Research Article

The effect of some medicinal and ornamental plant extracts against *Fusarium oxysporum*

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Abstract: During the past decade, natural plant products as environmentally safe option have received attention for controlling phytopathogenic diseases. Investigation of plants containing natural antimicrobial metabolites for plant protection has been recognized as a desirable method of disease control. The fungus *Fusarium oxysporum* causes diseases such as root rot, damping off and Fusarium wilt and it infects many plant species and crops. Methanolic crude extracts of 30 plant species belonging to 17 families collected from the west of Iran were screened for antifungal activity against *F. oxysporum* during 2012. Bioassay of the extracts was conducted by agar dilution method with five replications. The inhibitory effect of the extracts was examined at concentration of 2000 ppm. Twenty out of 30 tested plant species (67%) showed inhibitory activity against mycelial growth of *F. oxysporum*. The most effective extracts with more than 50% inhibition belonged to *Haplophyllum perforatum* and *Calendula officinalis.* High number of plants with antifungal activity in this experiment showed that the flora in the west of Iran could be regarded as a rich source of plants with antifungal activity. Therefore, further screening of other plant species, identifying active fractions or metabolites and *in vivo* application of active extracts are in progress.

Keywords: Agar dilution, *Calendula officinalis, Fusarium oxysporum, Haplophyllum perforatum*, Methanolic extract

Introduction

Plants are the major source of food, medicines and many other useful products for humans. Various insects, bacteria, viruses, fungi and other pests reduce their productivity and lead to a huge loss to mankind. At least 14% of crops and 20% of major foods and cash crops are lost due to plant diseases (Agrios, 2005; Oerke *et al*., 1994). As compared to other plant parasites, fungi have the greatest impact with regard to diseases and crop production losses. In agriculture, chemical fungicides are becoming ineffective due to the development of new physiological races of the pathogens (Ocamb *et al*., 2007). Although the most popular and effective method of protecting the plants against the fungal attack is the use of chemical fungicides (Rabea and Steurbaut, 2010), these fungicides usually have environmental mal effects, in particular, toxic residues in food, and other effects 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 (Rai and Carpinella, 2006). On the other

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hand, some synthetic fungicides are difficult to biodegrade, and hence they can accumulate in the soil, plants, water, and consequently cause toxicity to humans and animals through the food chains (Tapwal *et al*., 2011). Therefore, the discovery of new and alternative management systems such as biocontrol is urgent (Singh *et al*., 1999).

Biological control of plant diseases is slow, gives less short term profits, but can be long lasting, inexpensive and harmless. Investigation of plants containing natural antimicrobial metabolites for plant protection has been identified as a desirable method of disease control (Kim *et al*., 2002; Rai and Carpinella, 2006). Plant metabolites and plant based pesticides appear to be good alternatives in plant disease management, as they are known to have minimal harmful impact on the environment and human in contrast to the synthetic pesticides (Varma and Dubey, 1999). Therefore, considerable researches on biocides that are cheap, environmentally safe, locally available and easily biodegradable have been carried out during last two decades (Kim *et al*., 2005; Saxena *et al*., 2005; Tegegne *et al*., 2008).

Antifungal activity of different natural substances, such as plant extracts has been investigated. For example, Bahraminejad *et al*. (2011) screened 63 plant species collected from western parts of Iran for inhibitory activity against *Rhizoctonia solani*, *Fusarium oxysporum* and *Cochliobolus sativus* using paper disc method. The result showed that the extracts of *Glycyrrhiza glabra*, *Rosmarinus officinalis, Avena sativa, Vaccaria pyramidata, Centaurea behen, Anagalis arvensis* and *Tribulus terrestris* had broad-spectrum antifungal activity. Jasso de Rodriguez *et al*. (2005) evaluated antifungal activity of *Aloe vera* pulp on mycelial growth of *Rhizoctonia solani* and *F. oxysporum*. They reported that this extract reduced colony growth rate of both fungi at concentration of 105 μl^{-1}. Curir *et al*. (2005) determined phytoalexin inhibitory effect involved in carnation against *F. oxysporum* causal agent of *Fusarium* wilt. *Thymus vulgaris* essential oil exhibited broad fungitoxic spectrum against eight fungal strains including *F. oxysporum* with concentration 0.7 µl/ml (Kumar *et al*., 2008). Another report indicated that *T. vulgaris* extract showed complete suppression on colony growth of *F. oxysporum* (Al-Rahmah *et al*., 2013). Arora and Kaushik (2003) screened 41 different plant extracts for their activity against soybean fungal pathogens such as *F. oxysporum.* They reported that ginger inhibit mycelial growth of this fungus.

