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Antifungal Activity of Essential Oils and Plant Extracts from *Sideritis germanicopolitana* BORNM. Growin in Turkey

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ABSTRACT

In this study, the antifungal effects of essential oils and methanol plant extracts of *Sideritis germanicopolitana* BORNM grown in Tokat province was screened *in vitro* against, *Rhizoctonia solani* J.G. Kühn 1858, *Sclerotinia sclerotiorum* (Lib.) de Bary, (1884), *Alternaria solani* and *Fusarium oxysporum* f. Sp. *Lycopersici*. The chemical composition of extracted were also identified. According to of GC/MS analysis, a total of 20 components were identified, including α-pinene (58.34%), α-Limonen (10.48%), β-pinene (8.24%) and manoyl oxide (4.79%) as basic components. Essantial oil showed antifungal activities *in vitro* against all tested fungi except *Rhizoctonia solani* J.G. Kühn 1858. These results revealed that essential oils and methanol extracts of *Sideritis germanicopolitana* BORNM. could be used in the future for controlling plant diseases.

Key words: Antifungal, *A. Solani*, *R. Solani*, essantial oil, plant extracts.

INTRODUCTION

Plants belong to family Lamiaceae is spread over a wide area in the world. There are 236 genera and 7133 species (Harley *et al*., 2004, Yilar *et al*., 2015). Turkey is a gene center for Lamiaceae family, which include various aromatic plants. The family contains 546 species, 45 genus and entirely 731 taxa with the regional plant ratio of 44.2% (Koyuncu *et al*., 2010; Belen, 2012; Gümüşçü, 2014). Genus *Sideritis*, a member of the Lamiaceae family, has more than 150 species which are deploy in temperate and tropical zone of the Northern Hemisphere (Tomas-Barberan *et al*., 1988). There are 46 species and 53 of the taxon and species, 40 of them endemic in Turkey (Davis, 1982). *Sideritis germanicopolitana* BORNM. is one endemic to our country(Anonymous, 2016).

Synthetic fungicides are used to control fungi. The successive use of fungicides considered as one of polutant environmental problem and also the fungal resistance against pesticides had up. Active molecules and new control strategies are presence to exterminate, the resistance problem. All over the world has accelerated efforts to use alternative methods against plant pathogens (Barnard *et al*., 1997; Misra and Pavlostathis, 1997). *Fusarium oxysporum* f. sp *lycopersici, S. sclerotiorum*, *A. solani* and *R. solani* as plant diseases incidents causing significant losses to plant production all over world. Fungal disease around the world each year cause a loss of 14% yield (Agarwal and Sinclair, 1987; Agrios, 1997). *Fusarium Oxysporum* f. sp. *lycopersici* causes crown, wilt and root rot in tomatoes (Jones *et al*., 1991). *Sclerotinia sclerotiorum* is a pathogen to more than 400 plant species causing disease in the world (Bolton *et al*., 2006). *Alternaria solani*, also, causing early blight diseases to tomatoes and potatoes. Wide yield losses, from 10% to 30% of Rhizoctonia disease have been reported by (Carling *et al*., 1989 and Read *et al*., 989).

In this study, the effect of essential oils and methanol extract of *Sideritis germanicopolitana* Bornm on mycelial growth of plant pathogens; *F. oxysporum* f. sp. *radicis-lycopersici, R. solani, S. sclerotiorum,* and *A. solani* was investigated.

MATERIALS AND METHODS

Plant materials

The experimental used *Sideritis germanicopolitana* BORNM plants collected from Tokat/Turkey in July 2015. Collected plants were air dried on papers in shadow then used.

Essential oil extraction

A weight of 50 g of aerial parts of plant material were cut in small pieces and placed neo-clevenger type apparatus. Boiling process was continued for 2 h. Essential oils were seperated carefully using a micropipette. Ligth yellow oil was obtained (250 mg, 0.5 % of total dry weight). Essential oils were kept at 4 º C in dark bottle until used (Telci *et al*., 2006).

GC-MS analysis

Essantial oil composition of plant material was determined using Perkin Elmer Clarus 500 GC-MS. Elution of components was done over BPX-20 capillary column (30 m x 0.25 mm and 0.25 m ID). Enjection port and GC transfer line temperature were both 250 ºC. Oven temperature program was as follows: intial oven temperature: 50 ºC, ramp to 120 ºC with 3ºC/minute heating rate, ramp from 120ºC to 220ºC with 5ºC/ minute heating rate and finally hold

for 0.67 minute. Total run time was 44 minutes. Helium was used as carrier gas at 1.0 mL/ minute flow rate at the split mode (50:1). For MS detection, EI ionization system was used at 70 eV energy.

