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Antifungal Effects of Anise (*Pimpinella anisum* L.) Essential Oil Against Some Storage Fungi *In Vitro*.

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Abstract : This study was conducted in 2016 at Dep. of Field Crops and Dep. of Plant Protection in Faculty of Agriculture, Damascus University. This study aimed to determine the antifungal activity of anise (*Pimpinella anisum* L.) essential oil at different concentrations against storage fungi (*Aspergillus niger, Penicillium digitatum* and *Fusarium solani*) *in vitro*. The result showed that yield of anise oil province was 3.8% v/w base on dry weight of ripe fruits of anise. The results showed that essential oil from anise oil at 300, and 500 µ/100ml of anise oil showed complete inhibition (100%) of mycelia growth of *A. niger* and *F. solani* on the artificial media, respectively**.** The median effective concentration (EC_{50}) values for inhibition of radial mycelia growth of *A. niger ,F. solani, and P. digitatum.* on PDA medium, were 67.58, 148.25 and 175.50 µl/ 100ml of anise oil. respectively. This indicate that *A. niger, F. solani*, were highly sensitive to anise oil. Therefore we recommended to use the oil of the ripen fruits of anise as environment friendly fungicides

Key word : Anise, Essential oil, Fungi, EC_{50}.

Introduction:

Several postharvest fungi such as *Fusarium solani, Botrytis cinerea, Penicillium digitatum, Aspergillus niger, Botrytis cinerea, Penicillium expansum* and *Penicillium italicum* are major infectious agents of fruits and vegetables especially in the postharvest stage[1]. Postharvest fungal attack of fruit often leads to problems relating to diminished fruit quality, nutritional value, deterioration of organoleptic characteristics and reduced shelf life[2,3,4]. Pathogenic fungi are controlled primarily through the use of synthetic fungicides; however, restrictions are being placed on the use of chemicals due to the perceived negative effects that pesticides may have on human health and the environment[5,6]. The increasing regulations on the use of synthetic fungicides, and the emergence of pathogen resistance to the most valuable fungicides being used, validate the search for novel control strategies[7,8,9]. In recent years, a number of plant extracts, their essential oils and their volatile components have been reported to have strong antifungal activity[10,11,12,13]. In general, antimicrobial activity of plant extracts have been well documented and in more recent times, studies on the effects of plant extracts and essential oils on plant pathogens have also received attention[14,15]. The antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world. For example, a large proportion of the South American population use extracts obtained from medicinal plants as remedies for many infectious diseases[9]. In the agricultural sector, plant extracts, essential oils and their components are gaining increasing interest due to their volatility, reasonably safe status, their eco-friendly and biodegradable properties and wide consumer acceptance[16,17].

Anise (*Pimpinella anisum* L.) a plant belonging to the Apiaceae (Umbelliferae) family. The plant is indigenous to Near East and widely cultivated in Mediterranean rim (Turkey, Egypt, Syria, Spain, Mexico, Chile, etc.). It has been used as an aromatic herb and spice since Egyptian times and antiquity and has been cultivated throughout Europe[18,19]. *P. anisum* is primarily grown for its fruits (aniseeds) that harvested in August and September. Aniseeds contain 1.5–5% essential oil and used as flavoring, digestive, carminative, and relief of gastrointestinal spasms[20]. Consumption of aniseed in lactating women increases milkand also reliefs their infants from gastrointestinal problems [21]. Study of the essential oil of *Pimpinella anisum* L. fruits by GC and GC-MS showed the presence of *trans*-anethole (93.9%) and estragole (2.4%). Other compounds that were found with concentration higher than 0.06% were (E)-methyleugenol, *α*-cuparene, *α*- himachalene, *β*-bisabolene, *p*-anisaldehyde, and *cis*-anethole [22]. Aniseed extracts and oil as well as some oil components, exhibited *in vitro* strong inhibitory activities against the growth of a wide spectrum of bacteria and fungi known to be pathogenic for man and other species[23,24]. An acetone extract of aniseed inhibited the growth of a range of bacteria including *Escherichia coli* and *Staphylococcus aureus*, and also exhibited antifungal activity against *Candida albicans* and other organisms[25]. The essential oils of aniseed and other aromatic plants showed a toxic activity against several soil-borne plant disease-causing fungi including *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Phytophtora capsici*; this activity was attributed to the phenolic fraction of the essential oils[26]. The essential oils of anise (500 ppm), fennel (2000 ppm) and other herbals showed a dose-dependent inhibitory effect on the growth of tested fungi including *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliformis* [27,28,29].

