Antifungal activity of Fagonia Arabica L. extracts against Fusarium oxysporum and Rhizoctonia solani

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ABSTRACT

Antifungal activity of methanol, ethanol and acetone extracts of *Fagonia arabica* were studied against soil born fungi (*Fusarium oxysporum* and *Rhizoctonia solani*). Antifungal activity of plant extracts was performed using well plate method. Results indicated that all solvent extracts of *Fagonia arabica* exhibited strong antifungal activity against *Fusarium oxysporum* and weak activity against *Rhizoctonia solani*. Ethanol extract was the most effective against tested fungi with the least minimum inhibition concentration. Preliminary phytochemical screening of plant indicated the presence of various phytochemicals as phenolic acids, flavonoids, tannins, saponins, alkaloids and steroids that could be used as antifungal agents against soil born fungi.

Key words: Antifungal activity, Fagonia arabica L- Fusarium oxysporum- Rhizoctonia solani.

الملخص

تمت دراسة النشاط المضاد للفطريات لمستخلصات (الميثانول- الايثانول- الاسيتون) لنبات الشويكه ضد فطرى الفيوزاريوم اوكسيسبوريوم والريزوكتونيا سولانى. تم اختبار النشاط المضاد للفطريات للمستخلصات باستخدام طريقة الحفره بالطبق. أوضحت النتائج ان كل مستخلصات المذيبات لنبات الشويكه أبدت نشاط قوى ضد فطر الفيوزاريوم اوكسيسبوريوم ونشاط ضعيف ضد فطر الريزوكتونيا سولانى. مستخلص الميثانول كان الأكثر فاعليه ضد الفطريات محل الدراسه بأقل تركيز مثبط لنمو الفطريات. بين المسح الفيتوكيميائى للنبات وجود العديد من المواد الكيميائية النباتيه مثل الأحماض الفينوليه والفلافونيدات و التانيين والصابونين والقلويدات والاسترويدات والتى يمكن استخدامها كعوامل مضادة للفطريات ضد الفطريات الممرضه بالتربه

الكلمات الدالة : النشاط المضاد للفطريات، نبات الشويكه، فيوزاريوم اوكسيسبوريوم،ريزوكتونيا سولانى.

INTRODUCTION

Fusarium oxysporum and *Rhizoctonia solani* are soil born plant pathogenic fungi that are able to cause damages in a wide range of economically important crops. In recent survey in the fungal pathologists' international community, *Fusarium oxysporum* was ranked fifth in a list of top ten fungal plant pathogens based on scientific and economic importance, (Dean et al, 2012). Both pathogens are able to attack a wide range of hosts causing a variety of disease as, root rot, stem rot, damping off and vascular wilt. Globally, chemical fungicides are intensively used in order to control various phytopathogens. Control of *Fusarium oxysporum* and *Rhizoctonia solani* is considered a challenge to phytopathologists due to their ability to survive in the soil in addition, the current limitations in synthetic pesticides application. The mean of fungicides used in Egypt during (2012-2016) was 4497.958 (metric ton active ingredients), Abdel-mageed,

(2017). Long term application of chemical fungicides would lead to adverse impacts on the national income, ecological ecosystems and public health. Elevated levels of pesticides residues in foods and crops could harm the agricultural exports, leading to financial loss and increase the environmental contamination and human health problems. Thereby; it is essential to search for ecofriendly alternatives that can help in decreasing dependence on chemical fungicides.

Plants are a great natural resource of phytochemicals that could play an important role in control of various pathogens including phytopathogenic fungi. *Fagonia arabica* is low shrub with wide spread ranging in distribution from the North Africa to south arabica and Pakistan, Bolous (2002). It is commonly found in the Mediterranean coastal strip, sandy plains and desert wadis. *Fagonia* species are commonly used in traditional medicine to treat cancer, fever, asthma, toothaches, urinary diseases, stomach problems and kidney diseases, Basit et al, (2019). Callus of *Fagonia arabica* collected from Sinai showed spectra against both gram positive and gram negative bacteria, Eman et al, (2010). So that, the aim of this study was to explore the antifungal potential of different extracts of *Fagonia arabica* collected from Sadat city-Egypt against plant pathogenic *Fusarium oxysporum* and *Rhizoctonia solani* followed by preliminary phytochemical screening of plant extracts.

