JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2015. Vol. **9**(1), p. 515-521

Anti-Fungal Evaluation of Medicinal Plant on Some Pathogenic Fungi

Hashem Al-Sheikh

Department of Biological Sciences, College of Science,
King Faisal University, Al-Hassa 31982, Saudi Arabia.

(Received: 06 April 2014; accepted: 19 August 2014)

Aqueous extracts of *Cuminum cyminum* and *Foeniculum vulgare* were investigated as antifungal against *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium moniliforme*. The data showed that all studied plant extracts concentrations have significant inhibition in the growth of the three tested pathogenic fungi. All concentrations of the two tested plants extract were found to be inhibitory to fungal growth and the rate of inhibition increased generally by increasing the concentration, 75% concentration in tested plants was the most effective concentration. Generally, the results represented that the extracts of *Cuminum cyminum* and *Foeniculum vulgare* had antifungal effect against the tested pathogenic fungi with different degrees.

Key words: Antifungal; Medicinal plants; Pathogenic fungi; Plant extract.

It is known that medicinal plants playing an important role for treatments of various human ailments since time immemorial. World Health Organization reported that, over than 80% of the world's population, or 4.3 billion people, are used traditional medicinal plant to provide them with primary health care[1]. Past medicinal plant research primarily focused on bioactive phytochemicals, however, many phototherapeutic compounds are found to be produced by associated microbes or through symbiotic association[2]. Medicinal plants harbor a distinctive microbiome due to their unique and structurally divergent bioactive secondary metabolites that are most likely responsible for the high specificity of the associated microorganisms[3].

A number of diseases in human, animals, insects and plants are caused by pathogenic fungi. Antifungal compounds are therefore important both for controlling serious fungal diseases of plants, and for preserving food and other organic materials against attack by fungi. Agrios, (2004)[4] reported that pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In addition, fungi produce mycotoxins which have allergic or toxic disorders among consumers. Harris *et al.*, (2001)5 performed that the synthetic fungicides are used for controlling phytopathogenic fungi, however, the uses of synthetic fungicides have harmful effects on environment and the human health. In recent years, natural plant products as environmentally safe option have received attention for controlling phytopathogenic diseases. Therefore, many considerable researches have been carried out during the last two decades to search for biocides that are environmentally safe and easily breakdown. Mohana and Raveesha, (2008)6 reported that the extracts of many medicinal plants have antimicrobial activity under laboratory conditions.

Several studies have been shown that plant extracts demonstrate antifungal effects as

* To whom all correspondence should be addressed.
Tel: 00966555487911 ;
E-mail: plxha@kfu.edu.sa;stophashem@hotmail.com

reported by several researchers[5,3,2,7]. The present work aims to study the antifungal effect of *Cuminum cyminum* and *Foeniculum vulgare* extracts on some pathogenic fungi isolated from Al-Hassa area.

MATERIALS AND METHODS

Medicinal plants

Two medicinal plants were selected for this study namely: *Cuminum cyminum* and *Foeniculum vulgare.* The taxonomic characters and important medicinal uses of these plants are shown in table (1)[8,9,10,11,12,13]. They have a variety of antioxidant and antimicrobial properties. *Cuminum cyminum* and *Foeniculum vulgare* were selected to search according to their availability and use in traditional medicine.

Isolation and identification of fungi

Three fungal species were isolated and selected for this study namely, *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium moniliforme*. The tested fungal species were isolated from the soil at Al-Hassa, Saudi Arabia. Samples were transferred to the laboratory and kept in a refrigerator (3-5 °C) until fungal examination. Laboratory processing for fungal isolation was done by different standard scientific methods. A modified method of that described by Johnson *et al*., (1959)[14] was used**.** The fungal colonies arising on the plates were examined daily and the fungi were identified to the genera and species by microscopic examination. For purification and identification of the fungal species the isolates were transferred to sterilized plates. The grown fungi were mounted on a slide, stained with lactophenol-cotton blue to detect fungal structures[15], covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics[16,17]. Identification of isolated fungi was carried out using the taxonomic keys of Booth 11 (1971)[18], Domsch *et al*., (1980)[19], Moubasher (1993)[20] and Pitt and Hocking (1997)[21].

