© by PSP Volume 28 – No. 3/2019 pages 2170-2175 Fresenius Environmental Bulletin

ANTIFUNGAL POTENTIAL OF ESSENTIAL OILS OF *SALVIA OFFICINALIS* AND *SALVIA TOMENTOSA* PLANTS ON SIX DIFFERENT ISOLATES OF *ASCOCHYTA RABIEI* (PASS.) LABR.

Melih Yilar*, Yusuf Bayar

Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Kirsehir, Turkey

ABSTRACT

This study was conducted to determine the antifungal activity of the essential oils of the *Salvia officinalis* and *Salvia tomentosa* plants on *Ascochyta rabiei* (Chickpea blight) which is an important chickpea disease. As a result of the study, *S. officinalis* and *S. tomentosa* plant essential oils were applied to the isolates of *A. rabiei* at the doses of 0 (Control), 1, 2, 4, 8, 10 µL petri^{-1}. Essential oils are impregnated on the filter papers sticked to the petri dish with a micropipette at the application doses and the lids were immediately covered with a parafilm and left to incubate at the temperature of 23 °C for 15 days. At the end of the 15-day incubation, the measurements of mycelium diameters of isolates in petri dishes were carried out with electronic calipers. As a result, *S. officinalis* essential oil inhibited mycelium growth of Isolate 2 and Isolate 3 by 100% at the dose of 10 µL petri^{-1}. *S. tomentosa* essential oil inhibited the mycelium growth of Isolate 3, Isolate 4, Isolate 5, Isolate 6 by 100%. A difference was found in the responses of *Anthracnose* isolates to plant essential oils and to application doses. *S. tomentosa* was found to be more effective on the isolates. The findings indicate that both of these essential oils can be used as an alternative to synthetic fungicides in controlling the *A. rabiei* pathogen.

KEYWORDS:
Antifungal activity, *Ascochyta rabiei*, essential oil, Salvia

INTRODUCTION

Being a protein source for people around the world, edible grain legumes meet the vegetable protein need of human nutrition by 22% and of animal nutrition by 38%. Turkey is one of the gene centers of legumes [1]. Chickpea, which is in the group of edible grain legumes, is the first plant to be cultivated [2] and it is one of the important plant protein sources rich in amino acids such as leucine, histidine, isoleucine, lysine, phenylalanine, trionin, valine, which are of importance in human nutrition [3]. In Turkey in 2016, the cultivation area of chickpeas was 360 ha and the production was 455 thousand tons [4]; however, this production amount is not at the desired level, which may be due to the fact that the producerss do not apply appropriate cultivating techniques and the problems, such as, failure to combat the disease, pests and weeds and resistant varieties are not as widespread as they should be.

The grain yield of chickpea is significantly affected by abiotic factors (drought and salinity, etc.). In addition, there are more than 50 plant pathogens known to affect the chickpea yield adversely [5]. Among these pathogens, *Ascochyta rabiei* (Chickpea blight) is widely seen in the production areas in Turkey as well as in the world [6] and causes significant yield losses. In addition to causing infection in all aboveground parts of the plant, this fungus causes circular necrotic stains on leaflets and cones. Under favorable environmental conditions and in the presence of susceptible hosts, this pathogen causes up to 100% product losses in chickpea production areas [7, 8].

One of the methods that producers use extensively in controlling *Ascochyta rabiei* pathogen is chemical spraying. Researchers have turned to alternative fighting methods due to the fact that the chemical drugs used in disease control have detrimental effects on the environment and human health and that the pathogen gains resistance to these drugs that were used. One of these methods is the use of herbal medicines. For this reason, researchers have begun and still continue to carry out studies on the use of herbal essential oils and extracts, which are environmentally and human-friendly, in controlling diseases, pests and weeds.

This study was conducted to investigate the effectiveness of *Salvia officinalis* and *Salvia tomentosa* plant essential oils against 6 different isolates of *Ascochyta rabiei* pathogen causing significant loss in chickpea production areas.

