

Antifungal Activities of Some Essential Oils Against *Fusarium oxysporum* of Rosemary and Sage Plants

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Abstract

Rosemary (*Rosmarinus officinalis* L.) is a common household aromatic and medicinal plant belonging to the *Labiatae* family and sage (*Salvia officinalis*) *Lamiaceae* family is aromatic, rather woody perennial low shrub. This study aimed some antifungal effects of plant essential oils were investigated against fungal disease agent *Fusarium oxysporum* on mycelial growth inhibition *in vitro* and disease development reserve *in vivo* conditions. The tissue pieces were aseptically transferred to petridishes containing a potato dextrose agar medium supplemented with streptomycin sulfate at the rate of three to five pieces per plate. *Fusarium oxysporum* was grown for 7-10 days on PDA at 27°C. The concentrations were tested at 20, 40, 60 and 80 µl. The same quantity of PDA *Fusarium oxysporum* also established in control. *F. oxysporum* was cut with a sterile cork borer from the edge of actively growing cultures on PDA plates. At 20 µl, 40 µl, 60 µl and 80 µl concentration, lemon grass (100%) growth inhibition was confirmed against *Fusarium* wilt followed by spearmint (85.00%) and minimum inhibition recorded 25.00% at *Eucalyptus*. As conclusion lemon grass and spear mint essential oils demonstrated the maximum antagonistic effect against the *Fusarium* wilt, whereas *Eucalyptus* inhibit the list amount of growth pathogen.

Keywords: *Fusarium oxporum*; Essential oil; Mycelia inhibition; *In vivo*; *In vitro*

Introduction

Rosemary (*Rosmarinus officinalis* L.) is a common household aromatic and medicinal plant belonging to the *Labiatae* family; it is endogenous to Europe, Asia and Africa, mainly around the Mediterranean Sea [1]. It is used for flavoring food, in cosmetic and in traditional medicine for choretics, hepatoprotective and anti-morigenic activity; conventionally it is used for relieving visceral muscle spasms in renal colic, menstrual pain, bronchial asthma and gastrointestinal colic. It also has some therapeutic value in the treatment of disorders like, peptic ulcers, inflammatory diseases, ischemic heart disease, cataracts, and cancer [2].

Sage (*Salvia officinalis*) belonging to *Lamiaceae* family it is a common garden medicinal and aromatic herb native of the northern Mediterranean and currently cultured in different country, family reported to include more than 900 species [3]. Sage essential oil protected liver pates from oxidation processes and the herb used for relieve toothache, treatment of chronic bronchitis and thought topically for inflammation of the mucous membranes of the mouth and throat [4].

One of economically important disease in European countries on sage plants *antracnosis* caused by *Colletotrichum dematium*, *ascochitosis*, *Ascochyta sclarea* and root rot caused by *Rhizoctonia solani*. Similar in Italy and Spain include *Phomopsis sclarea*, *Phodosphaera inequalis*, *Erysiphae polygوني* and *Sclerotinia sclerotiorum* the major disease of sage. In California, USA major sever pathogen of sage seedlings infected by *Fusarium* was observed [5].

Although synthetic fungicides are effective, but continuous spaying has disturbed biological control by natural enemies and led to outbreaks in diseases, extensive development of resistance to mixture types of fungicides [6,7]. Synthetic fungicides have unfavorable environmental effects of have brought about the need for the development of new types of selective control the new alternatives broadly accepted plant product or botanicals fungicides. Due to their bioactive chemicals content essential oil bearing and botanical control of substitute currently used disease control agents [8,9].

The primary symptom of *Fusarium* wilt is observed by discoloration of the older leaves and change red to brown lesions on the stems. Above the ground symptoms include loss of turgour and leaf chlorosis, starting from the lower leaves, sometimes followed by leaf abscission and plant death. It may be manifested on only one side of the host plant [9]. Disease Plants are frequently under developed, growing slowly and change their color when compared to health ones.

Fusarium oxysporum caused by soil borne fungi and can survival in soil for many years as a resistant spore. It is spread by means of wind, rain, irrigation water, farm implements and crop residue. The soil-borne fungus is the causal agent of vascular wilt, a disease that affects a large variety of economically important crops worldwide [10].

