Scientific Papers. Series A. Agronomy, Vol. LIX, 2016
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ANTIFUNGAL ACTIVITY OF *Liquidambar orientalis* L., and *Myrtus communis* L. AGAINST SOME PLANT PATHOGENIC FUNGI

Abdurrahman ONARAN[1], Yusuf BAYAN[2]

[1]Gaziosmanpaşa Universty, Faculty of Agriculture, Department of Plant Protection, 60250, Tokat, Turkey
[2]Ahi Evran University, Faculty of Agriculture, Deparment of Plant Protection, 40100, Kirsehir, Turkey

Corresponding author email: abdonaran@hotmail.com

Abstract

As a result of the negative effects of pesticides used in the agricultural field, studies to find alternative methods have to be developed. In this study, Liquidambar orientalis L. (leaf and resin) and Myrtus communis L. (leaf) of methanol extracts of antifungal activities were determined against plant pathogens such as Fusarium oxysporum f. sp. lycopersici (FOL) (Sacc.) W.C. Snyder and H. N. Hans, Alternaria solani (Ell. and G. Martin), Botrytis cinereapers.:Fr, Rhizoctonia solani Kühn, and Sclerotinia sclerotiorum Lib De Bary which are caused intensive yield loss on tomatoes, strawberries, potatoes and cucumbers both in Turkey and in the world, In order to examine the used plant extracts, percentages of mycelium inhibition (MGI) values were calculated to compare with the positive control (80% Thiram), which is a standard fungicide. Also, the antifungal activities of plant extracts were evaluated statistically. 50 mg, 100 mg, 200 mg and 400 mg/mL of plant extract doses were used. L. orientalis and M. communis were shown distinguished antifungal activity. Plant extract of M. communis showed a strong antifungal effect against the tested fungi when compared with L. orientalis. As a result, nowadays the natural bio-pesticides used are cheap and eco-friendly; therefore they have potential in the control against plant pathogens.

Key words: *plant pathogens, plant extracts, antifungal activity, Liquidambar orientalis, Myrtus communis.*

INTRODUCTION

The chemical control of plant diseases have caused severe problems, so that, has been accelerated to work on new effective alternative control methods , particularly in the developed countries. As a result of using pesticides, natural environment was affected, as well as human health (Delen and Tosun, 1997). There are many studies on the fungicide, herbicide and insecticidal effects of components and essential oils within the plants and their biological activities (Dudai et al., 1999; Gören et al., 2002; Cavanagh and Wilkinson, 2002; Dulger and Hacıoğlu, 2008; Almedia et al., 2010; Kalkışım, 2012; Kordali et al., 2013).
L. orientalis is an endemic tree from Altingiaceae family which grown in Fethiye and Mugla, Turkey (Anonim, 2015a). *M. communis* is aplant from *Myrtaceae* family, also named as Mersin or Murt and it is in the form of bush. It is commonly seen in places where Mediterranean climate is dominant, especially in coastal areas (Anonim, 2015b).
Fusarium oxysporum f.sp. *lycopersici* (FOL), *B. cinerea*, *R. solani*, *A. solani* and *S. sclerotiorum* are responsible for plant diseases that cause significant yield losses both in Turkey and worldwide. *FOL* is known as crown and root rot disease in tomato (Can et al., 2004). *B. cinerea* causes significant yield loss of fruit on strawberry (Williamson et al., 2007). *S. sclerotiorum* is known as white mold on more than 400 plant species. It causes diseases on stems, fruits and roots of cucumbers (Yanar and Onaran, 2011). The known as early blight disease of *A. solani* is widely seen on tomatoes (Yazıcı et al., 2011). One biotic factor that causes significant yield losses in potato crops is *R. solani*, responsible for soft decay of roots and bumps (Yanar et al., 2005).
In this study, the antifungal activities of the methanolic plant extracts from different parts of *L. orientalis, M. communis* against five different plant pathogens of were determined.