Identification of oil components was accomplished by comparison of their mass spectral fragmentation patterns with available mass library (WILLEY and NIST). For analysis: 20 mg of essential oils were diluted with 1.2 mL acetone and 1 μL of final solution directly injected to instrument

Plant Extracts

Weighed 100 grams of ground plant materials was put, into a 1 liter Erlenmeyer flask and then add 600 ml of methanol. Mixture was left for 24 hours at room temperature, then after at 120 rpm in an orbital shaker. Then extract was filtered using paper filter. The Solvent was removed using a rotary evaporator at 40 ° C in organic solvents. The remaining extract was used to prepare a stock solution with 1% DMSO identified. Different concentrations to be used in the study (50, 100, 200 mg /100 ml PDA) was prepared (Kadioglu and Yanar 2004).

Fungal Cultur

The plant pathogen fungi used in this study were acquire from stock cultures of Phytopathology laboratories of Department of Plant Protection, Faculty of Agriculture, Ahi Evran University. Fungal cultures were used in the study after being recultured for 7 days at 25±2 °C in 90 mm petri dishes containing 20 ml of potato dextrose agar (PDA).

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

These assays were carried out to determine the effects of *S. germanicopolitana* BORNM. essantial oil and methanol extract against *A. solani, Fusarium oxysporum* f. sp *radicis- lycopersici, S. sclerotiorum* and *R. Solani*. Different volumes of plant extracts was added to PDA before its solidifying to obtain final concentrations of 500, 1000 and 2000 ppm. The PDA was poured into_60-mm Petri plates (10 mL plate-1). Then an agar disc (5 mm in diameter) of *A. solani, Fusarium oxysporum* f. sp *radicis-lycopersici, S. sclerotiorum* and *R. solani* were inoculated on the medium and the plates and incubated for 7 days at 25 °C. Since essential oils have a low solubility in water, they were used in the gas phase. A dedicated volume of each oil was entrench on a piece of filter paper that was glued to the cover of every Petri dish. The cover was closed and immediately sealed with parafilm. Essantial oil doses of 0 (control), 0,5, 1, 3 and 5 μL/petri dish were applied. Later the fungal cultures were left for incubation at 25± 2 °C for 7 days. Fungal development was saved after 7 days. Inhibition in the development in fungal growth was calculated using the following formula (Pandey *et al.*, 1982). PDA without essential oils and extracts was used as a negative control and synthetic Thiram 80% (hektas) was used as a positive control. All experiments were repeated twice and each in four replicates.

$$I=100\times[(DC - DT)/DC]$$

I: Inhibition percentage compared to the control (Mycelium development)
DC: Mycelium development in the control
DT: Mycelium development in essential oil applications

Statistical analysis

Data were analyzed by using One Way procedure of ANOVA (Windows version of SPSS, release 15.00). The differences between doses were compared using DUNCAN Multiple Range Test of $p<0.05$.

RESULTS AND DISCUSSION

World studies in agricultural areas showed that plant pathogenic fungi are considered as a reason significant for product losses through causing infections to plants (Dellavalle *et al.*, 2011; Agrios, 1997). Synthetic fungicides are usually used to control plant pathogenic fungi. But especially, synthetic pesticides reason environmental pollution because of their slow disintegration. This reason, researchers have focused on plant-based drugs. Most studies showed biological activity medicinal of aromatic plants. Biological activities of different extracts and essential oils from other species of Lamiaceae family, which involved *Sideritis* as well, were shown. *Sideritis* species had been reported by researchers to have antifungal and antibacterial activities (Digrak *et al.*, 2001; Bouchra *et al.*, 2003; Gulluce *et al.*, 2003; Souza *et al.*, 2005; Kilic, 2006).

Essential Oil Composition

Data in Table (1) show the *S. germanicopolitana* BORNM. chemical components of the essential oils of the plant were determined by GC / MS (Table 1). A total of 20 components, the basic including α-pinene (58.34%), α-Limonen (10.48%), β-pinene (8.24%) and Manoyl oxide (4,79%) were identified in essential oil. The total essential oil components were determined of 98.24 %.

Antifungal Activity of the Essential Oil and methanol extracts.

