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Antifungal Effect of Bay Laurel (*Laurus nobilis*) and Myrtle (*Myrtus communis* L) Essential Oil on Chickpea Blight (*Ascochyta rabiei*)

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Abstract: The present study was conducted with the aim to investigate, antifungal activities *in vitro* conditions of the essential oil retrieved from laurel (*Laurus nobilis*) and myrtle **(***Myrtus communis* L.) on three different isolates of the chickpea blight disease (*Ascochyta rabiei*). The antifungal activity study was conducted in 60 mm in diameter petris with 10 ml PDA environment, and the plantation of fungi was done in the middle of the petri in a diameter of 5 mm. The effected of essential oil was added to sterile blotting paper attached on the lids of the petris in 0,5, 1, 2, 4 ve 8 µl doses with the help of a micropipette. The petri lids covered with parafilm were incubated in 23 ° C for 14 days. Measurements were done after the incubation period and the percentage of mycelial growth was calculated. The results of the study revealed that the essential oil obtained from *Laurus nobilis* inhibited the disease growth of isolates-1, isolates-2 and isolates 3 in the dose of 8 µl by 72,53%, 67,37 % and 45,8%, respectively. It was found that the essential oil obtained from *Myrtus communis* L inhibited the disease growth of isolates-1, isolates-2 and isolates 3 in the dose of 8 µl by 45,19%, 57,35% and 39,36%, respectively. Furthermore, it was established that the fungus growth was inhibited in different degrees in the different doses. It was concluded that the essential oil of *Laurus nobilis* and *Myrtus communis* L possess a high rate of antifungal activities *in vitro* conditions.

Keywords: *Ascochyta rabiei***,** Essentials oil, *Laurus nobilis, Myrtus communis* L

INTRODUCTION

Chickpea blight is registered as a disease in more than 35 countries and causes great loss in quality and crop. If appropriate conditions are met for the growth of the disease the crop loss can reach 100% [1, 2]. While various methods were suggested to ensure the control of the disease, one of the outstanding methods is the use of fungicide. Due to the residual and environmental damage of the chemical medicines used, researchers maintain to work on the development of herbal medicine [3] and the efficiency of these chemicals on herbal diseases.

The Lauraceae family consists of 45 species and around 1000 types. Whereas the Laurus species has two types, *Laurus nobilis* L. (Defne) and *L. canariensis* Wild, and only the *Laurus nobilis* L. type can be found in Turkey [4,5] The essential oil of the laurel plant is not only used as an aroma in the food and cosmetic industries but also in the production of soap. Its dry leaves are also used for the making of tea. The essential oil of laurel was reported to be antimicrobial, analgesic, anti-inflammatory and anti-tumour [6-9].

Myrtus communis L. is a perennial bush plant. It can be found in various provinces such as Adana, İstanbul, Sinop, Ordu, Hatay and Antalya [10]. A large variety of studies exists on the exploration of the chemical components of the *M. communis* L. essential oil [11, 12]. Furthermore, studies were conducted on the biological activities of *M. communis.* The antimicrobial, antibacterial, antiviral, insecticidal and antifungal activities were analyzed. Antifungal activities were determined in the *C. albicans* [13]; *R. solani* [14]; pathogens. Nevertheless, a thorough review of the literature yielded no study on chickpea anthracnose isolates.

The present study was conducted with the aim to explore antifungal influence *in vitro* conditions of the essential oil of *Myrtus communis* L. (myrtle) and *Laurus nobilis* L. on three different isolates of the chickpea blight disease (*Ascochyta rabiei*).

MATERIALS AND METHODS

The plant *Laurus nobilis* L. was gathered in Alanya- Antalya and the plant *Myrtus communis* L. was collected in Demre-Antalya in 2016 by extracting the of the ground surface. The essential oil was obtained with the use of the hydro-distillation method by a Clevenger apparatus after the collected plant material was dried at room temperature without direct sunlight. The essential oil obtained was kept in a fridge at 4°C until used.

The development of fungi cultures

The fungi cultures were obtained from the stock cultures in the Phytopathology laboratories of the Ahi Evran University. The factor was developed in the potato dextrose agar (PDA). The factor was developed by two-week incubation at 25±2 ºC temperature through a 12-hour dark and 12-hour light period. This environment was used in the study.

The exploration of the antifungal influence of essential oil in in-vitro conditions

Approximately 10 ml PDA were prepared in 60 mm diameter petri lids in order to explore the antifungal influence of the anthracnose disease. The sterile paper was attached on the prepared petri lids. 5mm diameter mycelium disks of the disease isolates were included in the middle part of the petri container. After, essential oil retrieved from *Myrtus communis* L. and *Laurus nobilis* L. were added with a micropipette on the attached paper in concentrations of 0,5, 1, 2, 4 and 8 µl petri^{-1}. The petri lids covered with parafilm were kept in incubation for 14 days at 25±2 ºC through a 12-hour dark and 12-hour light period. The growth of the disease was measured with a calliper after the incubation period. The applications were replicated four times and repeated biologically. The disease growth percentage was calculated by comparing the inhibition of growth and the inhibition of growth of the control group based on Pandey *et al*. [15]

$I=100\times(dc-dt)/dc$

I: percentage of disease growth inhibition
dc: control of disease development
dt: disease growth treated with essential oil

***Statistic Analysis*:** All calculations were done through Statistical Package for the Social Sciences 15 (SPSS) [16]. The p-value $p<0.05$ was accepted as significant in the variance analyses.