In this study, a destructive phytopathogenic fungus, *F. oxysporum* was considered to test the antifungal activity of plant species. It is known that the genus *Fusarium* is a soil borne, necrotrophic, plant pathogenic fungus with many species that cause serious plant diseases around the world. *F. oxysporum* is cosmopolitan phytopathogen causing root rot, damping off and *Fusarium* wilt (Li *et al*., 1996; Ovadia *et al*., 2000; Hanson and Jacobsen, 2009). It consists of more than 120 *formae speciales* according to the hosts they infect. Each of them can be subdivided into physiological races with characteristic patterns of virulence on different host varieties (Webster *et al*., 2008).

Given the effect of the plant species origin and genetic diversity on chemical composition, studies screening for novel antifungal compounds in plants grown in different parts of the world are needed. Regarding the importance of screening plant crude extracts as first step of the project and the importance of bioactive crude extracts as eco-friendly agents, collected plants from the west of Iran were screened against *F. oxysporum*. The objective of this research as a part of larger screening program was to assess the antifungal activity of the extracts obtained from 31 randomly-collected plant species in Kermanshah and Hamadan provinces, west Iran, with a vast range of climatic conditions and rich plant diversity.

Materials and Methods

Plant material and fungi

Thirty plant species from 17 families were collected during 2012 from various parts of the provinces of Kermanshah and Hamadan. Except *Centaurea imperialis* and *Sambucus nigra* L. which were collected from Kurdistan province and Behshahr (located in Mazandaran province), respectively (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 herbarium of the Agricultural College of Razi University and the scientific names were checked in the International Plant Names Index (http://www.ipni.org/ipni/). Each sample was cleaned, air dried in the shade and ground to a fine powder with a coffee grinder. *F. oxysporum* was provided by 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 methanol. Methanolic extracts were obtained as described by Bahraminejad *et al*. (2008). The final residues were dissolved in 50% methanol and a sample of the extract at a concentration of 100 mg/ml was provided for bioassay.

Table 1 *In vitro* screening for anti-*Fusarium* activity of plant extracts.

Plant	Family	Location	Part used
Allium hirtifolium Boiss.	Alliaceae	Tuiserkan	Leaf
Celosia argentea cristata	Amaranthaceae	Kermanshah	Shoot
Dahlia sp.	Asteraceae	Kermanshah	Total
Centaurea imperialis Hausskn-ex Bornm	Asteraceae	Kurdistan	Shoot
Calendula officinalis L.	Asteraceae	Kermanshah	Shoot
Chrysanthemum sp.	Asteraceae	Kermanshah	Shoot
Cineraria grandiflora	Asteraceae	Kermanshah	Shoot
Cousinia stenocephala Boiss.	Asteraceae	Kerend gharb	Total
Gaillardia grandiflora Hort.	Asteraceae	Kermanshah	Shoot
Onopordum sp.	Asteraceae	Sarpole zahab	Shoot
Tagetes erecta L.	Asteraceae	Kermanshah	Shoot
Zinnia elegans	Asteraceae	Kermanshah	Shoot
Sambucus nigra L.	Caprifoliaceae	Behshahr	Shoot
Vaccaria pyramidata Medik.	Caryophyllaceae	Sarpole zahab	Total
Elaeagnus angustifolia L.	Elaeagnaceae	Homail	Flower
Euphorbia sp.	Euphorbiaceae	Sarpole zahab	Total
Onobrychis sp.	Fabaceae	Sarpole zahab	Total
Vitex pseudonegundo	Lamiaceae	Sarpole zahab	Leaf + Inflorescence
Allium noeanum Reut.	Liliaceae	Sarpole zahab	Leaf
Fumaria officinalis L.	Papaveraceae	Kermanshah	Total
Pinus eldarica Medw.	Pinaceae	Kermanshah	Leaf
Plantago lanceolata L.	Plantaginaceae	Sarpole zahab	Total
Portulaca oleracea L.	Portulacaceae	Sarpole zahab	Total
Malus floribunda	Rosaceae	Kermanshah	Shoot
Rosa sp.	Rosaceae	Kermanshah	Shoot
Citrus grandis	Rutaceae	Market	Fruit
Haplophyllum perforatum (MB.) Kar.& Kir.	Rutaceae	Tuiserkan	Total
Antirrhinum majus L.	Scrophulariaceae	Kermanshah	Shoot
Bellardia sp.	Scrophulariaceae	Tuiserkan	Total
Verbascum sp.	Scrophulariaceae	Tuiserkan	Shoot

