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Fungicidal and Fungistatic Activity of Some Plant Essential Oils against *Alternaria solani* the Causal of Tomato Early Blight.

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ABSTRACT

The effects of essential oils from different plants were studied in vitro for fungicidal and fungistatic activity towards *Alternaria solani* the causal fungus of early blight disease of tomato. Complete inhibition of fungal growth caused by pepper mint, lemongrass, thyme and sweet basiloils at concentration of 2% followed by caraway, geranium and eucalyptus oils at concentration of 4%. Neem oil had a lesser effect in this concern that it could inhibit completely the fungal growth only at the highest concentration used of 6%.At concentrations of 4 and 6%,pepper mint, lemongrass and neem oils showed fungicidal effect. In contrast, caraway, thyme and eucalyptus oils showed fungistatic effect. Both geranium and sweet basil have fungistatic effect at concentration of 4% and turned to be fungicidal with increasing their concentration up to 6%.

Keywords: *Alternaria solani,* essential oils, early blight, tomato, fungicide, fungistatic.

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INTRODUCTION

Early blight is a very common disease of both potato and tomato. It causes leaf spots and tuber blight on potato, and leaf spots, fruit rot and stem lesions on tomato. The disease can occur over a wide range of climatic conditions and can be very destructive if left uncontrolled, often resulting in complete defoliation of plants. In contrast to the name, it rarely develops early, but usually appears on mature foliage. Early blight is caused by the fungus, *Alternaria solani* (Ellis & G. Martin), which survives in infected leaf or stem tissues on or in the soil. This fungus is universally present in fields where these crops have been grown. Although the use of crop rotation, certified disease-free seeds and resistant varieties, are important control measures to minimize infection, it is usually necessary to apply fungicide sprays to fully protect plants from early blight.

Looking for fungicide alternatives that have fungicidal effect on disease incident and development is considered valuable in safety application and environmental pollution concern. Recently, there has been increasing interest in essential oils as possible substitutes for conventional synthetic pesticides. This has been due to concern over ecosystem pollution and pesticide resistance in pests and pathogens [1]. There are several examples of previous studies which have tested the fungicidal properties of various essential oils [2,3,4].Essential oils are also considered a promising alternative with many having antifungal properties. However, very high concentration is needed when applied to real food systems [5,6].Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use[7]. In this regard, lemongrass (*Cymbopogon citrates* L.) oil was reported to be antifungal activity against several plant pathogens. Also, thymol is an essential oil component from thyme (*Thymus capitates* L.) and has been used as medicinal drug, food preventative, and beverage ingredient [8] as well as plant diseases of several fruits and vegetables [9,10,11]. Moreover, [12] reported that carnation, caraway, thyme, peppermint and geranium essential oils have been found to have inhibitory effects against the mycelial growth of *Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii* and *Macrophomina phaseolina* under *in vitro* conditions.

The purpose of the present study was to evaluate the fungicidal and fungistatic effect of some essential oils on the *in vitro* growth of fungus *Alternaria solani.*

MATERIALS AND METHODS

Sources of essential oils, fungi and culture media

Pure-grade of the essential oils, *i.e.* peppermint (*Mentha piperita*), caraway (*Carum carvi*), thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*) Neem (*Melia azadirachta*), Thyme (*Thymus vulgaris*), geranium (*Geranium viscosissimum*), Sweet basil (*Ocimum basilicum*) and eucalyptus (*Eucalyptus globulus*) were obtained from Cairo Company for oils and aromatic extractions CID, Egypt. These essential oils were stored in dark bottles at 4ºC for further studies.

A highly pathogenic isolate of *Alternaria solani* the causal o early blight disease of tomato was used in this study. This isolate proved it's highly pathogenicity in previous work under greenhouse of Plant Pathology Dept., National Research Centre, Egypt.

Potato dextrose agar (PDA) was used as culture media for growth of *Alternaria solani.*

Effect of plant essential oils on growth

Some essential oils (Table, 1), *i.e.* Peppermint, Caraway, Lemongrass, Neem, Thyme, Geranium, Sweet basil and Eucalyptus at different concentrations, *i.e.* 1,2,4 and 6% were evaluated against the *Alternaria solani* fungal growth. The essential oils were prepared by dissolving in 0.5 ml of 0.1% Tween 80 before use. Certain volumes of each essential oil were added to melted PDA medium and carefully mixed by gentle swirling to ensure the equal oil concentration before pouring into 9 cm diam. Petri dishes. After agar was solidified, a 5 mm disk from the edge of an actively growing colony of *Alternaria solani* was placed in the centre of each Petri dish. A separate PDA plates free of essential oils was used as control treatment. All Petri dishes were incubated at 25±1°C and daily the radial growth were observed until respective colonies completely covered the medium

in control set,then the diameter of mycelial growth was measured in (mm). Reduction percentage in fungal growth was calculated in relative to its growth in control treatment following the formula:

$$\text{Percentage of mycelial inhibition} = \frac{dc - dt}{dc} \times 100$$

Where dc is mean colony diameter of control sets and dt is mean colony diameter of treatment sets.