Preparation of seed extract from some medicinal plants

The aqueous extracts of *Cuminum cyminum* and *Foeniculum vulgare* were prepared by soaking 50 g of finely ground plant seeds in 150 ml of distilled water for 24 hours. The mixtures were strained through cheesecloth. Different concentrations of extract were prepared such as:

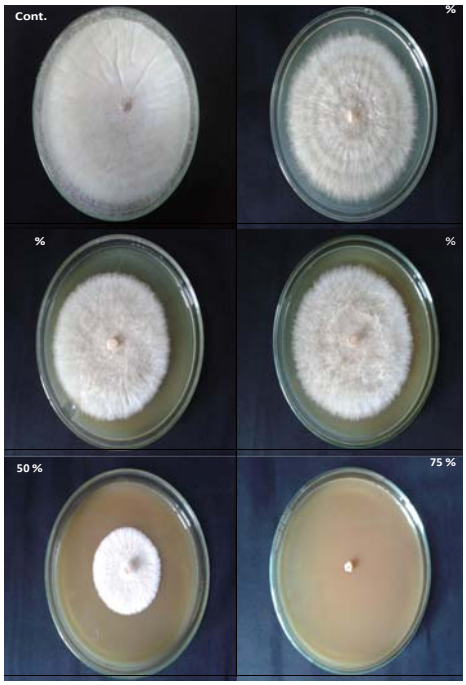

Plate. 1. The activity of *Cuminum cyminum* extracts on the mycelia growth of *Rhizoctonia solani*

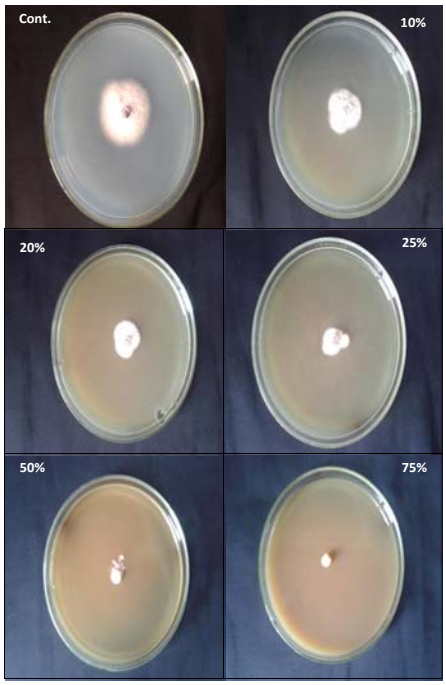

Plate. 2. The activity of *Cuminum cyminum* extracts on the mycelia growth of *Fusarium oxysporum*

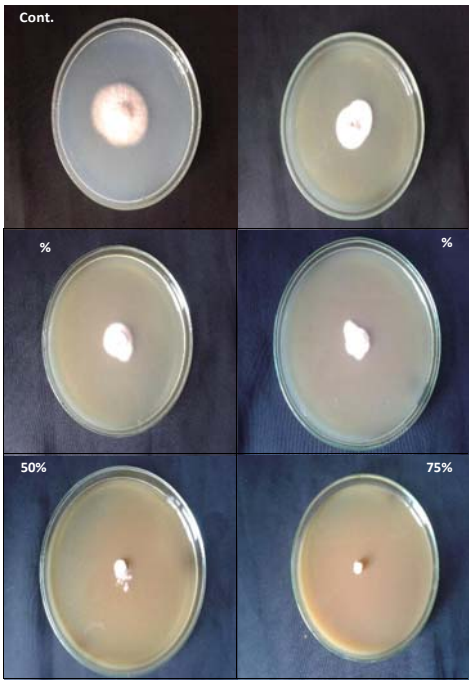

Plate. 3. The activity of *Cuminum cyminum* extracts on the mycelia growth of *Fusarium moniliforme*