TABLE 1
The antifungal activity of *Salvia officinalis* and *Salvia tomentosa* essential oils on the mycelium growth of *A. rabiei* isolates

Essential oils	Doses µlpetri $^{-1}$	Mycelium growth(mm)					
		isolate 1	isolate 2	isolate 3	isolate 4	isolate 5	isolate 6
Salvia officinalis	Control	44.42ab*±0.36	33.22a±0.64	32.80a±0.89	45.68a±0.63	38.35a±1.61	45.32a±1.02
	1	37.63b±2.48	29.13a±1.64	29.87b±0.09	39.28ab±3.67	33.01ab±1.75	39.16ab±1.34
	2	32.02b±5.62	24.01b±2.20	22.26c±0.10	32.4bc±2.54	28.59b±1.39	34.8bc±3.88
	4	29.12b±3.90	21.41bc±1.65	20.39d±0.07	27.09c±2.08	27.82b±1.02	30.52c±2.82
	8	17.59c±0.98	17.42c±1.28	14.89e±0.42	14.97d±2.39	17.35c±2.03	21.72d±2.35
	10	12.52c±1.03	0.00d±0.00	0.00f±0.00	8.09d±4.06	16.00c±2.90	16.75d±2.26
Salvia tomentosa	Control	45.42a±0.51	43.60a±0.61	42.90a±0.63	41.21a±0.44	34.15a±0.61	38.74a±0.65
	1	33.89b±0.54	38.22b±0.52	42.13a±0.60	35.37b±0.68	30.82b±0.55	27.88b±0.59
	2	29.24c±0.62	34.33c±0.52	37.57b±0.54	28.38c±0.49	21.78c±0.63	22.65c±0.60
	4	27.22d±0.59	32.58d±0.51	27.12c±0.61	22.22d±0.51	16.36d±0.54	17.41d±0.59
	8	20.18e±0.60	26.54e±0.62	19.56d±0.65	20.77d±0.60	10.12e±0.61	16.23d±0.60
	10	18.62e±0.55	15.77f±0.48	0.00e±0.00	0.00e±0.00	0.00f±0.00	0.00e±0.00

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

MATERIALS AND METHODS

Obtaining Plant Material and Essential Oil. The plant material, *Salvia officinalis* and *Salvia tomentosa*, was collected from Tokat province in 2014-2015 by taking the aboveground parts. The essential oils were obtained from the plant materials dried in the shade at room temperature using the hydro-distillation method with the help of Clevenger apparatus. The obtained essential oils were stored in the refrigerator at 4 °C until use.

Development of Fungus Cultures. The *Ascochyta rabiei* isolates used in the experiment were obtained from stock cultures in the Phytochemical Laboratory of the Department of Plant Protection, Agricultural Faculty of Ahi Evran University. The *A. rabiei* isolates obtained from stock cultures were transferred to the PDAs and were incubated at 22±1 °C for 12 hours in the dark period and for 12 hours in the light period. The mediums developed in 7 days were used in studies.

Determination of Antifungal Effect of the Essential Oil in *In-vitro* Conditions. To determine antifungal activity, about 10 ml of PDA medium was prepared in 60 mm diameter petri dishes for the development of disease agents. 5 mm-diameter mycelium discs were transferred to the middle of the petri dishes that paper was pasted. *M. spicata* essential oil in the concentrations of 0 (Control), 1, 2, 4, 8 and 10 µl petri^{-1} was added onto the paper pasted with a micropipette. Petri dishes were covered with a parafilm and incubated for 15 days at a temperature of 23±1 °C for 12 hours in the dark and for 12 hours in the light period.

At the end of this period, the development of fungal mycelium (colony) was measured by electronic calliper. The applications were conducted 4 times with 2 repetitions. The percent inhibition of different doses of essential oil was calculated by comparing the mycelium development in the essential oil-containing petri with that of the control petri [9].

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

MGI: Mycelium Growth Inhibition rate (%)

dc: Mycelium development in the control petri

dt: Mycelium development in the petri containing essential oil

Statistical Analysis. The significance levels of the differences between the treatments were determined by analysis of variance (ANOVA) and the means were compared using the DUNCAN test. The statistical analyzes were conducted using SPSS software program (Ver.15.0, SPSS). The data obtained in the experiment were analyzed by Probit analysis and LC_{50}, LC_{90} values were calculated using the SPSS 15 analysis program.

RESULTS AND DISCUSSION

The infections caused by plant pathogenic fungi cause loss of yield in agricultural products, both in the field and after the harvest. In addition, they lead to significant damage due to the fact that they are also the infection sources in the following years. Determination of environmental and human health effects of chemical medication, which is one of the methods used to prevent these losses, and resistance problems caused by in disease agents made it difficult for producers to work on controlling diseases. For this reason, researchers have begun to work on natural compounds that may be alternative to synthetic pesticides. It has been reported in various studies that plant extracts and essential oils have a significant effect on plant pathogens [10-14].