Bioassay

In agar dilution method, a concentration of 2000 ppm of the extract was prepared in one ml 50% methanol. The potato dextrose agar medium (PDA) was sterilized at 121 °C for 20 min and 1 atmosphere pressure. The prepared extract was added to culture medium when the temperature of the medium decreased to about 40 °C. The culture media plus (or amended with) one ml 50% methanol was considered as a control. The culture media immediately was poured into plates. A 6 mm diameter plug of 7 day fungal colonies was placed at the centre of the plates. Plates were incubated at 25 ± 4 °C and diameter of colony was measured until the control plates or one of the treatments was completely covered by the mycelial growth. The experiments were performed in five replicates. Percentage of inhibition of growth for the fungus was calculated based on conventional formula (Sarkar *et al*., 2003).

IP = [(C-T)/C] × 100, IP = percentage of mycelial growth inhibition; C = mean diameter (mm) of the control; T = mean diameter (mm) of tested concentration

Results and Discussion

Figures 1 and 2 reveal that mycelial growth of *F. oxysporum* was affected by the tested extracts. Twenty out of 30 (67%) screened plant species reduced the mycelial growth of the fungus. Maximum mycelial growth inhibition (more than 50%) was recorded for the extracts of *Haplophyllum perforatum* and *Calendula officinalis.* Some of the extracts not only reduced mycelial growth but also changed its appearance (Fig. 2). Seven plant species (23%) measurably enhanced the mycelial growth of the fungus the most active of which was *Celosia argentea cristata* with 30% stimulatory effect. Extract of *Dahlia* sp. showed very low stimulatory effect (≤ 1%) and the extracts of *Elaeagnus angustifolia* L. and *Bellardia* sp. had neither inhibitory nor stimulatory effect on the mycelial growth of *F. oxysporum*. Results indicated the presence of antifungal compounds in different plant extracts (Fig. 1), which was in agreement with the results reported by authors who tested the plant extracts on different plant pathogens using paper disc method (Bahraminejad *et al*., 2011; Bahraminejad *et al*., 2012; Bahraminejad, 2012; Bazie *et al*., 2014) and agar dilution method (Bahraminejad *et al*., 2013).

The strong inhibitory effect of *H. perforatum* and *C. officinalis* indicated that the extracts of these species have antifungal effect with possible potential for the control of different fungal diseases in plants. Therefore, more research would be of value on the activity of these plants against other plant pathogenic fungi.

The genus *Haplophyllum* from *Rutaceae* comprises about 50 species and is distributed from Africa to Eurasia. About 30 species of this perennial plant grow in Iran and 14 of them are endemic to it. *Haplophyllum* contains several quinoline alkaloids (Staerk *et al*., 2009) and lignan lactones (Sheriha *et al*., 1987). In this study, it was shown that *H. perforatum* collected from Tuiserkan contains strong antifungal activity. Our results are in accordance with the previous findings reported by Cantrell *et al*. (2005), Bahraminejad *et al*. (2011), Bahraminejad (2012) and Bahraminejad *et al*. (2012) who also demonstrated antifungal activity of this plant extract. Cantrell *et al*. (2005) concluded that quinoline alkaloids especially flindersine are responsible for the observed antifungal activity. As stated by Bahraminejad *et al*. (2011) there was weak inhibitory effect (WI) for methanolic extract of *H. perforatum* when paper disc method was used. The differences in the toxicity of the extracts in the two methods could be due to their solubility in medium and results might be influenced by the solubility of the active substances in the solvent and media so that higher solubility of the most active plant extracts in the medium could give better diffusion and ultimately strong activity while lower solubility of the most active plant extracts could show weak diffusion and weak activity when paper disc method is used. Therefore, showing the activity of the extracts would be more accurate when agar dilution method is used.

