude extracts as first step of the project and the importance of bioactive crude extracts as ecofriendly agents, collected plants from the west of Iran were screened against the *P.aphanidermatum*. Therefore, objective of the research reported here was, as a part of larger screening program, to assess the anti-*Pythium* activity of extracts from 97 randomly-collected plant species in Kermanshah and Hamadan and then evaluate the efficacy of the some selected plant crude extracts against the *P. aphanidermatum* in green house condition.

MATERIALS AND METHODS

Plant material and fungi

Ninty-seven plant species from 35 families were collected from the various parts of the provinces of Kermanshah and Hamadan in western Iran (Table 1). As a part of a wider screening program, plants were randomly collected to increase the chance of finding plants with bioactive extracts. The plants were identified by Razi University, College of Agriculture at Herbarium and the scientific names were checked in the International Plant Names Index (http://www.ipni.org/ipni/plantnamesearchpage.do). Each sample was cleaned, air dried in the shade and ground to a fine powder with a coffee grinder.

Pythium aphanidermatum was provided by the Plant Pathology Laboratory, Campus of Agriculture and Natural Resources, Razi University.

Preparation of plant extracts

The powdered plant materials were extracted at room temperature using water and methanol. Aqueous extraction was achieved by adding 100 ml distilled water to 5 g of plant powder and brought to the boil (once boiled). The suspension was allowed to stand for 4 h before being filtered. The extract was then concentrated using a rotary evaporator. A sample of extract at concentration of 100 mg/ml was bioassayed, as described in bioassay section. Methanolic extracts were also obtained as described [6]. The obtained residues were dissolved in 45% methanol in distilled water and a sample of extract at concentration of 100 mg/ml for bioassay was provided.

Bioassay of plant crude extract in laboratory condition

Fungal bioassay was performed as previously described [5] using the paper disc method to reveal any inhibitory effect of plant crude extracts. Each autoclaved filter paper disc (6 mm diameter) was loaded with 5×10 µl of the crude extract at the concentration of 100 mg/ml (equal to 5 mg/disc). The discs were dried between each application. Negative control discs were prepared with 5×10 µl of the appropriate solvent, sterile water or 45% methanol. Positive control discs at concentration of 1 mg/disc were prepared with mancozeb. Five millimeter in diameter plug of each fungus was transferred to potato dextrose agar (PDA) media and incubated at 25˚C in the dark until mycelia reach to approximately 25 mm from the edge of the plate. Loaded paper discs were placed on the growth medium about 10 mm from the margin of the growing mycelia. After addition of the paper discs, the plates were further incubated at 25˚C and radius zone of inhibition (distance between the centre of the paper disc and end of clear zone from three different directions) was recorded. Each plate was examined for any inhibitory effect every two hours. Four replicates were prepared for all extracts and controls and the experiment repeated twice.

Greenhouse test to control *Pythium aphanidermatum*

Seeds of cucumber (five seeds per plostic pot) were soaked in a 5% sugar solution for 30 seconds after surface-disinfestations with 1% sodium hypochlorite. The sticky seeds were fully mixed with the dried powder of the extracts in the target treatment. The proportion of the extract was about 10% of the weight of the seeds. Soil infestation was conducted by mixing the pasteurized soil of each plastic pot (10×10 cm) with 0.5 g of the inoculum prepared on a mixture of hemp seeds and sand. Extract-treated or non-treated seeds were sown in each plastic pot containing infested or non-infested soil. Therefore, treatments were in five groups; 1. Extract-treated seeds sown in infested soil 2. Extract-treated seeds sown in non-infested soil (to determine possible side effects of the extract) 3. Non-treated seeds sown in the infested soil (positive control) and 4. Non-treated seeds sown in non-infested soil (negative control) 5. Fungicide-treated seeds in infested soil. Non-infested pots were received hemp-sand mixture instead of inoculums. The experiment was performed in six replications.

Disease assessment was initiated following seedling emergence. The number of decayed seeds and seedlings exhibiting damping off was recorded daily and the number of unsurvived seedlings that calculated three weeks after sowing date was recorded as disease severity.

