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Study on Effect of Some Medicinal Plant Extracts on Growth and Spore Germination of *Fusarium oxysporum* schlecht. *In vitro*

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Abstract: In this study, antifungal activity of *Menthapiperita* L., *Cinnamomumzeylanicum*Blume, *Alliumhirtifolium*Boiss.and*Allium sativum*L. were investigated against *Fusarium oxysporum* schlecht. Inhibition of the growth rate and spore germination was evaluated after 8 and 10 days in PDA and PDB media. The results showed the plant extracts and their main components had inhibitory activities on the growth rate and spore germination of this fungus. The results exhibited *Cinnamomumzeylanicum*, *Menthapiperita*, *Allium hirtifolium* and *Allium sativum* at 1000, 500, 500 and 500 ppm respectively gave the largest inhibition on the growth rate of *F. oxysporum*in PDA media. Extract of *Cinnamomumzeylanicum*, *Menthapiperita*, *Allium hirtifolium* and *Allium sativum* showed maximum inhibition of the spore germination at 1000, 1000, 1000 and 500 ppm after 8 days, while the least spore germination was occurred at 1000, 100, 100 and 25 ppm after 10 days in PDB media.

Key words: Antifungal activity · *Fusarium oxysporum* · Plant extracts · Inhibition

INTRODUCTION

Fusarium oxysporum schlecht is one of the most important soil pathogen that presents in the rhizosphere of plants. Most *F. oxysporum* strains live saprophytically on organic substrates in soil. Some of soil-born strains are plant pathogens and causing plant disease. Some of them are biocontrol agent. The species is very complex group and based on the pathogenicity toward particular plant species take place into physiological races and form specialis [1].

There are several ways for controlling of cucumber damping-off includingbiological control [2-3], chemical control [4] and plant extracts [5-7]. Chemical fungicide traditionally been used against fungal plant pathogen. Frequency use of fungicide leaded to the development of resistant population of the pathogen against various chemical fungicide groups. The other side, the toxic properties of fungicides limited the use of these compounds [8].

Some plant species were assayed for pharmacological and biological activity such as antibacterial and antifungal activity [9-11]. Fungicidal properties of plant species depended on various plant products including oil, gums, resin, saponin, organic acid and alkaloids [12-17].

Khalil and Dababneh [18] investigated the inhibitory effect of four medicinal plant extracts against *Rhizoctonia solani*, *F. oxysporum*, *Verticillium* sp. and *Penicillium* sp.. *Vartemia iphionodes* showed maximum inhibitory for *Verticillium* sp. (44.8%), followed by *R. solani* (42.9%), *F. oxysporum* (42.7%) and *Penicillium* sp. (18.2%) at 1000 ppm.

The effect of *Zatariamultiflora* and *Satureja hortensis* essential oil were evaluated on the growth rate and ability of mycotoxin production by *F. graminearum* in PDA and PDB. The results indicated the essential oils had inhibitory activity on this fungus and mycotoxin production [19]. Asalem *et al.* [20] studied antifungal activity five plant species against three important pathogens including *Altrenaria solani*, *R. solani* and *Macrophomina phaseolina*. *Adhatoda zylanica* showed maximum inhibition against *M. phaseolina* whereas, *Dodonaea viscosa* had the most effective on *A. solani* and *R. solani*. Dababneh and Khalili [21] evaluated five Jordanian medicinal plants against five pathogenic fungi. The highest growth inhibition of all fungi was observed with *Achilleasantolina* at 1000 ppm. The

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growth inhibition of *F. oxysporum* and *R. solani* was 42.2 and 42.0% respectively.

Morsy, *et al.* [22] investigated the growth rate of five plant pathogen fungi such as *F. oxysporum, F. solani, Sclerotiumrolfsii, R.asolani*and *M. phaseolina*. The results stated plant extracts of onion and garlic reduced the growth rate of *F. oxysporum*, *F. solani*and *S. rolfsii*. The highest inhibition effect on *F. oxysporum* was observed when garlic extract was applied at 1.5ml/plate.