MATERIALS AND METHODS

Plant Materials. Plant species of *L. orientalis* and *M. communis* were collected from Muğla and Antalya province in Turkey 2014 (Table 1). The plant parts were air-dried at room temperature for three weeks in dark conditions. The dried plant parts were milled to a fine powder in a mill.

Table 1. List of plant species

Scientific name	Family	Part used
Liquidambar orientalis L.	*Altingiaceae*	Leaf, Resin
Myrtus communis L.	*Myrtaceae*	Leaf

Fungi Cultures. The plants pathogenic fungi (Table 2) tested in this research were obtained from the stock cultures of laboratory of phytoclinic, Department of Plant Protection, Faculty of Agriculture, University of Ahi Evran, Turkey. Plant pathogens were grown on Petri dishes on PDA and incubated at 25±2ºC for 7 days.

Table 2. List of plant pathogens

Plant pathogens	Origin
Fusarium oxysporum f. sp. lycopersici (Sacc.) W.C. Snyder and H.N. Hans	Tomato
*Botrytis cinerea pers.:*Fr	Strawberry
Rhizoctonia solani Kühn.	Potato
Alternaria solani (Ell. And G. Martin)	Tomato
Sclerotinia sclerotiorum (Lib.) de Bary	Cucumber

Plant Extracts. Powdered plant materials (100g each one) were extracted with methanol by incubated on orbital shaker (Lab. Corporation Group, Model-SI-300) at 120 rpm for 72 h (30°C). After that it was evaporated to dryness in a rotary evaporator (Heildolph Group, Model-Hei-Vap Presicion). The concentrate was then diluted with 50% Acetone. Each plant extract was used at 50, 100, 200 and, 400 mg/ml (Kadıoğlu et al 2004).

In vitro Antifungal Activity. Plant extracts were added to PDA at 40°C to give the final concentrations of 50, 100, 200 and 400 mg/mL for each extract. Seven-day-old agar discs (5 mm in diameter) bearing the desired fungus was transferred in the Petri plates. These fungus cultures were incubated at 25±2°C for 7 days. Mycelial growth was recorded daily (Onaran and Yılar, 2012). Commercial fungicide [Thiram 80% (Hektaş, group)] was used as a positive control. 50% Acetone was used as a negative control. Experiment was set up in four replications and repeated twice.

The percentage of mycelial growth inhibition was calculated accordingly the formula mentioned by Pandey et al. (1982):

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

I: Mycelial growth inhibition

dc: the mycelial growth in control

dt: the mycelial growth in treatment

Statistical Analysis. The data were analyzed by using Analysis of Variance (ANOVA) test. Differences between means were determined by the TUKEY test (at the 0.05 probability level). The software SPSS 13.0 was used to conduct all the statistical analysis.

RESULTS AND DISCUSSIONS

The antifungal activity of plant extracts against *FOL, B. cinerea, R. solani, A. solani* and *S. sclerotiorum*, expressed as Mycelial growth inhibition (MGI) (Figures 1-3 and Table 3).

No 100% inhibition was observed in any of the plant extracts used. But, compared to control, inhibition of mycelial growth occurred in generally, as the concentration increased. No activity was observed in all tested concentrations of *L. orientalis* leaf extracts against *FOL* and *S. sclerotiorum* (Figure 1). On the other hand, antifungal effect was observed in *L. orientalis* leaf extract against *B. cinerea, R. solani* and *A. solani* with different MGI values. *B. cinerea* was exposed to inhibition at 72.96-80.65%. The effect of plant extracts against *R. solani* was shown the lowest values (MGI between 10.61 and 28.32%). MGI values for *A. solani* were between 27.91 and 48.8% (Figure 1).