It is revealed by this study that the effectiveness of *Salvia officinalis* and *Salvia tomentosa* essential oils against the isolates of *A. rabia* pathogen which is an important chickpea disease. The antifungal activity performed by the essential oils

on the isolates is shown in Table 1, 2 and 3 and in Figure 1 and Figure 2.

Salvia officinalis essential oil had different levels of effect on *A. rabiei* isolates. This effect varied depending on the increasing doses of *S. officinalis* essential oil and on the isolates. Isolate 2 and Isolate 3 are the most sensitive, while Isolate 5 and Isolate 6 were the isolates that showed the highest resistance at the highest dose of the essential oil. *S. officinalis* essential oil inhibited mycelium growth of Isolate 2 and Isolate 3 by 100% compared to the control (Table 1; Figure 1).

S. tomentosa essential oil was found more effective than *S. officinalis* on A. rabiei isolates. When compared to the control, *S. tomentosa* essential oil inhibited the mycelium growth of Isolate 1 and Isolate 2 by 59.00% and 63.85%, respectively, while it inhibited the mycelium growth of other *A. rabiei* isolates (Isolate 3,4,5,6) by 100% (Table 1, Figure 2).

In dose-response trials, the values LC_{50} and LC_{90} values *S. officinalis* essential oil on the isolates of *A. rabiei* (Isolate 1, 2, 3, 4, 5, 6) was found 6.57, 5.78, 5.48, 5.70, 7.85, 7.57; 13.11, 10.75, 10.19, 11.23, 15.48, 14.85, respectively (Table 2) .

In the dose-response trials in the current study, the LC_{50} and LC_{90} values of *S. tomentosa* essential oil on *A. rabiei* isolates were found 1.15, 8.45, 6.03, 5.40, 4.60, 4.39; 16.77, 15.98, 9.89, 10.52, 8.81, 9.90, respectively (Table 3).

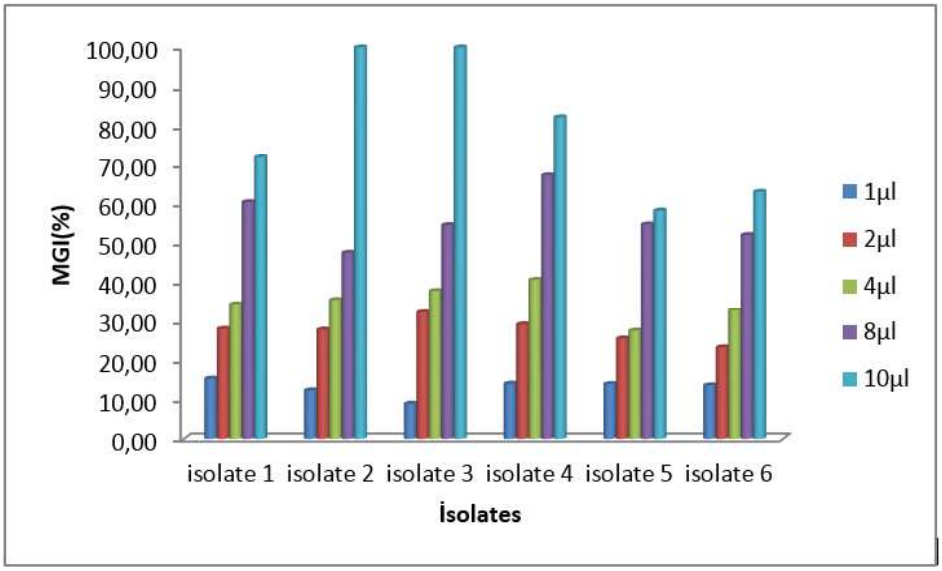

FIGURE 1
The inhibition rates of *Salvia officinalis* essential oil on *A. rabiei* isolates (%)