The inhibitory effect of *Thymus vulgaris*, *Satureja hortensis*, *Anthum grareolens* and *Mentha sativa* were investigated against *F. graminearum*. The result stated the essential oils reduced the growth rate and mycotoxim production [23].

Efficacy of Mancozeb and garlic extract were evaluated against *Alternaria alternate*, *F. oxysporum*, *Aspergillus* sp., *Rhizopus* sp. and *colletoterichum graminicola* on sorghum and groundnut seed. The results showed that garlic extraction was effective on seed germination and mycelia weight of the fungi [24].

The objectives of this study were to determine the effect of *Mentha piperita* L., *Cinnamomum zeylanicum*, *Allium hirtifolium* and *Allium sativum* L. on the growth rate and spore germination of *F. oxysporum*.

MATERIALS AND METHODS

This work was conducted in Department of Plant Pathology, Damghan Unit, Azad University during 2011-2012 to determine the antifungal activity of some medicinal plants, i.e. *C. zylanicum*, *M. piperita*, *A. sativum*and *A. hirtifolium*, against *F. oxysporum*, cucumber isolate. Pure culture of *F. oxysporum*was obtained from Plant Pathology department of Agriculture College, Tehran University.

Preparation of Plant Extracts: The bark of *C. zylanicum*, aerial parts of *M. piperita* and bulb of *A. sativum*and *A. hirtifolium* were selected and dried at 40°C for 24h. These plant materials were ground by a blender and 50 grams of their powders were subjected to soxhlation that is exhaustively extracted by ethanol. In the end of soxhlation, the solvent (ethanol) was evaporated at lower temperature under reduced pressure in rotary flash evaporator to get the crude extract [25-26]. The extracts were stored in dark vials at 4°C for future uses.

Extracts Analysis: Analysis of extracts was carried out by GC-MS chromatography (Agilent GC/ MS Model 5975) as recommended by manufacturer. This instrument was fitted with HP-5MS capillary column (30cm×0.25mm i.d., film thickness 0.25µm). The identity of components was ascertained based on the spectra and compared with library and literature data. The oven temperature program was 60-270°C (2.5°C per min.) and the carrier gas was helium with velocity 1ml/min.

Effect of Plant Extract on *F. oxysporum* Mycelia Growth: The effect of *C. zylanicum*, *M. piperita*, *A. sativum*and *A. hirtifolium* extracts on diameter growth of *F. oxysporum* on Potato Dextrose Agar (PDA) was evaluated according to reference documents [27]. The plant extracts were added to PDA (at 45°C) to give a final concentration 25, 50, 100, 500 and 1000ppm for each extract and then the resulting media were poured in petri dishes (8cm in diameter). Ethanol was added to medium in control plates. Then, inoculum discs (5 mm in diameter) from two days growing cultures of *F. oxysporum*were placed in the center of petri plates containing PDA and extracts. Each treatment was tested on 4 plates as replications. The plates were incubated in 27°C. After seven days (when the fungus overgrow on control plates), radial growth of *F. oxysporum*was recorded for each plate. The percentage of fungal growth inhibition was calculated as Pandey *et al.* [28] formula:

Growth inhibition% = [(growth in control – growth in sample)/growth in control] × 100.

The values reported for minimum inhibitory concentration were the lowest concentrations of extracts on which the fungus grew a little or any after seven days.

Fungistatic or Fungicide Effect of the Extracts: The method of Thomson [29] was used to assess the fungistatic or fungicide nature of the extracts on *F. oxysporum*. For each extract, a plug of plate medium on which the fungus grew a little or any removed and transferred to a new PDA medium without extract. If the fungus grows in the new medium after 7-10 days, the extract would be evaluated as fungistatic.