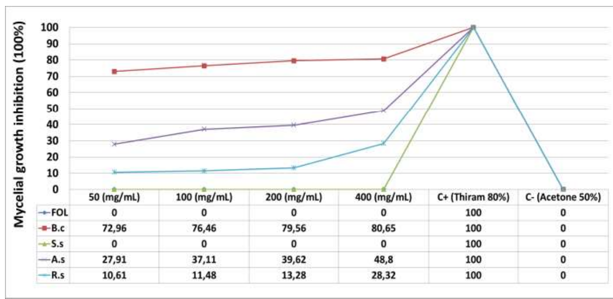

Figure 1. The effect of *L. orientalis* leaf extract on the mycelial growth inhibition of plant pathogenic fungi

Against FOL; *L. orientalis* resin extracts of 200 mg/ml and 400 mg/ml, were found between 27.93% and 39.75% values respectively (Fig. 2). The dose of 400 mg/ml of *M. communis* leaf extracts against *FOL* was observed value of 23.31% antifungal activity. Similarly, *M. communis* leaf extract at 400 mg/ml dose was determined against *S. sclerotiorum* rate of 82.40% antifungal activity (Figure 3). The dose of 400 mg/ml of *L. orientalis* resin extract against *S. sclerotiorum* was found value of 59.50% (Fig. 2). Other used plant extracts against *FOL* and *S. sclerotiorum* did not indicate any effects on doses (Figures 2-3).

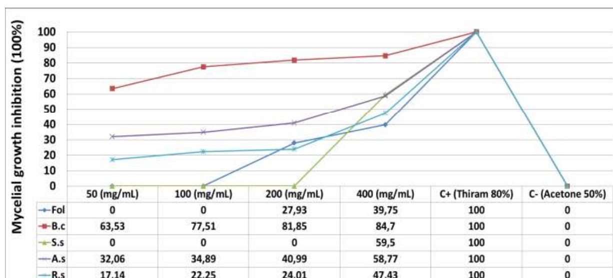

Figure 2. The effect of *L. orientalis* resin extract on the mycelial growth inhibition of plant pathogenic fungi

B. cinerea was the most affected pathogens from plant extracts in all pathogenic fungi species. This was followed by *A. solani*, *R. solani*, *FOL* and *S. sclerotiorum* respectively. The highest antifungal activity against *B. cinerea* was found in *L. orientalis* resin extract (84.70%) and of the same plant in leaf extract value was observed at 80.65%. This was followed by *A. solani* of *M. communis* leaf extract (60.71%), *R. solani* with 55.74% (*M. communis* leaf extract) and 47.43% (*L. orientalis* resin extract) respectively (Figures 1-3).

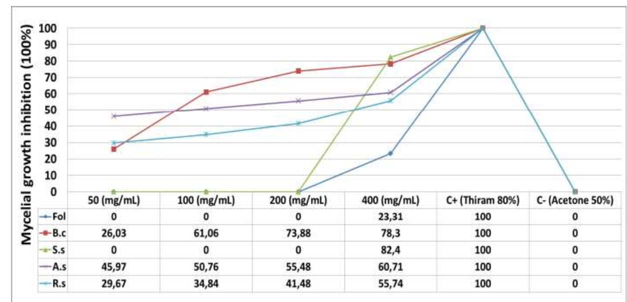

Figure 3. The effect of *M. communis* leaf extract on the mycelial growth inhibition of plant pathogenic fungi

Generally, antifungal activity of tested plant extracts varies with plant part and concentration. The antifungal effect increased with concentration. Different parts of the same plant have shown different antifungal effects. In the study, the commercial fungicide Thiram 80% was used as a positive control and showed 100% MGI against all pathogens. Acetone 50% was used as a negative control and had no effect on mycelial growth for all pathogens.

The antifungal activity of tested plant extracts were shown from the highest to the lowest were *M. communis* leaf extract, *L. orientalis* resin extract and *L. orientalis* leaf extract respectively (Table 3).

The plant extracts were evaluated in vitro conditions, on pathogens mycelial growth. The least effected was found the mycelial growth on *FOL* between 60.00 and 36.15 mm. Mycelial growth in the other plant diseases, for *S. sclerotiorum* between 60.00 and 24.32 mm, for *R. solani* between 45.64 and 22.60 mm, for *A. solani* between 38.97 and 21.24 mm and for *B. cinerea* between 44.38 and 9.18 mm were determined at different levels (Table 3).