RESULTS

Bioassay of plant crude extract in laboratory condition

Ten aqueous and 15 methanolic plant extracts screened *in vitro* (from 186 extracts) showed varying levels of anti-*Pythium* activity, expressed as radius inhibition zone from 6.90 to 12.25 mm (Table 1). Of the 97 species tested, 17 (17.5%) showed activity against mycellial growth of *Pythium aphanidermatum*. Inhibition of fungal growth was recorded with extracts of *Centaurea sp., Papaver dubium, C.behen, C.depressa, Hypericum perforatum, C.iberica, Juglans regia, Vaccaria pyramidata, Mespilus germanica, Verbascum sp., Avena sativa, Alhagi camelrum, H. scabrum, Ġlycyrrhiza glabra, Haplophylum perforatum, Xanthium strumarium* and *Portulaca oleraceae* . The maximum inhibition was obtained when the fungus was exposed to 5 mg per paper disc of methanolic extract of *Centaura* sp. (12.25±0.75 mm) similar to 1mg per paper disc of mancozeb as a positive fungicide (12.58±0.42 mm). Interestingly, It was found that all species of *Centaura* inhibited the mycellial growth of the tested fungus. The inhibitory effect of some plant extracts on mycellial growth of *P.aphanidermatum* was shown in Figure 1.

Efficacy of seed treatment of extracts on disease incidence caused by *P. aphanidermatum*

Seed treatments by different extracts (10% w/w) were superior in reducing the disease severity as compared to the fungus-infected control. Among the different treatments, seed treatments of *G. Glabra, P.oleraceae, C.behen, A. camelorum* and *Verbascum* sp. extracts reduced disease severity caused by *P.aphanidermatum* from 70% for

fungus-infected control to 43%, 43%, 46%, 53% and 56%, respectively (Figure 2). No side effect of the extracts on the seedlings was observed when the extract treated and non-treated seeds were compared.

Table 1. *In vitro* screening for anti-*Pythium aphanidermatum* fungal activity (mean ± standard error, n=4) of plant extracts at 5 mg/paper disc

Plant	Family	Location	Part Used	Solvent	Zone of Inhibition
Amaranthus retroflexus L.	Amaranthaceae	Kermanshah	Shoot	W	NI
				M	NI
Ixiolirion tataricum Hall (Pall.)	Amaryllidaceae	Kerend gharb	Total	W	NI
				M	WI
Artedia squamata L.	Apiaceae	Sarpole zahab	Total	W	NI
				M	WI
Bupleurum kurdicum Boiss**.**	Apiaceae	Sarpole zahab	Total	W	WI
				M	WI
Cuminum cyminum L**.**	Apiaceae	Kermanshah	Seed	W	ND
				M	NI
Dorema aucheri Boiss.	Apiaceae	Kerend gharb	Shoot	W	ND
				M	NI
Echinophora platyloba DC.	Apiaceae	Tuiserkan	Leaf	W	NI
				M	NI
Foeniculum vulgare Mill.	Apiaceae	Kermanshah	Shoot	W	NI
				M	NI
Ferulago angulata Boiss.	Apiaceae	Kerend gharb	Flower	W	NI
				M	WI
Johrenia aromatica Rech.f..	Apiaceae	Kerend gharb	Shoot	W	WI
				M	NI
Oliveria decumbens Vent.	Apiaceae	Sarpole zahab	Total	W	NI
				M	NI
Prangos ferulacea Lindl**.**	Apiaceae	Kerend gharb	Shoot	W	NI
				M	NI
Torilis sp.	Apiaceae	Sarpole zahab	Total	W	WI
				M	NI
Aristolochia bottae Jaub. & Spach	Aristolochiaceae	Serkan	Total	W	NI
				M	NI
Asparagus officinalis L.	Asparagaceae	Kermanshah	Shoot(no fruit)	W	NI
				M	NI
Carduus arabicus Jacq.	Asteraceae	Kermanshah	Shoot	W	NI
				M	NI
Centaurea behen L.	Asteraceae	Garaban	Total	W	7.58±0.33*
				M	9.58±0.45
Centaurea iberica Sennen&Elias	Asteraceae	Sarpole zahab	Total	W	NI
				M	9.33±0.31
Centaura sp.	Asteraceae	Harsin	Shoot	W	12.25±0.75
				M	ND
Centaura depressa M.Bieb	Asteraceae	Tuiserkan	Total	W	8.83±0.98
				M	9.53±0.38
Crupina crupinastrum Vis.	Asteraceae	Kermanshah	Total	W	NI
				M	WI
Cynara scolymus L.	Asteraceae	Kermanshah	Fruit	W	NI
				M	NI
Echinops ritrodes Bunge	Asteraceae	Sarpole zahab	Shoot	W	NI
				M	NI
Gundelia tournefortii L.	Asteraceae	Sarpole zahab	Total	W	NI
				M	NI
Silybum marianum (L.) Gaertn.	Asteraceae	Kermanshah	Leaf+ root	W	NI
				M	NI
Taraxacum sp.	Asteraceae	Kermanshah	Shoot	W	NI
				M	WI
Xanthium strumarium L.	Asteraceae	Kermanshah	Leaf	W	ND
				M	7.10±0.22
			Fruit	W	6.90±0.45
			Stem	M	WI
			Root	M	NI
Anchusa italica Retz.	Boraginaceae	Kermanshah	Total	W	NI

