

Laboratory Study of the Effect of Methanolic and Water Extracts of Aromatic Plant Seeds in the Growth of the Two Pathogenic Fungi *Fusarium oxysporum* and *Aspergillus niger*

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Abstract

A Study was carried out to evaluate the ability of the methanolic and water extractions of anise seeds (*Pimpinella anisum* L.) to inhibit the growth of the pathogenic fungi *Fusarium oxysporum* and *Aspergillus niger*. The results showed a good inhibition ability of the methanolic and water extractions on the growth of the studied fungi. The inhibition ability differed due to the used concentrations. When the methanolic extraction was used by concentrations (2, 4, and 6) mg/ml at the seventh day, the inhibition ability was (42.5, 50.6 and 64.4)% of the fungus *F. oxysporum*, respectively. And was (23.6, 42.7 and 55.1)% of the fungus *A. niger*, respectively. Whereas when the water extraction was used by the same concentrations, the inhibition ability was (59.2, 65.3 and 70)% respectively for the fungus *F. oxysporum*, and (46.1, 58 and 61)% for the fungus *A. niger*, respectively.

Keywords: *Pimpinella anisum*, *Fusarium oxysporum*, *Aspergillus niger*, Methanolic extraction, and water extraction.

I. INTRODUCTION

The officinal plants seem to be an important source of active biotic compounds that have medicinal value to many diseases [12], the officinal plants were used since ancient ages as protecting substances for alimental materiality because of their characteristics against pollution in addition to being antiseptic [11]. Anise plant *Pimpinella anisum* L. was one of the important officinal plants belong to Apiaceae, spreading in Mideterean region, including substances can be used for their medicinal characteristics [18]. It is useful as antifungal, antibacterial and antiviral compound.

Seeds of anise plant was used in Pharmacia and alimental industries and industry of beautification substances. This plant aggravate attention of many consumptives because of their effective as antioxidation [16].

The anise plant is used as essential stimulant, and against bowel swelling, and is considered as the importance aromatic plants. It is annual locally herb, from regions of the middle east like Iran, Turkey, India, Egypt and some other hot

countries in the world. Anise plant prefers warm climatic conditions during growth season, and planes especially in semi equatorial countries. To success its planting, temperatures would be ranged from 8-23°C, with rainfall between 1000- 1300mm [19]. The top height for this plant reach to 30- 70 cm. Their flowers are very small and white. leaves in the basic portion are simple and their tall ranged between 1.3- 5.1 cm, whereas leaves in the apex portion are complex divided to sever leaves. Fruit look like as egg or pear. Seeds are very small and curved their tall about 0.5cm, gray to brown [15].

Cells of seed include the oil, aloron and crystals of calcium oxalates [2]. Oil percentage in the seed was ranged between 1.3 – 3.7 % [1]. The basic compounds to the oil that extracted from anise seeds are trans- a estragole (2.4%), nethole (93.9%), and the compounds that founded with amount higher of 0.06% are (E)- methyeugenol, α - cuparene, α - himachalene, β - bisabolene, p- anisaldehyde and cis-anethole ([17]; [1]).

The genus *Fusarium* is one of the important fungi that caused the vascular wilt for many plants and economically crops. it is wideworld especially in fields [8]. *Fusarium* is facultative parasite fungus, live as saprophyting or pathogenic on plant tissues, have a wide range of hostes [21]. *F. oxysporum* is the responsible of most of *Fusarium* diseases [7].

Also, *Aspergillus niger* is found with high frequency in the soils, and considered as common opportunist saprophytic fungi that have important role in the environmental and economic system of human, and some kinds of it cause disease for human or animal or plants, and caused big economic loser [6].

Because of the medical importance of anise plant and including of many active compounds that inhibit the pathogenic fungi, this research was done to evaluate the in vitro inhibition ability of extractions of seeds of anise plant against *F. oxysporum* and *Aspergillus niger*.

II MATERIALS AND METHODS

The search was performed in Tishreen University, Syria, during 2016.

A. The Fungi Isolates

We obtained *F. oxysporum* from plants showed the symptoms of infection of vascular wilt (yellowing and general wilt), and identifying the species with keys of classification of ([10]; [4]). Isolates cultured on PDA medium, on 26±2°C for 7 days. Then saved on 4°C until use.

The fungus *A. niger* was isolated from onion plants that showed growth of the fungus [9] and identifying depending on [3].

B. Preparation of Extractions of Anise Seeds

1) Methanolic Extract:

[14] method was followed, where 20g of seeds were grinded, and the flour was added in Soxhlet extractor, then 200ml of Methanol added to it on 40°C for 24 hours. Filtering was done with filtering paper Whatman No. 1. The process was repeated several times to get enough amount of extract. The extract finally was dried by rotary evaporator on temperature does not over 50°C to get the extract in dry form. It saved on refrigerator on 4 °C in dark closer container until use.

2) Water Extract

100g of anise seeds were added in electric mixer, and 1L of cold distiller water was added for 15 minutes. The mixer was moved by hot plate magnetic stirrer (45- 50 °C) for 48 hours discontinuously. The mixer then was filtered by filtering papers. The extract was added in centrifuge with fast 3000 cycle / minute, the extract we obtained from this sept was exposition to centrifugation three times to remove settlings. final extract then filtered using filtering papers Whatman No. 1. Then added in aqueous bath (60°C). then saved on 4°C until use.