For all treatments three replications were used and the experiments were repeated three times.

Table 1. Botanical plant classification and main active principles of their essential oil*

Common name	Scientific name	Family	Major active component
Peppermint	*Mentha piperita*	*Lamiaceae*	Menthol, menthone, menthyl acetate, viridiflorol, ledol
Caraway	*Carum carvi*	*Umbelliferae*	Carvone, limonene, carveol, pinen, thujone
Lemongrass	*Andropogon citratus*	*Gramineae*	Citrol or citral, about 70 % up to 85 %
Neem	*Meliaa zadirachta*	*Meliaceae*	Ssterols,nimbin, nimbidin and nimbinene plus bitter principles, tannins and flavonoids
Thyme	*Thymus vulgaris*	*Labiatae*	Thymol, carvacrol, geraniol, thymol methyl ether, α -terpinene
Geranium	*Geranium viscosissimum*	*Geraniaceae*	Geraniol, Citronellol, Tannins
Sweet basil	*Ocimum basilicum*	*Labiatae*	Volatile oils (up to 28 percent methyl cinnamate)
Eucalyptus	*Eucalyptus globulus*	*Myrtaceae*	Volatile oil (l,8-cineole- terpineole, a-pinene, p-cymene - aldehydes, ketones and alcohols; Polyphenolic acids (caffeic, ferulic, gallic, protocatechuic) and Flavonoids (eucalyptin, hyperoside, rutin)

*herb information (www.holisticonline.com/Herbal-Med/_Herbs/h280.htm

Evaluation of fungistatic activity

Essential oils were directly assayed to each fungus with the two highly concentration tested 4 and 6%. A control was used for each case by not exposing the fungus to any essential oil. Assays of fungistatic activity were done using method previously described [13] by adding a 1 cm diameter of each fungal growth in the center of a Petri dish containing PDA culture medium. Four filter paper discs (Whatman No3, 5 mm diameter) impregnated with proposed dilutions of different tested essential oils were placed around the fungi at the four edges of Petri dish. Another set of inoculated Petri dishes containing filter paper discs impregnated with sterilized distilled water was used as control treatment. All inoculated treated plates were incubated at 25±1ºC. After seven days when the fungal colony achieved full growth in control treatment, the filter paper in essential oils treatments were picked up and left for incubation again for another seven days. Then the diameter of fungal inhibition zone was measured. These activities were considered to be positive (fungicidal) when inhibitory action (inhibition zone) of the fungal growth was observed and negative (fungistatic) when the fungal grew over the place previously occupied with filter paper (similar to control treatment).

Statistical analysis

Tukey test for multiple comparisons among means was utilized as described by [14].

RESULTS AND DISCUSSION

The essential oils evaluated in this work have a great variety of phytochemicals (Table 1) that could be considered as responsible for a larger or smaller antifungal activity. Those phytochemicals are as follows: 1. Menthol, menthone, menthyl acetate, viridiflorol, ledol in peppermint essential oil; 2. Carvone, limonene,

carveol, pinen, thujone in caraway essential oil; 3. citrol or citral in lemongrass oil; 4.sterols, nimbin, nimbidin and nimbinene plus bitter principles, tannins and flavonoids in neem oil; 5. Thymol, carvacrol, geraniol, thymol methyl ether, α -terpinene in thyme essential oil; 6. Geraniol, Citronellol, Tannins including gallic acid in geranium essential oil; 7. Volatile oils (up to 28 percent methyl cinnamate) in sweet basil oil and 8. Volatile oil (l,8-cineole- terpineole, a-pinene, p-cymene - aldehydes, ketones and alcohols; Polyphenolic acids (caffeic, ferulic, gallic, protocatechuic) and Flavonoids (eucalyptin, hyperoside, rutin) in eucalyptus oil (C.f. herb information: www.holisticonline.com/Herbal-Med/_Herbs/h280.htm).