Plant	Family	Location	Part Used	Solvent	Zone of Inhibition
				M	WI
Onosma sp.	Boraginaceae	Bide sorkh	Total	W	NI
				M	NI
Trichodesma zeylanicum R.Br.	Boraginaceae	Tuiserkan	Shoot	W	NI
				M	NI
Alysum strigosum Soland	Brassicaceae	Tuiserkan	Total	W	NI
				M	NI
Conringia orientalis L.	Brassicaceae	Kermanshah	Total	W	NI
				M	NI
Goldbachia laevigata DC.	Brassicaceae	Kermanshah	Total	W	NI
				M	NI
Isatis lusitanica L.	Brassicaceae	Bisetun	Total	W	NI
				M	WI
Matthiola arabica Boiss.	Brassicaceae	Jairan Bolagh	Total	W	NI
				M	NI
Nasturtium officinale W.T.Aiton	Brassicaceae	Sarpole zahab	Total	W	NI
				M	WI
Neslia apiculata Fisch., C.A.Mey. & Avé-Lall.	Brassicaceae	Kermanshah	Total	W	NI
				M	NI
Sameraria stylophora Boiss.	Brassicaceae	Biseton	Total	W	NI
				M	WI
Sisymbrium sp.	Brassicaceae	Sarpole zahab	Total	W	NI
				M	NI
Dianthus orientalis Donn	Caryophyllaceae	Tuiserkan	Total	W	NI
				M	NI
Vaccaria pyramidata Medik.	Caryophyllaceae	Sarpole zahab	Total	W	7.71±0.58
				M	8.71±0.57
Chenopodium album L.	Chenopodiaceae	Kermanshah	Shoot	W	NI
				M	NI
Kochia scoparia (L.) Schrad.	Chenopodiaceae	Kermanshah	Total	W	NI
				M	WI
Cuscuta sp.	Cuscutaceae	Kermanshah	Total	W	NI
				M	NI
Dipsacus sp.	Dipsaceae	Tuiserkan	Total	W	NI
				M	NI
Alhagi camelorum Fisch.	Fabaceae	Sarpole zahab	Total	W	NI
				M	8.17±0.24
Pisum sativum L.	Fabaceae	Sarpole zahab	Total	W	NI
				M	NI
Glycyrrhiza glabra L.	Fabaceae	Kermanshah	Shoot	W	7.41±0.08
				M	7.36± 0.23
Melilotus officinalis Lam.	Fabaceae	Kermanshah	Total	W	NI
				M	NI
Prosopis stephaniana (Willd.)	Fabaceae	Sarpole zahab	Total	W	NI
				M	NI
Scorpiurus muricatus L.	Fabaceae	Sarpole zahab	Total	W	NI
				M	WI
Vicia ervilia L. (Willd.)	Fabaceae	Harsin	Total	W	NI
				M	NI
Geranium sp.	Geraniaceae	Sarpole zahab	Total	W	NI
				M	NI
Hypericum scabrum L.	Hypericaceae	Tuiserkan	Total	W	ND
				M	7.75±0.44
Hypericum perforatum L.	Hypericaceae	Homail	Total	W	9.44±0.63
				M	7.58±0.19
Juglans regia L.	Juglandaceae	Serkan	Fruit peel	W	8.42±0.46
				M	8.79±0.64
Lallemantia sp.	Lamiaceae	Kerend gharb	Total	W	NI
				M	NI
Lamium amplexicaule L.	Lamiaceae	Sarpole zahab	Total	W	WI
				M	NI
Stachys inflate Benth	Lamiaceae	Tuiserkan	Shoot	W	NI
				M	WI
Stachys lavandulifolia Vahl	Lamiaceae	Tuiserkan	Shoot	W	NI
				M	NI