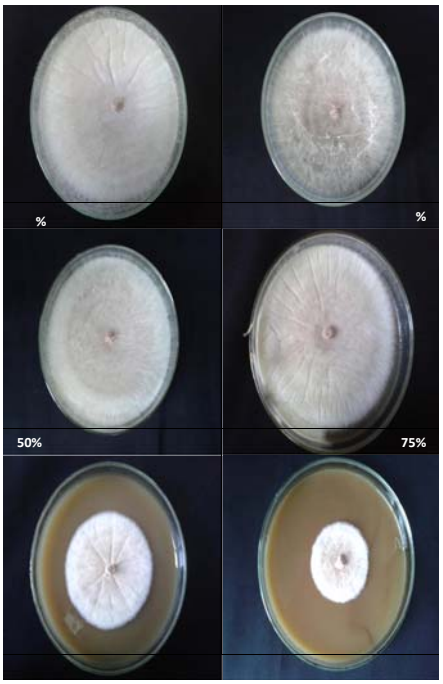

Plate. 4. The activity of *Foeniculum vulgare* extracts on the mycelia growth of *Rhizoctonia solani*

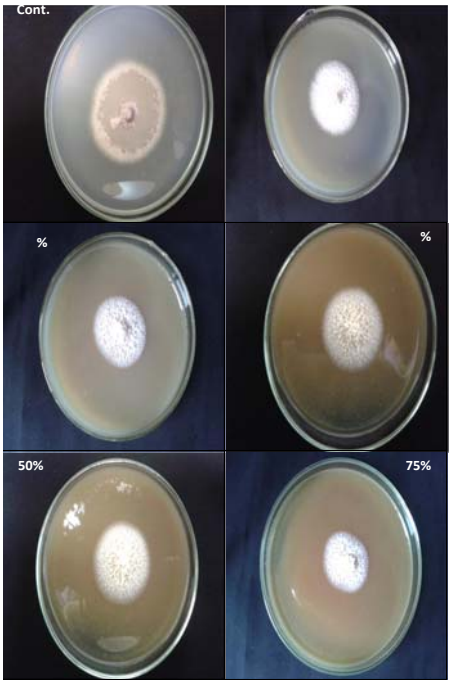

Plate. 5. The activity of *Foeniculum vulgare* extracts on the mycelia growth of *Fusarium oxysporum*

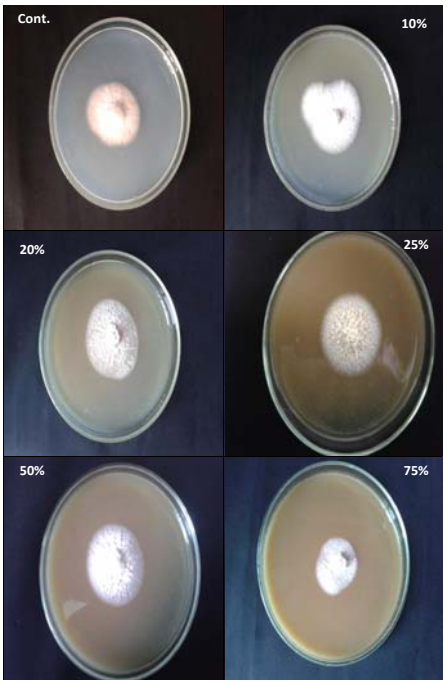

Plate. 6. The activity of *Foeniculum vulgare* extracts on the mycelia growth of *Fusarium moniliforme*

10, 20, 25, 50 and 75%. The media (PDA) were autoclaved at 121°C for 15 minutes and then poured into 9 cm diameter petri dishes. After solidification, three plates of each seed extract agar medium were inoculated by placing 3.0 mm diameter discs. Samples were incubated for 4 days at 30° C. The seed extract free samples were used as controls. Percentage inhibition of mycelia growth by plant extracts were calculated according to the following equation:

$$\text{Inhibition of myceliagrowth}(\%) = \frac{(\text{Diameterofcontrol}) - (\text{diameteroftest})}{(\text{Diameter ofcontrol}) \times 100}$$

RESULTS AND DISCUSSION

Antifungal compounds from plants origin are most suitable being less toxic and more environmentally compatible by nature. From past many years number of plants have been extracted and screened for their antifungal activities and valuable results have been achieved 22,23,24. In this respect, the present study was aimed to evaluate the antifungal activity of *Cuminum cyminum* and *Foeniculum vulgare* 11 extracts, against some plant pathogenic fungi isolated from Al-Hassa area (*Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium moniliforme*).

