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THE ANTIFUNGAL AND PHYTOTOXIC EFFECT OF DIFFERENT PLANT EXTRACTS OF *SALVIA VIRGATA* JACQ

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ABSTRACT

This study was conducted in order to investigate the phytotoxic and antifungal activity of the methanol and n-hexane extracts obtained from the surface parts of the *Salvia virgata* Jacq. plant collected in Kırşehir province. In the experiments, *S. virgata* extracts were prepared and used in 125, 250, 500, 1000 ppm doses. The seeds of *Lactuca sativa* L., *Lepidium sativum* L., and *Triticum vulgare* L., plants were placed as 25 seeds for each of Petri dishes with a 9-cm diameter, which two layers of filter paper were placed, and it was humidified thoroughly for control purpose with distilled water and plant extracts (125, 250, 500, 1000 ppm) and then they were left for incubation for 3 weeks at 24 ^{o}C±1 conditions. At the end of the period, the germination percentages, root-shoot developments and wet and dry weights of the seeds were determined. In the antifungal studies, the plant methanol and hexane extracts were added to the PDA environments prepared in the way for their final concentration to be 125, 250, 500, 1000 ppm doses. In the study, Thiram (80%) fungicide was used as the negative control (only PDA) and positive control. The extract and Thiram-added PDA were transferred to the petri dishes with 60-mm diameter. The mycelium of the pathogens was transferred to these petri dishes and left to incubation for 7 days. According to the trial results, the seed germinations of the *S. virgata* methanol and n-hexane extracts inhibited garden cress, wheat, and lettuce at the rate of 79.45%, 18.67%, 88.57%, and 78.08%, 82.86%, 100%, respectively. The mycelium developments of *Rhizoctonia solani* Kühn, *Alternaria solani* (Ell. And G. Martin) *Fusarium oxysporum* f sp *radicis lycopersici*, and *Verticillium dahliae* pathogens were inhibited by 1000 ppm dose of methanol and n-hexane extracts; 0%, 28.17%, 38.77%, 0% and 2.43%, 36.04%, 37.0%, and 72.22%.

KEYWORDS:
Antifungal activity, extract, phytotoxic effect, *Salvia virgata*

INTRODUCTION

Due to the severe problems that occurred as a result of the chemical control against the plant diseases performed for long years, the studies to find alternative methods especially starting in developed countries have accelerated. As a result of using pesticide intensely, the natural balance was deteriorated and it has begun to threaten the environment and human health [1].

It is important to find environment-friendly methods that can substitute chemicals in the control of plant diseases and are suitable for the integrated control principles. Also, in order to maintain the agriculture, it becomes a necessity to research methods alternative to chemical control and transfer them into applications. One of these alternative methods is to determine the herbal compounds and use them in the control of the plant diseases, pests, and weeds. In the previous studies, there are many studies on the fungicidal, herbicidal, and insecticidal effects of the compounds and essential oils in plants and their biological activities [2-5]

Lamiaceae family with its 250 genera and 7133 species has a wide spreading area [6-7]. Salvia L., one of the largest genera of the Lamiaceae family includes approximately 1000 species [8]. *Salvia virgata* Jacq., included in the Salvia genus, is a perennial species and its length reaches to 160 cm. The plant can spread in various areas like the empty fields and roadsides and in all regions of Turkey [8]. *S. virgata* is an important plant with high-quality used in the medical practices and it is used as wound-healing and against the skin disorders among people [9-10]. It also has antioxidant, antimicrobial, and antibacterial effect [11-12].

This study was conducted to determine the antifungal activity of different extracts obtained from *Salvia virgata* Jacq. plant on *Rhizoctonia solani Kühn, Alternaria solani* (Ell. And G. Martin), *Fusarium oxysporum* fsp *radicis lycopersici,* and *Verticillium dahliae*, which are important plant pathogenic fungi, and their phytotoxic effect on *Lactuca sativa* L., *Lepidium sativum* L., *Triticum vulgare* L. cultivated plants.