Plant	Family	Location	Part Used	Solvent	Zone of Inhibition
Salvia multicalus Vahl.	Lamiaceae	Tuiserkan	Shoot	W	NI
				M	WI
Fritillaria imperialis L.	Liliaceae	Kerend gharb	Total	W	WI
				M	NI
Gagea sp.	Liliaceae	Tuiserkan	Total	W	NI
				M	NI
Muscaria neglectum	Liliaceae	Tuiserkan	Total	W	NI
				M	NI
Ornithogalum sp.	Liliaceae	Kerend gharb	Total	W	NI
				M	NI
Abutilon theophrasti Medik.	Malvaceae	Kermanshah	Total	W	NI
				M	NI
Malva neglecta Waller.	Malvaceae	Kermanshah	Shoot	W	NI
				M	NI
			Leaf+Stem	W	NI
				M	WI
Orobanche alba Rchb.	Orobanchaceae	Tuiserkan	Total	W	NI
				M	NI
Olea europaea L.	Oleaceae	Sarpole zahab	Leave+stem	W	NI
				M	WI
Syringa vulgaris L.	Oleaceae	Kermanshah	Shoot	W	ND
				M	NI
Papaver dubium L.	Papaveraceae	Tuiserkan	Total	W	9.08±0.17
				M	9.67±0.12
Acantholemon sp.	Plumboginaceae	Tuiserkan	Total	W	NI
				M	NI
Aegilops triuncialis L.	Poaceae	Tuiserkan	Shoot	W	NI
				M	NI
Avena sativa	Poaceae	Kermanshah	Root	W	ND
				M	8.50±0.63
Bromus tomentellus Boiss.	Poaceae	Tuiserkan	Total	W	NI
				M	NI
Digitaria sanguinalis	Poaceae	Kermanshah	Shoot	W	NI
				M	NI
Echinochloa crus-galli L.	Poaceae	Kermanshah	Total	W	NI
				M	NI
Melica persica Kunth	Poaceae	Tuiserkan	Total	W	NI
				M	NI
Phalaris sp.	Poaceae	Sarpole zahab	Total	W	WI
				M	NI
Setaria viridis L. (P.Beauv)	Poaceae	Kermanshah	Shoot	W	NI
				M	NI
Stipa barbata Desf.	Poaceae	Tuiserkan	Total	W	NI
				M	NI
Taniterum sp.	Poaceae	Tuiserkan	Shoot	W	NI
				M	NI
Portulaca oleraceae L.	Portulacaceae	Sarpole zahab	Shoot	W	NI
				M	7.0±0.22
Anagallis arvensis L.	Primulaceae	Sarpole zahab	Total	W	WI
				M	WI
Ranunculus arvensis L.	Rananculaceae	Kerend gharb	Total	W	NI
				M	NI
Mespilus germanica Thunb.	Rosaceae	Kermanshah	Leaf	W	ND
				M	9.33±0.36
Callipeltis cucullaria (L.) DC.	Rubiaceae	Tuiserkan	Total	W	NI
				M	NI
Haplophyllum perforatum (MB.) Kar. and Kir.	Rutaceae	Tuiserkan	Total	W	7.27±0.66
				M	WI
Linaria chalepensis Mill. (L.)	Scrophulariaceae	Sarpole zahab	Total	W	NI
				M	NI
Scrophularia striata Boiss.	Scrophulariaceae	Sarpole zahab	Total	W	NI
				M	NI
Verbascum sp.	Scrophulariaceae	Tuiserkan	Total	W	8.67±0.19
				M	7.17±0.35
Veronica anagallis-aquatica L.	Scrophulariaceae	Sarpole zahab	Total	W	NI

Plant	Family	Location	Part Used	Solvent	Zone of Inhibition
				M	NI
Nicotiana tabacum L.	Solanaceae	Super market	Leaf	W	NI
				M	NI
Hyoscyamus reticalatus L.	Solanaceae	Garaban	Total	W	NI
				M	WI
Valerianella sp.	Valerianaceae	Tuiserkan	Total	W	NI
				M	NI
Tribulus terrestris L.	Zygophyllaceae	Sarpole zahab	Shoot	W	NI
				M	NI
			Root	W	NI
				M	NI
Urtica dioica L.	Urticaceae	Serkan	Total	W	NI
				M	NI
Mancozeb (fungicide)					12.58±0.42