Table.1. Taxonomic characters and important medicinal uses of the plants selected for this investigation

Common name	Scientific Name	Medicinal importance
Cumin	Cuminum cyminum	Cuminum cyminum (Cumin) is aromatic plants within the Apiaceae family that is used in foods, fragrances, and medical preparations (liqueurs, mouthwashes, toothpastes, soaps, and perfumes). They are used as antispasmodic, carminative, and appetite stimulating agents (Iacobellis et al., 2005)8. Cumin seeds have been found to possess significant biological activities, such as antibacterial (Morton, 1976)9, antifungal, anti- carcinogenic (Gagandeep et al., 2003)10, anti-diabetic, anti-thrombotic (Dhandapani et al., 2002)11, and antioxidant properties (Ferrie et al., 2011)12.
Fennel	Foeniculum vulgare	Foeniculum vulgare (Fennel). is a biennial medicinal and aromatic plant belonging to the family Apiaceae (Umbelliferaceae). It is a hardy, perennial–umbelliferous herb with yellow flowers and feathery leaves. It grows to a height of up to 2.5 m with hollow stems. Fennel is highly aromatic with a characteristic aniseed flavour. Ethnobotanical data currently available on wild useful plants. Roots, young shoots, leaves, flowering stems, mature inflorescences and fully ripened and dried seeds are commonly used for homemade remedies, being useful in the treatment of several complaints, specifically those of the digestive system. Fennel is also highly recommended for diabetes, bronchitis and chronic coughs, for the treatment of kidney stones, and is considered to have diuretic, stomachic and galactogogue properties (Lillian et al., 2010)

Table.2. Effect of different plant extracts on the linear growth (cm) of *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium moniliforme*

Plant	Fungalspecies	Extractconc. (%)					
		Cont.	10	20	25	50	75
Cuminum cyminum	*Rhizoctonia solani*	9	7.35	5.56	5.76	3.53	0
	Fusarium oxysporum	3.2	2.26	1.63	1.23	0.7	0
	Fusarium moniliforme	4	2.06	1.86	1.4	0.33	0
Foeniculum vulgare	*Rhizoctonia solani*	9	9	8.96	8.26	5.93	4.56
	Fusarium oxysporum	5.2	3.7	3.7	4.3	4.1	3.1
	Fusarium moniliforme	4	4	4	4	4	2.9

Table (2), Figure (1) and Plates (1-6) summarizes the antifungal activities of the *Cuminum cyminum* and *Foeniculum vulgare* against the three testes pathogenic fungi (*Rhizoctonia solani*, *Fusarium moniliforme* and *Fusarium oxysporum*) at different concentrations. The data clearly revealed that *Cuminum cyminum* extracts cause growth inhibition on the three tested fungi, however the inhibition rate of growth of tested fungi was found to be varied with different concentrations. On the other hand, the results also shows that *Foeniculum vulgare* extracts has inhibited effects on *Rhizoctonia solani* and *Fusarium oxysporum* growth, whereas *Fusarium moniliforme* didn't show any significant difference between vary concentrations except the