MATERIALS AND METHODS

Plant material. ***Salvia virgata*** **Jacq.** plant used in the trial was collected in Kırşehir province in 2018 vegetation period. The plant materials collected in the blooming stage were dried in the shade in the laboratory environment and they were ground in the electrical grinder. The plant diagnosis was performed by Faculty Member Dr. Melih Yılar.

Fungus cultures. In the study, *Rhizoctonia solani* Kühn, *Alternaria solani* (Ell. And G. Martin), *Fusarium oxysporum* f sp *radicis lycopersici* (Jarvis & Shoemaker), and *Verticillium dahliae* (Kleb) plant pathogens were used. These plant pathogens were obtained from the stock cultures in Ahi Evran University, Faculty of Agriculture, Plant Protection Department, laboratory of Phytopathology.

Preparation of Extracts. 500 gr ground plant material was put into 2-liter Erlenmeyer and 1000 ml sterile distilled water was added on it. This solution was put into the shaker for extraction at room temperature for 24 hours. After the extraction, the solution was filtered from the filter paper. Methanol and hexane in the solutions were removed using a rotary evaporator. The final solution was kept at +4ºC until it was used in the trial.

The antifungal activity of the plant extract under in vitro conditions. The materials obtained were dissolved by acetone-water mixture and the stock solution was obtained. Among the original solutions, the ones with the final concentration of 125, 250, 500, and 1000 ppm were added to the PDA environments cooled until 45-50 ºC [14]. For control, fungi were only inoculated in the petri dishes containing PDA. In addition, a fungus with Thiram active ingredient was used as the positive control in the trials. These PDA media in different doses were poured as 10 ml in the petri dishes with a 60-mm diameter. Mycelium discs with 5-mm diameter taken from the plant pathogen cultures developed 7-10 days before the trials were inoculated in the petri dishes containing PDA medium with extract. The fungal cultures were left to incubation for 7 days at 25±1 ºC in the growth cabin after the inoculation. This study was duplicated with 4 iterations. The diameters of mycelium developing in the petri dishes were measured with a digital caliper device. The inhibition percentage of the extracts for the mycelium development was calculated according to the following formula:

I:100x(dc-dt)/dc

Percentage of inhibition on the mycelium development

dc: The mycelium development in control

dt: The mycelium development in the application [15].

Phytotoxic effect study. These studies were triplicated in the petri dishes with a 90-mm diameter. The seeds of the test plants were distributed homogeneously (25 for each) in the petri dishes in which 2-layer blotting paper was placed. The plant extracts in different concentrations (250, 500, and 1000 ppm) and the distilled water for control purpose were humidified by adding 5 ml in the petri dishes. The plant extracts with different concentrations (250, 500 and 1000 ppm) and pure water for control purpose were added in 5ml to Petri dishes and humidified. The petri dishes were left to incubation for 3 weeks at averagely 24 ºC ±1 ºC for 12-hour light and 12 hour- dark. At the end of this period, the seed germination rates of the test plants and the root and shoot height were determined. The trial was repeated twice [16].

Statistical Analysis. The significance levels of the differences between the treatments were determined by using the analysis of variance (ANOVA) during the trials. The means were compared using the DUNCAN test. The statistical analyses were performed using SPSS -15 computer program.

RESULTS AND DISCUSSION

It was determined that *Salvia virgata* plant methanol and hexane extracts had a phytotoxic activity on garden cress, wheat, and lettuce and an antifungal activity on *A.solani, V.dahliae, F. oxysporum f sp. Radicis lycopersici, and R.solani* pathogens. Tables 1, 2, 3, and 4 and Figures 1 and 2 summarize the results obtained.

The phytotoxic effect of *S. virgata* extract on the cultivated plants varied depending on the extract, dose and test plant. The hexane extract of *S. virgata* reduced the seed germination and the root and shoot development of garden cress, wheat, and lettuce at a statistically significant level compared to the control (Table 1). Accordingly, the hexane extract inhibited the seed germinations of garden cress, wheat, and lettuce at the rate of 78.08%, 82.86%, and 100%, compared to the control. The hexane extract inhibited the root and shoot lengths of garden cress and lettuce at the rate of 100%, it inhibited the root length of wheat at the rate of 83.88% and the shoot length of wheat at the rate of 82.86% compared to the control. Although the effect in the plant methanol extract realized at a lower level compared to the hexane extract, it had a high level of phytotoxic effect on the test plants. Accordingly, the methanol extract reduced the seed germination of garden cress, wheat, and lettuce at the rates of 79.45%, 18.67%, and 88.57%, respectively, reduced the root elongation at the rates of 94.77%, 70.18%, and 83.21%, respectively and reduced the shoot elongation at the rates of 94.26%,