Mycelial growth inhibition was observed at 200 mg/ml of resin extract of *L. orientalis* against *FOL* at 43,24 mm, and in 400 mg/ml doses against *FOL* at 36,15 mm and *S. sclerotiorum* at 24.32 mm. The leaf extract of *M. communis* was shown the mycelial growth inhibition in 400 mg/ml doses against *FOL* at 40.01mm and *S. sclerotiorum* at 10.59 mm. The mycelial growth inhibition was displayed similar values for *FOL* and *S. sclerotiorum* at other all plant extracts. But for *B. cinerea, R. solani* and *A. solani*; different antifungal activity values were exhibited by all plant parts and doses (Table 3).

Tablo 3. The antifungal activity of plant extract on the mycelium growth of plant pathogenic fungi

Plants	Parts	Concentration (mg/mL)	[1]Plant Pathogens				
			FOL	**B.c**	**S.s**	**A.s**	**R.s**
C+	Thiram	80%	0.00^{2}±0.00^{e3}	0.00±0.00^{j}	0.00±0.00^{e}	0.00±0.00^{v}	0.00±0.00^{t}
C-	Acetone	50%	60±0.00^{a}	60±0.00^{a}	60±0.00^{a}	54.06±0.00^{a}	51.06±0.00^{a}
[2]***L.o***	Leaf	50	60±0.00^{a}	16.22±1.26$^{d-ı}$	60±0.00^{a}	38.97±1.49$^{c-ı}$	45.64±0.56^{a-f}
		100	60±0.00^{a}	14.12±0.79$^{d-ı}$	60±0.00^{a}	34.00±0.73^{h-m}	45.20±0.82^{b-g}
		200	60±0.00^{a}	12.26±0.68$^{f-ı}$	60±0.00^{a}	32.64±0.47$^{ı-o}$	44.28±0.52$^{b-ı}$
		400	60±0.00^{a}	11.61±0.86^{g-j}	60±0.00^{a}	27.68±0.89^{m-t}	36.6±1.14^{nop}
	Resin	50	60±0.00^{a}	21.88±3.51^{c-h}	60±0.00^{a}	36.73±1.21^{f-k}	42.31±0.55^{d-m}
		100	60±0.00^{a}	13.49±2.19$^{e-ı}$	60±0.00^{a}	35.20±1.97^{g-l}	39.70±0.41^{g-o}
		200	43.24±3.95^{bc}	10.89±0.65^{h-j}	60±0.00^{a}	31.90±1.10^{j-p}	38.80±0.57$^{ı-o}$
		400	36.15±2.69^{d}	9.18±1.22$^{ı-j}$	24.32±9.24^{c}	22.29±1.44^{stu}	26.84±1.31^{rs}
M.c	Leaf	50	60±0.00^{a}	44.38±3.32^{b}	60±0.00^{a}	29.21±0.68^{l-r}	35.91±1.28^{op}
		100	60±0.00^{a}	23.36±5.70^{c-g}	60±0.00^{a}	26.62±2.70^{n-t}	33.27±0.75^{pq}
		200	60±0.00^{a}	15.67±0.21$^{d-ı}$	60±0.00^{a}	24.07±0.55^{q-u}	29.88±1.17^{qr}
		400	46.01±2.64^{b}	13.02±0.40$^{e-ı}$	10.59±0.50^{d}	21.24±0.53^{tu}	22.60±0.81^{s}

[1]Plant pathogens; *F. oxysporum f. sp lycopersici*=**FOL**, *B. cinerea*=**B.c**, *S. sclerotiorum*=**S.s**, *A. solani*=**A.s**, *R. solani*=**R.s**. [2]Plants . ***L.o*: *Liquidambar orientalis;* **M.c**:*Myrtus communis*. C+=Positive control, C-=Negative control
[3]Means in the same column by the same letter are not significantly different to the test of TUKEY.