*, *mean of radius inhibition zone (mm) ± standard er ror; W, water; M, methanol; NI, no inhibition; WI, weak inhibition; ND, not done.*

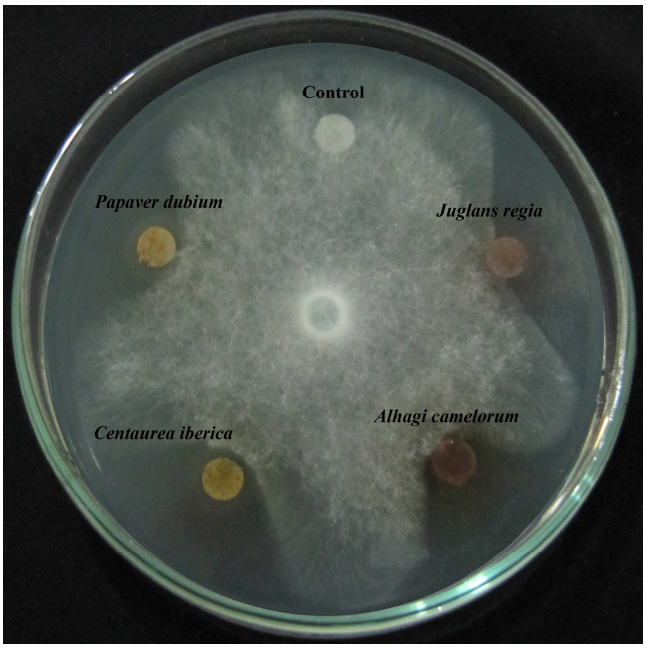

Figure 1. Inhibitory effect of plant crude extracts (5 mg/paper disc) on mycelial growth of *Pythium aphanidermatum*. Control is paper disc loaded by solvent (45% methanol)

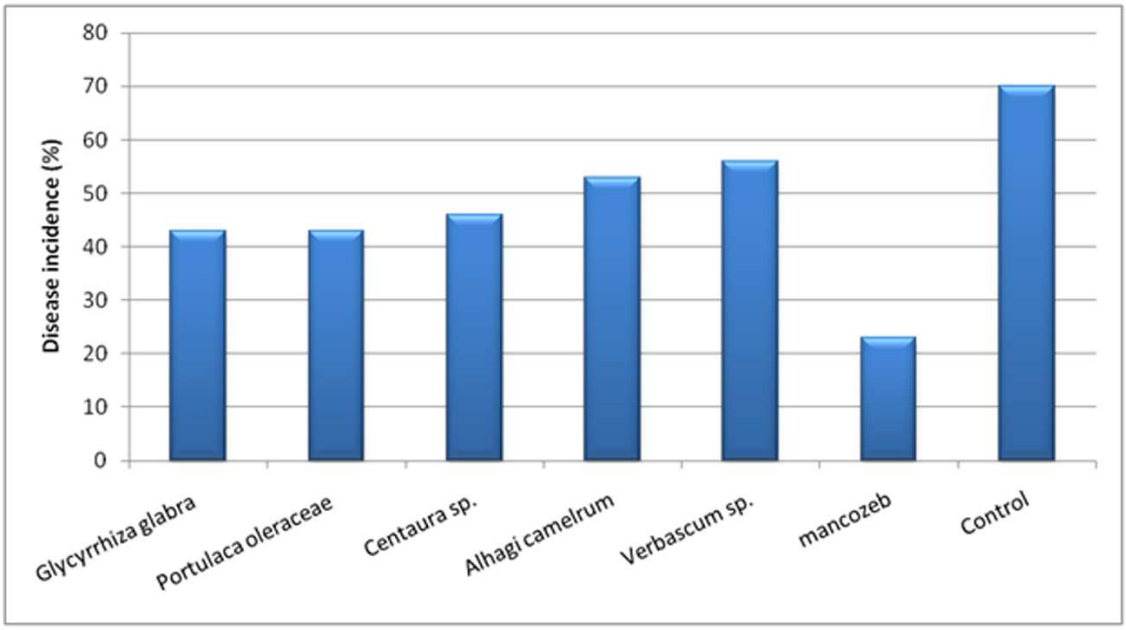

Figure 2 Efficacy of seed treatment with different plant methanolic extracts on damping off disease incidence caused by *Pythium aphanidermatum* in cucumber, three weeks after sowing date.

