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# Antifungal Activities of the Essential Oils on Post-harvest Disease Agent *Penicillium digitatum*

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**Abstract:** Antifungal activities of the essential oils obtained from oregano (*Origanum syriacum* var. *bevanii*), fennel (*Foeniculum vulgare*), Artemisia (*Artemisia annua*), laurel (*Laurus nobilis*) and lavender (*Lavandula stoechas* subsp. *stoechas*), growing in the Eastern Mediterranean Region of Turkey, were investigated against *Penicillium digitatum,* causal agent of green mould rot of citrus. Different concentrations of the essential oils on conidial germination and germ tube elongation were determined *in vitro*. The results indicate that essential oils of *O. syriacum* and *F. vulgare* were strongly inhibitory to conidial germination of *P. digitatum* compare with other essential oils used. Complete inhibition of conidia by essential oils of *Origanum* and *Foeniculum* was observed at concentrations of 64 and 352 μg $mL^{-1}$, respectively. Essential oils of *Lavandula*, *Artemisa* and *Laurus*, however, failed to inhibit conidial germination completely at all concentrations used (32 to 352 μg $mL^{-1}$). All essential oils were found to inhibit the germ tube elongation in a dose-dependent manner. Similarly, *Origanum* and *Foeniculum* oils, at 64 and 352 μg $mL^{-1}$ concentrations, were found to inhibit the germ tube elongation completely. Essential oils of *Lavandula, Laurus* and *Artemisia*, at the highest concentration used (352 μg $mL^{-1}$), reduced the germ tube elongation by 69, 58 and 28%, respectively. Microscopical observations revealed that both *Origanum* and *Foeniculum* oils significantly altered the morphology of *P. digitatum* hyphae.

**Key words:** Antifungal activity, essential oil, post harvest disease, *Penicillium*

## INTRODUCTION

Economic losses caused by post harvest pathogens are greater than is often realized and the avoidable losses between the farm gate and the consumer are big concern. Green mould decay caused by *Penicillium digitatum* accounts for most of the post harvest losses of economically important vegetable and fruits including citrus[1]. In many countries including Turkey, these diseases are primarily controlled by the extensive use of fungicides, such as ortho-phenyl phenate, imazalil and thiabendazole as pre- or post harvest treatments[2]. However consumer demands for pesticide-free organic food and the development of pathogenic strains that are resistant to currently used fungicides and ineffectiveness of such pesticides necessitates the development of environmentally friendly alternative methods for post harvest diseases[3]. During last few years, considerable research efforts have been developed to identify effective alternative methods for controlling diseases of fruit, vegetable and crop plants. Alternative to synthetic chemicals that are of potential use in post harvest disease control include antagonistic microorganism (Biological Control Agent, BCA) such as yeast and bacteria, natural plant- and animal-derived products with fungicidal properties and induced natural resistance of plants. The use of biologically based compounds, such as essential oil obtained from medicinal plants, was suggested as a feasible approach for reducing post harvest diseases in harvested fruits and vegetable[4]. The use of such alternative method for controlling post harvest diseases will help to combat fungicide resistant strains of pathogens and to avoid pesticides residues from the environment and commodities thus minimizing effects on non target microorganisms. Essential oils of certain botanical herbs possess antimicrobial activity and have been demonstrated to control post harvest decays of fruits efficiently[5-10].

The objective of this study was to evaluate the *in vitro* antifungal activities of essential oils derived from

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oregano (*Origanum syriacum* L. var. *bevanii*), fennel (*Foeniculum vulgare*), Artemisia (*Artemisia annua*), laurel (*Laurus nobilis*) and lavender (*Lavandula stoechas* L. subsp. *stoechas*) against green mould rot of citrus, *P. digitatum*. The effects of essential oils on the morphology of fungal hypha were also determined.

## MATERIALS AND METHODS

**Plant material and isolation of essential oils:** The essential oils tested were extracted by water stream distillation using a Clevenger apparatus from the whole flowering parts of oregano (*O. syriacum* var. *bevanii*), fennel (*F. vulgare*), Artemisia (*A. annua*), laurel (*L. nobilis*) and lavender (*L. stoechas* subsp. *stoechas*). After extraction, the essential oils were dried over anhydrous sodium sulphate and were stored in a refrigerator at 5°C.