In similar studies, other researchers have stated that the plant extracts used in our study were effective against the different plant pathogens at different values. Resin extract of *L. orientalis* 28x10^{-3} mg/mL air concentration had showed antifungal effect against *Phytophthora cactorum*, *Cryphonectria parasitica* and *Fusarium circinatum,* and 17x10^{-3} mg/mL air concentration was effective on *P.cactorum* and *F.circinatum pathogens,* whereas 7x10-3 mg/mL and 3.5x10-3 mg/mL air concentrations were only effective against *P.cactorum* pathogen (Lee et al., 2009). Similarly, plant extracts of *L. orientalis* in different doses and parts antimicrobial activity values was determined (Özcan et al., 2005; Oksay and Sarı 2005). Another study has evaluated the *n*-hexane, methanol, ethanol, ethyl-acetate and water extracts of M. communis to be finding antibacterial and cytotoxic activities. The antibacterial activities of extracts were tested on bacteria *Escherichia coli ATCC 29998, E.coli ATCC 25922, E.coli ATCC 11230, Staphylococcus aureus ATCC 6538P, S. aureus ATCC 29213, S. epidermidis ATCC 12228, Salmonella typhimurium CCM 5445, Enterobacter cloacae ATCC 13047, Enterococcus faecalis ATCC 29212, Pseudomonas aeruginosa ATCC 27853* and on fungus *Candida albicans ATCC 10239*. *E. coli ATCC 29998, E. coli ATCC 11230, S. epidermidis ATCC 12228, S. typhimurium CCM 5445* and *P. aeruginosa ATCC 27853* were shown different antifungal activities. Only the methanol extract obviously displayed antibacterial effect on ATCC 25922. None of the extracts was effective on ATCC 13047 and ATCC 29212 (Mert et al., 2008).

CONCLUSIONS

The plant species used in our study were determined to have different levels of antifungal effect. These differences change between 84.70% and 0.00% depending on mycelium inhibition rates; different results appeared according to the plant species and in different parts of plants. The result obtained after using different parts of different plants have caused varied results in mycelium inhibition values. Antifungal activities against all test organisms have reduced mycelium growth at the level of observable in a dose dependent manner. Hopeful results were obtained from the use of plant extracts controls. According to these results, the antifungal activities of the extracts obtained from different parts of plants to be used against plant pathogens (*FOL, B. cinerea, S. sclerotiorum, A. solani* and *R. solani*) were revealed in our study. One of the most important results is provided alternative control methods by plant based bio pesticides against other commercial uses chemicals pesticides. In this manner, the disadvantages caused by chemical control will be minimized.

ACKNOWLEDGEMENT

This study was supported by University of Ahi Evran with the Project Number PYO. ZRT.4001.14.001.

REFERENCES

Almeida L.F.R., Frei F., Mancini E., Marttino L.D., Feo V.D., 2010. Phytotoxic Activities of Mediterranean Essential Oils. Molecules, 15; 4309-4323.

Anonim., 2015a. Anadolu sığla ağacı (*Liquidambar orientalis*). Erişim Tarihi:22.05.2015. http://tr.wikipedia.org/wiki/Anadolu_s%C4%B1%C4%9Fla_a%C4%9Fac%C4%B1

Anomim., 2015b. Mersin (*Myrtus communis*). Erişim Tarihi:22.05.2015.
http://tr.wikipedia.org/wiki/Mersin_%28bitki%29

Can C., Yücel S., Korolev N., Katan T., 2004. First report of fusarium crown and root rot of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Turkey. Plant Pathol. 53(6):814.

Cavanagh H.M.A., Wilkinson J.M., 2002. Biological Activities of Lavender Essential Oil. Phytotherapy Research, Phytother Res, 16; 301-308.