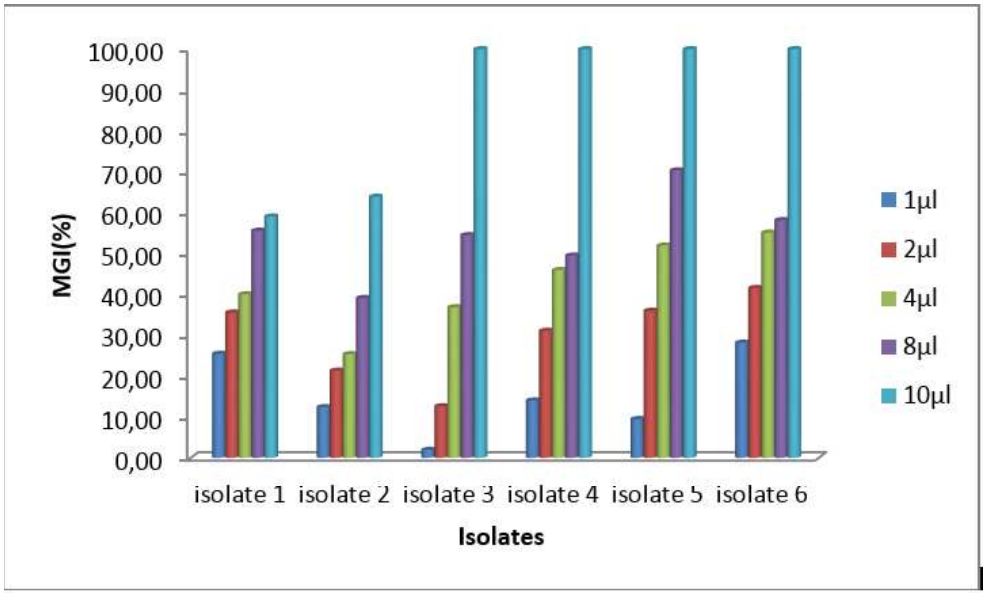

FIGURE 2
The inhibition rates of *Salvia tomentosa* essential oil on *A. rabiei* isolates (%)

TABLE 2
The dose-response results of *S. officinalis* essential oil on *A. rabiei*

Antracnose isolates	LC_{50}	LC_{90}	Slope + Standard error	X^2
isolate 1	6.57 (4.82-9.45)	13.11 (10.01-21.46)	0.20±0.016	18.21
isolate 2	5.78 (2.87-12.11)	10.75 (7.38-31.70)	0.26±0.018	61.83
isolate 3	5.48 (2.73-10.60)	10.19 (7.08-26.59)	0.27±0.019	59.57
isolate 4	5.70 (4.17-7.75)	11.23 (8.83-16.74)	0.32±0.017	18.28
isolate 5	7.85 (5.72-12.75)	15.48 (11.31-29.86)	0.68±0.016	18.84
isolate 6	7.57 (5.69-11.27)	14.85 (11.19-25.45)	0.18±0.016	16.22

TABLE 3
The dose-response results of *S. tomentosa* essential oil on *A. rabiei*

Antracnose isolates	LC_{50}	LC_{90}	Slope + Standard error	X^2
isolate 1	1.15 (3.80-31.7)	16.77(10.52-124.30)	0.133±0.015	32.73
isolate 2	8.45(6.36-13.28)	15.98(11.84-29.19)	0.17±0.016	16.62
isolate 3	6,03(4.00-9.07)	9.89(7.51-17.48)	0.33±0.21	44.31
isolate 4	5.40(2.02-13.01)	10.52(7.01-39.63)	0.25±0.018	68.69
isolate 5	4.60(2.35-7.77)	8.81(6.30-18.14)	0.30±0.20	48.26
isolate 6	4.39(0.39-12.65)	9.90(6.30-56.49)	0.23±0.018	70.66

The difference that occurs in both sage species arises from the differences in the proportions and the compounds of the essential oil compositions that the species contain. The main component of the *S. tomentosa* species collected from Tokat province was determined β-Thujene, while the main component of *S. officinalis* essential oil was determined 3-Thujanone [15]. Salvia species are rich in terms of biological activity. In the studies carried out on biological activity, it was reported by researchers that Salvia species have antibacterial [16], insecticidal [17], antioxidant [18], antiviral [19], antimicrobial [20], antifungal [21] and herbicidal effects [22]. Salvia species contain phenolic compounds and flavonoids having phytotoxic characteristic in their constituents [23].

In similar studies, researchers investigated the activities of different plant essential oils and extracts on *A. rabiei*. Zerroug et al. [24] reported that *A. rabiei* never developed mycelium at doses of 1.5, 3, 6 mg/ml of *Saccocalyx satureioides* Coss. Et. Dut. plant essential oil. In similar studies, plant extracts were investigated for their activity on *A. rabiei* mycelium development.