Effect of Plant Extracts on Spore Germination: The antifungal activity on mentioned medicinal plant extracts on spore germination of *F. oxysporum*was tested using potato dextrose broth (PDB). The plant extracts were added to PDB (at 45°C) to give a final concentration 25, 50, 100, 500 and 1000ppm for each extract. Water was added to medium in control Erlenmeyer flask. Then, three

inoculum discs (5mm in diameter) from two days cultures of the fungus were put in each flask. Each treatment was replicated three times and inoculated media were incubated in 27°C on shaker (150rpm). Eight days after treatment (when produced spores of the fungus were germinated in control flasks), the evaluation on spore germination was started using a light microscope (X= 10 × 40 in magnification). Besides, the number of germinated spores in each treatment was counted again 10 days after treatments. The Percentage of spore germination was calculated according to following formula:

Spore germination% = (No. of spores germinated/total no. of spores examined) × 100.

Statistical Analysis: Data regarding two parameters (concentration and medicinal plants) were analyzed statistically using SAS program with completely randomized design (CRD). Inhibition of radial mycelial growth was examined using analysis of variance (ANOVA) and means were compared by the test of least significant difference (LSD 0.05).

RESULTS

Effect of Plant Extract on *Fusariumoxysporum* Mycelia Growth: Different doses (25, 50, 100, 500 and 1000ppm) of the extracts from four medicinal plants (C. zylanicum, *M. piperita*, *A. sativum* and *A. hirtifolium*) were tested against *F. oxysporum* to determine their antifungal activity in *in vitro* tests. The results of analysis of variance revealed that all tested medicinal plants caused significant inhibition of mycelia growth of the fungi (Table 1). Furthermore, data analysis showed the differences between extracts, between doses, as well as between their interaction are significant ($p<0.01$). The comparison of means showed maximum inhibition of *F. oxysporum* growth was found at highest doses, 500 or 1000ppm (Table 2). It was followed by the concentration 100, 50 and 25ppm of the plant extracts as compared to control which showed least inhibition on mycelia growth. The extract of *C. zylanicum* at highest dose (1000ppm) was most effective in reducing fungus growth followed by 500ppm of *M.piperita*,*A. hirtifolium* and *A. sativum*, respectively. Growth inhibition varies from 23.19 to 5.71% in different concentrations of *C. zylanicum* extracts. In the lowest concentration (25ppm) of different extracts, maximum inhibition of mycelia growth was obtained by *A. hirtifolium*.

Effect of Plant Extracts on Spore Germination: In the present work, the effect of different concentrations of extracts from four medicinal plants was studied on spore germination of *F. oxysporum* eight and ten days after treatment. The results in table 3 show that all treatment concentrations of plant extracts significantly reduced spore germination of *F. oxysporum* in PDB compared with control. In other words, analysis of variance showed the differences between extracts, doses, times of counting, as well as their interaction are significant ($p<0.01$). The comparison of means showed the extent of spore germination usually decrease by increasing the concentration of extracts (after 8 or 10 days) and the maximum inhibition was usually observed at highest doses of extracts, 1000 or 500ppm (Table 4 and 5).

Table 1: ANOVA table for the effect of different concentrations of some medicinal plant extracts in inhibition of *Fusariumoxysporum* mycelia growth

Source of variances	Degree of freedom	Sum of square	Means of square	F test
Extract	3	59.611	19.87	26.96**
Concentration	5	4139.31	827.86	1123.18**
Extract ×Concentration	15	649.13	43.27	58.71**
Error	69	50.85	0.73	
Total	92	4908.23		
CV	6.64%			

P value<0.01

Table 2: Inhibition percent of mycelia growth of *F. oxysporum* by different concentrations of *C.zylanicum*, *M. piperita*,*A. sativum* and *A. hirtifolium* extracts on PDA

	Concentrations				
Plant extract	25	50	100	500	1000
C. zylanicum	6.16d	5.71d	18.62b	15.77c	23.19a
M. piperita	13.62c	14.54c	18.45b	21.30a	13.74c
A. sativum	8.64d	14.91c	17.42b	20.27a	17.87b
A. hirtifolium	14.08d	13.85d	14.89c	20.38a	16.49b

Means within each column having the same letters are not significantly different (Duncan 5%).