C. officinalis, belonging to the family of *Asteraceae*, is a medicinal plant with yellow to orange flowers, mostly found in the Mediterranean region and in central and southern Europe, western

Asia and United States and has been cultivated as a food and medicinal plant since the Middle Ages (Gazim *et al*., 2008; Singh *et al*., 2011). It has a high economic value as herbal medicine and is widely used in cosmetics, perfumes, pharmaceutical preparations and in food (Gazim *et al*., 2008). *C. officinalis* contains esquiterpenes glycosides, saponins, xanthophylls, triol triterpenes, flavonoids and volatiles (Gazim *et al*., 2008) and a high number of carotenoids such as flavoxanthin, lutein, rubixanthin, β-carotene, g-carotene and lycopene (Pintea *et al*., 2003). It has been reported to possess many pharmacological activities, which include antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral (Singh *et al*., 2011). Calendula reduces inflammation, promotes digestion and prevents the overgrowth of yeasts and used as an antiseptic (Rashmi and Goyal, 2011). The *in vitro* antifungal activity of *C. officinalis* flower extracts has been investigated. The extracts of these plant species showed high level of activity against *Aspergillus niger*, *Rhizopus japonicum*, *Candida albicans*, *Candida tropicallis* and *Rhodotorula glutinis* (Kasiram *et al*., 2000). Results of Tiwari *et al*. (2011) indicated that root extract of *C. officinalis* was highly effective against both Gram-positive and Gram-negative organism. In their study, preliminary phytochemical screening of the extracts showed the presence of Alkaloids, terpenoids, flavonoids, sterols, carbohydrates and tannins. Rashmi and Goyal (2011) stated that all parts of *C. officinalis* showed significant anti microbial activity. Gazim *et al*. (2008) tested *in vitro* activity of the essential oil from *C. officinalis* flowers using disc diffusion methods. Their results showed that the essential oil of this plant was effective against all 23 human clinical fungi strains tested.

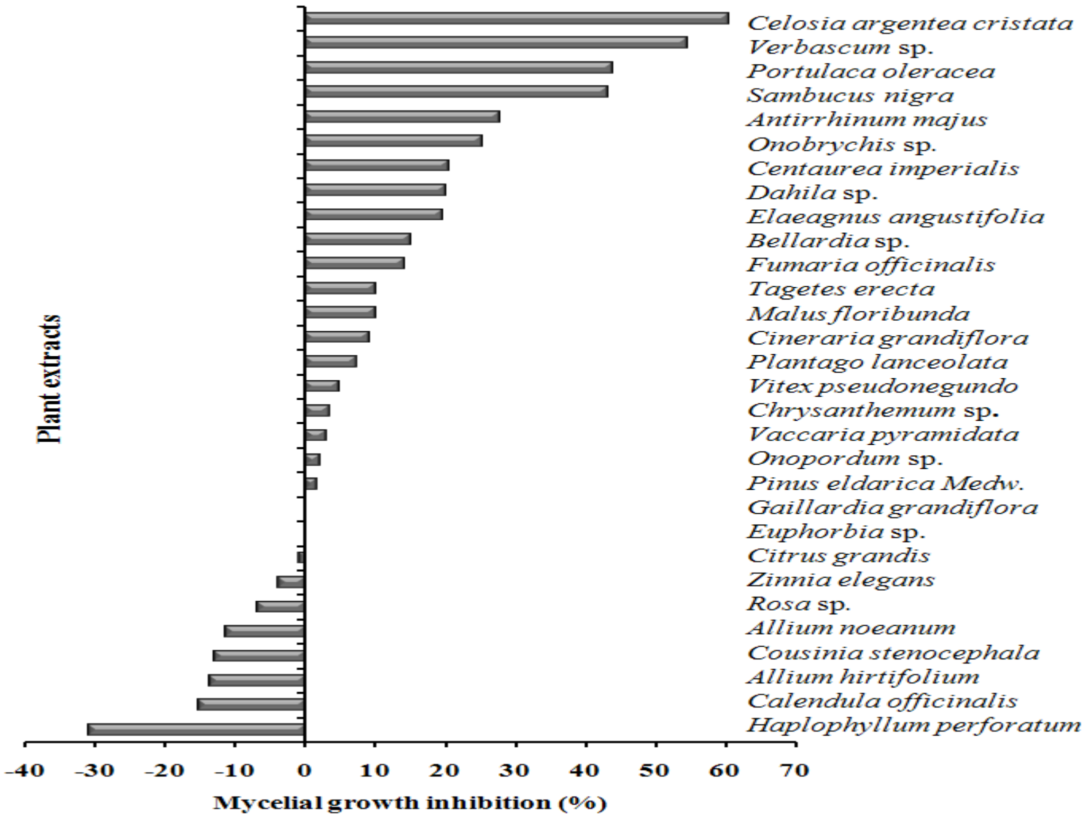

Figure 1 Antifungal activity and stimulatory effects of different plant extracts against *Fusarium oxysporum*.

