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USE OF ESSENTIAL OILS AND EXTRACTS FROM *SATUREJA* AND *ORIGANUM* SPECIES AS SEED DISINFECTANTS AGAINST *XANTHOMONAS AXONOPODIS* PV. *VESICATORIA* (DOIDGE) DYE

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ABSTRACT

The aim of this study was to test for antibacterial efficiacy and potential use as seed disinfectants of extracts and essential oils of *Satureja hortensis, Satureja spicigera, Origanum onites* and *Origanum rotundifolium* against *Xanthomonas axonopodis* pv. *vesicatoria* causes of bacterial leaf spot of pepper and tomato. According to the in-vitro test results, the essential oils showed a strong antibacterial activity based on the zone of inhibition against pathogen on Petri plates. The inhibasyon zones and MIC values of the essential oils ranged from 29-42 mm and 15.62-125 µl/mL, respectively. However, the extracts showed weak or no antibacterial activity. According to the results on petri plate assays of *O. rotindufolium* and *S. spicigera* essential oils, some of the concentrations (31.25 and 62.50 µL/mL) had toxic effect on the pepper seed germination, but the concentration of the oil with 15.62 µL/mL had not toxic as compared to only the pathogen application. This application caused a decrease on the number of the infected seedlings with *X. axanopodis* pv. *vesicatoria.* Furthermore, this decrease was statistically significant compared to both streptomycin sulfate and only pathogen applications. On the other hand *O. onites* hexane extract had a toxic effect on the pepper seed germination, but all concentration of the acetone extract and chloroform extracts (except 20 mg/mL) had no toxicity. According to the results on pot assays, essential oils used in the best germination and disease control was observed at lower doses. In the application of the *O. onites* extracts onites the best germination and best disease control have changed according to the dose used. Our results showed that some of the concentrations of the essential oils and/or extracts from *S. hortensis*, *S. spicigera*, *O. onites* and *O. rotindufolium* can be used as a seed disinfectant and as potential control agents for management of the bacterial disease.

KEYWORDS:
Antibacterial activity, essential oil, plant extract, *Origanum*, *Satureja*, *Xanthomonas*

INTRODUCTION

Bacterial diseases caused by *Xanthomonas* have devastated various host plants, leading to considerable losses in productivity and quality of harvests [1]. *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye, the causal agent of bacterial leaf spot of pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum*), occurs worldwide in regions of pepper and tomato production [2]. The disease is characterized by necrotic lesions on leaves, steams, and fruits. In warm and rainy weather, bacterial spot may cause severe defoliation of the plants, which results in reduced yield, and diseased fruits may not be suitable for fresh-market sale.

In recently, a lot of studies related to antimicrobial activities of the extracts and essential oils of plants have been made, followed by the genus *Satureja* [5,6,7,8,9,10,11] and Origanum [3,12,13,14,15,16,17,18]. In addition, there are some studies related to various plant species which have more or less antagonistic activity against plant pathogenic bacteria *Xanthomonas* sp. [8,19,20,21,22,23]. But, it is also known that antimicrobial effects or biological activities of the essential oils and extracts of plants may be subjected to a change, based on the variations in the chemical composition of an essential oil that may be observed due to the origin, the locality, the environmental conditions, and the stage of development of the collected plant material [5]. Furthermore, there is no report available in the literature on the in vivo antibacterial activity of the essential oils and extracts from genus *Satureja* and *Origanum* against plant pathogenic bacteria of *Xanthomonas* spp.

Seed-borne diseases can be spread with seeds,

and control of them using commercial disease management methods are extremely difficult [24]. Therefore, the use of healthy seeds is the most important factor for controlling the seed-borne diseases. Seed disinfection is also an important problem in organic agriculture. Therefore, this study was carried out to assess the in vitro and in vivo antibacterial efficacy of the essential oils and/or extracts obtained from *Satureja hortensis* L, *Satureja spicigera* (K.Koch.) Boiss, *Origanum onites* L. and *Origanum rotundifolium* Boiss. as a seed disinfectant against *X. axonopodis* pv. *vesicatoria* on the Petri plate and pot assays.