**Test microorganism:** The fungal isolate used in the study was isolated from infected citrus fruit and maintained on Potato Dextrose Agar (PDA, Merck). The culture was stored at 4°C and sub-cultured once a month. Spore suspension was prepared from 2-week-old PDA culture. The spores were removed from the surface of the culture, suspended in 5 mL of sterile distilled water containing 0.05% (v/v) Tween 80 and filtered through sterile steel filter with 50 μm mesh. Spore concentration was determined using a haemocytometer and adjusted to $5x10^4$ spores $mL^{-1}$.

**Determination of antifungal activities of the essential oils:** Essential oils obtained from the oregano, fennel, Artemisia, laurel and lavender were used for assessing its contact effects towards spore germination and germ tube elongation. PDA was autoclaved and cooled in a water bath to 40°C. Stock solutions of essential oils were filter sterilized through a 45 μm Millipore filter and added to sterilised water containing 0.05% (v/v) Tween 80. The oil prepared as above was subsequently mixed with sterile molten PDA to obtain final concentrations of 32 to 352 μg $mL^{-1}$. The PDA was poured into petri dishes (≈20 mL/plate), which were then seeded with an aliquot of 200 μL of *P. digitatum* conidial suspension at the concentration of $10^4$ spores $mL^{-1}$. All inoculated Petri dishes were incubated for 24 h, at 25°C. Approximately 200 spores of *P. digitatum* were evaluated for germination rate and germ tube length per treatment within each replicate using microscope eyepiece graticule. Each treatment was replicated three times and the experiment was repeated twice.

The growth values were obtained and then converted in to the inhibition percentage of spore germination and germ tube elongation in relation to the control treatment. SPSS statistic program was performed for all calculations and the significance was determined by means of Duncan's Multiple Range Test ($p<0.01$).

**Light microscopical observations:** An agar block (10 mm) of 5-day-old culture of pathogen was placed in the centre of PDA plate with the facing the medium and pre-incubated at 25°C for 2 days. After 2 days of pre-incubation, different concentrations of essential oils (32 to 352 μg $mL^{-1}$ air) were dropped onto covers of Petri dishes, sealed by parafilm to prevent releasing essential oils from the Petri dish and incubated at 25°C for further 3 days. A thin layer of agar disc was then aseptically removed one-day intervals and processed for light microscopy. Agar discs of 1 mm thickness were cut from growing edges, placed in a drop of 50% glycerol on a microscope glass slides and subsequently covered with glass cover. Observations were made under light microscope (Olympus BX-50, Tokyo, Japan).

## RESULTS

The results indicate that essential oils of *O. syriacum* and *F. vulgare* were strongly inhibitory to conidial germination of *P.digitatum* compare with other essential oils used. Complete inhibition of conidia by essential oils of *Origanum* was observed at the concentration of 64 μg $mL^{-1}$ (Table 1). The germination of conidia was, however, totally inhibited relatively the higher concentration of fennel oil (352 μg $mL^{-1}$). As it was seen in Table 1, essential oils of *Lavandula, Artemisa* and *Laurus* showed either weak or no inhibitory effect on conidial germination at all concentrations used (32 to 352 μg $mL^{-1}$). All essential oils were, however,

Table 1: Antifungal activity of plant essential oils, used at various concentrations, on conidial germination of *Penicillium digitatum*

| | Conidial germination (%) | | | | |
|---|---|---|---|---|---|
| Conc. (μg $mL^{-1}$) | *O. s* | *F. v* | *L. s* | *L. n* | *A. a* |
| 0 | 100 | 100 | 100 | 100 | 100 |
| 32 | 8 | 95 | 100 | 100 | 100 |
| 64 | 0 | 87 | 100 | 100 | 100 |
| 96 | 0 | 85 | 100 | 100 | 100 |
| 128 | 0 | 80 | 100 | 100 | 100 |
| 160 | 0 | 73 | 100 | 100 | 100 |
| 192 | 0 | 61 | 100 | 100 | 100 |
| 224 | 0 | 49 | 95 | 100 | 100 |
| 256 | 0 | 32 | 92 | 100 | 100 |
| 288 | 0 | 17 | 90 | 100 | 100 |
| 320 | 0 | 5 | 83 | 100 | 100 |
| 352 | 0 | 0 | 78 | 97 | 100 |

