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Antifungal Activities of some *Salvia* Species Extracts on *Fusarium oxysporum* f. sp. *radicis- lycopersici* (Forl) Mycelium Growth *In-vitro*

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ABSTRACT

The antifungal effects of essential oils and plant extracts (water, ethanol and methanol) of *Salvia cryptantha* Montbret et Aucher ex Bentham, *Salvia tomentosa* Mill., *Salvia officinalis* L.(cultural form) grown in Tokat province was screened against *Fusarium oxysporum* f. sp. *radicis- lycopersici*. The essential oils and plant extracts of *S. officinalis*, *S. cryptantha* and *S. tomentosa* were determined to find the most effecient against *F. oxysporum* f. sp. *radicis- lycopersici in vitro*. Different volums of either essential oils and plant extracts were mixed with the sterile PDA to obtain various concentrations. The suplemented PDA were inoculated with agar disc (5 mm in diameter) of *Fusarium oxysporum* f. sp. *radicis- lycopersici* pathogens (from 7 day-old PDA cultures) were inoculated on medium. They were incubated at 25±2 ºC for 7 days. Then the bloking fungal development was calculated.The highest effects on the development of mycelium of *F. oxysporum* f. sp. *radicis- lycopersici* has shown blocking rate of 62,71% with a *S. officinalis* essential oils, this was followed by *S. tomentosa* and *S. cryptantha*. Similar results were observed in plants extracts. The highest effects on the development of mycelium of *F. oxysporum* f. sp. *radicis- lycopersici* showed *S. cryptantha* plant extracts and this S. *officinalis* and *S. tomentosa* has followed.

Key words: Antifungal activity, essential oils, *F. oxysporum* f. sp. *radicis- lycopersici, Salvia* species.

INTRODUCTION

Diseases, pests and weeds corrupting products in agricultural areas causing significant losses. For this reason, pesticides are heavily used in order to reduce the losses caused by these pests. However, studies to this date have revealed that increased pesticide use causes several problems. Plant metabolites and plant-based medicines are thought to be less harmful to human health and the environment compared to synthetic pesticides and studies have been conducted to this end (Kordali *et al.,* 2007). Therefore, studies on the effects of various plant extracts and essential oils on plant diseases have become prominent. Lamiaceae family that involves numerous aromatic plants, is among these studied plant groups.

Salvia species, a member of Lamiaceae family, is one of the most important ones in this group. It is reported to include around 95 different members according to the most recent studies in Turkey (Celep *et al.,* 2009). It has an important place in the flora of Turkey and its endemism rate (51%) is also quite high (Davis, 1982 and Poyraz and Koca, 2006). Most of *Salvia* species are commonly used in food, drug, cosmetics and perfumery industry (Bağcı and Kocak, 2008). Lamiaceae plants, involving *Salvia* species are rich especially in terms of terpenoid compounds and also contain flavonoids, essential oils, phenolic compounds and some quinonoids (Durling et al., 2007; Bisio *et al.,* 2011; Al-Qudah *et al.,* 2014). For this reason, A large number of studies, carried out on *Salvia* species suggested that it has numerous biological activities such as antibacterial activities (Kawahara *et al.,* 2004), antifeedant activities (Fraga *et al.*, 2005), antioxidant activities (Lakhal *et al.,* 2013), cytotoxic activities (Lee *et al.,* 2010), antiviral activities (Tada *et al.,* 1994), antifungal activities (Abu-Darwish *et al.,* 2013), antimicrobial activities (Paknejadi *et al.,* 2012), and herbicidal activities (Bouajaj *et al.,* 2013 and Rowshan and Karimi, 2013).

In the present study, efficacy of plant extracts and essential oils of *Salvia* species against the important plant pathogen *Fusarium oxysporum* was studied.

MATERIALS AND METHODS

Plant materials

Salvia species; *S. officinalis*, *S. tomentosa* and *S. cryptantha,* used in the experiment were collected from the province of Tokat, Turkey in 2012-13 vegetation periods by harvesting the shoot system in flowering phase. Harandsted plants were dried on papers in a dark room, ground in an electric mill and kept in plastic containers to be used in the experiment.

Extraction of essential oils

Essential oils of the plants were obtained by hydro-distillation method using a Schilcher device. Disteled water was added to weighed plant samples (1:10 w/v) and boiled for 2 hrs. Obtained essential oils were maintained until used in the experiment (Telci *et al.,* 2006).