Table 3: ANOVA table for the effect of different concentrations of some medicinal plant extracts in inhibition of *F. oxysporum* spore germination

Source of variances	Degree of freedom	Sum of square	Means of square	F test
Extract	3	9229.68	3076.56	373.97**
Time	1	9040.84	9040.84	1098.95**
Concentration	5	93794	18758	2280.21**
Extract×Time	3	6856.74	2285.58	277.82**
Extract×Concentration	15	13556	903.74	109.85**
Time×Concentration	5	5532.7	1106.54	134.5**
Extract×Time×Concentration	15	10846	723.12	87.9**
Error	94	773.31	8.22	
Total	141	149636		
CV	4.5%			

P value<0.01

Table 4: Inhibition percent of spore germination of *F. oxysporum* by different concentrations of *C. zylanicum*, *M. piperita*,*A. sativum* and *A. hirtifolium*extracts 8 days after treatment in PDB

	Concentrations					
Plant extract	0	25	50	100	500	1000
C. zylanicum	8.33c	93a	92.33a	85b	85.33b	96.33a
M. piperita	8.66e	59.33d	68.33c	79.33b	93a	97.33a
A. sativum	10.66d	57.66c	73b	88.66a	91a	94.66a
A. hirtifolium	7.66c	74.33b	73.33b	94.33a	95a	91.33a

Means within each column having the same letters are not significantly different (Duncan 5%).

Table 5: Inhibition percent of spore germination of *F. oxysporum* by different concentrations of *C. zylanicum*, *M.piperitaA. sativum* and *A. hirtifolium*extracts 10 days after treatment in PDB

	Concentrations					
Plant extract	0	25	50	100	500	1000
C. zylanicum	5c	84.33b	89.66b	85.66b	89.33b	95.66a
M.piperita	6e	33.66d	65.33c	92.33a	87b	93.66a
A. sativum	6.66d	57.33b	70a	75.66a	40c	42.66c
A. hirtifolium	5.66e	70.33a	73.33a	32.33b	22c	15d

Means within each column having the same letters are not significantly different (Duncan 5%).

It was followed by the concentration 100, 50 and 25ppm of the plant extracts as compared to control which showed least inhibition on spore germination. Exceptionally in 10 days after treatment for *A. sativum* and *A. hirtifolium* extracts, the maximum inhibition doses were 100 and 50ppm, respectively. Eight days after treatment, the extract of Peppermint at highest doses (1000ppm) was most effective extract in reducing spore germination followed by 1000ppm of *C. zylanicum*, 500ppmof *A. hirtifolium* and 1000ppm *A. sativum*, respectively (Table 4). Also, inhibition of spore germination varies from 97.33 to 57.66% (eight days after treatment). In the lowest concentration (25ppm) of different extracts, maximum inhibition of spore germination was obtained by *C. zylanicum* and then *A. hirtifolium*.

Table 6: Anlysis of *C. zylanicum* extract by GC/MA chromatography

Component	Retention time (min.)	Percentage
2-propenal,3-phenyl	26.49	16.20
Cinnamic aldehyde	27.70	57.73
Pz-prope-1-01,3-pheylnn	29.11	1.37
4-(1-Hydroxyethyl)benzaldehyde	30.00	2.12
Alpha-copaene	32.91	6.85
ZH-1-Benzopyran	36.59	6.85
Alpha-amorpen	38.96	2.07
Alpha-muurolene	40.43	1.85
Beta-cadinene	41.82	4.47
Para methoxycinnamic aldehyde	42.55	5.61

Table 7: Anlysis of Peppermint extract by GC/MA chromatography.