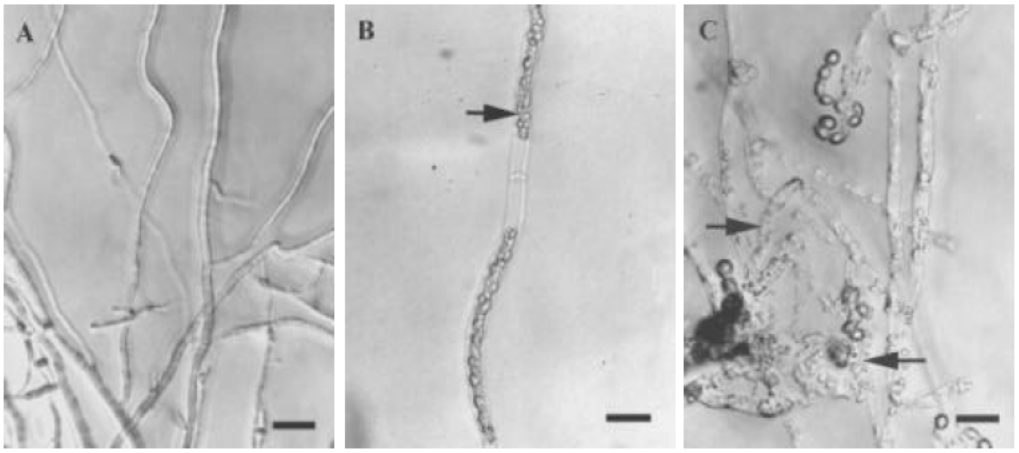


Fig. 1: Effect of essential oil of oregano on hyphal morphology. (A) Shows healthy hyphae growing on control plate. (B) and (C) Show contact effects of oregano and fennel essential oils, respectively, on hyphal morphology. Note alterations in cytoplasmic content such as cytoplasmic coagulation and vesiculation (arrow) in plate (B) and hyphal shrivelling and organelle depletion (arrows) in plate (C), in comparison to hyphae on control plate (A). Bar=10 μm

Table 2: Antifungal activity of plant essential oils, used at various concentrations, on germ tube elongation of *Penicillium digitatum*

| | Length of germ tube (μm) | | | | |
|---|---|---|---|---|---|
| Conc. (μg mL$^{-1}$) | *O. s* | *F. v* | *L. s* | *L. n* | *A. a* |
| 0 | 980a | 980a | 980a | 980a | 980a |
| 32 | 210bC | 610bB | 970aA | 970aA | 985aA |
| 64 | 0cC | 530bB | 920abA | 950aA | 960aA |
| 96 | 0cC | 480cB | 880bA | 940aA | 970aA |
| 128 | 0cC | 410cB | 860bA | 910aA | 960aA |
| 160 | 0cC | 320dB | 810bcA | 870abA | 950aA |
| 192 | 0cD | 230eC | 790cB | 820bB | 960aA |
| 224 | 0cD | 160fC | 720cdB | 760bcB | 940aA |
| 256 | 0cD | 90gC | 680dB | 710cB | 890abA |
| 288 | 0cD | 40hC | 510eB | 670cB | 830bA |
| 320 | 0cD | 15hC | 450efB | 520deB | 780bcA |
| 352 | 0cE | 0iD | 310fC | 420eB | 720cA |

Mean values followed by different small or capital letters within the column or row, respectively, are significantly different according to Duncan Multiple Range Test ($p<0.01$)

found to inhibit the germ tube elongations in a dose-dependent manner. Similarly, *Origanum* and *Foeniculum* oils, at 64 and 352 μg mL$^{-1}$ concentrations, respectively, were found to inhibit the germ tube elongation completely. Essential oils of *Lavandula*, *Laurus* and *Artemisia*, at the highest concentration used (352 μg mL$^{-1}$), reduced the germ tube elongation by 69, 58 and 28%, respectively (Table 2).