Preparation of water, methanol and ethanol extracts of plant samples

Water extracts

Dried herbal materials were powdered by grinding them in a plant grinding mill. 400 gr of ground plant material was placed in a glass container containing

1000 ml of disteled water and shaked for 24 hrs at 120 rpm in an orbital shaker and then solid residues were removed using filter papers. Solid residues were completely removed using centrifuge for 15 min at 5000 rpm.

Methanol and ethanol extracts

100 gr from each plant material were put in 1 liter erlenmayers and methanol, and ethanol was added as 600 ml of each. Mixtures were shaked for 24 hrs at room temperature, at 120 rpm, in an orbital shaker. The extract was then filtered using paper filters. Methanol and ethanol were removed by evaporating at 32-40°C. Remaining extract was used to prepare a stock solution with disteled water (Kadioglu and Yanar 2004).

Fungus cultur

The plant pathogen fungus used in this study was obtained from stock cultures found at Phytopathology laboratories of Department of Plant Protection, Faculty of Agriculture, Gaziosmanpasa University, Turkey. Fungus culture was used after being developed for 7 days at 25±2°C in 60 mm Petri dishes containing 10 ml of Potato Dextrose Agar (PDA).

***In- vitro* antifungal activity of plant essential oils and extracts**

PDA prepared to be used in the experiment was autoclaved and chilled to 40°C. Essential oils were mixed with melted sterile PDA at the concentrations of 0, 100, 500, 1000 and 2000 ppm. PDA was poured into 60 mm Petri dishes (as 10 mm). Different plant extracts obtained (water, ethanol and methanol) were mixed with melted sterile PDA to have final concentrations of 1, 3, 7, 10 and 20%, and then poured into 60 mm Petri dishes (as 10 ml). Mycelium discs (5 mm in diameter) obtained from the 7-day fungus culture were placed in the centre of Petri dishes. After inoculation, fungus culture was left for incubation at 28°C for 7 days. Fungal development was recorded after 7 days (Hadizadeh *et al.,* 2009). Inhibition in the development was calculated using the following formula (Pandey *et al.,* 1982):

$$I = \frac{DC - DT}{DT} \times 100$$

Where:- I: Inhibition percentage compared to the control (Mycelium development), DC: Mycelium development in the control and DT: Mycelium development in essential oil applications.

PDA without essential oils and extracts was used as a negative control and synthetic Propineb fungicide (0.4 g/200 mL PDA) was used as a positive control. The experiments were repeated twice and replicated four.

Statistical Analysis

Analysis of variance (ANOVA) was used to determine the significance leandls of differences between experiment treatments, and averages were compared using the DUNCAN test. Statistical analyses were carried out using the SPSS software.

RESULTS AND DISCUSSION

Essential oils and plant extracts of the three different *Salvia* species (*S. officinalis*, *S. tomentosa* and *S. cryptantha*) were found to be significantly effective on *F. oxysporum* mycelium. Essential oil from *S. officinalis*, one of the *Salvia* species used in the trial had the highest impact on *F. oxysporum* f. sp. *radicis-lycopersici* mycelium development (62.71% blocking rate), followed by *S. cryptantha* (53.39%) and then *S. tomentosa* (29.44%) (Table 1). An increase in the effect of blocking effect of plant extracts on *F. oxysporum* was observed depending on the dosage increase and extract used. While, the water extract of *S. officinalis* had the highest effect, followed by plant essential oil, ethanol and methanol extracts. The water extract of *S. officinalis* had a blocking rate of (65.29%) at the highest dosage, (58.63%) for the ethanol extract and (53.84%) for the methanol extract (Table 1). Onaran *et al.* (2014) indicated that *Thymus fallax* Fish & Mey., *Origanum vulgare* L. and *Mentha dumetorum* Schult plant essential oils blocked *F. oxysporum* mycelium development to a significant degree. In another study Hadi *et al.* (2013) reported that *Mentha piperita* L. extracts blocked *F. oxysporum* spore germination and mycelium development.

Among *S. cryptantha* plant essential oil and extracts, the water extract had a complete blocking effect on *F. oxysporum* (100%), followed by the methanol and ethanol extracts (67.66 - 67.77%), and the essential oil (53.40%) (Table 1). The ethanol extract of *S. tomentosa* had the highest blocking rate on *F. oxysporum* mycelium development compared to the control with 77.64%. The methanol extract blocked *F. oxysporum* mycelium development with a rate of 62.68%, the water extract with 41.47% and the essential oil with 29.44% (Table 1). However, differences regarding this effect were identified depending on the dosage of application and plant extracts and essential oils used. It was reported in different studies that *Salvia* species had anti-fungal effect on *Fusarium* species. *Salvia sclarea* essential oil was effective on *F. tricintum* and *F. sporotrichioides* species (Džamič *et al.,* 2008). *Salvia sclarea* essential oil was also effective on *F. oxsporum* f. sp. *dianthi* development (Pitarokili *et al.,* 2002), *Salvia tigrina* ethanol extract was effective on *F. oxysporum* (Dulger and Hacioglu, 2008). It was also found that *S. officinalis* plant was effective on *Candida* spp. and *Aspergillus niger* (Badiee *et al.,* 2012 and Abu-Darwish *et al.,* 2013), *Plasmopara*

