Screening of some wild plants as antifungal agent against phytopathogenic fungi

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ABSTRACT

The present study tested the antifungal activity of twenty wild plants grown at the desert area of Sadat city, Menoufiya governorate, Egypt against three phytopathogenic fungi (Fusarium oxysporum, Fusarium solani and Rhizoctonia solani). Antifungal activity of plant extracts was performed using poison food technique. Aqueous extracts showed variability in inhibition of mycelial growth at 10%concentration. Among tested extracts Artemisia herb alba, Convolvulus arevensis, Conyza discordis, Deverra tortuosa, Fagonia arabica, Gymnocarpus decandrum, Limonium sinuatum, Mathiola livida, Pulicaria undulata, Rumex vesicarius, and Typha domnegensis were the most effective plants in reducing mycelial growth. Aqueous extract of Artemisia herb alba recorded the highest percent of inhibition in the radial growth of tested fungi that exceeded 45% in case of fusarium oxysporum.

Key words: Antifungal, wild plants, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani

الملخص

اختبرت الدراسة الحاليه النشاط المضاد للفطريات لعدد عشرين نبات بري نامي فى صحراء مدينة السادات –محافظة المنوفيه- مصر- ضد الفطريات الممرضة للنبات وهى (فيوزاريوم اوكسيسبوريوم،فيوزاريوم سولانى وفطر الريزوكتونيا سولانى) وتم اختبار النشاط المضاد للفطريات للمستخلصات النباتيه باستخدام تقنية الPOISON FOOD . أظهرت المستخلصات المائية تباين فى منع النمو الخيطى عند تركيز 10%. ومن بين المستخلصات التى تم اختبارها كانت مستخلصات نبات الشيح،اللبلاب ،والبرنوف،وشبت الجبل والشويكه، والقصيص والعويذران المتعرج والشجاروالجثجاث والحميض والبردى هى الأكثر فاعليه فى تثبيط النمو الفطرى، و سجل المستخلص المائى لنبات الشيخ أعلى نسبه تثبيط للنمو الخيطى للفطريات المختبره والتى تجاوزت 45% فى حالة فطر الفيوزاريوم اوكسيسبوريوم.

الكلمات الدالة : التضاد الفطرى، نباتات بريه، فيوزاريوم اوكسيسبوريوم،فيوزاريوم سولانى، ريزوكتونيا سولانى.

INTRODUCTION

Phytopathogenic fungi are the main disease organisms of plants, being responsible for the major losses of world agricultural production. They are consider a challenge to food security because they are able to destroy major crops globally and contaminate food and feed with mycotoxins that are detrimental to animal and human health. (Bebber and Gurr, **2015**) Various methods can be employed for control of fungal infection however, application of chemical fungicides are the most common method. Thereby; Fungicides are ranked first in the amount of

pesticides used in Egypt in the last five years. (Abdel Megeed, 2017) Increased application of fungicides could lead to adverse impacts on the heath of aquatic and terrestrial ecosystems and potentially became a risk to soil fertility. To ensure the sustainability of agriculture production systems, it is important to found a balance between control of fungal disease and safety of ecosystems. Therefore continued research, including plant based products is required to provide effective biological products that are cheap, less toxic, and effective. (Martinez, 2012) Egyptian plant flora is very rich with plant species that had been investigated for their antimicrobial activity against human and animal pathogens. However, few have been investigated for use in food and crop protection. Considering that this work aimed to screen the antifungal activity of twenty wild plant grow in non-reclaimed area in Sadat desert for future use as potential sources of active antifungal compounds.

MATERIAL AND METHODS

1 - Collection of plant material

Twenty fresh plant specimens which are grown wildly in the Deseret of Sadat city were collected during the winter season, (2018). Plants were washed with tap water to remove the dust. Then, samples were air-dried in the shade followed by grinding to a fine powder for further study. Pressed voucher herbarium specimen was processed for each of the studied plants and deposited in the Herbarium of Natural Resource Survey Department, Environmental studies and research institute, University of Sadat city, Menoufiya governorate, Egypt.

2- Extraction

Twenty gram of the powdered plant materials were macerated in 200 ml of hot distilled water for 24 h .then, filtered and autoclaved at 120 for 20min and kept at 4 c° until used.

3- Antifungal activity of plant extracts

3.1- preparation of fungal inoculum

The fungal inoculums were prepared from seven day old cultures grown in potato dextrose agar medium (PDA). Petri dishes were flooded with 10 ml distilled water and the conidia were scrabed using sterile spatula. The spore density of each fungus was adjusted to obtain final concentration approximately10^5 spores /ml.