51.45%, and 71.77%, respectively compared to the control. In the studies conducted on the Salvia species, it has been reported that *S. officinalis* water extract inhibits the seed germination and development of lettuce [17] and corn [18]. Also, it has been reported that *S. miniata* inhibits the germination of *Papaver rhoeas* and *Avena sativa* [19] and *S. leucophylla* essential oil inhibits the seed germination of *Brassica campestris* [20]. The phenolic compounds that were determined to have phytotoxicity and biological activity effect include *S. virgata* plant [21]. The methanol extract of this plant has a total phenolic content of 195.22 (mg GAE/g extract) [22].

In the results of the present study, it was also determined that *S. virgata* hexane and methanol extracts had a phytotoxic effect on the cultivated plants. Previous studies revealed that the salvia species displayed biological activity and contained phenolic compound, which supports this study. In accordance with the present study and previous studies, *S. virgata* plant has the potential to demonstrate high allelopathic effect and therefore, it may be a natural herbicide.

It was determined that *Salvia virgata* methanol and hexane extracts varied according to the extract, application dose, and plant pathogen and it had an antifungal potential. *S. virgata* methanol extract inhibited significantly the mycelium developments of *V. dahliae* and *F. oxysporum f sp radicis lycopersici* pathogens compared to the control. However, it was found that it had no effect on the mycelium development of *A. solani* and *R. solani* (Table 3, Figure 1).

TABLE 1
The phytotoxic effect of the *Salvia virgata* hexane extract on the test plants.

Doses	*Lepidium sativum* L.			*Triticum vulgare* L.			*Lactuca sativa L.*		
	GR* (%)	SL (mm)	RL (mm)	GR* (%)	SL (mm)	RL (mm)	GR* (%)	SL (mm)	RL (mm)
Control	97.33a[y] ±2.66	26.11a± 1.05	41.72a± 8.02	100.00a± 1.33	102.94a ±8.75	101.08a± 17.14	93.33a± 2.66	12.54a± 1.25	17.58a± 4.33
250ppm	57.33b± 7.06	11.47b± 5.75	12.89b± 5.63	98.66a± 1.33	39.59b ±20.46	42.24ab± 24.62	60.00b± 2.30	4.97bc± 3.32	4.05b± 3.42
500ppm	38.66c± 1.33	10.65b± 4.27	11.19b± 9.63	92.00a± 8.00	37.61b± 18.51	35.22b± 22.57	41.33c± 1.33	3.32c± 3.46	3.42b± 2.67
1000ppm	21.33d± 3.52	0.00b± 0.00	0.00c± 0.00	74.66b± 6.66	17.64b± 8.99	16.29b± 8.76	0.00d± 0.00	0.00c± 0.00	0.00b± 0.00

*GR: Germination; SL: Shoot length; RL: Root length; [y] the means with different letters in the same column are different at the significance level of $p<0.05$ according to DUNCAN.

TABLE 2
The phytotoxic effect of the *Salvia virgata* methanol extract on the test plants.

Doses	*Lepidium sativum* L.			*Triticum vulgare* L.			*Lactuca sativa L.*		
	GR* (%)	SL (mm)	RL (mm)	GR* (%)	SL (mm)	RL (mm)	GR* (%)	SL (mm)	RL (mm)
Control	97.33a[y] ±2.66	26.11a± 1.05	41.72a± 8.01	100.00a± 1.33	102.94a± 8.75	101.08a± 17.14	93.33a± 2.66	12.54a± 1.25	17.58a± 4.33
250ppm	66.66b± 4.80	22.11a± 5.47	30.06b± 13.31	92.00a± 6.11	72.15b± 0.65	45.26b± 10.52	66.66a± 5.81	10.86ab± 0.39	11.94ab ±2.60
500ppm	40.00c± 4.62	2.72b± 2.72	4.64c± 4.64	89.33a± 3.52	50.72c± 3.04	40.23b± 7.23	25.33b± 13.92	5.52ab± 3.33	6.11ab± 4.28
1000ppm	20.00d± 2.30	1.31b ±0.66	2.18c± 1.18	81.33b± 4.80	49.97c± 5.72	30.14b± 16.26	10.66b± 7.01	3.54b± 2.43	2.95b± 2.08