Component	Retention time (min.)	Percentage
Sabinene	9.16	1.27
Limonene	11.74	14.14
Trans-sabinene hydrate	13.83	1.60
Cis-beta-terpineol	21.64	6.49
Menthol	24.86	47.19
Beta-bourbonene	33.23	3.36
Trans-caryophyllene	35.29	4.56
2-Hexadeceh-1-01	59.04	1.01
Hexadecanoic acid	65.48	2.13
Isomenthole	71.88	2.53
9,12,15-octadecatrien-1-01	73.47	5.13
4,6-bio(4-methoxyphenyl)-z-meth	81.23	4.21
9-methoxy-11-methyl-5-methylimin	86.28	1.69
Campesterol	91.87	1.29
Neophytadinene	97.57	1.21
Gamma-sitostrol	101.29	2.19

Table 8: Anlysis of *A. hirtifolium* extract by GC/MA chromatography

Component	Retention time (min.)	Percentage
Glycine	4.33	1.69
Heptanoic acid	10.47	1.18
Tridecanal	52.63	1.23
Hexodecanoic acid	65.78	18.69
Hexadecanoic acid, ethyl ester	66.64	1.66
9-octadecenoic acid	73.75	33.17
Ethyl linoleate	74.03	1.43
Ethyl oleat	74.32	3.69
(4s)-4-hydro	80.16	1.21
Piperidino-5-4hydroxyphenoxy	80.46	9.18
Hexadecanoicacid,z-hydroxy	88.00	5.46
Oleic acid,3-hydroxypropyl ester	94.68	6.07
2,2-bis-trideuteriomethyl	94.97	4.45
Stigmasterol,22,23-dihydro	101.30	10.99

Table 9: Anlysis of *A. sativum* extract by GC/MA chromatography

Component	Retention time (min.)	Percentage
Methanamine	43.39	3.17
2,3,4,5,6-D5-Ailine	7.41	0.97
2-Benzyl-2-methyl-1	8.38	0.89
Disulfide	14.39	1.36
Phenol	15.15	0.94
Methyl 2-propenyl	17.74	0.93
Beta-d-allopyranoside	18.19	0.74
2-3-Dihydro-Benzofuran	23.91	1.95
Pyridine	24.72	1.03
Trislfide,di-2-propenyl	27.95	1.09
2-Methoxy-4-vinylphenol	29.14	1.99
Cyclopentanol	44.39	0.64
Methyl-(2-Hydoxy-3-Ethoxy-Benzyl)	49.01	0.66
Benzylbenzoate	54.80	0.73

Table 9: Continued

Hexadecanoic acid	65.68	24.45
2-12 octadecadienoic acid	73.33	24.95
Dimethylamino	79.24	0.76
Bicyclo	86.47	2.17
Cyclotetracosane	92.66	0.67
Nonadecene	94.92	1.82
Hexadecadien-1-01 acetate	96.21	0.67
Hexacosene	99.68	1.36
Ethylcholest	101.32	9.95
17-pentariacontene	101.89	2.19
Tetracosan-1-01	103.30	5.44
Alpha-amyria	104.78	4.18
Stigmastan-3,5-dien	106.90	1.41

Extract Analysis by Chromatography: Analysis of extracts was carried out by GS/MS chromatography to determine their constituents. The quantitative composition of components in C. *zylanicum*, *M. piperita*, *A. hirtifolium* and *A. sativum* extracts were shown in tables 6, 7, 8 and 9, respectively.

DISCUSSION

The study demonstrated the plant extract such as *C. zylanicum*, *M. piperita*, *A. sativum*and *A. hirtifolium*, and their concentration had considerable effect on the growth rate and spore germination of *F. oxysporum*. The ethanolic extracts of *C. zylanicum* indicated considerable antifungal activity against *F. oxysporum*. This activity could be attributed to the presence of *Cinnamic aldehyde* (57.73%). Similar studies have been carried out by different researcher on antifungal activity of plant extract. The effect of plant extracts and essential oils from *Rosmarinusofficinalis*, *Thymus vulgaris*, *C. zeylanicum* and *Syzigium aromaticum* was evaluatedon the mycelial growth and spore germination of *F. oxysporum*f. sp. *cubense in vitro*. The results indicated that plant extracts and essential oils of *C. zeylanicum*and *S.aromaticum*were effective at 500 ppm against the mycelialgrowth [30]. Boniface *et al.* [31] investigated the antifungal activity of *C. zeylanicum*essential oil. The results proved the oil had fungicidal properties against *F. oxysporum* and *Penicilium digitatum*. The essential oils was mainly composed *Cinnamic aldehyde* (37.6%), ciannamic acetate (23.7%), ciannamyl benzoate (14.6%) and other compounds.

