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Isolation and identification of fungi infected seeds of some medicinal plants

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Abstract

This investigation was designed to isolate the fungi which associated the seeds of some medicinal plants. A samples of the seeds safflower and fennel were collected from plant garden belonging to the Department of Drugs and Medicinal Plants, Pharmacy College, University of Baghdad, the division of medicinal plants and herbal spices for Industrial Crops, Ministry of Agriculture. Twelve different fungal genera were isolated and identified as *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Fusarium monilliforme, Mucor, Rhizopus, Penicillium, Chaetomium, Nigrospora, Phoma*. It was observed that *Fusarium oxysporum* showed the highest percent of frequency on the cultures of safflower seeds which was 30% and 15.52% on fennel seeds, while *Alternaria alternata* percent was 22.7% only found on fennel seeds. On the both species of *Aspergillus flavus, Aspergillus niger* the percents of frequency were 15.25, 17.25% on safflower, and were 15.69, 6.83 %on Fennel seeds, respectively. The results showed that the percentage of Seeds rot on safflower and fennel which treated with fungi *F. oxysporum, A. alternate, C. lunata* were (60, 30 and 10 %) (40, 40 and 20 %) respectively, and Seedlings damping off on safflower and fennel which treated with fungi *F. oxysporum, A. alternate, C. lunata* were (20, 10 and 10%) (30, 50 and 10%) respectively and was 0% for the control treatment.

Keywords: Safflower, Fennel, seeds, fungi, isolation.

Introduction

Safflower (*Carthamus tinctorius* L.) belongs to the family Asteraceae. Safflower has been grown mainly for orange-red dye (carthamin) extracted from its flower used in food coloring and flavoring. Safflower seed oil is highly rich in linoleic acid (unsaturated fatty acid) which makes it highly suitable for human consumption. The whole plant of *C. tinctorius* possesses many pharmacological activities like antifibrosis, antidiabetic, antitumor, anti-inflammatory, hepatoprotective, anti-hperlipidemic, anticoagulant, and antioxidant activities. Regardless of it numerous uses, this crop is under the category of minor and neglected crop, therefore additional research work is required for its commercialization (Sanskriti *et al.,* 2014). Many studies point to that fennel (*Foeniculum vulgare* Mill: Apiaceae) is of great importance and is used in the pharmaceutical, food, cosmetic and healthcare industries. Fennel is one of the oldest spice plants which widely grows in arid and semi-arid and due to its economic importance and pharmaceutical industry usage, it is one of the world's most dimension medicinal herb. This plant has anti-inflammatory, antispasmodic, antiseptic, carminative, diuretic and analgesic effect and is effective in gastrointestinal disorder treatment. Also with its anti-ulcer and anti-oxidant properties it is used to treat neurological disorders. Since a long time it has been used for medicinal purpose especially in China and India but it is yet proved to be a multi-purpose usage spice (Abe and Ohtani, 2013; Jamshidi *et al.,* 2012; Birdane *et al.,* 2007). Medicinal plants are a rich source of ingredients that can be used in the pharmaceutical industry and numerous benefits. And that the production of seedlings of medicinal plants free of diseases and pests are very important and must be oriented to the study of seeds and pathogens that could affect them and stand on the damage that may be caused to these plants. Researcher (Ghosal *et al.,* 1977) found that the seeds of safflower (*Carthamus tinctorius* Linn.), infected with fungus *Fusarium oxysporum* f. sp. *carthami*. Mentioned (Gayathri *et al.,* 2014) that the reason for weakness in the production of safflower is the lack of quality seeds at planting time and seeds infecting by a number of fungal diseases, As well as there are a lot of fungi infect the seeds of these plants as record by (University of California, 2000) that the pathogen

(*Septoria apiicola*) is spread in infected fennel (*Foeniculum vulgare* Mill.) seeds and caused Late Blight disease and also survive in plant refuse. Damping-off disease is caused by a soil borne fungus *Rhizoctonia solani* that attacks germinated seedlings that have not yet emerged or have just emerged. Fennel crop suffer from many diseases causing by fungi pathogens, such as *Rhizoctonia solani, Pythium aphanidermatum, Cercospera* sp., *Sclerotinia sclerotiorum, Alternaria alternate* and *Fusarium oxysporum* (Khare *et al.,* 2014). The objective of this study was to isolate and identify fungal species from field-collected samples of seeds of safflower and fennel.