C. Effect of methanolic and water extracts of anise seeds on growth of the fungi *F. oxysporum* and *A. niger*:

The concentrations 2, 4 and 6 mlg/ ml of each both of methanolic and water extracts were prepared in glasses flasks containing PDA before

hardening. PDA was infused in Petri dishes after adding the concentrations by 4 replicates of each concentration/ extract. control treatment was prepared as above without adding extract. center of each dish was inoculated by 1 cm piece taking from edges of 7 days old colony of *F. oxysporum*. dishes were incubated on 25±2°C for 7 days. surface radial growth was recorded in the third, fifth and seventh days after incubation using two perpendicular diameters previously drawn at the bottom of each Petri dish. percentage of inhibition was calculated using the following counteraction:

Inhibition Rate %= {(mean of diameter of control colony – mean of diameter of treatment colony)/ mean of diameter of control colony}. the experiment was repeated with the fungus *A. niger* as done above.

D. Statistic Analysis

Experiments were performed using completely random, the differences between treatments were compared by One-way repeated analysis of variance (ANOVA). LSD were performed post comparisons. The level of probability was set at P<0.05.

III RESULTS

A. Effect of Methanolic Extract of Anise Seeds on Growth of the Fungi *F. oxysporum* and *A. niger*:

The results showed clear effect of methanolic extract with the used concentrations on growth of the studied fungi, with superiority of 4 and 6 mlg/ ml. It was noticeable existence direct relation between inhibition rates and increasing the concentration. Also same relation was found between inhibition rate and time (Table 1, 2). A diameter of colony of the fungus *F. oxysporum* was decreased in the third day of experiment from 3 cm in control treatment to 2.5, 2.0 and 1.6 cm for concentrations 2, 4 and 6 mlg/ml, respectively. Significant differences between treatments were increased by time. In the seventh day, the fungal growth in control treatment was 8.7cm, where in extract treatments was 5, 4.3 and 3.1 cm for concentrations 2, 4 and 6 mlg / ml, respectively, and was 6.8, 5.1 and 4 cm for *A. niger* compared with control (8.9)cm.

Table 1: The Effect of Methanolic Extract of Anise Seeds by Concentrations (2, 4 And 6) Mlg/ Ml on Growth of Fungus *F. Oxysporum* (Cm) on PDA After 3, 5 And 7 Days Post Incubation

| Time (day) | Concentrations (mlg/ml) | | | control | LSD (p<0.05) |
|------------|-------------------------|-------|------|---------|--------------|
| | 2 | 4 | 6 | | |
| 3 | 2.5bc* | 2.0ab | 1.6a | 3c | 0.5 |
| 5 | 3.8c | 2.9b | 2.2a | 6e | |
| 7 | 5b | 4.3b | 3.1a | 8.7c | |

*Means followed by different uppercase letters in a line (isolate) indicate significant differences

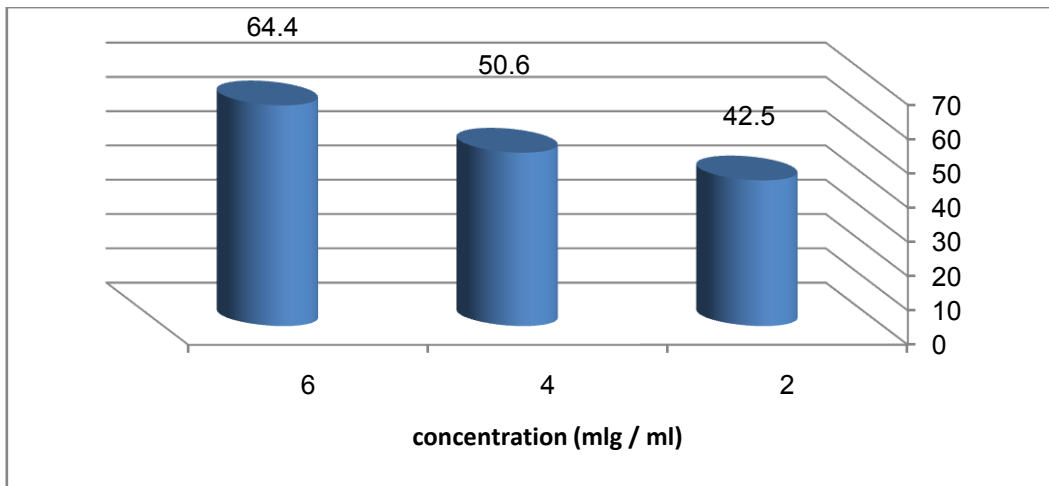


Fig 1. Inhibition Rate % of the Fungus *F. oxysporum* When Usage of Methanolic Extract of Anise Seeds After 7 Days of Incubation