Mentha piperita extracts exhibited remarkable antifungal activity against *F. oxysporum*. The species extract included Menthol (47.19%) and Neophytadinene (1.21%) which compound had antifungal properties.

Farshbaf Moghaddam *et al.* [32] investigated composition and antifungal activity of *M. piperita* oil on *F. oxysporum* f. sp. *ciceri*., *Macrophomina phaseolina* and *Dreschlera oryzea*. Essential oil analysis with GS/MS showed that main compounds of oil include menthol (19.76%), menthan-3-one (19.31%), menthofuran+isomenthone (9.12%), 1, 8-cineole+beta phellandren (8.8%) and menthol acetate (5.63%). Twenty-one components were identified in the essential oil of *M. piperita*. The essential oil revealed a good activity against *Fusarium oxysporum* at a concentration of 0.2% of the essential oil. TLC-bioautography yielded three subfractions that prevented fungal growth, suggesting the presence of antifungals. The results indicated that several fungitoxics were responsible for the antifungal activity of *M. piperita*, with the principal ones being menthone, neomenthol, menthol and carvone [33].

The ethanolic extracts of *A. sativum*and *A. hirtifolium*revealed considerable antifungal activity against *F. oxysporum*. Okigbo *et al*. [34] investigated the fungi toxic effects of *A. sativum* against six plant pathogenic fungi such as *F. oxysporum*, *F. solani*, *Botryodiplodia theobromae*, *M. phaseolina*, *Penicillium oxalicum* and *Aspergillus niger*. The obtained results revealed *A. sativum* had effective inhibition (25.2-86.9%) on the mycelial growth of all tested fungi. Jacob and Sivaprakasan [35] and Arya *et al.* [36] evaluated the antifungal activity of the extracts of various plant species against *Fusarium pallidoroseum* and the results stated extracts of garlic bulbs and Bignonia leaves inhibited the mycelial growth of *Fusarium pallidoroseum*.

Bowers and Locke [37] studied antifungal activity of various plant extract against *Fusarium solani*f. sp. *melongenae* and the results showed that the extract of *Allium sativum*exhabited maximum inhibition in spore germination followed by *Datura stramonium*, *Artemisia* spp. *Mentha spicata* and *Juglans regia*. Efficacy of fungal activity of various plant extracts were studied against the spore germination of *Alternaria brassicae* causing *Alternaria* blight in rapeseed and mustard. The results indicated that garlic demonstrated maximum inhibition in spore germination [38].

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REFERENCES

1. Armstrong, G.M. and J.K. Armstrong, 1981. Formaespeciales and races of *Fusarium oxysporum* causing wilt diseases. In: P.E. Nelson, T.A. Toussoun and R.J. Cook, editors. Fusarium: Diseases, biology and taxonomy. University Park, USA: The Pennsylvania State University Press. pp: 392-399.
2. Kim, H.K. and H.J. Jee, 1988. Influence of rhizosphere antagonists on suppression of cucumber wilt, increased cucumber growth and density fluctuation of *Fusarium oxysporum* f.sp. *cucmerinum* Owen. Korean J. Plant Pathol., 4: 10-18.
3. MohamedGehad, M., 1996. Studies on biological control of some soil-borne of cucumber wilt, increased cucumber growth and density fluctuation of of root-rot disease of sunflower. Pakistan J. Botany, 30: 45- 50.
4. EL-Shami Mona, A.E., A.A. Farahat, F.A. Fadl and M.M. El-Zayat, 1988. Greenhouse studies on soil fungi attacking vegetable cucurbits in the field and fungicidal treatments against *Fusariumoxysporum* f. sp. *niveum*. Agric. Res. Rev., 66(2): 265-275.
5. Shalaby, M.S. and M.M. Atia, 1996. Biological and Chemical control of watermelon and cantaloupe root- rot in the reclaimed soils. Zagazig J. Agric. Res., 22: 1113-1130.
6. Ehteshamul, H.S., M.J. Zaki, A.A. Vahidy and A.B. Abdil Gaffer, 1998. Effect oforganic amendments on the efficacy of *Pseudomonas aeruginosa*in the controlof root-rot disease of sunflower. Pakistan J. Botany, 30: 45-50. (C.F. Rev. Pl. Pathol., 78: 1486).
7. Tohamy, M.R., A.Z. Aly, T.H. Abd-El-Moity, M.M. Atia and L. Abd-El-MoneimMaisa, 2002. Evaluation of some plant extracts in control damping-off and mildew diseases of cucumber. Egypt. J. Phytopathol., 30(2): 71-80.
8. Lin, K.C., 1981. Resistance of ten tree species to sulfur dioxid. Bulletin, Taiwan Forestry Research Institute, pp: 349.
9. Al-Mughrabi, K., 2003. Antimicrobial activity of extracts from leaves, stems and flowers of *E. macroclada* against plant pathogenic fungi. Phytopathol. Mediterranea, 42: 245-250.
10. Ismail, M., Z. Iqbal, B. Ahmad, S. Zakiri and U. Naiz, 2003. Biological and pharmacological properties of two indigenous medicinal plants, Rheum emodi and Paeoniaemodi. Pak. J. Biol. Sci., 6: 984-986.