In controlling A. rabiei, the usability of the n-hexane extract of *Datura Metel* [25], the water, ethanol and n-hexane extracts of the leaf, fruit, root and shoot shell of *Syzgium cumulus* [26], the organic solvents of allelopathic trees [27], the organic solvent extracts of *Tagetes erectus* plant [28] and Chenopodium album extracts [29] has been revealed.

CONCLUSION

The researches that carried out on the potential of the secondary metabolites, such as, plant essential oils and extracts to be an alternative to pesticides in fighting against the pathogens, diseases, pests and weeds in economically important plants continue to be the primary studies. This study revealed that *S. officinalis* and *S. tomentosa* essential oils have a high level of antifungal effect on the *A. rabiei* isolates. It was determined that this effect differs according to isolates and sage species. Today, these studies have become more valuable owing to the fact that the fungicides used for disease control create resistance problem in plant pathogens and in addition to that their detrimental effects on the environment and people have been revealed. In the current study, it has been suggested that *S. officinalis* and *S. tomentosa* essential oils could be used in controlling the disease.

REFERENCES

[1] Gülümser, A. (2016) Situation of Pulse in Turkey and World. Journal of Field Crops Central Research Institute. 25(Special issue-1), 292-298.

[2] Biçer, B.T., Akıncı, C., Eker, S. (2017) Determination of Cold Stress, Anthracnose Disease and Seed Cooking Traits of Chickpea Winter Genotypes. El-Cezeri Journal of Science and Engineering. 4(3), 355-364.

[3] Üstün, A.S., Dolar, S. (2001) Changes in the Relative Water Content, Dry Matter and Proline Amount in the Ascochyta Blight (*Ascochyta rabiei* (Pass.) Labr.) Resistant and Susceptible Chickpea Cultivars. Journal of Agricultural Sciences. 7(1), 119-124.

[4] Anonymous (2017) Crop Production Statistics, Crop Production Statistics, Dry pulses (Dried leguminous vegatables) http://www.tuik.gov.tr/ (Date of access: 1.11.2017).

[5] Akalın, M., Yanar, Y., Akdağ, C. (2011) Reaction of Chickpea Genotypes against *Ascochyta rabiei* (Pass.) Labr. Causal Agent of Ascochyta blight Disease. Journal of Agricultural Faculty of Gaziosmanpasa University. 28(1), 21-26.

[6] Türkan, M., Dolar, F.S. (2007) The Use of Spectrophotometric Method to Determine Solanapyrones Production of *Ascochyta rabiei* (Pass) Labr., Causal Agent of Chickpea Blight. Journal of Agricultural Sciences. 13(4), 405-408.

[7] Singh, K.B. and Reddy, M.V. (1990) Patterns of resistance and susceptibility to races of Ascochyta rabiei among germ plasma accessions and breeding lines of chickpea. Plant Disease. 74, 127- 129.

[8] Bayraktar, H., Dolar, F.S., Maden, S. (2007) Mating type groups of *Ascochyta rabiei* (Teleomorph: *Didymella rabiei*), the causal agent of Chickpea Blight in Central Anatolia. Turkish Journal of Agriculture and Forestry. 31, 41-46.

[9] Pandey, D.K., Tripathi, N.N., Tripathi, R.D. and Dixit, S.N. (1982) Fungitoxic and phytotoxic properties of essential oil of *Hyptis suaandolens*. Z. Pflanzenkrankheiten Pflanzenschutz. 89, 344–349.

[10] Dižamič, A., Sokovič, M., Ristič, M., Grujič-Jovanovič, S., Vukojevič, J., Marin, P.D. (2008) Chemical composition and antifungal activity of *Salvia sclarea* (Lamiaceae) essential oil. Arch. Biol. Sci, Belgrade. 60(2), 233-237.

[11] Yılar, M., Bayan, Y., Onaran, A. (2016) Chemical Composition and Antifungal Effects of *Vitex agnus-castus* L. and *Myrtus communis* L. Plants. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 44(2), 466-471

[12] Bayan, Y. (2016) Chemical Composition and Antifungal Activity of the Plant Extracts of Turkey Cardaria Draba (L.) Desv. Egyptian Journal of Biological Pest Control. 26(3), 579-581.

[13] Onaran, A., Bayan, Y. (2016) Antifungal activity of *liquidambar orientalis* l., and *myrtus communis* l. against some plant pathogenic fungi. Scientific Papers-Series A, Agronomy. 59, 360-364.