The produce from plant resulting compound are concentrated hydrophobic liquid including volatile aromatic compounds that have antifungal activity describe as essential oil. Plant extracted essential oils as have been used for since disease and insect pest due to for their broad spectrum antimicrobial activity and defense mechanism against plant pathogens. Several plant based extracts and plant essential oils have been reported to be effective antimicrobials against soil-borne fungi [11,12].

Chemical control have control a limited number of species, including the specific target of disease and insect pest species, integrated pest management the most excellent management alternative than chemical control. In recent years, organic pest management has great contribution for the control of fungus due to it is simply exchangeable organic material and have great role for confirm successful pest management strategy [13,14].

In the last study several reports on the antifungal activities of essential oils against plant pathogenic fungi *in vitro* conditions plant derivate essential oil its reach source biochemical activity alternative to currently used disease control agents. In the present study, antifungal effects of plant essential oils resulting from leaf part were investigated beside fungal disease agent *Fusarium oxysporum* effect of the essential oils on mycelial growth inhibition *in vitro* and disease development inhibition *in vivo* conditions.

Materials and Methods

Extraction method of essential oils

Plant extract were collected from Wondo Genet Agricultural Research Center. Above ground Air-dried plant materials (200 gms) of *Cymbopogon nardus* L., *Mentha spicata* L. and *Eucalyptus citrodora* Hook. The essential oils were obtained by steam distillation for 3 h using Clevenger-type apparatus (Ildam, Ankara). The extracted oils were stored at 4°C in a clean amber glass bottle for further used.

Isolation of *Fusarium oxysporum*

The infected tissues along with adjacent small unaffected tissues were cut into small pieces of 2-5 mm squares and were transferred by using flame-sterilized forceps to sterile petridishes containing 0.1% mercuric chloride solution. The tissue pieces were surface-sterilized in this solution for 30-60 sec and washed in sterile water two or three times. Clorox (10%) were used to sterilize the surface. The tissue pieces were aseptically transferred to petridishes containing a potato dextrose agar medium supplemented with streptomycin sulfate at the rate of three to five pieces per plate. 7-10 days on PDA at 27°C *Fusarium oxysporum* were grown and stock cultures obtained from single spore was preserved on PDA and sub-cultured kept at 4°C.

Efficacy of essential oils on mycelial growth *in vitro* conditions

This experiment carried out efficacy of essential its contact and volatile phase belongings towards mycelial growth of *Fusarium oxysporum* described by Sarwar et al. [15]. To see efficiency of contact effects, Eo were discrete as an emulsion in water using ethanol (0.1% v/v) and added to PDA. The concentrations were tested at 20, 40, 60 and 80 µl. The controls received the same quantity of PDA *Fusarium oxysporum*. The fungus was cut with a sterile cork borer from the edge of actively growing cultures on PDA plates. The culture media develop in the dark and were sealed with parafilm to prevent loss of essential oil vapors and incubated at 27°C. After ten day fungal and oil inoculation the mean radial mycelial growth of the pathogen was determined by measuring the diameter of the colony in two directions at right angles when the plate surface of the control Petri was covered by fungus. Mean inhibition percentage of mycelial growth in relation to the control treatment by using the formula, MGI (%) = $\frac{(dc-dt)}{dc} \times 100$, dc and dt represent mycelial growth diameter in control and treated Petri plates, respectively.

Efficacy of essential oil on fungal growth *in vivo* conditions

Cymbopogon nardus L. and *Mentha spicata* L. essential oil, due to its better efficacy those were selected for greenhouse trial to see the efficacy of the essential oil on *Fusarium oxysporum*. All experiments were arranged in a completely randomized design with five replications. (20, 40, 60, and 80 mg/l) concentrations of essential oil were prepared by dissolving the requisite amounts in distil water (0.1% v/v) solution. Three month sage and rosemary plants were sprayed with these emulsions (10 ml for each plant) uniformly using with a manually operated glass knapsack sprayer.

Statistical analysis

Analysis made using SAS software version: 9.2 and the data from two sovereign experiments were analyzed separately.

Results and Discussion

From examined essential oil it was practical observed that at 20 µl, 40 µl, 60 µl and 80 µl absorption of essential oil of lemongrass oil showed the maximum inhibition the mycelial growth, pursue by *spear mint* and *Eucalyptus* has the minimum effective.