RESULTS AND DISCUSSIONS

The present study explored the antifungal activities in three different anthracnose isolates of different doses of essential oil retrieved from *Myrtus communis* L. and *Laurus nobilis* L. on chickpea blight (*Ascochyta rabiei*), which causes fundamental crop loss. The influence of *Myrtus communis* L. and *Laurus nobilis* L. essential oil on the mycelial growth of the anthracnose isolates is presented in Table 1, Table 2, Figure-1 and Figure-2.

It was noted that the essential oil retrieved from *Myrtus communis* L. inhibited in different rates the mycelial growth of the anthracnose isolates (Table-1).

Table-1: The effect of the *Myrtus communis* L. essential oil on the mycelial growth of the anthracnose isolates

Doses (µl pedri^{-1})	*Myrtus communis*		
	Isolate 1	Isolate 2	Isolate 3
Control	38.38a±0.60	39.14a±0.61	44.45a±0.53
0,5	36.70a±0.69	35.45b±0.68	42.31b±0.54
1	34.46b±0.52	27.81c±0.57	41.48b±0.56
2	32.64c±0.66	26.26c±0.47	39.15c±0.55
4	28.41d±0.49	22.51d±0.40	31.39d±0.65
8	20.58e±0.50	16.57e±0.69	26.64e±0.47

* Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)

It was found that the *Myrtus communis* L. essential oil had the greatest influence on isolate 2, followed by isolate 1 and isolate 3. The effect of the doses on the isolates showed statistically different levels of significance based on $p<0,05$. Negative influence increased depending on the increased doses. The percentages of the influence of the *Myrtus communis* L. essential oil on the mycelial growth of the isolates was presented in Figure-1.

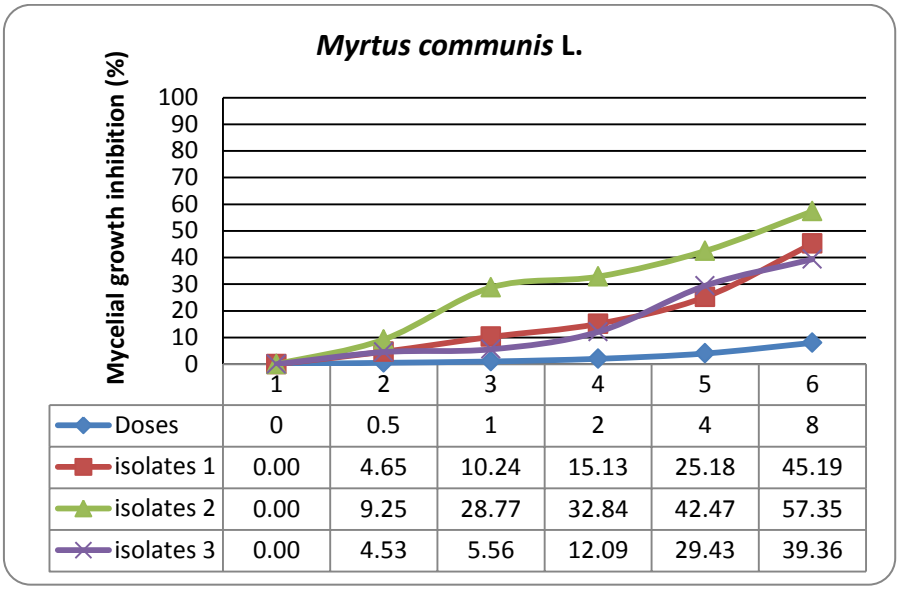


Fig-1: The effect of *Myrtus communis* L. essential oil on the mycelial growth inhibition of anthracnose isolates

The essential oil retrieved from *Myrtus communis* L was found to inhibit the mycelia growth of isolate-1, isolate-2 and isolate-3 for 8 µl petri^{-1} doses by 45,19%, 57,35% and 39,36%, respectively. The most sensitive isolate to the essential oil was revealed to be isolate 2, followed by isolate-1 and isolate-3. The biological activities of *M. communis essential oil* and extracts were reported by various researchers in previous studies. It was claimed that the *M. communis* essential oil showed weak antifungal activities for *Fusarium solani* and *Rhizoctonia solani*; whereas for *Alternaria alternata* and *Botrytis cinerea* it revealed to have activities by 47,2% and 77,2%, respectively [14,17]. *Myrtus communis* demonstrates antioxidant, antibacterial, insecticidal and cytotoxic activities [18]. In addition, Yilar *et al.* [19] remarked that the M. *communis* essential oil has a fundamental antifungal effect on the mycelia growth of plant pathogens *Verticillium dahliae*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, *Sclerotinia sclerotiorum* and *Rhizoctania solani*.