MATERIALS AND METHODS

Antibacterial activity assays. Antibacterial activity assays were carried out by disc diffusion method [25] with a minor modification on Triptic Soy Agar (TSA, Merck, Germany) medium. The essential oils and the extracts were prepared by dissolving using 10% dimethylsulfoxide (DMSO), and then were sterilized by filtration by 0.45 µm Millipore filters. Bacterial cultures were grown in Triptic Soy Broth (TSB, Merck, Germany) and adjust spectophotometrically $1x10^8$ CFU/ml by turbidity. Their suspension (100 µL) containing $1x10^8$ CFU/ml of bacteria spread by a sterile swab on TSA medium. The disks (6 mm in diameter) were impregnated with 12.5 µL of the essential oils (1/1 mL DMSO) and 1.25 mg of the extracts (10 mg/mL DMSO) solutions. The disks were kept out for evaporization of the organic solvents. Then, they were put in the middle of the inoculated plates. The bacterial cultures were incubated at 27 ± 2 ºC for 48 h, and then inhibition zones were measured in diameter (mm) around of the discs. Furthermore, bactericidal and bacteriostatic activities were also determined. The Triptic Soy Agar samples taken from inhibited areas around the disks were put into nutrient broth without essential oils, extracts and pure compounds, and incubated at 27 ± 2 ºC for for 48 h. After 48 h, ıt was observed that there was no bacterial growth in the broth culture and it was considered as bactericidal effect or not bactericidal. All the tests were made in triplicate.

Determination of minimum inhibition concentration (MIC). Minimum inhibition concentrations (MICs) of the essential oils, extracts and the pure compounds were tested by a two-fold serial dilution method [4]. The dilutions of the essential oils and carvacrol (500µL/mL) were prepared by diluting 10% DMSO to achieve a decreasing concentration ranging from 500 µL/ml to 3,125 µL/mL. However, solutions of the extracts and thymol were prepared by diluting 10% DMSO at the concentrations ranging from 100 mg/mL to 10 mg/mL. 100 µL of suspension containing $1x10^8$ CFU/ml of bacteria spread on TSA plates. The blank disks (Oxoid) were impregnated with 12.5 µL of the solutions tested. Then, they were put in the middle of inoculated TSA plates. The bacterial cultures were incubated at 27 ± 2 ºC for 48 h. The least concentration of each the treatments showing a clear zone of inhibition were taken as the MIC. 10% DMSO was used as negative control. Each test was repeated at least twice.

Seed surface disinfection with sodium hypochlorite. The pepper seeds were surface disinfected to avoid the presence of any saprophytic and/or pathogenic microorganisms on the seed surface. Seed disinfection was performed by dipping the seeds for 3 min in 3% sodium hypochlorite and washing four times in sterilized and distilled water (sd H_2O). The seeds were left to dry on sterile Whatman filter paper sheets were stayed an overnight in the laminar flow hood for using further studies.

Coating procedure of pathogenic bacterium on the seeds. Pathogen bacterium *X. axonopodis* pv. *vesicatoria* strain Xcv 110c were grown in 50 ml flasks containing 20 mL of TSB medium on a rotary shaker (Gerhardt, laboshake) at 27 °C for 24 h. Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and appropriately diluted to $1x10^8$ CFU/ml in sdH_2O. Approximately, 0.2 g of sucrose (10 mg/mL) was added to each Erlenmeyer flasks, and 90 g of the surface-sterilized seeds were soaked separately in this suspension. The seeds were incubated in the flasks by shaking at 80 rpm for two days at 28 ºC to coat the seeds with the pathogen. After shaking, the seeds were taken out and air-dried on sterile Whatman filter paper sheets were stayed an overnight in the laminar flow hood.