The oils of the *S. germanicopolitana* BORNM. showed inhibition percentages at different statistically significant levels ($p<0.05$) against the tested plant pathogenic fungi (Table 2). The oil 5μl dose of *S. germanicopolitana* BORNM was

Table 1. The chemical composition of the *S. germanicopolitana* BORNM. essential oil

RT	RI	Components	%
4.00	951	Thujene	0.45
4.26	965	α-pinene	58.34
4.61	977	Camphene	0.12
5.34	1040	β-pinene	8.24
5.49	1048	Myrcene	2.60
6.18	1083	3-caren	3.35
6.56	1100	α-terpinen	0.19
6.89	1116	α-Limonen	10.48
7.00	1122	β-phellandrene	1.35
7.44	1142	Ocimene	0.10
7.94	1164	γ-Terpinen	0.34
8.90	1203	Terpinolene	2.78
9.42	1126	Linalool	0.22
15.26	1465	Decenal	0.12
22.95	1810	β-Caryophyllen	2.74
24.99	1913	Caryophyllen	0.28
25.56	1943	Germacrene D	0.47
26.07	1970	γ-Elemene	1.14
29.68	2167	Caryophyllen oxide	0.14
39.14	2276	Manoyl oxide	4.79
Total			98.24

Table 2. Antifungal activity of essantial oils of *S. germanicopolitana* BORNM. against some plant pathogens

Dose (µl/petri)	Percentage of growth inhibition			
	Alternaria solani	*Fusarium oxysporum* f. sp. *radicis- lycopersici*	*Sclerotinia sclerotiorum*	*Rhizoctonia solani*
Control	0.00^{b*}	0.00^{c}	0.00^{c}	0.00^{a}
0.5	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{a}
1	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{a}
2	0.00^{b}	19.71^{b}	10.6^{b}	0.00^{a}
5	33.02^{a}	47.54^{a}	41.38^{a}	0.00^{a}

Table 3. Antifungal activity of methanol extracts from *S. germanicopolitana* BORNM against some plant pathogens

Dose (mg)	**Plant pathogens			
	S.S	A.S	Fol	R.S
P.C (Thiram %80) 300mg/100ml	$100±0.0^{a*}$	$100±0.0^{a}$	$100±0.0^{a}$	$72.15±1.94^{a}$
N.C (DMSO %1)	$0.00±0.0^{e}$	$0.00±0.0^{e}$	$0.00±0.0^{e}$	$0.00±0.0^{c}$
50	$26.81±0.90^{d}$	$28.62±0.87^{d}$	$24.58±0.58^{d}$	$0.00±0.0^{c}$
100	$40.74±0.95^{c}$	$43.01±0.61^{c}$	$33.62±0.83^{c}$	$0.00±0.0^{c}$
200	$80.01±0.25^{b}$	$63.61±1.74^{b}$	$44.76±1.72^{b}$	$32.94±^{b}$

*The averages with different letters in the same column are significantly different at the level of $p<0.05$.
**Plant pathogens; *F. oxysporum f. sp lycopersici*: Fol, *S. sclerotiorum: S.s, A. solani:* A.s, *R. solani*:R.s.; P.C: positive control; N.C; Negative control (Dimetil sülfoksit, DMSO).

Fusarium oxysporum f. sp. radicis- *lycopersici, Sclerotinia sclerotiorum (*Lib.) and *Alternaria solani* pathogens inhibition percentages of 47.54%, 41.83% and 33.02% respectively. The oil of *S. germanicopolitana* BORNM had no effect on the development of mycelium inhibition *Rizoctani solani.*

Sideritis species, the chemical compound is present in the exhibit biological activity. Studies on *Sideritis* species, were determined to be rich in certain compounds such as myrcene, β-caryophyllene, α-pinene, sabinene (Tabanca *et al.*, 2001; Todorova *et al.*, 2000)

The antifungal activity of plant methanol extract against four fungi species is summarized in (Table 3). The results revealed that the methanol extract showed antifungal activity with varying levels. The antifungal activity of plant extracts was against *Sclerotinia sclerotiorum, Alternaria solani, Fusarium oxysporum* f. sp. *radicis- lycopersici* and *Rhizoctania*

Solani. The percentages of mycelial growth inhibition were 80.01%, 63.61%, 44.76% and 32.94% respectively.

The most active *Sideritis trojana* and *Sideritis bilgerana* methanol extracts were found to be against clotrimazole-resistant *Candida Albicans* (Dulger *et al*., 2006). Ethanolic and methanolic extracts of the genus *Sideritis* spp., other than *S. syriaca*, which exert their antimicrobial effect mostly on Gram-positive bacteria (Gonzalez-Burgos & Carretero, 2011).

Through this study, antifungal activity of essential oils and methanol extracts of *S. germanicopolitana* BORNM on *F. oxysporum* f. sp *lycopersici, R. solani*, *S. sclerotiorum*, *A.solani* was investigated.

The *S. germanicopolitana* BORNM in our study were determined to extract and essential oil have different levels of antifungal effect. These differences change between 80% and 0.00% depending on mycelium inhibition rates; different results appeared according to the plant extract and essential oil. The extract antifungal activities against all test organisms have reduced mycelium growth. Promising results were getting from the use of plant extract and essential oil controls

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