Cuminum cyminum ***Foeniculum vulgare***

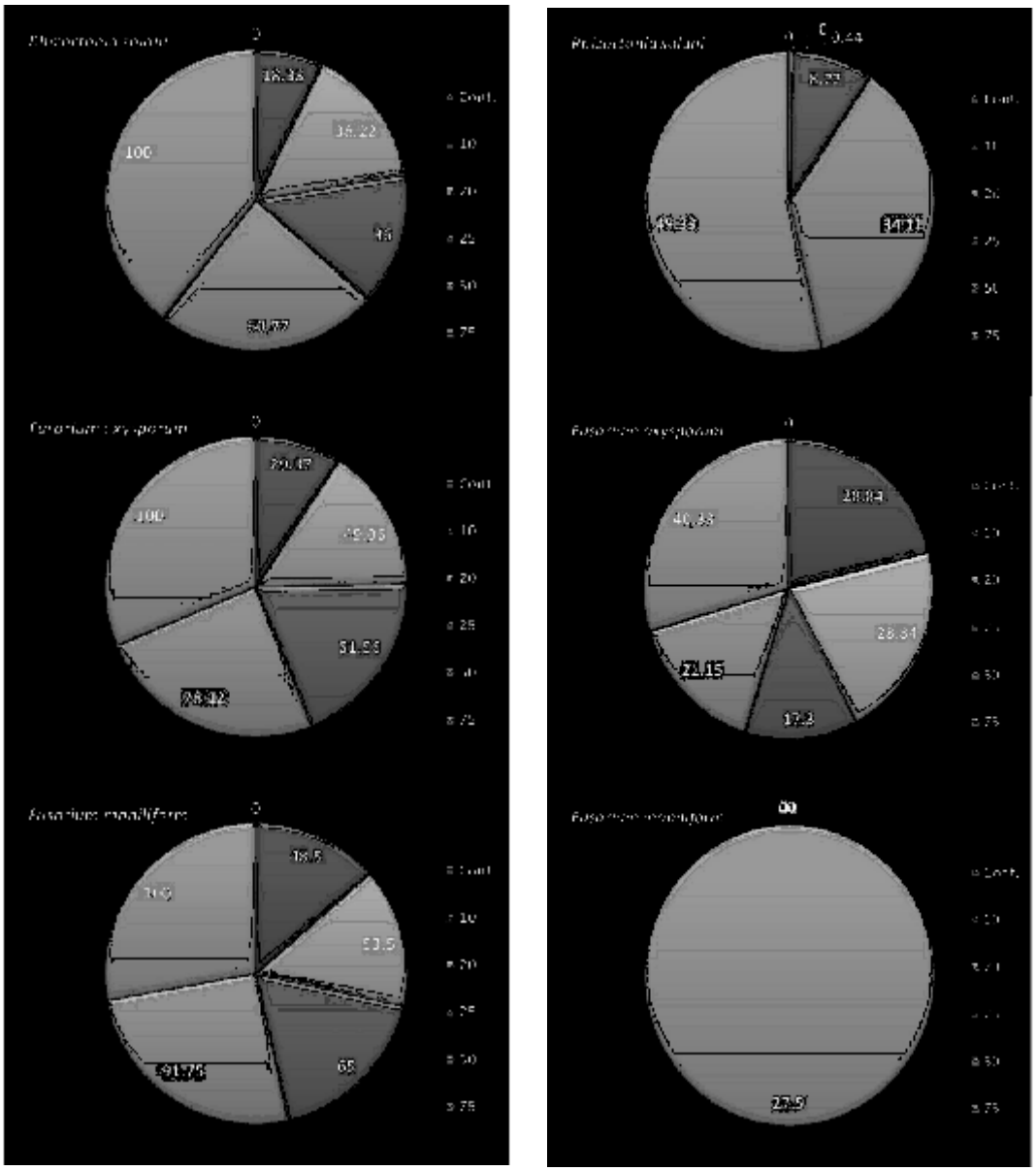

Fig. 1. Antifungal activities of *Cuminum cyminum* and *Foeniculum vulgare* extracts against *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium moniliforme* (Inhibition percent)

concentration of 75% (Table 2). The data of Figures (1) performed the effect of different extracts of *Cuminum cyminum* and *Foeniculum vulgare* on the growth (inhibition percent) of the three selected fungi. The data show that all investigated concentrations of the two tested plants extracts were found to have inhibitory effect on fungal growth; however the rate of inhibition increased generally by increasing the concentration. The data also show that the most effective concentration of tested plants extract was 75%. Generally, the obtained data indicated that the extracts of *Cuminum cyminum* and *Foeniculum vulgare* had different degrees of antifungal activity against the three tested pathogenic fungi (Table 2 and Figure 1).