At 20 µl, 40 µl, 60 µl and 80 µl concentration, 100% growth inhibition was recorded against *Fusarium* wilt by lemongrass followed by spearmint (85.00%) and minimum 25.00% for *Eucalyptus*. All concentration of lemon grass essential oil showed entirely inhibited the growth of mycelial. Essential oils of mint were found to be antifungal 40 µl, 60 µl, 80 µl and 20 µl respectively (Figures 1a-1d).

In this study examination discovered that the three essential oils tested, had antifungal activity from minimum to maximum against *F. oxysporum* on sage and rosemary plants. From all tested essential oil treatments inhibited mycelial growth of *F. oxysporum* sage in dual culture. *F. oxysporum* was inhibited by *Citronel oil*, *spearmint oil* and *lemon grass oil*, by 100, 85 and 25% respectively. Present study it has determined that lemon grass presented highly activity to *Fusarium* pathogen.

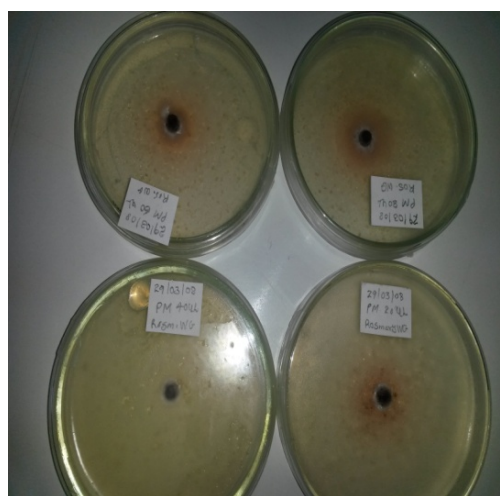


Figure 1a: Paper mint.

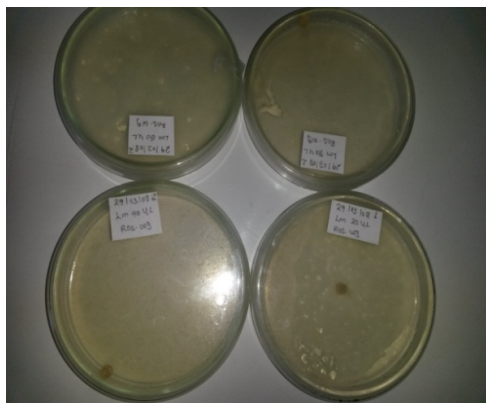


Figure 1b: Lemon grass.

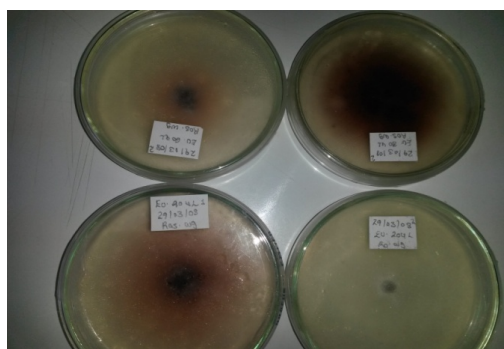


Figure 1c: *Eucalyptus globules*.

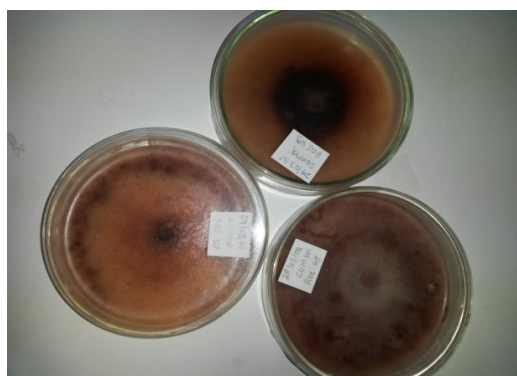


Figure 1d: Control.