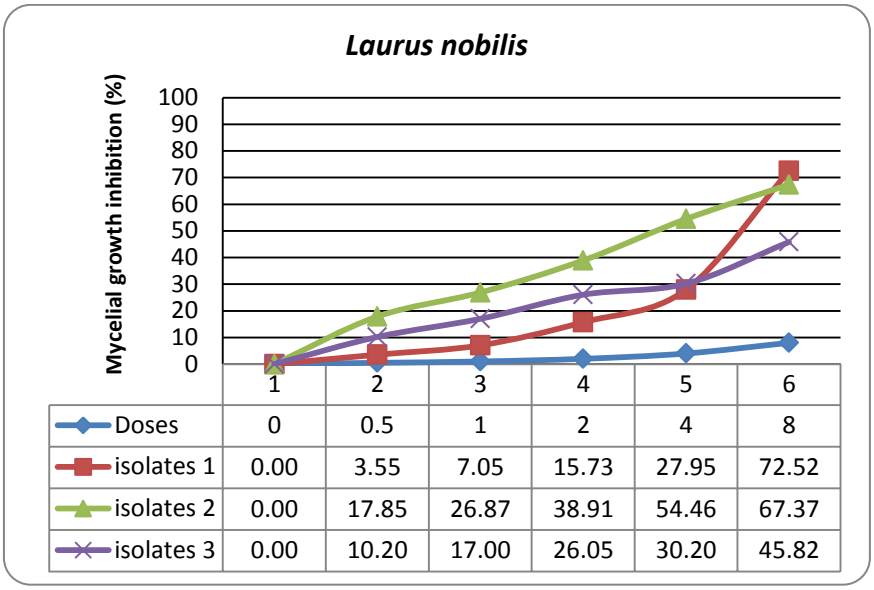
The effect of *Laurus nobilis* L. essential oil on the mycelia growth of the isolates was presented in Table 2. A statistically significant difference was found between the doses and the isolates ($p<0.05$).

Table-2: The effect of *Laurus nobilis* L. essential oil on the mycelia growth of the anthracnose isolates

Doses (µl pedri^{-1})	*Laurus nobilis*		
	Isolate 1	Isolate 2	Isolate 3
Control	38.38a±0.60	39.20a±0.56	44.54a±0.65
0,5	36.68b±0.54	32.15b±0.57	39.53b±0.55
1	35.55b±0.57	28.54c±0.63	36.72c±0.53
2	32.25c±0.52	23.65d±0.62	32.52d±0.54
4	27.54d±0.49	17.52e±0.56	31.41d±0.60
8	10.54e±0.46	12.55f±0.53	24.22e±0.68

* Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)

It was found that that the effect of the essential oil on isolate 1 was considerably high and that the mycelia growth based on the increasing application doses was affected much greater than the other isolates. This isolate was followed by the mycelia growth of isolate 2 and isolate 3. The mycelia growth of isolate 3 was affected the least by the essential oil application among the other isolates.



	1	2	3	4	5	6
Doses	0	0.5	1	2	4	8
isolates 1	0.00	3.55	7.05	15.73	27.95	72.52
isolates 2	0.00	17.85	26.87	38.91	54.46	67.37
isolates 3	0.00	10.20	17.00	26.05	30.20	45.82

Fig-2: The effect of *Laurus nobilis* essential oil on the mycelial growth inhibition of anthracnose isolates

The effect of *L. nobilis* essential oil is presented in Figure-1. The 8 µl doses of the obtained essential oil inhibited the disease growth of isolate-1, isolate-2 and isolate-3 by 72,53%, 67,37% and 45,82%. The mycelia growth of isolate 1 was affected the most by the fungi of the essential oil. This isolate was followed by isolate 2 and isolate 3.

Similar studies conducted on *L .nobilis* also reported biological activities. Antifungal activities were found of the *L. nobilis* essential oil on Candida spp [20]; *Glomus deserticola*, *G. intraradices* mycorrhizal fungi [21]. Furthermore, antimicrobial and antioxidant activities of the *L. nobilis* plant were revealed by researchers [22, 23]. Considering similar studies, it was reported by researchers that *M. communis* L. and *L. nobilis* essential oils and extracts showed considerably high activities against fungi and bacteria. These results are in accordance with and support our study.

CONCLUSION

A thorough literature review yielded no study on the effect of essential oil of *M. communis* L. and *L. nobilis* on anthracnose isolates. As a conclusion, the essential oils of *M. communis* L. and *L. nobilis* showed significant activities against anthracnose isolates. This study may be presented as an initial study on the probable use of essential oils against plant pathogens.

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