[14] Salhi, N., Saghir, S.A.M., Terzi, V., Brahmi, I., Ghedairi, N., Bisatti, S. (2017) Antifungal Activity of Aqueous Extracts of Some Dominant Algerian Medicinal Plants. BioMed Research International. 2017, 6p.

[15] Yılar, M. (2014) Determination of Antifungal and Bioherbicidal Activities of common Salvia Speciees in Tokat Province. PhD Thesis. Gaziosmanpaşa University, Graduate School of Natural and Applied Sciences, Department of Plant Protection. Tokat

[16] Delamare, A.P.L., Moschen-Pistorello, I.T., Artico, L., Atti-Serafini, L., Echeverrigaray, S., (2007) Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chemistry. 100, 603–608.

[17] Karakoç, Ö.C., Tüfekçi, A.R., Demirtaş, İ., İpek, A. (2013) Insecticidal activity of *Salvia tchihatcheffii* and *Salvia cryptantha* volatile oils and extracts on two important storage pests. Journal of Agricultural Sciences. ISSN : 1308-3945. 6(1), 155-158.

[18] Ghorbani, A., Esmaeilizadeh, M. (2017) Pharmacological properties of Salvia officinalis and its components. Journal of Traditional and Complementary Medicine. 7(2017), 433-440.

[19] Özçelik, B., Orhan, İ.E., Kan, Y. (2011) Determination of antiviral activity and cytotoxicity of selected Sage (*Salvia* L.) species. FABAD Journal of Pharmaceutical Sciences. 36, 155-160.

[20] Cui, H., Zhang, X., Zhou, H., Zhao, C., Lin, L. (2015) Antimicrobial activity and mechanisms of *Salvia sclarea* essential oil. Botanical Studies. 56(16), 8p.

[21] Rus, C.F., Pop, G., Alexa, E., Şumalan, R.M., Copolovici, D.M. (2015) Antifungal Activty and Chemical Composition of *Salvia officinalis* L. Essential Oil. Research Journal of Agricultural Science. 47(2), 186-193.

[22] Khedher, M.R.B., Khedher, S.B., Chaieb, I., Tounsi, S., Hammami, M. (2017) Chemical Composition and Biological activities of *Salvia officinalis* essential oil from Tunisia. EXCLI Journal. 16, 160-173.

[23] Özcan, M.M., Özkan, G. (2015) Determination of antioxidant activity and total phenol contents of two Salvia extracts. Indian Journal of Traditional Knowledge. 14(2), 226-230.

[24] Zerroug, M.M., Laouer, H., Strange. R.N. and Nicklin, J. (2011) The Effect of Essential Oil of Saccocalyx Satureioides Coss. Et Dur. On the Growth of and the Production of Solanapyrone a by Ascochyta Rabiei (Pass.) Labr. Advances in Environmental Biology. 5(2), 501-506.

[25] Shafique, S., Shafique, S. (2008) Antifungal activity of n-hexane extracts of *Datura metel* against Ascochyta rabiei. Mycopath. 6(1-2), 31-35.
[26] Jabeen, K., Javaid, A. (2010) Antifungal activity of *Syzygium cumini* against *Ascochyta rabiei*-the cause of chickpea blight. Natural Product Research. 24(12), 1158-1167.
[27] Jabeen, K., Javaid, A. (2008) Antifungal activity of aqueous and organic solvent extracts of allelopathic trees against *Ascochyta rabiei*. Allelopathy Journal. 22(1), 231-237.
[28] Shafique, S., Shafique, S., Bajwa, R., Akhtar, N., Hanif, S. (2011) Fungitoxic Activity of Aqueous and Organic Solvent Extracts of *Tagetes erectus* on Phytopathogenic Fungus-*Ascochyta rabiei*. Pakistan Journal of Botany. 43(1), 59-64.
[29] Sherazi, A.Z., Jabeen, K., Iqbal, S., Yousaf, Z. (2016) Management of *Ascochyta rabiei* by *Chenopodium album* Extracts. Planta Daninha, Viçosa-MG. 34(4), 675-680.

Received: **16.11.2018**
Accepted: **29.01.2019**

CORRESPONDING AUTHOR

Melih Yilar
Ahi Evran University,
Faculty of Agriculture,
Department of Plant Protection,
Kırşehir – Turkey

e-mail: melih.yilar@ahievran.edu.tr