*GR: Germination; SL: Shoot length; RL: Root length; [y] the means with different letters in the same column are different at the significance level of $p<0.05$ according to DUNCAN.

TABLE 3
The mycelium development effect of *Salvia virgata* methanol extract on plant pathogenic fungi.

Doses	*A. solani*	*V. dahliae*	*F. oxysporm* f sp. *radicis lycopersici*	*R. solani*
Control[+]	0.00b*±0.00	0.00e±0.00	0.00f±0.00	0.00b±0.00
Control[-]	60.00a±0.00	60.00a±0.00	51.18a±0.00	60.00a±0.00
125ppm	60.00a±0.00	60.00a±0.00	47.10b±0.36	60.00a±0.00
250ppm	60.00a±0.00	53.82b±3.11	44.47c±0.53	60.00a±0.00
500ppm	60.00a±0.00	42.69c±0.88	40.74d±0.58	60.00a±0.00
1000ppm	60.00a±0.00	36.74d±2.77	36.76e±0.73	60.00a±0.00

*The means with different letters in the same column are different at the significance level of $p<0.05$ according to DUNCAN.

As a result of the percentage effect study, 1000 ppm dose of the methanol extract inhibited the mycelium development of *V. dahliae* and *F. oxysporum f sp. radicis lycopersici* pathogens at the rates of 38.77% and 28.17%, compared to the control. However, it was determined that it had no effect on the mycelium development of *A. solani* and *R. solani* pathogens (Figure 1).

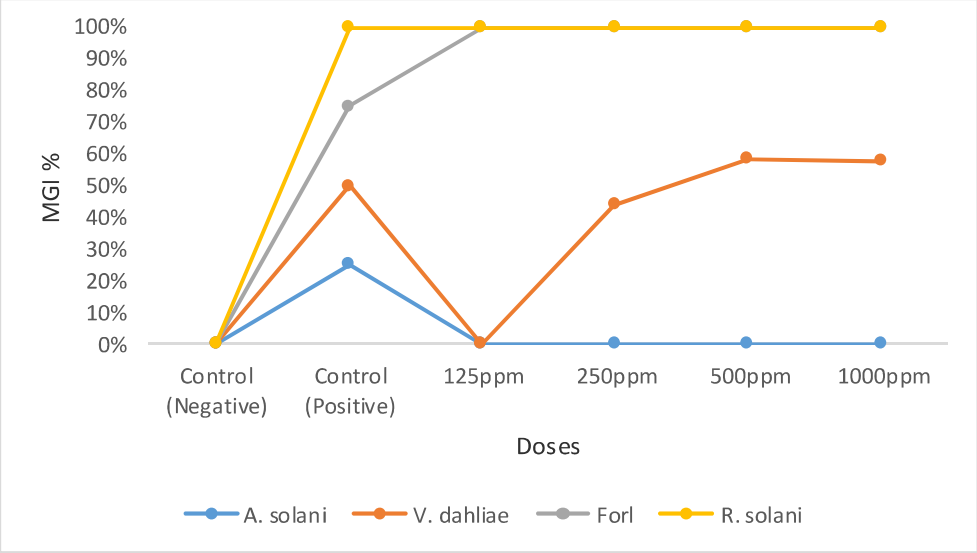

FIGURE 1
The % effect of *Salvia virgata* methanol extract on the mycelium growth inhibitions (MGI) of the plant pathogenic fungi.

TABLE 4
The mycelium development effect of Salvia virgata hexane extract on the plant pathogenic fungi.

