

## ISOLATION AND IDENTIFICATION OF FUNGI WHICH INFECT FENNEL *Foeniculum vulgare* Mill. AND ITS IMPACT AS ANTIFUNGAL AGENT

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### ABSTRACT

The study included isolation and diagnosis of fungi that infect *Foeniculum vulgare* Mill planted in the Department of Drugs and Medicinal Plants, Pharmacy College - University of Baghdad, different symptoms such as wilting and yellowing, stunting on the plants were observed fungi: *Alternaria alternata*, *Rhizoctonia solani*, *Phoma herbarum* and *Fusarium oxysporum*, The disease incidence ranging between 5-10%. Studied the effect of *Foeniculum vulgare* plant seeds extract against *Alternaria alternata*, *Rhizoctonia solani*, *Phoma herbarum* and *Fusarium oxysporum*, where tested the concentrations 0,2.5 and 5% of alcoholic extract of fennel seeds showed effectiveness noticeable trend all fungal isolates and showed significant differences between different levels of concentrations, at  $P = 0.01$  and  $P = 0.05$ .

### INTRODUCTION

The studies are looking for new products with little residues in order to comply with food safety standards, and Various industries are now looking to alternative sources for a number of reasons, including natural and environmentally friendly antimicrobials, antibiotics, antioxidants and Crop protection agents (1), the use of fungicides is more harmful in the post-harvest period because of the short time between treatment and consumption, However, these chemicals will be terminates in the near future due to their potential adverse impact on the environment. Some fungus have shown resistance against Fungicides, such as Benzimidazoles, Imazalil and Prochloraz due to repeated usage and some of them such as *Mucor* and *Rhizopus* are not sensitive and need especial fungicides to be controlled. (2).

*Foeniculum vulgare* Mill, commonly known as Fennel an aromatic and medicinal Plant, it is refer to family Umbelliferae (Apiaceae). It is an indigenous herb Mediterranean sea, is a very popular spice as well as an important traditional Chinese medicine. (3,4). Is an annual, biennial or perennial herbs (5). The *F.vulgare* is believed to be one the oldest medicinal plant, because it has been known from the time the first prophet, Adam (6). Fennel is chiefly known as culinary herb but it is a commonly used household remedy for various medicinal purposes (7,8). An analysis of fennel shows it to consist of moist 6.3%, protein 9.5%, fat 10 %, minerals 13.4%, fiber 18.5% and carbohydrates 42.3%. it is mineral and vitamin contents are calcium, phosphorous, iron, sodium, potassium, thiamine riboflavin, niacin, and vitamin C. (9). Recently, essential oils of the fruits of Egyptian fennel showed high antimicrobial and antioxidant activities, 18 major monoterpenoids in essential oils as trans-Anethole, estragole, fenchone and limonene were highly abundant in all examined oils. (10) It is a well-known medicinal plants within a broad stimulant, diuretic, carminative and sedative and galactagogic, emmenagogic, expectorant and antispasmodic (4). Essential oil of fennel has been reported to possess antifungal activity (11). Fennel essential oils could also be used as possible bio fungicides alternative to synthetic fungicides against phytopathogenic fungi as it has been reported to reduce the mycelial growth and germination of *Sclerotinia sclerotiorum* (12).

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Fennel crop suffer from many diseases causing by fungi pathogens, such as *Rhizoctonia solani*, *Pythium aphanidermatum*, *Cercospora sp.*, *Sclerotinia sclerotiorum*, *Alternaria alternata* and *Fusarium oxysporum* (13).

Our study aimed to evaluate the effectiveness of inhibitory alcoholic extract against fungi that infect the plant because of the available benefits to *Foeniculum vulgare* and its effectiveness.