The microscopic observation of *P. digitatum* mycelium derived from 3-day-old co-culture of two efficient essential oils (*Origanum* and *Foeniculum*)/fungus showed degenerative changes in the hyphal morphology (Fig. 1). Following exposure of the growing hyphae to essential oils (3 days after treatment), the mycelium appeared degraded, with large vesicles inside the cell walls (Fig. 1B). In many cases, the mycelium cells had either no cytoplasm or the cytoplasm was depleted of organelles (Fig. 1C). Under the influence of the oils, the growth of the fungus was suppressed and the hyphae structure was modified.

## DISCUSSION

Volatile compounds from plants, especially essential oils, have antimicrobial activity against a variety of food borne, human and plant pathogens[11]. The results of this study confirm that essential oils from *O. syriacum* var. *bevanii* and *F. vulgare* possess strong antimicrobial activity on conidial germination and germ tube elongation of fungal pathogen *P. digitatum*. Essential oils from oregano and fennel oils and their main components such as thymol, carvacrol, estragole and t-anethole have been previously reported to have antimicrobial activity against variety of plant pathogenic fungi and bacteria[7-9,12-16]. To our knowledge, this study was the first study showing antimicrobial activities of essential oils of *O. syriacum* and *F. vulgare* against *P. digitatum*. Other tested essential oils also exhibited antimicrobial activity but is weaker than both fennel and oregano essential oils. In many studies, essential oils of *Lavandula, Artemisa* and *Laurus* have been demonstrated possess some antifungal and antibacterial activities[18-24]. Although these oils were reported to inhibit conidium germination and germ tube elongation of the other post harvest disease agent such as *Botrytis cinerea*[25], complete inhibition of conidial

germination was not affected at concentrations up to 352 μg $mL^{-1}$. Similar negative results was also reported by Daferera *et al.*[26]. Essential oils of *L. angustifolia* also failed to inhibit conidial germination of *P.digitatum*. They further reported that essential oil of *L. angustifolia* was more effective in the inhibition of germ tube growth than of hyphal growth as in the case of result obtained in this study.

The microscopic observation of *P. digitatum* mycelium derived from 3-day-old co-culture of two efficient essential oils (*Origanum* and *Foeniculum*)/fungus showed degenerative changes in the hyphal morphology. Following exposure of the growing hyphae to essential oils (3 days after treatment), the mycelium appeared degraded, with large vesicles inside the cell walls. In many cases, the mycelium cells had either no cytoplasm or the cytoplasm was depleted of organelles. Under the influence of the oils, the growth of the fungus was suppressed and the hyphae structure was modified. Similar observations with different essential oils were reported by Fiori *et al.*[27] who showed that essential oils of *Achillea millefolium, Cymbopogon citrates, Eucalyptus citriodora* and *Ageratum conyzoides* caused markedly damaged on the invading hyphae of *Didymella bryoniae* compared to non-treated controls. The results obtained by observations with light microscopy are also in accordance with those of Zambonelli *et al.*[13] who verified that essential oils of *Thymus vulgaris, Lavandula* and *Mentha piperita* caused degeneration of hyphae as well as the cytoplasmic emptying of *Colletotrichum lindemuthianum* and *Pythium ultimum* var. *ultimum*. The aqueous extract of *Allium sativum* also caused morphological alterations on the hyphae of *Pythium ultimum. R. solani* and *C. lindemuthianum* and *F. solani* presented hyphae with smaller diameters when they were treated with *Allium sativum*[28].

In conclusion, this study demonstrates the possibility of protecting stored food materials against post harvest fungi in a closed storage conditions by applying the *Origanum* and *Foeniculum* essential oils. The essential oils of *Origanum* and *Foeniculum* may be considered as potential alternatives for synthetic fungicides, or lead compounds for new classes of synthetic fungicides against *P. digitatum*. The procedures provides natural, non-toxic economically feasible and effective antifungal agent. However further experiments are needed to obtain information regarding the antimicrobial activities of these oils *in vivo* conditions.

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