Doses	*A. solani*	*V. dahliae*	*F. oxysporm* fs p. *radicis lycopersici*	*R. solani*
Control^{+}	0.00e*±0.00	0.00d±0.00	0.00e±0.00	0.00c±0.00
Control^{-}	60.00a±0.00	60.00a±0.00	51.18a±0.00	60.00a±0.00
125ppm	57.72a±2.28	46.21b±1.62	38.45b±1.06	60.00a±0.00
250ppm	53.52b±2.08	37.10c±1.61	36.68bc±0.79	60.00a±0.00
500ppm	47.52c±0.44	36.14c±6.16	34.70c±0.51	60.00a±0.00
1000ppm	38.16d±0.20	16.67d±2.60	32.21d±0.73	58.54b±10.16

*The means with different letters in the same column are different at the significance level of $p<0.05$ according to DUNCAN.

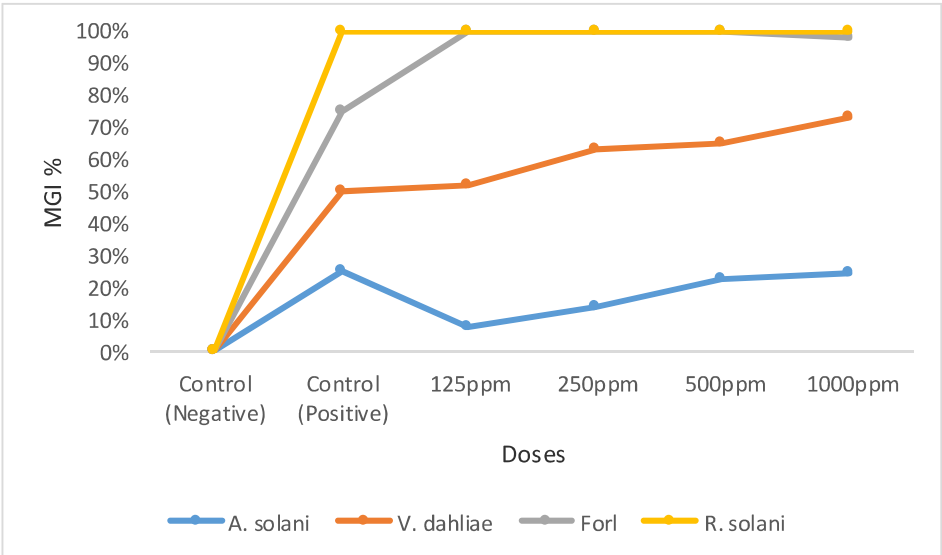

FIGURE 2
The % effect of *Salvia virgata* hexane extract on the mycelium growth inhibitions (MGI) of the plant pathogenic fungi

S. virgata hexane extract inhibited the mycelium development of all the pathogens compared to the control. It was determined that *R. solani* was the most tolerant pathogen to the hexane extract and *V. dahliae* was the most sensitive pathogen to the hexane extract (Table 4, Figure 2).

1000 ppm dose of the hexane extract inhibited the mycelium development of *V. dahliae, F. oxysporum f sp. radicis lycopersici, A. solani, and R. solani* pathogens at the rates of 72.22%, 37.01%, 36.04%, and 2.43%, respectively, compared to the control (Figure 2).

In previous studies, it was reported that the plant extracts and their essential oils had a significant effect on the plant pathogens [24-25]. *Salvia* species exhibit many activities such as antifungal, antibacterial, antioxidant, herbicidal ones [18, 26-31]. However, it was not determined that *Salvia virgata* had an effect on *A. solani, V. dahliae, F. oxysporum* f sp. *radicis lycopersici* and *R. solani* pathogens, which were the plant pathogenic fungus. Also, the present study revealed the results parallel with the ones reported in previous similar studies.

CONCLUSION

Consequently, it was revealed that *Salvia virgata* methanol and hexane extracts had a phytotoxic effect on garden cress, wheat, and lettuce plants and an antifungal effect on *A. solani, V. dahliae, F. oxysporum* f sp. *radicis lycopersici,* and *R. solani,* which were significant plant pathogenic fungi. The effect of *Salvia virgata* methanol and hexane extracts on the plant pathogens was determined with this study for the first time.

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