11. Hoffman, B.R., A. Delas, K. Blanleo, N. Wiederhold, R.E. Lewis and L. Williams, 2004. Screening of antibacterial and antifungal activities of ten medicinal plants from Ghana. Pharm. Biol., 42: 13-17.
12. Sofowora, A., 1984. Medicinal plants and Traditional Medicine in Africa, Johnwiley, New York, pp: 256-257.
13. Asthana, A., K. Dixit, N.N. Tripathi and S.N. Dixit, 1989. Efficacy of*Ocimum* oil against fungi attaching chilli seed during storage. Trop. Sci., 29: 15-20.
14. Chaturvedi, R., A. Dikshit and S.N. Dixit, 1987. Adenocalymmaalliacea, a new source of a natural fungitoxicant. Trop. Agric., 64: 318- 322.
15. Daoud, A.S., N.A. Qasim and N.M. Al-Mallah, 1990. Comparison study on the effect of some plant extract and pesticides on some Phytopathogenic fungi. Mespotamia J. Agric., 22: 227-235.
16. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews., 12: 564-582.
17. Al-Mughrabi, K.T., T. Aburajai, G.H. Anfoka, W. Shahrour, 2001. Antifungal activity of olive cake extracts. Phytopathol. Mediter., 40: 240-244.
18. Khalil, A. and B.F. Dababneh, 2007. Inhibition of Phytopathogenic Fungi by Extracts from Medicinal Plants in Jordan. Journal of Biological Sciences, 7: 579-581.
19. Lahooji, A., M. Mirabolfathy and R. Karami-Osboo, 2010. Effect of *Zataria multiflora* and *Satureja hortensis* essential oils, thymol and carvacrol on growth of *Fusarium gramineum* isolates and deoxynivalenol production. Iran. J. Plant Path., 46(1): 11-13.
20. Aslam, A., F. Naz, M. Arshad, R. Qureshi and C.A. Rauf, 2010. *In vitro* antifungal activity of selected medicinal plant diffusates against Alternariasolani, Rhizoctoniasolani and *Macrophomina phaseolina*. Pak. J. Bot., 42(4): 2911-2919.
21. Dababneh, B.F. and A. Khalil, 2007. The Inhibitory Effect of Extracts from Jordanian Medicinal Plants Against Phytopathogenic Fungi, 6(2): 191-194.
22. Morsy, S.M., E.A. Drgham and G.M. Mohamed, 2009. Effect of Garlic and Onion Extracts or their Intercropping on Suppressing Damping-off and Powdery Mildew Diseases and Growth Characteristics of Cucumber. Egypt. J. Phytopathol., 37(1): 35-46.
23. Hoseiniyeh Farahani, S.H., M. Mirabolfathy, H. RezaieDanesh and R. KaramiOsboo, 2012. Effect of Five essential oils on zearalenon production and growth of Fusariumgraminearum. Plant Pest and Pisease, 80(1): 81-94.
24. Syed, D.Y.N., T. Mengesteab, N. Robiel, W. Robiel and Z. Tekle, 2012. Efficacy of garlic extract and mancozeb against seed borne fungal pathogen of farmer saved sorghum (*Sorghum bicolor*) and groundnut *(Arachishypogaea)* seeds. Greener Journal of Agricultural Sciences, 2(2): 31-36.
25. Momeni, T., 2001. Plant extractes. Iran. Shahid Farhad Reza Publication, pp: 264.
26. Shariat, S., 1993. Selection of medicinal plants. Extractedactive ingredients from medicinal plants andidentification andevaluation of them. Iran. Mani publication, pp: 292.
27. Grover, R.K. and J.D. Moore, 1962. Toximetric studies of fungicides against brown rot organism - *Sclerotinia fructicola* and *S. laxa*. Phytopathology, 52: 876-80.
28. Pandey, D.K., N.N. Tripathi, R.D. Tripathi and S.N. Dixit, 1982. Fungitoxic and Phytotoxic properties of the essential oil of *H. suaveolens zeitschrift* fur pflanzenkraneaten and pflanzenschutz, 89: 344-349.
29. Thompson, D.P., 1989. Fungitoxic activity of essential oil components on food storage fungi. Mycologia, 81: 151-153.
30. Fernando, P.M., L.C. Ferreira, J.L. Silva, L.P. Pacheco and P.E. Souza, 2013. Influence of Plant Extracts and Essential Oils against Panama Disease (*Fusarium oxysporum* f. sp. *cubense*) in Banana Seedlings. Journal of Agricultural Science, 5(4): 63-74.
31. Boniface, Y., S. Philippe, H.R. de Lima, N.J. Pierre, A.G. Alain, T. Fatiou and S. Dominique, 2012. Chemical composition and Antimicrobial activities of *Cinnamomum zeylanicum* Blume dry Leaves essential oil against Food-borne Pathogens and Adulterated Microorganisms. International Research Journal of Biological Sciences, 1(6): 18-25.
32. Farshbaf Moghaddam, M., R. Omidbaigi, V.M. Pourbaig and A. Ghaemi, 2004.Composition and antifungal activity of peppermint (*Mentha piperita*) essential oil. Iranian Journal of Pharmaceutical Research, 3(2): 68-69.