Materials and Methods

1- Sampling: Seed samples of two plants fennel (*Foeniculum vulgare*) and safflower (*Carthamus tinctorius*) seeds were collected from the Department of Drugs and Medicinal Plants, Pharmacy College, University of Baghdad, seeds which were harvested in the previous season and brought to the laboratory and kept in bags and stored in the refrigerator.

2- Isolation and Diagnosis: The samples brought to the lab, washed and sterilized with a solution of sodium hypochlorite 1.5% for of 2-3min and washed with sterilized distilled water three times and dried by filter papers and transferred to PDA dishes as 5 seed of each dish and left in the incubator on the degree of 25±1°C and follow-up appearance of mycelia growth. The fungi associated with plant Safflower have been diagnosed to the species level depending on the morphological characteristics of developing colonies in dishes, conidia and conidiophores were diagnosed by using to the taxonomic basis approved and use taxonomic keys contained in (Barnett, 1965; Booth, 1971; Domsch *et al.,* 1980; Ellis, 1971) after purification, sub-culture and transfer from tip of the mycelium by sterile needle, estimated the density of fungi by calculate the percentage of frequency each fungi according to the following equation:

The percentage of frequency

$$= \frac{\text{The no.pieces colonized by the fungus} \times 100}{\text{Total no.cultured pieces}}$$

3 -Test the seed germination treated with isolated fungi: The germination rate fennel and safflower Seeds was estimated after contamination for the seeds by fungi: *Alternaria alternata, Fusarium oxysporum, Curvularia lunta*, seeds planting (20 seeds / dish) after one week from the date of contaminating soil fungi in ceramic dishes container 25cm diameter the soil is composed of (sand: peat moos) (1:2) sterile by Autoclave at a temperature of 121°C and under pressure 15 pounds/square inch, the each treatments of Fennel and Safflower seeds were prepared as follows:

1- The seeds planted in soil contaminated with the suspension solution of spores of *F. oxysporum* concentration of 1×10^5 spore/ml.

2- The seeds planted in soil contaminated with the suspension solution of spores of *C. lunata* a concentration of 1×10^5 spore/ml.

3- The seeds planted in soil contaminated with the fungus *A. alternata* by 1/2 dish of pure culture. Treatments left in the field until the seeds germinate and each treatment replicated three time with the use only sterile water in control treatment, the percentage of seed germination and seedling damping off was account after 10-30 days from seed germination according to the following equations:-

The percent of seed germination = $\frac{\text{the no.of grown seed}}{\text{total no.of seeds}} \times 100$

The percent of damping off = $\frac{\text{no.of dead seedlings}}{\text{no. of total seedlings}} \times 100$

Result and Discussion

1-Isolation and identification: A total 12 fungal species were isolated, *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Fusarium monilliforme, Mucor, Rhizopus , Penicillium, Chaetomium, Nigrospora, Phoma.* It was observed in Table (1) that *Fusarium oxysporum* showed the highest percent of frequency on the cultures of safflower seeds which was 30% and 15.52% on Fennel seeds. The *Alternaria alternata* percent was 22.7% only found on fennel seeds. On the both species of *Aspergillus flavus, Aspergillus niger* the percents of frequency were 15.25, 17.25 % on safflower and were 15.69, 6.83 %on Fennel seeds, respectively. The rest of fungi *Curvularia lunata*, *Chaetomium* sp. and *Rhizopus* sp. Their frequency percents on safflower seeds were highest than fennel seeds.Similar results have been recorded by (Sumanth and Waghmare, 2010), on seeds of Indian spices were *Aspergillus niger* and *Aspergillus flavus* the most dominant fungi, and (Raghuwanshi and Deokar, 2002) refers that the *Aspergillus, Alternaria*, *Fusarium* and *Rhizopus* were predominant fungi and the intensity of the *Penicillium* and *Curvularia* was low.