Material and methods

1 - Plant material and extraction

Aerial parts of *Fagonia arabica* was collected from desert area in Sadat city, Egypt at the flowering stage. Fifty grams of air dried plant material were soaked in 500ml of solvents (methanol, ethanol, and acetone) individually. Extracts were shaken for 24 h with continuous agitation at 150rev/min then the extract filtered through filter paper (whatman no. 1). The solvents were removed by using rotary vacuum evaporator with water path at 40c°. Finally residues were collected and incubated at 4c° for further use.

2- Microorganisms

Fungal isolates were isolated from infected plant organs at faculty of agriculture Menoufia University, *Fusarium oxysporum* f. sp. Faba bean vascular wilt, and *Rhizoctonia solani* f. sp faba bean root rot

3-preparation of fungal inoculum

The fungal inoculums were prepared from 6-10 old day cultures grown in potato dextrose agar medium (PDA). The spore density of each fungus was adjusted to obtain final concentration approximately10^5 spores /ml**.**

4- Antifungal activity

Antifungal activity of solvent extracts were determined using well plate method as described by Haggag et al, (2017) with slight modifications where fungal cultures of *Fusarium oxysporum* and *Rhizoctonia solani* were spread over PDA plates by spread plate technique. One well (15mm) was made into each plate with the help of a sterile cork borer.100µl of (100 mg/ml) methanol, ethanol and acetone extracts of *Fagonia arabica* were added to separated wells. Wells containing the corresponding solvents served as negative control while fungicide Mancozeb: 64% Manganese ethylene_bis (dithiocarbamate), (polymeric) complex with zinc salt and 28% inactive matter as 72% wettable powder was used as positive control. The plates were incubated at 25C° for 48-72 hours. Each experiment was repeated three times and the mean of inhibition zone around the wells was calculated in mm.

5- Determination of minimum inhibition concentrations (MICs)

Extracts of *Fagonia arabica* were dissolved in acetone to a concentration of 20 mg/ml. Then, 100 µl of plant extracts were serially diluted up to 50% with water in 96 well micro-liter plates. 100 µl of fungal cultures transferred into fresh potato dextrose broth was added to each well and appropriate solvents were included as blank. As an indicator of fungal growth, 40 µl of 0.2 mg/ml of *p*-iodonitrotetrazolium violet (Sigma®) dissolved in water was added to each of the microplate wells. The covered microplates were incubated for 2 days at 25 °C. The minimum inhibition concentration was recorded as the lowest concentration of the extract that inhibited fungal growth after 24 h, Mahlo et al, (2013)**.**

6-Determination of minimum fungicidal concentrations (MFCs):

MFCs were determined by inoculating 20µl from the wells with concentration equal to and higher than minimum inhibition concentration on potato dextrose agar and incubated over night at 25c°. MFCs values were determined as the concentration that totally inhibited the growth of fungal colonies, Arikan, (2007).

7- Phytochemical screening

The extracts were subjected to various qualitative chemical tests to determine the presence of various phyto-constituents like alkaloids, flavonoids, saponins, phenols, steroids, and tannins, (Shahid-Ud-Duaula and Bashir, 2009; Boxi et al., 2010; Adegoke et al., 2010).

7.1- Test for flavonoids

A few drops of concentrated hydrochloric acid were added to 5ml of plant extract. Immediate development of a red colour was taken as an indicator to the presence of flavonoids.

7.2- Chemical test for saponins

0.5 g of plant extracts were dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponins. (Frothing test)

7.3- Chemical test for steroids

2 ml of acetic anhydride and 2 ml conc. $H_2SO_4$was added to 5 ml of the extract. Change of colour from violet to blue confirms the presence of steroids. (Liebermann–Burchard reaction)

7.4- Chemical test for tannins and phenolic compounds

Few drops of acetic acid were added to 5ml of plant extracts. Red colored solution indicated the presence of tannins and phenolic compounds.