3.2- Antifungal assay

All the collected plants (Table 1) were subjected to antifungal activity assay according to the method described by Mohana and Raveesha, (2007). PDA medium with 10 % aqueous extract of all plants were prepared and sterilized at 121°C, 15 lb/inch2 pressure for 15 minutes. 15 ml of each media was separately poured into petri plates, allowed to cool and solidify. After complete solidification of the medium, 5mm disc of seven day old culture of *F. oxsporum*, *F. solani* and *R. solani* was inoculated in to PDA at the center of the Petri dishes. The plates were incubated at 25 ± 1 °C for seven days. The Petri dishes containing media devoid of the extract but with same amount of distilled water served as control. After incubation the colony diameter

was measured in mm, Singh and Tripathi, (1999). Each treatment was repeated four times. The fungi toxicity of the extract in terms of percentage inhibition of mycelial growth was calculated using the formula: - Percent inhibition = C - T / C X 100, where C = Average increase in mycelial growth in control plate and T = Average increase in mycelial growth in treatment plate.

4- Statistical analysis

All values of antifungal effect were expressed as the mean +standard deviation (SD) of radial growth on treated plates with four replicates for each treatment. Data were analyzed by one way analysis of variance (ANOVA) using computer SPSS 15.software package. Differences on statistical analysis of data were considered significant at $p<0.05$

RESULTS AND DISCUSSION

1- Plant collection

Aerial parts of twenty wild taxa were randomly collected from non-reclaimed desert areas of Sadat City (Table 1). Authentication was carried out in herbarium of faculty of science, Menoufiya University. Collected plants are belonging to 14 families distributed as (Asteraceae (3), Brassicaceae (2), Zygophyllacea (2), Poaceae (2), Aizoaceae (1), Convolvulacea (1), Apiacea (1), Solanacea (1), Plumbaginacea (1), Portulacea (1), Polygonacea (1), Fabacea (1) and Typhaceae (1)).

2-Antifungal screening

The present study screened the antifungal activity of aqueous extracts of the twenty wild plants on the growth of *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani*. Effects of aqueous extracts on the radial growth of tested pathogens are showed in table 2. Where, aqueous extracts showed variability in inhibition of mycelial growth at 10%concentration. All treatments succeeded in reducing the mycelial growth of tested fungi but statistical analysis of results indicated that among tested plant extracts only eleven extract caused significant inhibition in mycelial growth of all tested fungi; (*Artemisia herb alba, Convolvulus arevensis, Conyza discordis, Deverra tortuosa, Fagonia arabica, Gymnocarpus decandrum, Limonium sinuatum, Mathiola livida, Pulicaria undulata, Rumex vesicarius,* and *Typha domnegensis).* Those are belonging to nine families including asteraceae and apiacea which are considered as rich source of antimicrobial compounds. In particular, the aqueous extract of *Artemisia herb alba* was found to be the most effective against all tested fungi. It caused the highest percent of inhibition in the radial growth of tested fungi that exceeded 45% in case of *fusarium oxysporum* (Figure.1). The high Antifungal efficiency of aqueous extracts of *Artemisia herb alba* was reported by Salhi *et al*, 2017 & 2019 against different pathogens (*Fusarium graminearum, Fusarium sporotrichioides,* and *Fusarium* spp. & *alternaria* spp) respectively. Also, extracts from *Pulicaria undulata* and *Fagonia arabica* proved strong activity against tested fungi. These findings are in agreement with results of (Ahmed and Ibrahim, 2018) that proved the antifungal effect of water extract of *pulicaria undulata* on *Asperigellus niger.*

Results showed that *Aizoon canariense, Portulaca oleracea* and *Spergularia salina* affected only *Fusarium oxysporum* and *Fusarium solani* and failed to cause significant inhibition on mycelial growth of *Rhizoctonia solani* (Table 2). Moreover, *Zygophllum coccineum, Zilla spinosa, Lophochloa pumila* extracts did not show any significant inhibition on mycelial

growth of all tested fungi (Fig.1). This findings are in agreement with early reports of (Mandeel and taha,2005; Panduraju et al,2009; Ajaib et al, 2015; Mehani et al,2016;El Sawi et al,2018).