Table 2. Average linear growth (mm) of *A. solani* isolates in response to different concentrations of some essential oils under *in vitro* conditions

Essential oil	Concentration (%)*	Av. linear growth (mm)	Reduction (%)**
Pepper mint	1.0	27.7 d	69.3 d
	2.0	0.0 f	100 a
	4.0	0.0 f	100 a
	6.0	0.0 f	100 a
Caraway	1.0	37.3 c	58.5 e
	2.0	17.7 e	80.4 b
	4.0	0.0 f	100 a
	6.0	0.0 f	100 a
Lemon grass	1.0	15.3 e	83.0 b
	2.0	0.0 f	100 a
	4.0	0.0 f	100 a
	6.0	0.0 f	100 a
Neem	1.0	44.7 b	50.4 e
	2.0	26.7 d	70.4 c
	4.0	10.7 e	88.1 b
	6.0	0.0 f	100 a
Thyme	1.0	15.3 e	83.0 b
	2.0	0.0 f	100 a
	4.0	0.0 f	100 a
	6.0	0.0 f	100 a
Geranium	1.0	36.7 c	59.3 e
	2.0	23.3 d	74.1 c
	4.0	0.0 f	100 a
	6.0	0.0 f	100 a
Sweet basil	1.0	25.0 d	72.2 c
	2.0	0.0 f	100 a
	4.0	0.0 f	100 a
	6.0	0.0 f	100 a
Eucalyptus	1.0	41.3 b	54.1 e
	2.0	22.7 d	74.8 c
	4.0	0.0 f	100 a
	6.0	0.0 f	100 a
Control		90.0 a	0.0 f

Mean values followed by the same letter are not significantly different at $p \leq 0.05$

* Concentrations of essential oils were calculated as (v:v) to the growth medium

** Reduction in fungal growth under different treatments used, calculated relative to fungal growth in untreated control

The present investigation has demonstrated the antifungal activity of all treatments tested. This work proves that some essential oils have potential and could be useful when evaluated against *A. solani*. From the earlier reports [15,16] it is evident that some of the plant products have antifungal compounds which do have the capacity to inhibit the fungal pathogens. Therefore, there is increasing interest in obtaining alternative antimicrobial agents for use in plant disease control systems. One of the main procedures used in the research of biologically active substances is a systematic screening for the interaction between microorganisms and plant products. This procedure has been a source of useful agents to control the microbial survival [17]. Plant products of a recognized antimicrobial spectrum could appear in food conservation systems as main antimicrobial compounds or as adjuvant to improve the action of other antimicrobial compounds [18]. Among other chemical products, aromatic plants possess essential oils resulting from secondary metabolism. These

substances have a great economic potential, especially in the food, pharmaceutical and perfumery sectors. Thus, the number of studies on the chemical composition and biological properties of these oils, as well as the taxonomic, environmental and cultivation factors that lead to variation in their quantity and quality, has been increasing [19].

In the present study the inhibitory effect of tested essential oils against the mycelial growth of tomato pathogenic fungus *A. solani* presented in Table (2). Fungal mycelial growth decreased significantly as the concentrations of essential oils were increased, to reach the fungal growth's minimum at the highest concentration used. Pepper mint, lemongrass, thyme and sweet basil showed highly effect that they caused complete inhibition to fungal growth at concentration of 2%. Meanwhile, caraway, geranium and eucalyptus oils showed the same effect at concentration of 4%. Neem oil had a lesser effect in this concern that it could inhibit completely the fungal growth only at the highest concentration used of 6%.