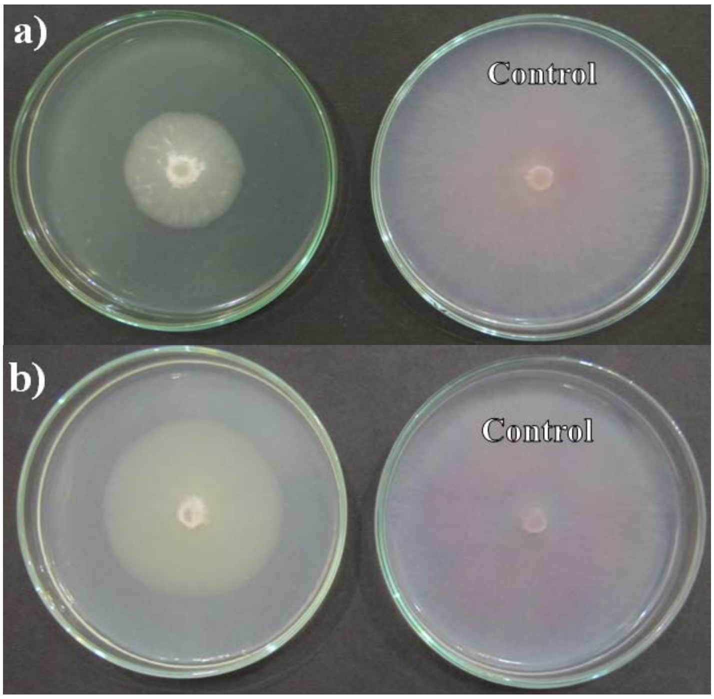

Figure 2 The anti-*Fusarium* activity of plant extracts (2000 ppm) used in potato dextrose agar: **a)** *Haplophyllum perforatum* and **b)** *Cousinia stenocephala.*

These results and the encouraging percentage of plants with antifungal activity (68% in this research) indicated that the flora in the west of Iran can be considered as rich sources of plants with antifungal activity. These findings persuaded us to continue screening more plant species. Moreover, they could form the basis for further investigation of fractionation for finding active fractions, the effect of natural habitat on the quality and quantity of active compounds, the amount of bioactive compounds in different plant parts and finally *in vivo* application of extracts will be considered.

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اثر عصاره تعدادی از گونه‌های گیاهان دارویی و زینتی بر رشد قارچ *Fusarium oxysporum*

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چکیده: در طول دهه‌های گذشته استفاده از فراوردههای طبیعی گیاه ان بهعنوان یک روش سازگار با محیطزیست، توجه زیادی را در کنترل بیمارگرهای گیاهی به خود جلب کرده است. امروزه، بررسی و کاربرد گیاهان حاوی متابولیت‌های ضدمیکروبی بهعنوان یک روش مطلوب در کنترل بیماری‌ها مورد توجه است. قارچ *Fusarium oxysporum* در بسیاری از گونه‌های گیاهی باعث بروز برخی بیماری‌ها ازجمله پوسیدگی ریشه، بوتهمیری و پژمردگی فوزاریومی می‌گردد. عصاره خام متانولی ۳۰ گونه گیاهی از ۱۷ تیره گیاهی جمع‌آوری شده از غرب ایران بهمنظور دارا بودن فعالیت ضدقارچی علیه *F. oxysporum* طی سال‌های ۱۳۹۰ و ۱۳۹۱ غربال شدند. بررسی فعالیت ضدقارچی عصاره‌ها توسط روش اختلاط با محیط کشت در پنج تکرار انجام گرفت. اثر بازدارندگی عصاره‌ها در غلظت ۲۰۰۰ پی‌پی‌ام آزمایش شد. بیست گونه گیاهی از ۳۰ گونه مورد مطالعه (۶۷ درصد) فعالیت بازدارندگی علیه رشد میسلیومی قارچ *F. oxysporum* نشان دادند. قوی‌ترین عصاره ها با بیش از ۵۰ درصد بازدارندگی متعلق به گونههای *Haplophyllum perforatum* و *Calendula officinalis* بود. تعداد فراوان گیاهان دارای فعالیت ضدقارچی در این آزمایش نشان می‌دهد که فلور غرب ایران می‌تواند بهعنوان یک منبع غنی از گیاهان با فعالیت ضدقارچی مدنظر قرار گیرد. بنابراین، غربال سایر گونه‌های گیاهی، شناسایی اجزاء یا متابولیت‌های فعال و کاربرد *in vivo* عصاره‌های مؤثر در حال مطالعه می‌باشد.

واژگان کلیدی: اختلاط با محیط کشت، *Calendula officinalis*، *Fusarium oxysporum*، *Haplophyllum perforatum*، عصاره متانولی