Results indicated the presence of antifungal compounds in different plant extracts, which was in agreement with the results reported by authors who tested the plant extracts on different plant pathogens using paper disc method[25,26]. The broad spectra of the inhibitory effect of *C. stenocephala* indicated that the extract of this species is potent antifungal plants with possible potential for controlling plants fungal diseases. Therefore, more researches would be of value on the activity of this plant against the other plant pathogenic fungi. The species in 11 the genus of *Cousinia* widely grow in Iran, Afghanistan, Pakistan, Himalaya and Central Asia[27]. *Cousinia* is the third largest group in Asteraceae and 220 out of 385 species of this genus recorded in Flora Iranica specifically grow in Iran 28. Besides, the number of species is continuously growing every year. The antibacterial activity of different species of *Cousinia* was documented earlier. Shahverdi *et al.*, (2007)[29] found that ethanolic extract of *Cousinia phyllocephala* is the strongest extract among seven tested Iranian species of *Cousinia* against Gram positive and negative bacteria. Recently, Bahraminejad *et al.*, (2013)[26] reported that the flora in the west of Iran can be regarded as a rich source of plants with antifungal activity.

CONCLUSION

Nowadays, application of chemical compounds is the most common method used in controlling plant disease. However, the natural active compounds found in plants appear to be safer and acceptable than synthetic chemical compounds. Considerably the alternative control methods are needed. The objective of this study was to investigate the antifungal effect of *Cuminum cyminum* and *Foeniculum vulgare* extracts on some pathogenic fungi isolated from Al-Hassa area. The obtained data indicated that the extracts of *C* had different degrees of antifungal activity against *Rhizoctonia solani*, *Fusarium moniliforme* and *Fusarium oxysporum*. The extract of *Cuminum cyminum* and *Foeniculum vulgare* could be used as antifungal agent against fungal diseases.

ACKNOWLEDGMENTS

This work was supported in part by grants (No. 150122 **&** No. 140042) from the Deanship of Scientific Research, King Faisal University (KFU). Financial support for this work is gratefully acknowledged. 11

REFERENCES

1. Jena, S.K. and Tayung, K. Endophytic fungal communities associated with two ethno-medicinal plants of Similipal Biosphere Reserve, India and their antimicrobial prospective. *Journal of Applied Pharmaceutical Science*, 2013; **3**(4): S7-S12. DOI: 10.7324/JAPS.2013.34.S2.
2. Köber, M., Schmidt R., Ramadan, E. M., Bauer, R. and Gabriele, B. The microbiome of medicinal plants: diversity and importance for plant growth, quality and health. *Front Microbiol.;* 2013; **4**(400): 1-9. Doi: 10.3389/fmicb.2013.00400.
3. Qi, X., Wang, E., Xing, M., Zhao W. and Chen, X. Rhizosphere and non-rhizosphere bacterial community composition of the wild medicinal plant Rumex patientia. *World J. Microbiol. Biotechnol.*, 2012; **28**(5) : 2257-65.
4. Agrios, G.N. Losses caused by plant diseases. P.29-45. *Plant Pathology.* 2004; Elsevier, Oxford, UK.
5. Harris, C.A., Renfrew, M.J., and Woolridge, M.W. Assessing the risk of pesticide residues to consumers:recent and future developments. *Food Additives and Contamination*, 2001; **18**: 1124-1129.
6. Mohana, D.C. and Raveesha, K.A. Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. *Journal*