In contrast to our findings, Alghanem et al, 2018 proved the antifungal activity of ethyl acetate and methyl alcohol extracts of *Zilla spinosa* on the growth of *candida albicans*, *Aspergillus fumigatus* and *Mucor* spp. These differences could be related to different extraction methods used and different test organisms. It is clear that the antifungal activity of different extracts depend mainly on plant species and the tested fungi. *Fusarium oxysporum* and *Fusarium solani* were more sensitive to plant extracts than *Rhizoctonia solani* which could be due to different mode of action or the ability of fungal species to overcome the effect of plant extract (Elmergawy *et al*, 2018). Moreover differences in antifungal activity of tested aqueous extracts may be related to their different phytochemical content such as (phenolic,flavonoids, tannins and terpenes) and their concentration. The antifungal effect of plant extract is probably related to the interaction of plant extract components with enzymes and proteins in fungal cell membrane. At the same time, Chen et al., (2018) found that the ethanol extract of *Curcuma longa* can inhibit the synthesis of ergosterol of *Fusarium graminearum* causing disruption in the synthesis of critical proteins and enzymes which may ultimately inhibit the growth of fungi.

Table 1: List of wild plants collected from desert area in Sadat City, Egypt.

	Scientific name	**Family**	**duration**
1-	*Aizoon canariense L.* *Syn. Glinus chrystallinus Forrsk.,*	Aizoaceae	perennial
2-	*Artemisia herb alba(Asso.)*	Asteraceae	perennial
3-	*Chloris virgata SW.*	Poaceae	Annual
4-	*Convolvulus arvensis L.*	convolvulaceae	perennial
5-	*Conyza discoridis(L)Desf.*	Asteraceae	perennial
6-	*Deverra tortuosa Desf.DC*	apiaceae	perennial
7-	*Fagonia Arabica L.*	Zygophyllaceae	perennial
8-	*Gymnocarpos decandrus forssk*	Caryophyllaceae	perennial
9-	*Hyoscyamus muticus L.*	solanaceae	perennial
10-	*Limonium sinuatum L.*	plumbaginaceae	annual
11-	*Lophochloa pumila (desf) bor*	Poaceae	annual
12-	*Matthiola livida (Delile)DC.*	Brassicaceae	annual

13-	*Portulaca oleracea L.*	portulaceae	annual
14-	*pulicaria undulata (L.) C.A.Mey*	Asteraceae	annual
15-	*Rumex vesicarius L.*	polygonaceae	annual
16-	*Spergularia salina L.*	caryoophyllaceae	annual
17-	*Trigonella stellate Forrsk.,*	fabaceae	annual
18-	*Typha domnegensis pers.*	Typhaceae	perennial
19-	*Zilla spinosa L.*	Brassicaceae	perennial
20-	*Zygophyllum coccnium L.*	zygophyllaceae	perennial

Table 2: Effect of plant extracts on radial growth of *Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani*.

	Radial growth (mean±SE)			
P	***Treatment***	***F. oxysporum RG*(cm)***	***F .solani RG (cm)***	***R.solani RG (cm)***
P1	*Aizoon canariense L.* *Syn. Glinus chrystallinus Forrsk.,*	**7.83±0.20ad**	**8.06±0.06 ajk**	**8.53±0.26ajkmno**
P2	*Artemisia herb alba(Asso.)*	**4.83±0.33 l**	**5.4±0.20 b**	**6.13±0.18b**
P3	*Chloris virgata SW.*	**8.13±0.18 abd**	**8.23±0.08 acjk**	**8.5±0.29ajkmno**
P4	*Convolvulus arvensis L.*	**7.83±0.16 ad**	**7.96±0.26 ajk**	**8.26±0.14acjkmn**
P5	*Conyza discoridis(L)Desf.*	**7.9±0.35 abd**	**8.03±0.41 ajk**	**7.86±0.29 cd**
P6	*Deverra tortuosa Desf.DC*	**7.9±0.12 adm**	**7.56±0.26 ad**	**8.13±0.18 adkm**
P7	*Fagonia Arabica L.*	**6.13±0.40 c**	**7.13±0.44 d**	**7.6±0.33 cde**
P8	*Gymnocarpos decandrus forssk*	**7.76±0.14 ad**	**7.7±0.15 ad**	**7.93 ±0.12 ad**
P9	*Hyoscyamus muticus L.*	**7.83±0.15 ad**	**8.3±0.20 ae**	**8.16±0.16 adf**
P10	*Limonium sinuatum L.*	**7.73±0.18 ad**	**7.76±0.14 ad**	**8.13±0.18 adg**