The aim of this study:

The aim of work was to define the effect of essential oil of anise fruits on fungi (*Fusarium solani*, *Penicillium digitatum* and *Aspergillus niger*) *in vitro*.

Materials and Methods:

Research was done at the Dept. Field Crops and Dept. Plant Protection, in Faculty of Agriculture, Damascus University, during 2016. The following fungi: *Fusarium solani*., *Penicillium digitatum* and *Aspergillus niger* were tested.

Plant material and isolation of essential oil:

The ripen fruits of anise (*P. anisum* L.) were collected during 2016 from Homs filed, Syria. Fruits were cleaned and air-dried in shade at ambient temperature. The dry fruits were blended by Electrical mill blender. The oils were taken from 100 g of the powders in hydro distillation method with the help of Clevenger set for three hours[23,30] Following the sample oils were dried with anhydrous sodium sulfate. The oils yields were calculated on a dried weight basis (V/W). The oil extracts were stored in sterile dark vials at+4°C for future uses.

Pathogen isolates:

Three fungi *Fusarium solani*, *Aspergillus niger* and *Penicillium digitatum*, were provided from the collection of the Department of Plant Protection, Faculty of Agriculture, Damascus University. The fungi were identified on the basis of morphological characteristics, viz. formation on media, mycelia and types of conidial characteristics.

Effect of anise (*P. anisum*) oil on mycelia growth:

Antifungal activity of oil extract of anise was tested by Poison Food Technique [31]. The oil was diluted in 2 ml of 95% ethanol and were then mixed and homogenized by Vortex Mixer with 100 mL of culture medium potato dextrose Agar (PDA), after autoclaving to achieve final concentrations of 0, 25, 50, 100,150 ,200, 250,300,400, and 500 µ/100ml , and addition 0.04% Tween 20 as an emulsifying agent. Control growth medium contained equivalent amounts of 95% ethanol and Tween 20. The amended medium was poured into sterilized petri dishes (9 mm) and allowed to gel. After solidification, each plate was inoculated with 5-mm disk of mycelia from the growing edge of a 7-day old colony. Inoculated plates were incubated at 25 °C with 12-h light. Radial mycelia growth was measured. Therefore, to compare the effect of the oils on the growth of various fungi mycelium the inhibitory activeness (%) was calculated using the formula[32]:

R = (Do - D) / Do × 100
R – inhibitory activeness (%).
Do – diameter of a control colony (cm).
D – diameter of a tested colony (cm).

Three replicates were done for each of the three pathogens and for each concentration tested and the experiment was repeated once.

The EC_{50} value for each fungus, which was defined as the concentration of anise oil causing 50 % inhibition of mycelia growth, was determined according to[33].

Statistical analysis:

The experiment was conducted using a completely randomized design. The results were processed by Gen.Stat 12 statistics program (2009). A *p*-value <0.01 was considered statistically significant.

Results and Discussion

Effect of anise (*P. anisum*) oil on mycelia growth of the tested fungi:

The essential oil isolated by water distillation were obtained in yield 3.8% v/w base on dry weight of sample for ripe fruits of anise. Our results agreement with many studies were reported on the yield of the essential oil from anise fruits, varies between 1.5% and 5% [20,34].

Results in table 1 showed that anise (*P. anisum*) oil was significantly inhibitory to all three pathogens at all the respective concentrations, compared to their controls. The highest mycelia inhibition was recorded for *A. niger* with a concentration of 300µl / 100 ml of anise oil gave a completely inhibition (100%) of the pathogen (Table 1 and fig. 1). While, the 500 µl/ 100ml of anise oil showed complete inhibition (100%) of mycelia growth of *F. solani* on the artificial media. In contrast, the anise oil didn't have completely inhibition of mycelia growth of *P. digitatum* at the maximum concentration (500µl/ 100ml). Overall, percentage of growth inhibition was significantly ($P < 0.01$) influenced by essential oil concentration. The rate of growth was influenced by concentration of the oil, as indicated by the decrease in colony diameter with increasing extract concentration (Table 1 and Fig. 1). Furthermore, data analysis showed the differences between fungi are significant ($p< 0.01$).

Reviewing the above-mentioned results, anise oil (*P. anisum*) had a significant effect on the growth of the pathogens tested. This finding agrees with earlier reports on the antifungal properties of anise oil [26,27]. The effect of anise oil on postharvest fungi was determined by direct exposure of pathogens to oil . In the case of *A. niger*, an 300µl/ 100ml of anise oil gave stronger activity than the other tested fungi (Table 1 and fig. 1). However, all studies do support the fact that anise oil do result in antimicrobial and fungi activity [23, 28]. However, the present study revealed that complete (100%) mycelia inhibition of *F. solani* could be achieved by using a 500 µl/ 100ml anise oil (Table 1). In recent years, a number of studies have been carried out concerning the application of essential oils as microbial agents [11,13,35]. Most of the information published on this subject has reported on the antifungal activity of essential oils and plant extracts exposed directly to fungus (as per method described above using amended media). The antifungal activity of anise oil due to contains of many compounds, such as anethole , γ-himachalene, estragole, β-bisabolene and phenolic compounds[21,22,26].