Similar results were also reported concerning the efficacy of essential oils as antifungal inhibitors. Report of [20] stated that essential oil of thyme (*Tymus vulgaris*) inhibited the mycelial growth in all of *Penicillium digitatum, Aspergillus flavus, Colletotrichum gloeosporioides, Pythium ultimum, Rhizoctonia solani* and *Bipolaris sorokiniana*fungi. The most important soilborne fungi, *Pythium ultimum, Colletotrichum gloeosporioides* and *Rhizoctonia solani*, had 100% complete inhibition of mycelial growth, and agreed with those obtained by [21], that showed *T. vulgaris* oil asvolatile compound inhibited mycelial growth of three phytopathogenic fungi such as *C. gloeosporioides, R.solani* and *F. oxysporum*. The essential oil of *T. vulgaris* inhibited various fungi including food spoilage, mycotoxin producing fungi and postharvest pathogenic fungi. These essential oil in high concentration had inhibitory on mycelial growth on *Penicillium digitatum*, *Aspergillus flavus* and *Bipolaris sorokiniana*, and confirm the experiment by [22] that showed thyme, summer savory and clove essential oil showed notable antifungal activities against *A. flavus* and has the highest antifungal activity and followed by summer savory and clove essential oil. The main constituents of *Tymus vulgaris* oil were thymol, p-cymen and a-terpinene were thought to be responsible for the antifungal activity. In addition, experiment showed essential oil of *Eucalyptus camaldulensys* inhibited the mycelial growth in all of the experiment fungi. Eucalyptus essential oil in *Pythium ultimum* and *Rhizoctonia solani* had 100% complete inhibition of mycelial growth, and agreed with those obtained by [23],that showed *Eucalyptus unigera* oil inhibited mycelia growth of three phytopathogenic fungi such as *C. gloeosporioides, R. solani* and *Pythium* spp. Also, [24] reported that 4% of the tested essential oils geranium, rose, lemon and mint have an inhibitory effect against the mycelial growth of *R. solani* and *F. oxysporum* f. sp. *Phaseoli* under *in vitro*, causing complete inhibition in fungal growth. Furthermore, [25] studied the biochemical reaction of onion, garlic, eucalyptus, caraway, fennel**,** black cumin**,** mustard**,** carnation**,** neemix and trilogy essential oils against mycelial growth of *R. solani* and *Pythium debaryanum in vitro.* They found that complete inhibition of both fungi was obtained by only carnation oil at 4%, however, considerable inhibition (more than 90%) was obtained with neemix and trilogy oils. Moreover, [12] stated that carnation, caraway, thyme, peppermint and geranium essential oils have been found to have inhibitory effects against the mycelial growth of *Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii* and *Macrophomina phaseolina* under *in vitro* conditions. Complete inhibition of fungal growth was observed with the use of 4% carnation and geranium oils. Mycelial growth of the tested fungi showed more sensitivity to high concentrations of thyme than to caraway and peppermint oils.

On the other hand, there is great interest to replace syntheticxeno-biotic with similar acting natural compounds. Itis important to determine secondary metabolites with fungicidal or fungistatic activity, since they allow the useof natural origin compounds that are generally species specific, have low environmental persistence, and are biodegradable. In the present study presented data (Table 3) revealed that Pepper mint, lemongrass and neem oils showed fungicidal effect on the growth of *A. solani* at the tested concentrations of 4 and 6%. Meanwhile, in contrast, caraway, thyme and eucalyptus oils showed fungistatic effect.

In this regard, it was interested to observe that both geranium and sweet basil have fungistatic effect at concentration of 4% and turned to be fungicidal with increasing their concentration to 6%. This observation could be due to increase in the active ingredients of Geraniol, Citronellol, Tannins and methyl cinnamate at the high concentration used of these essential oils. In this regards, [26] studied the fungistatic activity of the essential oils of Chilean Monimiaceae, boldo (*Peumus boldus*), tepa (*Laureli opsisphilippiana*), and laurel (*Laureli asempervirens*) against *Rhizoctonia solani*, *Pythium irregulare*, *Ceratocystis*, *Phragmidium violaceum* and *Fusarium oxysporum.* They found that the essential oil from *L. sempervirens* showed the highest fungistatic activity with significant differences in dose as well as exposure. They added that *Phragmidium*

violaceum was the most sensitive strain and *P. irregular* the most resistant one of all the essential oils. Finally they conclude that the 1,2-dimethoxy-4-(2-propenyl)-phenol compound, known to be one of recognized toxic activity, was found only in *L. philippiana* and could be attributed to fungistatic activity referring to previous report [27].

Table 3. Fungicidal and fungistatic effect of some essential oils against the growth of *A. solani* under *in vitro* conditions

Essential oil	Concentration (%)	Effect type
Pepper mint	4	**L**
	6	**L**
Caraway	4	**S**
	6	**S**
Lemon grass	4	**L**
	6	**L**
Neem	4	**L**
	6	**L**
Thyme	4	**S**
	6	**S**
Geranium	4	**S**
	6	**L**
Sweet basil	4	**S**
	6	**L**
Eucalyptus	4	**S**
	6	**S**