The maximum inhibition zone recorded at 10 days, 20 days and 30 days, similarly from 20 μ l, 40 μ l, 60 μ l and 80 μ l minimum mycelial growth rate (25%) were found with all concentration. Similarly, Hadi and Kashefi, showed that Cinnamon extract was effective against *Fusarium oxysporum*. Soylu et al. [16] Reported *Mentha sp.* extract to cause inhibition development of *Fusarium oxysporum* f. sp. *cumini*, supports our findings. Ghorbany et al. [17] tested that the Cinnamon oil create that the oil had antifungal activity against *Penicillium*

digitatum and *Fusarium oxysporum*. Cumin, Thymus and Rosemary suppressed the growth of *Fusarium oxysporum* f. sp. *cicer* mycelial it was reported by Boniface et al. [18] Plant derivative extract has antifungal activity was reported against fungi such as *F. oxysporum* [19] *Phytophthora nicotianae* [20]. It has been reported that combined treatment with rosemary oil, clove oil and thyme oil can reduce tomato *Fusarium* wilt much more efficiently in comparison with control effect with single treatment by clove oil, thyme oil, or rosemary oil [21].

The efficacy of plant extract against the pathogen was in the orderly of clove oil greater than lemongrass followed by mint and *Eucalyptus*. Clove oil not only for the control of single fungi it has broad-spectrum activities to suppress several plant pathogenic fungi [22].

In our five-year examination, rosemary and sage plant highly attacked by *Fusarium* wilt and *Phoma* spp. This is probably due to major contamination in the field as continues harvesting effect of manner and also irrigation problem. From the previous study most of the aromatic and medicinal plants attacked by *Fusarium* and *Phoma* sp (Figures 2a and 2b).



Figure 2a: Treated.



Figure 2b: Untreated.

This study show that root rot, vascular wilt, damping off of medicinal plant, which cause by soil borne fungi, are very significant disease in medicinal plants. Thus, it is probable medicinal plants will be planted in the vast number of fields. It is necessary that researcher consider and survey different kind of control methods or prevention of soil borne fungi disease. Moreover, control of pathogen agents of medicinal plants is different from other agricultural plants. In future, regarding to problems of chemical medicine, medicinal plants maybe will be used more than now and medicinal plant will be very important

plant in future. So, it is a nice suggestion that research project concentrate on nonchemical methods or integrated pest management in order to decrease consuming of chemical and prevent adverse effects of them on human and nature [23].

Two main soil-borne fungal pathogens, *Fusarium* and *Phoma* species have been reported to cause wilting diseases in a variety of

plants including Rosemary as an economic tree. Recently, these soil-borne pathogens have been severely increased due to development of Rosemary plantation in the country. These soil-borne pathogens have caused root rot and wilting disease that resulted yield losses on infected plants all over the world [24] (Tables 1-3).

S No.	Treatments	10-days% Inhibition				20-days% Inhibition				30-days% Inhibition			
		20 µl	40 µl	60 µl	80 µl	20 µl	40 µl	60 µl	80 µl	20 µl	40 µl	60 µl	80 µl
1	Spear mint	100	100	100	100	88.89	90	86.67	91.11	77.8	85.6	87	81
2	Lemon grass	100	100	100	100	100	100	100	100	100	100	100	100
3	<i>Eucalyptus globules</i>	80.55	67.77	60.55	58.33	27.78	27.78	19.44	08.33	5.5	0	0	0
4	Control	0	0	0	0	0	0	0	0	0	0	0	0

Each value represents the mean of five replicates. Inhibition zone was compared using least significant difference at $\leq 5\%$.

Table 1: Growth inhibition of treated with different essential oils.