DISCUSSION

Results indicated the presence of antifungal compounds in different extracts (Table 1) which in agreement with the results reported by other researchers on different pathogens [3,21,33].

Anti-*Pythium* activity of all tested *Centaurea* species in present study indicated that these plants are potent antifungal plants with possible potential for the control of damping-off diseases in cucumber. The antifungal activity of the plants in this genus was reported earlier [3,4,18]. Nine different compounds isolated from the aerial parts of *C. thessala* ssp. *drakiensis* and *C. attica* ssp. *attica* were shown to be effective against fungi [30]. Therefore, more research on the activity of the plants in this genus against the other plant pathogenic fungi would be of value.

The broad antimicrobial activity of the plant species was shown to be related to the presence of saponins, alkaloids and tannins [22]. The antifungal activity of *A. sativa* probably may be due to presence of saponins in their content [10, 21]. The inhibitory effect of *X. strumarium* could be due to the presence of sesquiterpene lactones [19]. In this study, it was shown that *H.perforatum* (Syn. *H. acutifolium*) collected from Homail represents antifungal activity. Our results are in accordance with the previous findings reported [7]. It has been found that this plant possesses antifungal activity and quinoline alkaloids especially flindersine are responsible to this activity.

While several plant extracts tested showed a high level of inhibition at a single concentration (5mg/paper disc), plant species with adequate material and different range of activity on *P.aphanidermatum* were selected for greenhouse experiment. So, seeds of cucumber were treated by the extracts of *C.behen*, *Verbascum* sp., *A. camelorum*, *G. Glabra* and *P.oleraceae*. The disease severity of different treatments were caculated. Results indicated that all plant extracts reduced the disease severity and none of them had side effects. Among the tested extracts *G. Glabra* and *P.oleraceae* were the most active extracts. Although all of the plant extracts showed activity, the order of their activity in greenhouse experiments was different from *in vitro* experiment. The difference between results of the two experiments could be because of the nature of the plant compounds and their interaction with soil compounds. As discussed by Kim et al. (2002), the efficacy of plant extracts on the control of plant pathogens in field experiments was rarely proven[19]. The results of this experiment and similar *in vivo* experiments could help to explore novel natural products and ultimately could contribute to sustainable agriculture. This will provide benefits to farmers, environment and whole society.

As Chitwood (2002) stated, the results of these kinds of research could help to develop new natural fungicide, chemically synthesized derivatives or to grow the plants with antifungal activity in a crop rotation program [9]. These results will also help to find out the active metabolites in active plants and subsequently help to use in reverse genetic engineering from metabolites to genes. Regarding to the allelopathic properties of oats (*Avena sativa*), oat can be grown in a crop rotation program to suppress and break the cycle of soil-borne plant pathogenic fungi [27]. Therefore, oat as unknown crop plant in Iran could help to reduce the severity of soil borne diseases. Moreover, as most of the plant species with inhibitory effect on *P.aphanidermatum* are known as weeds, we can conclude that weeding and returning these plant species to the soil to retain the soil healthy is a way to reduce phytopathogenic fungi and success in a long-term crop production program.

These results and the acceptable percentage of the plants with antifungal activity (17.5% in this study) indicated that the flora in the west of Iran can be regarded as a rich source of plants with antifungal activity. These findings encouraged us to continue screening more plant species for antifungal agents.

The results of this study may form the basis of further investigation on fractionation for finding active fractions and the effect of origin of growing on the quality and quantity of active compounds. Therefore, further investigations are being conducted on *G. Glabra* and *P.oleraceae* as they showed more inhibition in *in vivo* experiment.

CONCLUSION

According to the results and discussion mentioned above, we can conclude that the flora in the west of Iran can be regarded as a rich source of plants with antifungal activity. Therefore, more research needed to find the plant with high level of toxicity against phytopathogenic fungi in laboratory and field conditions. Moreover, decision on how we can use the results of these kinds of studies depends on the nature of the plant with antifungal activity. Here, we could advise that weeding and returning these plant species to the soil is a way to reduce phytopathogenic fungi and success in a long-term crop production program. If we would like to retain soil and environment healthy, we must reduce the usage of pesticides and find alternatives. As a regard to the research results, it is possible to continue screening , explore biocides as alternatives and use in IPM program.

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