### MATERIALS AND METHODS

- 1-Sampling: The samples of *Foeniculum vulgare* were collected from the Department of Drugs and Medicinal Plants, Pharmacy College - University of Baghdad, which showed symptoms of yellowing and wilting and death of the seedling, collected the samples of *F. vulgare* seeds harvested in the previous season and brought to the laboratory and kept in the refrigerator degree  $1 \pm 25\text{ }^{\circ}\text{C}$ .
- 2-Isolation: washed the plant samples collected which are leaves, stems and roots, with water, cut off to parts of a length of 2-5 mm, and sterilized with sodium hypochlorite 1.2% and then washed with sterilized distilled water and dried by sterilized filter papers and then transferred to the Potato Dextrose Ager plate and incubated in the incubator at  $1 \pm 25\text{ }^{\circ}\text{C}$  until the pathogen growth emergence.
- 3- Morphological Diagnosis: purified fungal isolates by transfer from the end of the isolated fungal culture by sterile needle the fungi mounted on slide, stained with lacto phenol-cotton blue and examined under microscope diagnosis based on morphological characteristics of the colonies and spores depending on taxonomic keys cited by (14,15) and preserved in Slants at  $4-5\text{ }^{\circ}\text{C}$ .
- 4-Estimation the disease incidence:: the survey have been done, in order to determine the infection percentage and follow from the first stages of the growing of the beginning of October until the harvest in June, *F. vulgare* was planted in two boards and the percentage of wilting infection and seedling damping - off calculated depending on the equation:  
The percentage of disease infection = No. of diseased plants / No. of all plants  $\times 100$
- 5-Assays the efficiency of *F. vulgare* alcoholic extract as antifungal:  
Adopted a *F. vulgare* seeds collected from the previous planting season after cleaned, washed and dried and milled by electric mill and preserved in a sterile polyethylene bags.

**Preparation alcoholic extract:** attended the alcoholic extract 5 gm of *F. vulgare* seeds powder was soaked in 50 ml of alcohol ethanol 95% overnight at room temperature and then the extract was centrifuged at (7000 rpm) for 20 minutes and the extract was filtered by using Whatman No 0.1 filter papers and the alcoholic extract of fennel seeds was stored at  $4\text{ }^{\circ}\text{C}$  till further use (16) To increase the required size of the alcoholic extract of the seeds of *F. vulgare* will be on the basis of (V/V) ratio of the same material.

**Testing the effectiveness of alcoholic extract:** alcoholic extract was added to the media PDA before solidification, three concentrations of extract were prepared, Zero, 2.5, 5%, shake the media before it pour (in order to homogenize) in a sterile Petri dish 9 cm in diameter and

leave at room temperature to next day, three replicates of each concentration were inoculated by placing 5 mm diameter discs of *Alternaria alternata*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Phoma herbarum* and incubated at temperature  $1 \pm 25\text{ C}^\circ$  until the mycelia growth appears and measuring the linear growth. The percentage inhibition of mycelia growth was calculated by using the formula (17):

$$\% \text{ inhibition of mycelia growth} = \frac{Dc. - Dt.}{Dc.} \times 100$$

Dc. = diameter of control, Dt. = diameter of test (treatment)

## RESULTS AND DISCUSSION

1-Isolation and morphological diagnosis: the fungi were purified, identify and diagnosis as *Alternaria alternata*, *Rhizoctonia solani*, *Phoma herbarum* and *Fusarium oxysporum*. The study (13) indicated that *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium moniliforme* are pathogens attack the fennel plant causing diseases such as Root rot, wilting and blight.

2-Estimation the fungal disease incidence:

It is clear in the Fig. 1. That the percentages of disease incidence in the months January, March and May (5,7,10%), respectively, the diseases more common in nurseries and be clear and severely when the condition is favorable, high moisture in soil and a little are exposed to sunlight.

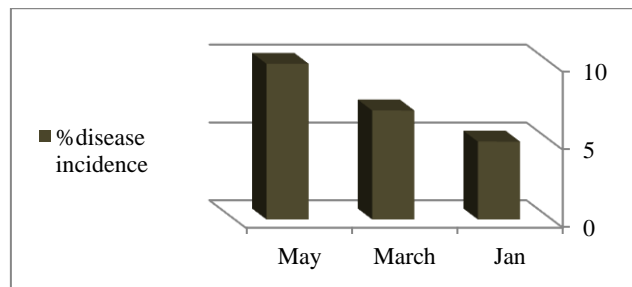


Figure (1): The Percentage of disease incidence of *Foeniculum vulgare*.

3-Testing the efficiency of *F.vulgare* alcoholic extract:

The antifungal activities of the tested plant extract (*Foeniculum vulgare*) were investigated at different concentrations Table (1) and Fig.2. The results clearly revealed that fennel extract could cause growth inhibition on the four tested fungi, although the rate of inhibition of tested fungi shows that two concentrations 2.5 and 5% were found to be inhibitory to mycelia growth and the rate of inhibition increased generally by increasing the concentration, and 5% concentration was the most effective on the mycelia growth of *Alternaria alternata*, *Rhizoctonia solani*, and *Phoma herbarum* and *Fusarium oxysporum*, were 1.31, 1.56, 1.13 and 1.65 cm, respectively Fig.1 compared to the treatment of control where the concentration was 0% the rates of fungal growth diameter was 2.4, 9, 2.5 and 2.63 cm, and shown the existence significant differences between mycelia growth rates in the dishes and between concentrations of alcoholic extract of *F. vulgare* and at  $P = 0.01$  and  $P = 0.05$ , the same results obtained from study (18) indicated the fennel (*F. vulgare*) seed is a potential source of natural antioxidant of the both water and ethanol seed extracts, and (19) showed that *Foeniculum vulgare* extract which was the one of the 49 medicinal plants extracts inhibited both fungal growth and production of AFs B1 and G1 producing of *Aspergillus parasiticus* and intentioned to use this plant as effective antimicrobial to protect foods and feeds from