Delen N, Tosun N., 1997. Türkiye'de Pestisit Kullanımının Toksikolojik Değerlendirilmesi. II. Ulusal Toksikoloji Kongresi, Antalya-Türkiye, 90-95.

Dudai, N., Poljakof-Mayber, A., Mayer A.M., Putıevsky, E., Lerner, H.R., 1999. Essential oils as allelochemicals and their potential use as bioherbicides. Journal of Chemical Ecology, 25; 5:1079-1089.

Dülger B., Hacıoğlu N., 2008. Antifungal Activity of Endemic *Salvia tigrina* in Turkey. Tropical Journal of Pharmaceutical Research, 7;3:1051-1054.

Gören A.C., Topçu G., Bilsel G., Bilsel M., Aydoğmuş Z., Pezzuto M.P., 2002. The Chemical Constituents and Biological Activity of Essential Oil of *Lavandula stoechas* ssp. *stoechas*. Z. Naturforsch, 57:797-800.

Kadıoğlu İ., Yanar Y., Asav U., 2004. Allelopathic Effects of Plant Extracts Against Seed Germination of Some Weeds, Asian J. of Plant Sciences, 3; 4: 472-475.

Kalkışım Ö., 2012. In vitro antifungal evaluation of various plant extracts against walnut anthracnose (*Gnomonia leptostyla* (Fr.) Ces et de Not.). J. Food Agrıc. Environ. 10 (3&4): 309-313.

Kordali S., Usanmaz A., Cakir A., Cavusoglu A., Ercisli S., 2013. In Vitro antifungal effect of essential oils from *Nepeta meyeri* Benth. Egypt. J. Biol. Pest. Control 23(2): 209-213.

Lee Y.S., Kim J., Lee S.G., Oh E., Shin S.C., Park I.K., 2009. Effects of plant essential oils and components from Oriental sweet gum (*Liquidambar orientalis*) on growth and morphogenesis of three phytopathogenic fungi. Pestic. Biochem. Phys. 93. 138-143.

Mert T., Fafal T., Kıvçak B., Öztürk, H. T., 2008. *Myrtus communis*'in Antimikrobiyal ve Sitotoksik Aktiviteleri. Ankara Ecz. Fak. Derg. 37 (3) 191-199.

Onaran A., Yılar M. 2012. Antifungal activity of *Trachystemon orientalis* L. aqueous extracts against plant pathogens. Journal of Food, Agriculture & Environment 10 (3&4), p. 287-291.

Oskay M., Sarı D., 2007. Antimicrobial Screening of Some Turkish Medicinal Plants. Pharmaceutical Biology. 45**,** 176-181.

Özcan M., Özkan G., Özçelik S., Sağdıç O., 2005. A study on inhibitory effect of sığla tree (*Liquidambar orientalis mill. Var. Orientalis*) storax againts several bacteria. Phytother.Res**.** 19:549-551.

Pandey D.K., Tripathi N.N., Tripathi R.D., Dixit S.N., 1982. Fungitoxic and phytotoxic properties of essential oil of Hyptis suaveolens. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 89:344-349.

Williamson B., Tudzynski B., Tudzynski P., Kan J.A.L., 2007. *Botrytis cinerea*: the cause of grey mould disease. Molecular Plant Pathology 8: 561-580.

Yanar Y., Yılmaz G., Cesmeli I., Coskun S., 2005. Characterization of *Rhizoctonia solani* isolates from potatoes in turkey and screening potato cultivars for resistance to AG-3. *Phytoparasitica* 33:370–376.

Yanar Y., Onaran A., 2011. Mycelial compatibility groups and pathogenicity of *Sclerotinia sclerotiorum* (Lib.) De Bary causal agent of white mold disease of greenhouse grown cucumber in Antalya-Turkey. African Journal of Biotechnology, 10: 3739–3746.

Yazici S., Yanar Y., Karaman I., 2011. Evaluation of bacteria for biological control of early blight disease of tomato. Afr. J. Biotechnol. 10:1573-1577.

View publication stats