33. Freir, M.M., G.N. Jham, O.D. Dhingra, C.M. Jardim and V.M. Moreira, 2012. Composition, antifungal activity and main fungitoxic components of the essential oil of *Menthapiperita*. Journal of Food Safety, 32(1): 29-36.
34. Okigbo, R.N., R.E. Okorie and R.R. Putheti, 2009. *In vitro* effects of garlic (*Allium sativum* L.) and african basil (*Ocimum gratissimum* L.) on pathogens isolated from rotted cassava roots. Interciencia, 34: 742-747.
35. Jocob, C.K. and K. Sivaprakasam, 1994. Evaluation of some plant extracts and antogonsists for the control of pre-emergence damping-off of brinjal (*Solamum melongena* L.) In: Crop Disease-innovative Techniques and management (Eds. Sivaprakaasam, K.). Kalayni Publisher, New Delhi, pp: 289-294.
36. Arya, A., R. Chauhan and C. Arya, 1995. Inhibition of growth of 200 pathogenic fungi by garlic extract.Mycologia, 67: 882-885.
37. Bowers, J.H. and Locke, 2000. Effect of botanical extracts on the population density of *Fusariumoxysporum* in soil and control of *Fusarium*wilt in the green house. Plant Dis., 3: 300-305.
38. Khurana, A.K., N. Metha and M.S. Sangwan, 2005. Variability in the Sensitivity of *Alternaria brassicae* isolates to plant extracts. J. Mycol. Plant Pathol., 35: 76-77.