(**L**) Lethal fungicidal effect. (**S**) Fungistatic effect

CONCLUSION

Plant essential oils are one of the promising safeand environmentally-friendly candidates for future use as alternatives to conventional synthetic pesticides for managing fungi as plant pathogens, food contaminantsand decays. Most of the formulations used in the present study *in vitro,* showed high efficacy on *A. solani* fungal growth. Among the candidates used, Pepper mint, lemongrass, thyme and sweet basil, theirmain active components, menthol, citrol, thymo and methyl cinnamate effectively inhibited mycelial growth through fungicidal and fungistatic actions. The active ingredients of the candidates and their isomers could be used as seed and soil treatments, and also as preventives of postharvest decay and as food preservatives. On the light of present work, it would also be interesting to study the effect of the essential oil on plant pathogenic important fungi in order to develop new anti-fungal or fungistatic agents for preventive treatment of such serious fungal diseases.

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REFERENCES

[1] Holmes GL and Eckert JW. Phytopathology1999; 89: 716–721.
[2] Plotto S, Roberts DD and Roberts RG. ActaHorticulture2003; 628: 737–745.
[3] Macias FA, Molinillo JMG, Varela RM and Galindo JCG. Pest Management Science 2007; 63:327–348.
[4] Neiri F, Mari M, Brigati S and Bertolini P. Plant Disease 2007;91: 30–35.
[5] Hammer KA, Carson CF and Riley TV. J. Appl. Microbiol. 2003; 95: 853-860.
[6] Ahmet C, Saban K, Hamdullah K and Ercan K. Bosse. Biochem. Syst. Ecol. 2005; 33: 245- 256.
[7] Ormancey X, Sisalli S and Coutiere P. Parfums, Cosmetiques, Actualites 2001; 157: 30-40.
[8] Mansour F, Ravid U and Putievsky E. Phytoparasitica1986; 14:137–142.
[9] Anglelini P, Pagiotti R, Menghini A and vianello B. Annals of Microbiology 2006; 56(1): 65-69.
[10] Feng W and Zheng X. Food control 2007; 18(9):112-1130.

[11] Klaric MS, Kosalec I, Mastelic J, pieckova E and Pepljnak S. Letters in Applied Microbiology 2007; 44(1):36-42.
[12] Abdel-Kader MM, El-Mougy NS and Lashin SM. J Plant Protection Res 2011; 51 (3): 302-309.
[13] Woodward B and Groot RDE. Forest Prod J 1999; 49(4):87-94.
[14] Neler J, Wassermann J and Kutner MH. Applied linear statistical models, regression, analysis of variance and experimental design. 2nd edition. Homewood, Illinois: Richard D. Irwin Inc. 1985; 1127 p.
[15] Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM, Lavekar GS.Afr J Tradition 2007; CAM 4 (3): 313–318.
[16] Bindu S and Kumar P. 2009. Int J Green Pharmacy 2009; 3: 63–65.
[17] Salvat A, Antonnacci L, Fortunato RH, Suarez EY and Godoy HM. Lett ApplMicrobiol 2001; 33: 93–297.
[18] Kaur J and Arora D. Int J Antimicrob Agents 1999; 12: 257–262.
[19] Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA and Petrovick PR. Farmacognosia: da Planta aoMedicamento. 5th ed. UFRGS/UFSC; Porto Alegre (RS)/Florianópolis (SC), Brazil: 2004.
[20] Katooli N, Maghsodlo R, Honari H and Razavi SI. Global Journal of Medicinal Plant Research 2012; 1(1): 1-4.
[21] Lee SO, Choi GJ, Jang KS and Kim JC. Plant Pathol J 2007; 23(2): 97-102.
[22] Omidbeygi M, Barzegar M, Hamidi Z and Naghdibadi H. Food Control J 2007; 18: 1518-1523.
[23] Huv JS, Ahn SY, Koh YJ and Lee CI. J Plant Pathol 2000; 16(5): 286-289.
[24] El-MougyNS, El-Gamal NG, Abdel-Kader MM. J Plant Protection Res 2007; 47 (3): 255–265.
[25] El-ToonyAME, Awad NGH andTadrous MFI. Egypt J ApplSci 2003; 18: 47–68.
[26] Bittner M, Aguilera MA, Hernández V, Arbert C, Becerra J and Casanueva ME. Chilean J AgricTural Res 2009; 69 (1): 30-37
[27] Pérez R and Ubera J. I CongresoIberoamericanosobreSeguridadAlimentaria (CIBSA) de la granja a la mesa, ida y vuelta, Sevilla, España. 8-10 May 2006. Red Española de SeguridadAlimentaria (SICURA).