7.5- Chemical test for alkaloids

0.5 g of the extract was stirred with 5 ml of the 1% aqueous hydrochloric acid on a steam bath. A few drops of dragendorff's reagent were used to treat 1 ml of the filtrate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids.

8- Statistical analysis

All experiments were conducted in completely randomized design with three repetitions for each treatment. All values of antifungal effect were expressed as the mean ±standard deviation (SD) zone of inhibition on treated plates. The statistical analysis of results was conducted by analysis of variance (ANOVA) using computer SPSS 15 software package. Differences on statistical analysis of data were considered significant at $p<0.05$

Results

1- Extraction of *Fagonia arabica L.*

Extraction of *Fagonia arabica* with polar solvents (methanol, ethanol and acetone) led to yellowish green and slight sticky materials. Ethanol extracted the highest amount of plant material with 89.1mg/g followed by methanol (84.32 mg/g) and acetone (51.43 mg/g). (Table 1)

Table (1): Extraction of *Fagonia arabica* with polar solvents.

Solvent	color	Consistency	Yield mg/g
Methanol	Yellowish Green	Slight sticky	84.32
Ethanol	Yellowish Green	Slight sticky	89.1
Acetone	Green	Slight sticky	51.43

ethod and compared with fung

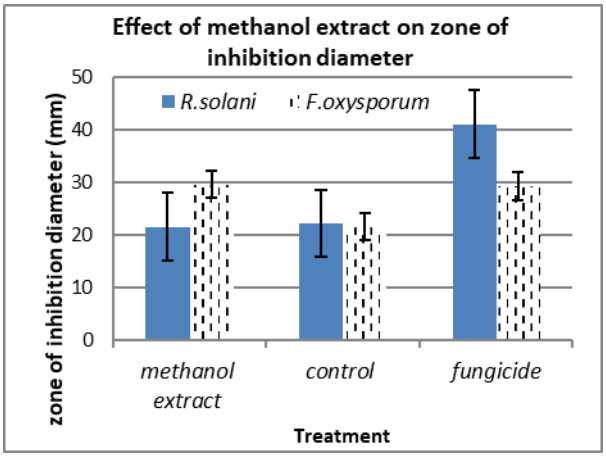
2- Antifungal activity of *Fagonia arabica L,*

The results of antifungal activity of different solvents of *Fagonia arabica* are shown in Table (2) and Figures (1, 2, and 3). Antifungal activity of extracts was detected by measuring zone of inhibition diameters in mm. All solvent extracts of *Fagonia arabica* showed potential antifungal activity against *fusarium oxysporum* compared with control. Furthermore, methanol, ethanol and acetone extracts exhibited similar effects against *f. oxysporum* with zone of inhibition (29.60, 33.33 and 33.00) mm respectively. However, the same solvent extracts of *Fagonia arabica* showed very weak activity against *Rhizoctonia solani* with zone of inhibition (22.33, 23.33 and 19.31) mm for methanol, ethanol and acetone extracts respectively.

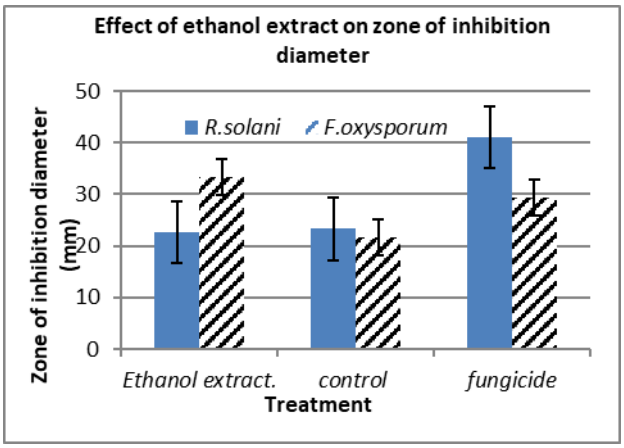
Table (2): Antifungal activity of different solvent extracts on *Fusarium oxysporum* and *Rhizoctonia solani*.