Table 2: The Effect of Methanolic Extract of Anise Seeds by Concentrations (2, 4 And 6) Mg/ Ml on Growth of Fungus *A. Niger* (Cm) on PDA After 3, 5 And 7 Days Post Incubation

| Time (day) | Concentrations (mg/ml) | | | control | LSD (p<0.05) |
|------------|------------------------|-------|------|---------|--------------|
| | 2 | 4 | 6 | | |
| 3 | 3.0ab | 2.1a | 2.0a | 3.4b | 1.02 |
| 5 | 4.5b | 3.8ab | 3.0a | 6c | |
| 7 | 6.8c | 5.1b | 4a | 8.9e | |

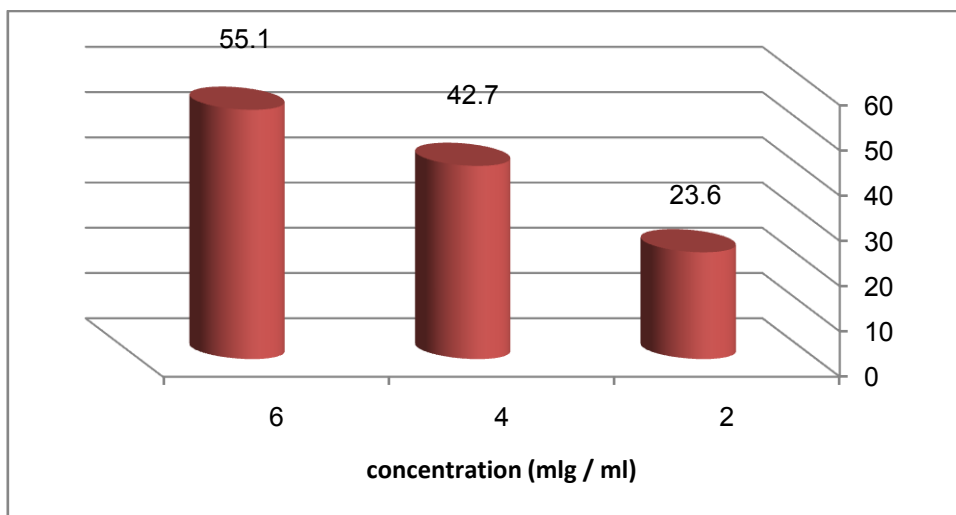


Fig 2. Inhibition Rate % of the Fungus *A. niger* when Usage of Methanolic Extract of Anise Seeds After 7 Days of Incubation

Inhibition rates in the seventh day by concentration 6 mg / ml were 64.4 and 55.1% for *F. oxysporum* and *A. niger*, respectively. Whereas 50.6 and 42.7 % by 4 mg /ml.

B. Effect of Water Extract of Anise Seeds on Growth of the Fungi *F. oxysporum* and *A. niger*:

The results showed effect of the water extract by used concentrations on inhibition the growth of the two fungi, it was notable that the concentration 6 mg / ml was the highest effective (Table 3, 4).

Table 3: Inhibition Rate % of Water Extract of Anise Seeds by Concentrations 2, 4 and 6 mlg/ml on Growth of the Fungus *A.niger*.

| Time (day) | Concentrations (mlg/ml) | | | control | LSD (p<0.05) |
|------------|-------------------------|------|-------|---------|-----------------|
| | 2 | 4 | 6 | | |
| 3 | 18.5b | 23ab | 25a | 0c | 5.2 |
| 5 | 38b | 40b | 50.4a | 0c | |
| 7 | 46.1c | 58ab | 61a | 0e | |

Table 4: Inhibition Rate % of Water Extract of Anise Seeds by Concentrations 2, 4 and 6 mlg/ml on Growth of the Fungus *F.oxysporum*.

| Time (day) | Concentrations (mlg/ml) | | | control | LSD (p<0.05) |
|------------|-------------------------|-------|-------|---------|-----------------|
| | 2 | 4 | 6 | | |
| 3 | 14b | 25.4a | 27.1a | 0c | 5.04 |
| 5 | 44b | 50a | 55a | 0c | |
| 7 | 59.2c | 65.3b | 70b | 0e | |

IV DISCUSSION

The results showed high capability of extracts of anise seeds of inhibition the growth of pathogenic fungi *F.oxysporum* and *A.niger*. These capability fit direct with time and increasing the used concentration.

[13] demonstrated that water extract of anise fruit and its oils have an inhibition ability against four kinds of ferment (*Candida albicans*, *C.parapsilosis*, *C. tropicalis* and *C. pseudotropicali*) and four kinds of dermal fungi (*Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis* and *M. gypseum*).

Also, reference [20] reported that methanolic extract of anise seeds showed high ability of inhibition the fungus *A.niger*.

The present results agree with research of [5] who explain that the efficacy of extracts due to existence of some compounds in the extracts, for example Anise oil that found in seeds consist of anothol about 85% that considered the active element in oil of seeds, also the oil include another chemical compounds like eugenol, methylchavicol, estragol and anisaldehyde.

Due to the encouraging results of this study we advance to evaluate the inhibition capability of extracts of anise seeds against another pathogenic fungi, wherein usage this extracts considered promising in plant pathogenic fungi control associated with decreasing chemical use and exchanged it by biotic origin substances.

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