P11	*Lophochloa pumila (desf) bor*	**8.46±0.31dehk**	**8.3±0.20 af**	**8.5±0.28 ah**
P12	*Matthiola livida (Delile)DC.*	**7.8 ±0.46aej**	**7.5±0.76 ad**	**7.93±0.29 ad**
P13	*Portulaca oleracea L.*	**7.9 ±0.30 aefj**	**8.06±0.34 ag**	**8.43±0.34 aim**
P14	*pulicaria undulata (L.) C.A.Mey*	**6.6±0.15 c**	**6.2±1.15 b**	**7.13±0.18 e**
P15	*Rumex vesicarius L.*	**7.4±0.32 a**	**7.6 ±0.20 ad**	**7.76±0.18 cd**
P16	*Spergularia salina L.*	**7.66±0.16 agj**	**8.0±0.05 ah**	**8.8±0.16 hij**
P17	*Trigonella stellate Forrsk.,*	**7.8±0.15 ahj**	**8.16±0.16 ai**	**8.63±0.18 fghik**
P18	*Typha domnegensis pers.*	**7.6±0.40 aij**	**7.8±0.41 ad**	**8.16±0.12 adkl**
P19	*Zilla spinosa L.*	**8.36±0.18dgijk**	**8.7±0.15efghij**	**8.63±0.18fghilm**
P20	*Zygophyllum coccnium L.*	**8.63±0.18bfkm**	**8.66±0.16efghik**	**8.76±0.12 fhiln**
C	**Control**	**9.0 k**	**9.0 cefi**	**9.0 hio**

RG: radial growth of fungus,Values are represented as means ± SD of three replicates, Means having the same letters in the same column are not significantly different at (p=0.05) level.

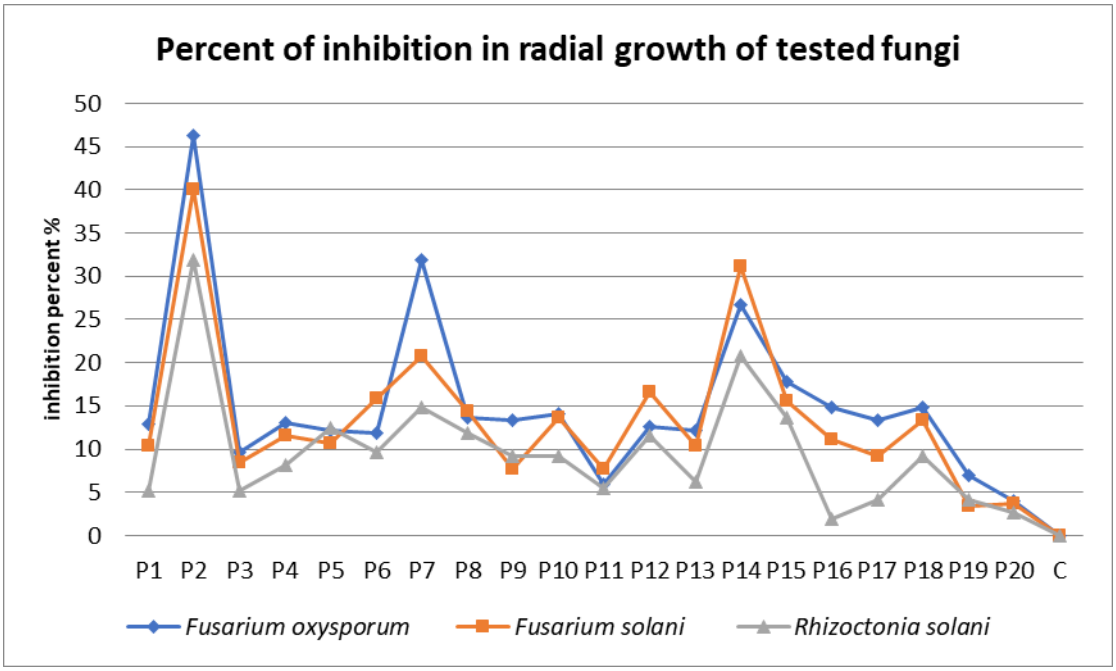

Figure 1: percent of inhibition in radial growth of *Fusarium oxysporum,Fusarium solani* and *Rhizoctonia solani*.

CONCLUSION

Aqueous extracts of *Artemisia herb alba, Pulicaria undulata, Gymnocarpus decandrum, Fagonia arabica, Mathiola lividia, Rumex vesicarius, Typha domnegensis Convolvulus arevensis, Conyza discordis, Deverra tortuosa* and *Limonium sinuatum* are potential sources of active antifungal compounds for formulation of new natural fungicides. Further studies are required for isolation of antifungal compounds from study plants and testing their effect on other phytopathogens.

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