	solvent	Inhibition zone in mm	
Treatment		***Fusarium oxysporum***	***Rhizoctonia solani***
Plant extract	**Methanol extract**	**29.60 +0.04 [a]**	**22.23+0.24 [A]**
	Ethanol extract	**33.33+0.23 [a]**	**23.33+0.24 [A]**
	Acetone extract	**33.00+0.24 [a]**	**19.31+0.04 [A]**
Negative control solvents	**Methanol**	**21.61+0.23 [b]**	**21.60+0.16 [A]**
	Ethanol	**21.60+0.23 [b]**	**22.60+0.23 [A]**
	Acetone	**16.63+0.04 [b]**	**18.02+0.17 [A]**
Fungicide Positive control		**29.33+0.11 [a]**	**41.06 +0.36 [B]**

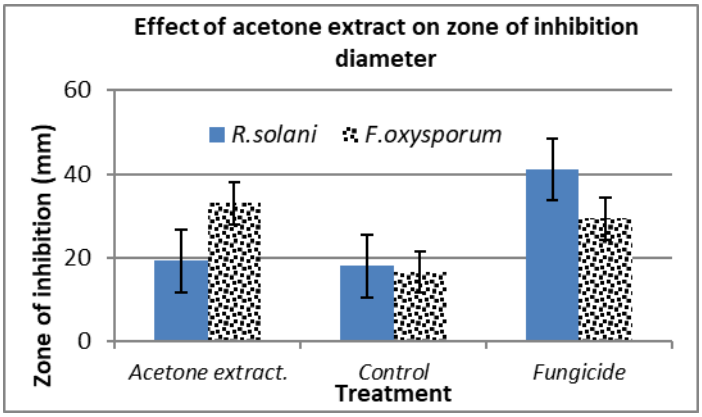
*Values are represented as means ± SD of three replicates, Means having the same letters are not significantly different at (p=0.05) level.



Figur1: Antifungal effect of methanol extract of *Fagonia arabica* on *Fusarium oxysporum* and *Rhizoctonia solani*.



Figur2: Antifungal effect of ethanol extract of *Fagonia arabica* on *Fusarium oxysporum* and *Rhizoctonia solani*.



Figur3: Antifungal effect of acetone extract of *Fagonia arabica* on *Fusarium oxysporum* and *Rhizoctonia solani*

3-Minimum inhibition concentration

Determination of minimum inhibition concentration involved a semi-quantitative test by dilution method which gives an approximation of the least concentration of plant extract that is required to inhibit fungal growth. Solvent extracts of *Fagonia arabica* exhibited different MICs and MFCs values on different fungal species. Results of MICs and MFCs are listed in Table 3. The minimum inhibitory concentrations (MICs) of different solvent extracts ranged between 0.75 and 2.5 mg/ml in case of *fusarium oxysporum* while it was 20mg/ml for all solvent extracts against *Rhizoctonia solani*. (Table 3)

4- Minimum fungicidal concentration

Minimum fungicidal concentrations (MFCs) were 5mg/ml for acetone extract against *fusarium oxysporum* and 2.5mg/ml for methanol and ethanol extract against the same fungus. However, all studied concentration of different solvent extracts of *Fagonia arabica* did not cause fungicidal effect on *Rhizoctonia solani*. (Table 3)

Table 3: Minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC) of solvent extracts

MIC/ MFC (mg/ml)		
Treatment	***F. oxysporum***	***R. solani***
Methanol extract	2.5/10	20/-
Ethanol extract	0.75/5	20/-
Acetone extract	2.5/10	20/-

MIC: minimum inhibition concentration, MFC: minimum fungicidal concentration, (-) not detected

5- Phytochemical analysis

The results of qualitative phytochemical screening of the methanol, ethanol and acetone extracts of *Fagonia arabica* are shown in Table 4. The results indicated the presence of

saponins, steroids ,tannins, phenolic acids, flavonoids and alkaloids in all extracts of *Fagonia arabica* while steroids was not detected in acetone extract.