Table (1): The percentage of frequency of fungi isolated from seeds of Fennel and Safflower.

Fungi	The percentage of frequency of fungi on Safflower	The percentage of frequency of fungi on Fennel
1-*Alternaria alternata*	-	22.7
2-*Aspergillus flavus*	15.25	15.69
3-*Aspergillus niger*	17.25	6.83
4-*Curvularia lunata*	10	8.62
5-*Fusarium monilliforme*	10	19.25
6-*Fusarium oxysporum*	30	15.52
7-*Mucor*	20	5.83
8-*Rhizopus*	13	9.75
9-*Penicillium*	20	3.70
10-*Chaetomium*	10.5	6.5
11-*Nigrospora*	-	3.00
12-*Phoma*	-	4.25

Table (2) indicates the difference in the presence of fungi isolated from the seed fungi that have been isolated from the seeds of both plants safflower and Fennel are: *Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium monilliforme, Fusarium oxysporum, Mucor* sp. The fungi that were previously only found in isolates of safflower seeds were *Alternaria alternate, Rhizopus stolonifer, Penicillium notatum, Nigrospora* sp. and the fungus *Chaetomium* sp. and *Phoma* sp. isolated from fennel only.

Table (2): the occurrence of fungi isolated from the seeds of Safflower and Fennel.

No.	Fungi	Safflower Seeds Isolates	Fennel Seeds Isolates
1	*Alternaria alternata*	+	–
2	*Aspergillus flavus*	+	+
3	*Aspergillus niger*	+	+
4	*Curvularia lunata*	+	+
5	*Fusarium monilliforme*	+	+
6	*Fusarium oxysporum*	+	+
7	*Mucor* sp.	+	+
8	*Rhizopus stolonifer*	+	–
9	*Penicillium notatum*	+	–
10	*Chaetomium* sp.	–	+
11	*Nigrospora* sp.	+	–
12	*Phoma* sp.	–	+

+ Positive, – negative.

3-Seeds germination treated with isolated fungi: The results showed that the percentage of Seeds rot before emergence and Seedlings damping off after emergence treated with fungi on Safflower and Fennel *F. oxysporum, A. alternate, C. lunata,* were (60, 30 and 10%) (40, 40 and 20%) and (20, 10 and 10%) (30, 50 and 10%) respectively and 0 % for the control treatment, (Table 3). Because *F. oxysporum* and *A. alternate* of soil fungi and they are the reason for the phenomenon of lack of seed germination and seedling death, *C. lunata* has little effect in reducing the rate of seed germination. The percentage of seedling damping off for fungi *C. lunata, A. alternate, F. oxysporum* was 10, 20 and 60%. The researchers (Chacko and Mohanan, 2002; Mohanan *et al.,* 2005) indicated to the fact that the infected seeds and vectors of pathogenic fungi may causes a reduction in the percentage of seed germination especially if they are not properly stored, and not collected in a timely manner and proper drying and good storage is necessary to protect the seeds from rotting and damage, If the soil is too moist, the root and crown will attacking by fungi diseases and can be a problem.

Table (3): Percentages of Seeds rot before emergence and Seedlings damping off after emergence of Fennel and Safflower seeds.

Treatments	Seeds rot before emergence%		Seedlings damping off after emergence %		Control	
	Safflower	Fennel	Safflower	Fennel	Safflower	Fennel
F. oxysporum	60	40	20	30	0	0
A. Aalternata	30	40	10	50	0	0
C. lunat	10	20	10	10	0	0

The study (Rajeswari *et al.,* 2012) pointed to mycoflora associated with safflower seed samples and they reported the occurrence of *Alternaria carthami, Alternaria alternata, Macrophomina phaseolina, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger, Curvularia lunata* and *Rhizopus* sp. *Alternaria carthami* was recorded to be (Prasad *et al.,* 2009) seed borne nature in safflower by using component plating technique and Maximum infection of *Alternaria carthami* was occurred on seed coat (76.6%) followed by endosperm (38.3%) and embryo (20.4%).

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