Treatments (%)	PH (cm)	Brno.	FLWPP (Kg)	FSW	EOC	EOY (lit.)	DS (%)	DC (%)
Spear mint 20	78.25 ^{ab}	70.75 ^{ab}	63.76 ^a	22.70 ^a	0.310 ^c	19.76 ^a	25.00 ^a	0.00 ^b
Spear mint 40	87.50 ^a	60.25 ^b	37.48 ^{cb}	17.70 ^a	0.170 ^f	6.37 ^{bc}	5.00 ^b	80.00 ^a
Spear mint 60	76.00 ^{ab}	68.50 ^{ab}	39.48 ^b	22.87 ^a	0.180 ^e	7.10 ^{bc}	25.00 ^a	0.00 ^b
Spear mint 80	76.00 ^{ab}	52.00 ^b	42.18 ^b	18.91 ^a	0.140 ^h	5.90 ^c	5.00 ^b	80.00 ^a
Lemon grass 20	53.75 ^b	49.25 ^b	34.05 ^{cbd}	18.81 ^a	0.310 ^c	10.55 ^b	5.00 ^b	80.00 ^a
Lemon grass 40	79.75 ^a	70.50 ^{ab}	23.94 ^{cd}	22.47 ^a	0.290 ^d	6.94 ^{bc}	5.00 ^b	80.00 ^a
Lemon grass 60	77.75 ^{ab}	48.25 ^b	30.99 ^{cbd}	18.24 ^a	0.160 ^g	4.96 ^c	5.00 ^b	80.00 ^a
Lemon grass 80	70.00 ^{ab}	62.75 ^{ab}	21.09 ^d	12.79 ^a	0.380 ^b	8.01 ^{bc}	5.00 ^b	80.00 ^a
control	76.75 ^{ab}	93.75 ^a	36.48 ^{cb}	13.04 ^a	0.540 ^{ab}	19.70 ^a	25.00 ^a	0.00 ^b
Isd	22.227	31.383	13.898	15.303	0	4.3188	0.02	0.02

The same later within the same column are not statistically different ($p < 0.05$). Where PH-Plant height, Brno-Branch number, FLWPP-fresh leaf weight per plant, FSW-Fresh stem weight, EOC-essential oil content, EOY-essential oil yield, DS-disease severity and DC disease control.

Table 2: Efficacy of essential oil on rosemary *Fusarium* wilts in green house condition.

Treatments (%)	PH (cm)	Brno.	FLWPP(Kg)	FSW	EOC	EOY (lit.)	DS (%)
Spear mint 20	87.75 ^{bac}	43.00 ^e	53.34 ^{ba}	37.02 ^{ab}	0.360 ^e	13.32 ^b	5.00 ^b
Spear mint 40	96.00 ^{ba}	45.25 ^e	55.25 ^{ba}	35.29 ^{bac}	0.400 ^c	14.11 ^b	5.00 ^b
Spear mint 60	73.75 ^c	47.50 ^{de}	42.23 ^b	32.44 ^{bc}	0.350 ^f	11.35 ^{cb}	5.00 ^b
Spear mint 80	88.00 ^{bac}	40.75 ^e	40.65 ^b	31.29 ^{bc}	0.430 ^b	13.45 ^b	5.00 ^b
Lemon grass 20	87.50 ^{bac}	67.75 ^c	41.72 ^b	33.09 ^{bc}	0.380 ^d	12.57 ^{cb}	5.00 ^b
Lemon grass 40	85.75 ^{bac}	66.00 ^{dc}	43.09 ^b	26.56 ^c	0.350 ^f	9.29 ^c	5.00 ^b
Lemon grass 60	96.25 ^{ba}	89.25 ^b	58.60 ^{ba}	34.70 ^{bc}	0.380 ^d	13.18 ^b	5.00 ^b
Lemon grass 80	102.00 ^a	112.00 ^a	71.16 ^a	43.74 ^a	0.470 ^a	20.56 ^a	3.00 ^c

control	77.25 ^{bc}	54.50 ^{dce}	41.73 ^b	31.83 ^{bc}	0.470 ^a	14.96 ^b	20.00 ^a
lsd	19.335	18.943	20.803	8.765	0	3.707	1.123

The same later within the same column are not statistically different (p<0.05). Where PH–Plant height, Brno- Branch number, FLWPP–fresh leaf weight per plant, FSW- Fresh stem weight, EOC-essential oil content, EOY-essential oil yield, DS-disease severity and DC disease control.

Table 3: Efficacy of essential oil on on sage *Fusarium* wilts in green house condition.

Conclusion

In conclusion lemon grass and spear mint essential oils showed the maximum anti-fungal activity against the *Fusarium* wilt on both sage and rosemary plants, whereas *Eucalyptus* inhibit the list amount of growth pathogen. If these antifungal activities also effective in field conditions, they can be used as option for the management of *Fusarium* wilt and used as alternative to chemical control which is environmentally safe. Therefore, developing commercial products containing several plant essential oils might be promising as eco-friendly strategy to control sage and rosemary *Fusarium* wilt.

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