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toxigenic fungus growth and subsequent (AF) Aflatoxin contamination. The researcher (20) the main constituents were E-anethol (92.9%), p-alylanisol (2.2%), Z- $\alpha$ -biosabolene (1.8%) for anise oil and E-anethol (71.2%), limonene (8.2%), Fenchone (8.53%), Methylchavicol (7.01%) for fennel oil and especial antifungal activities arise from interaction of components or complexity of the oils. The study (10) suggests that antioxidant activity is mostly related to the concentration of trans-anethole. The major component the volatile oil was trans-anethole (70.1%) and showed complete zone inhibition against *Aspergillus niger*, *Aspergillus flavus*, *Fusarium graminearum* and *Fusarium moniliforme* (21).

Aqueous and ethanol extract of *F.vulgare* were used showed significant inhibition of growth of *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* (22). The aqueous and alcoholic seed extract of *F. vulgare* were evaluated for their antifungal activity against *A. alternata*, *Mucor rouxii* and *Aspergillus flavus* (Neet et al. 2013). Concentrations of 10, 20 and 30% extracts of *F. vulgare* reduced the fungal biomass production in all tested pathogenic fungal species especially in initial growth stage (23).



Figure (2): The activity of *F.vulgare* alcoholic extract on the mycelia growth of *Alternaria alternata*, *Rhizoctonia solani*, *Phoma herbarum* and *Fusarium oxysporum* (from right to left), at the concentration 0, 2.5, 5 % ( from up to down).

Table (1): Effect of extract of *F. vulgare* plant on the linear growth (cm) of *Alternaria alternata*, *Rhizoctonia solani*, and *Phoma herbarum* and *Fusarium oxysporum*.

Fungi species	Ethanol extract concentrations		
	5%	2.5%	0%
1- <i>Alternaria alternata</i>	1.31	2	2.4*
2- <i>Rhizoctonia solani</i>	1.56	4.8	9
3- <i>Phoma herbarum</i>	1.13	1.81	2.5
4- <i>Fusarium oxysporum</i>	1.65	2.13	2.63

\* The three rate readings with significant differences at probability 0.05 and 0.01, and value of LSD fungi = 7.3, LSD conc. = 8.2 and LSD fungi x conc. = 5.5.

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Table (2): The percentage inhibition of mycelia growth by fennel seed extract of *Alternaria alternata*, *Rhizoctonia solani*, *Phoma herbarum* and *Fusarium oxysporum*.

Fungi species	inhibition of mycelia growth%	
	Extract conc.2.5%	Extract conc.5%
1- <i>Alternaria alternata</i>	14.06	45.30
2- <i>Rhizoctonia solani</i>	25.55	70.55
3- <i>Phoma herbarum</i>	13.33	46.66
4- <i>Fusarium oxysporum</i>	21.41	32.85

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### عزل وتشخيص الفطريات التي تصيب نبات *Foeniculum vulgare* Mill وتأثيره بذوره كمضاد فطري

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#### الخلاصة

شملت الدراسة عزل وتشخيص الفطريات التي تصيب نبات حبة الحلوة *Foeniculum vulgare* Mill، المزروعة في قسم العقاقير والنباتات الطبية، كلية الصيدلة - جامعة بغداد، وشوهدت عليها حالات من أعراض الاصفرار والذبول والتقزم، وتم تشخيص الفطريات:

*Fusarium oxysporum* و *Alternaria alternata*, *Rhizoctonia solani*, *Phoma herbarum* وتراوح بين ٠.٥-١٪. كما وتم دراسة تأثير المستخلص الكحولي لبذور نبات *Foeniculum vulgare* ضد *Alternaria*, *Rhizoctonia*, *Phoma* و *Fusarium*، حيث اختبرت التراكيز ٠.٥، ٢.٥ و ٥٪ من المستخلص الكحولي لبذور نبات حبة الحلوة وأظهر فعالية ملحوظة اتجاه جميع العزلات الفطرية من خلال ظهور فروقات معنوية ما بين المعاملات ما بين الفطريات والتراكيز عند احتمال  $P = 0.01$  و  $P = 0.05$ .