of Agricultural Technology, 2008; **12**: 119-137.
7. El-Said A.H.M. and El-Hady G. Antifungal Activities of *Cuminum cyminum* and *Pimpinella anisum* Essential Oils. *Int.J.Curr.Microbiol.App.Sci* 2014; **3**(3): 937-944.
8. Iacobellis, N., Cantore, P. , Capasso, F. and Senatores, F. Antibacterial Activity of *Cuminum cyminum* L.and *Carum carvi* L. Essential Oils. *J. Agric. Food Chem.,* 2005; **53**: 57-61.
9. Morton, J. F. Herbs and Spices. New York: Golden Press. 11: 1976.
10. Gagandeep, S., Dhanalakshi, E., Mendiz, A., Rao, R., and Kale, R. Chemopreventive effects of *Cuminum cyminum* L. in chemically induced forestomach and uterine cervix tumors in marine model system. *Nutrition and Cancer,* 2003; **7**: 171-180.
11. Dhandapani , S., Subramanian, V., Rajagopal, S. and Namasivayam, N. Hypolipidemic effect of *Cuminum cyminum* L. on Alloxan- induced diabetic Rats. *Pharmacological Research,* 2002; **46**: 251-255.
12. Ferrie, M., Bethune, T., Arganosa, G. and Waterer, D. Field evaluation of doubled haploid plants in the Apiaceae: dill (*Anethum graveolens* L.), caraway (*Carum carvi* L.), and fennel (*Foeniculum vulgare* Mill.). *Plant Cell Tiss Organ. Cult.* 2011; **104**, 407-413.
13. Lillian, B., Ana Maria C. and Isabel C.F.R. The nutritional composition of fennel (*Foeniculum vulgare*): Shoots, leaves, stems and inflorescences.LWT - *Food Science and Technology,* 2010; **43** (814–818).
14. Johnson, L.F., Curl E.A., Bond J. H. and Fribourg H. A. Methods for studying soil microflora. Plant diseases relationships. Burgess Publ. Co.Minn. U.S.A 1959.
15. Basu, P.K. Production of chlamydospores of *phytophthora megasperma* and their possible role in primary infection and survival in soil. *Can. J. Plant Pathol.*, 1980; **2**: 70-75.
16. Ronhede, S., Jenesen, B., Rosendahl, S. Kragelund, B.B., Juhler R.K. and Amand J. Hydroxylation of the herbiside isoproturon by fungi isolated from agricultural soil. *Appl. Environ. Microb.*,2005; **71**(12): 7927-7932.
17. Rajankar P.N., Tambekar, D.H. and Wate, S.R. Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of purna river basin. *Res. J. Agric. Biol. Sci.*, 2007; **3**(6): 701-703.
18. Booth C. The Genum. Commonwealth Mycological Institute, Key, Surrey, UK 1971.
19. Domsch, K.W., Gams W. and Anderson T.N. : Compendium of soil Fungi. Academic Press., London 1980.
20. Moubasher, A.H. (1993): Soil Fungi in Qatar and other Arab Countries. Center for Scientific and Applied Research, University of Qatar, Doha, Qatar, pp: 566. 11
21. Pitt, J.I. and Hocking, A.D. Fungi and Food Spoilage. 2nd Edn., Gaithersburge, Chapman and Hall, Maryland, 1997; pp: 593.
22. Bansal, R.K., and Gupta, R.K., Evaluation of plant extracts against *Fusarium oxysporum*, wilt pathogen of fenugreek, *Indian Phytopathology*, 2000; **53**: pp 107-108.
23. Zaker, M. and Mosallaneja, H. d. Antifungal activity of some plant extracts on *Alternaria alternata*, the causal agent of *Alternaria* leaf spot of potato. *Pak. J. Biol. Sci.*, 2010; **13**: 1023-1029.
24. Dwivedi, S.K. and Dwivedi, N. Antifungal activity of some plant extracts against guava wilt pathogen. *International journal of environmental sciences*, 2012: **3**(1):412-420.
25. Bahraminejad, S. In vitro and In vivo antifungal activities of Iranian plant species against *Pythium aphanidermatum*. *Ann Biol Res*, 2012; **3**(5):2134-214.
26. Bahraminejad, S., Reza, A., Ghasemi, S. and Fathi, N. Inhibitory effect of some Iranian plant species against three plant pathogenic fungi. *International Journal of Agriculture and Crop Sciences*. 2013; **5**(9): 1002-1008.
27. Shinwari, Z.K. and Qaiser M. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot*, 2011; **43**: 5-10.
28. Ghaffari, S.M., Garcia-Jacas, N., and Susanna, A. New chromosome counts in the genus *Cousinia* (Asteraceae) from Iran. *Bot J Linn Sos*, 2006: **151**:411-419.
29. Shahverdi, A.R., Khorramizadeh, M.R., Attar F., Saada, F., Vahid, S. and Ghahramann A. Concomitant chemopreventive and antibacterial effects of some Iranian plants from the genus *Cousinia* (Asteraceae). *Braz. J. Pharmacogn.* 2007; **17**(3):325-330