[36]An investigation into the effectiveness of anise oil for the control of pathogenic bacteria and fungi has demonstrated that anethole is a major component of essential oils derived from anise. Where, the anethole compound induced growth inhibition and morphological changes in filamentous fungi (*Mucor* and *Fusarium* sp.) via metabolic blockade of cell wall biosynthesis based on the uncompetitive inhibition of chitin synthase [37].

Table 1. Inhibition percent of mycelia growth of the tested fungi by different concentrations of anise (*P. anisum*) oil in vitro.

Concen. µ /100ml	*A. niger*	*F. solani*	*P.digitatum*
	% Inhibition		
0	0^{x}	0^{x}	0^{x}
25	25.50^{t}	17.50^{v}	10.75^{w}
50	43.87^{p}	29.32^{s}	20.26^{u}
100	67.89^{k}	38.89^{r}	29.25^{s}
150	78.56^{i}	50.25^{o}	41.58^{q}
200	89.25^{e}	66.89^{m}	59.56^{n}
250	98.39^{b}	75.89^{j}	67.58^{l}
300	100^{a}	86.23^{f}	78.96^{h}
400	100^{a}	93.25^{d}	84.69^{g}
500	100^{a}	100^{a}	95.23^{c}
L.S.D $_{(0.01)}$	between the Concen.	between the fungi	interaction
	1.52	**1.29**	**2.16**

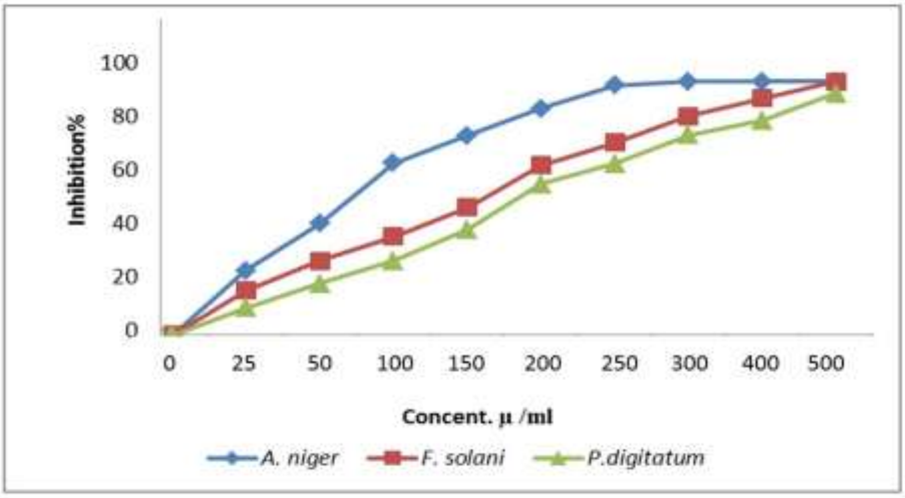

Fig. 1: Effect of different concentrations of anise (*P. anisum*) oil on inhibition percent of mycelia growth of tested fungi in vitro.

Values of EC_{50} of the tested fungi by anise (*P. anisum*) oil:

The effective concentration at which 50% pathogen inhibition (EC_{50}) resulted from the use of anise oil was calculated in table 2. The EC_{50} values for inhibition of radial mycelia growth of *A. niger* , *F. solani* and *P. digitatum* on PDA medium, were 67.58, 148.25 and > 175.50 µl/ 100ml of anise oil. This indicate that *A. niger* and *F. solani* were highly sensitive to anise oil.

Table 2. Values of EC_{50} of the tested fungi by anise (*P. anisum*) oil in vitro.

fungi	*A. niger*	*F. solani*	*P.digitatum*
	Values of EC_{50} (µ /100ml)		
Oil extract	67.58	148.25	175.50

Conclusions:

1. Fungi of different species have different response to the concentrations of anise oil.

2. The strength of the effect of oil depended on the concentrations: the greater the concentration the stronger was the inhibitory effect.
3. Essential oil of anise had the fungicidal effect on all the tested fungi, which the most strongly affected on *A. niger* and *F. solani* then *P. digitatum.*

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