Table 4: Qualitative phytochemical analysis of plant extracts

Fagonia arabica extracts	saponins	steroids	tannins	Phenolic acids	flavonoids	alkaloids
Methanol extract	++	+	++	++	++	++
Ethanol extract	++	+	+++	+++	++	++
Acetone extract	+	-	++	++	++	++

(-): Absence, (+): Less presence, (++): Moderate presence, (+++): High presence.

Discussion

Recent years witnessed an increased interest with natural wild plants as a great source of bioactive compounds that could be used as antimicrobial agents against human and plant pathogens. In the current study, *Fagonia arabica* was used as a natural source of bioactive compounds that could be used as potential antifungal agents against plant pathogen *Fusarium oxysporum* and *Rhizoctonia solani*. Efficiency of plant extracts as antimicrobial agents depend mainly on many factors. These factors include their content of secondary metabolites, their solubility, PH, volatility, diffusion potential in growth medium and the type of organism under investigation. Using different solvents in extraction of *Fagonia arabica* played an important role in the yield of extraction. Ethanol was the most effective solvent in extraction of phytochemicals from *Fagonia arabica.* Therefore, it is recommended as the optimal solvent to obtain high content of phytochemicals from *Fagonia arabica*.

Studying the antifungal effect of methanol, ethanol and acetone extracts of *Fagonia arabica* on *Fusarium oxysporum* and *Rhizoctonia solani* indicated the high susceptibility of *Fusarium oxysporum* to plant extracts. Statistical analysis of data indicated that all solvent extracts of *Fagonia arabica* exhibit significant inhibition against *Fusarium oxysporum* only. In case of *Fusarium oxysporum*, all solvent extracts of *F. arabica* showed zones of inhibition equal to that of the commercial fungicide used as positive control. In addition, differences in zones of inhibition between different solvents were not significant on the same fungus. All extracts of *F. arabica* was able to induce fungistatic and fungicidal effects on *Fusarium oxysporum* at tested concentrations, while *Rhizoctonia solani* was more resistant to low concentrations of plant extracts. High concentration (20mg/ml) of *F. arabica* was able to inhibit the growth of *Rhizoctonia solani* in growth media but failed to cause fungicidal effects. Ethanol extract of *F. arabica* showed the least minimum inhibition concentration among tested extracts which could be related to its hydrophilic nature that in turn effect on its diffusion in the growth media making active phytochemicals more available to assayed fungi. The present study results are in correlation with earlier work of El-Amier and abo-Aisha, (2019) that showed the high antimicrobial activity of crude extracts of aerial parts of *F. arabica* collected from Wadi Hagul,

North Eastern desert,Egypt against *Candida albicans* due to their high content of total phenols and tannins.

Secondary metabolites involve abroad range of compounds that can play an important role in the biological activity of wild plants. Phytochemical screening of *Fagonia arabica* extracts showed their high content with phenolic acids, tannins and flavonoid compounds in addition to their content with alkaloids and saponins. Similar phytoconstituents have been detected in studies of Syed et al, (2013) and Abobaker, (2017). Presence of different bioactive constituents in solvent extracts of *Fagonia arabica* contribute toward their antifungal activity. Ethanol extract showed the highest presence of phenolic acids that in turn decreased its minimum inhibition concentration against tested fungi. Early study of Rongai et al, (2015) indicated that plant extracts with good antifungal activity generally had a high level of total phenolic content. Toxicity of phenolic compounds to microorganisms could be related to their hydroxyl groups that cause enzyme inhibition or disrupting the membrane integrity of fungal cell.

Conclusion

The results obtained from this work showed that solvent extracts (methanol, ethanol and acetone) of *Fagonia arabica* collected from Sadat city, Menoufiya governorate, Egypt exhibit fungicidal effect against *Fusarium oxysporum* and fungistatic effect against *Rhizoctonia solani*. Moreover, extracts of *Fagonia arabica* are rich with phytochemicals that could be used as antifungal agents. Further studies are needed for purifying and characterizing of the bioactive compounds in plant and for promoting their use in agriculture to reduce chemical fungicides application.

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