Table (1): Effects on rmycelium growth rate of of different *Salvia* spp. on *F. oxysporum* f. sp. *radicis-lycopersici*

	Doses	*S. officinalis*	*S. cryptantha*	*S. tomentosa*		Doses	*S. officinalis*	*S. cryptantha*	*S. tomentosa*
Essential oil	Kontrol	51.66a*±3.40	51.66^{a} ±3.40	51.66^{a} ±3.40	Methanol extract	Kontrol	51.66^{a}±3.40	51.66^{a}±3.40	51.66^{a}±3.40
	100µl	31.67^{b} ±0.69	35.57^{b} ±3.18	46.20^{ab}±2.45		1%	26.24^{b} ±0.31	24.61^{b} ±1.17	39.10^{b}±0.23
	500 µl	29.72^{b}±0.95	34.26^{b} ±1.46	42.45^{b} ±0.50		3%	25.79^{b} ±0.43	23.72^{b} ±0.85	30.48^{c}±1.28
	1000 µl	27.09^{b}±0.66	29.75^{bc}±0.39	42.33^{b} ±0.41		7%	25.75^{b} ±0.25	23.62^{b} ±0.80	27.29^{c} ±0.48
	2000 µl	19.26^{c}±0.69	24.07^{c} ±0.77	36.45^{c} ±1.49		10%	23.88b^{c} ±0.34	21.58^{b} ±0.95	26.58^{c} ±0.15
	Propineb	0.00^{d}±0.00	0.00^{d} ±0.0	0.00^{d} ±0.00		20%	21.37^{c}±0.37	16.70^{c} ±0.42	19.28^{d}±0.10
						Propineb	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{e}±0.00
Water extract	Kontrol	51.66^{a}±3.40	51.66^{a}±3.40	51.66^{a}±3.40	Ethanol extract	Kontrol	51.66^{a}±3.40	51.66 ^{a}±3.40	51.66 ^{a}±3.40
	1%	29.33^{b}±1.12	36.09^{b} ±0.86	51.60^{a} ±0.78		1%	39.76^{b} ±1.34	25.38^{b} ±0.64	31.09^{b} ±0.07
	3%	28.60^{b} ±0.22	28.95^{c} ±0.91	47.60^{b} ±1.05		3%	39.79^{b} ±0.37	23.04^{bc} ±0.35	23.67^{c} ±0.23
	7%	27.81b^{c}±0.19	26.93^{c} ±0.10	35.89^{c}±2.40		7%	23.17^{c}±1.02	19.59^{cd} ±0.49	22.75^{c} ±0.20
	10%	26.88b^{c}±0.51	18.68^{d} ±0.53	31.55^{cd} ±0.57		10%	23.01^{c} ±0.23	18.76^{d} ±0.31	20.72^{c} ±0.19
	20%	23.84^{c}±0.25	0.00 ^{e}±0.00	30.23^{d} ±1.12		20%	17.93^{c} ±3.69	16.64d ±0.30	11.55^{d} ±0.09
	Propineb	0.00^{d}±0.00	0.00^{e}±0.00	0.00^{e}±0.00		Propineb	0.00^{d}±0.00	0.00^{e}±0.00	0.00^{e}±0.00

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

viticola (Dagostin *et al.,* 2010), *Alternaria* spp. (Mahmoudi and Ahmadi, 2013); while *S. cryptantha* and *S. tomentosa* showed antimicrobial and antibactericidal effects (Haznedaroglu *et al.,* 2001). These findings are similar to the obtained results. Biological activities of *Salvia* species are a result of the compounds contained by plants. Because it was found in numerous studies conducted on *Salvia* species that these plants were rich in camphor, linalool, eucalyptol (1,8-cineole), borneol compounds (Pandey 2009 and Okamoto *et al.,* 2011), and phenolics (Lu and Foo 2000 and Yumrutas *et al.,* 2011).

This study and similar previous studies showed that *Salvia* species are effective on *F. oxysporum* f. sp. *radicis-lycopersici* mycelium development. Considering the environmental damage caused by fungicides commonly used against plant diseases, obtained findings of this study may give some lights to future studies in this field.

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