Pepper seeds treated with essential oils and extracts. As stated above, the seeds were surface disinfected and coated with the pathogen (*X. axonopodis* pv. *vesicatoria* strain Xcv 110c). Then, the seeds were treated with the essential oils and extracts on two different ways. The concentrations of the essential oils for *S. spicigera* and *O. rotindufolium* (15.62, 31.25 and 62.50 µL/mL), and for *O. onites* hexane, acetone and chloroform extracts (5, 10, 15 and 20 mg/mL) were prepared by dissolving in 10% DMSO: distilled-water in 10 mL flasks.

In one of the applications, the seeds were directly soaked in the extracts suspensions, and then incubated by shaking at 80 rpm for 3 h at 28 ºC until the seeds were uniformly coated with the suspensions. In the other application, the seeds were indirectly applied with the essential oil suspensions. Sterile Whatman no. 1 filter papers were placed on bottom of each of Petri dishes (9cm x 1.5cm deep). The seeds surface disinfected and coated with the

pathogen put on this filter paper sheets in the Petri plate. And then, Whatman no. 1 paper was stuck onto the top of Petri dishes from inside and then impregnated with the oil suspensions using an automatic pipette. Thus, there was no direct contact between the oil and the seeds. Petri dishes were sealed with parafilm to prevent evaporation of oil and incubated by shaking at 80 rpm for 1 h at 28 ºC.

Determination of the germination percentage and number of infected seedlings of pepper seeds treated with the essential oils and extracts on Petri plate assays. As stated above, the seeds were surface disinfected, coated with the pathogen (*X. axonopodis* pv. *vesicatoria* strain Xcv 110c) and then the seeds were treated with the essential oils and extracts on two different ways. Seeds were left to dry on sterile Whatman filter paper sheets were stayed an overnight in the laminar flow hood. Two sterile Whatman filter papers were placed on the bottom of each Petri dish (9 cm-1.5 cm deep) and 10 seeds were transferred on the filter papers. Then, 10 mL of sd. H_2O was added to each Petri plate. The Petri dishes containing 10 seeds were sealed with parafilm to prevent evaporation of water were incubated in growth chamber on supplied with 12 h of fluorescent light and humidity of 80% at 23 ± 2 ºC. The treated seeds were allowed to germinate in Petri dishes. The assays were arranged in a completely randomized design with three repetitions including the controls. The percentage of germinated seeds per treatment was determined by counting the number of germinated seeds after 7 days. If at least 2 mm of radicle had emerged, the seeds were considered germinated. If no, a few water-soaked lesions on the cotyledons in the seedling stage were observed after 7 days, these seedlings were considered as infected with pathogenic bacterium and vice versa. Streptomycin sulfate (500 µg/mL), only sterilized seed+pathogen and sterilized seed+no pathogen were used as controls.

Statistical analysis. SPSS software programme version 10.0 was used for statistical analysis. Analysis of variance (ANOVA) was used to determine the effects of treatment on disease incidence and growth measurements. The means were compared using Duncan's multiple range tests. Results were expressed as average ± standard errors (SE).

Determination of the effect of the essential oils and extracts on the percentage of germinated pepper seeds, **disease severity and growth promotion on pot assays.** As stated above, the seeds were surface disinfected, coated with the pathogen, and then treated with the essential oils and extract two different ways. In the both applications, the seeds were left to dry on sterile Whatman filter paper sheets were stayed an overnight in the laminar flow hood. We planted 10 seeds per plastic pots (6.5 x 6.5 cm) containing garden soil and sand (1:1). The experiment was conducted in a growth chamber with an average temperature of 23 ± 2 ºC, relatively humidity of about 60 % and photoperiod of approximately 12-14 h day light. The seedlings were watered once every two days. Pots were arranged in a randomized block design with three repetitions. Three pots of each treatment comprised a replicate.

The percentage of germinated seeds per treatment was determined by counting the number of germinated seeds twenty days after planting. Disease severity was evaluated 45 days after planting using a 1 to 5 scale, in which 1 = no disease, 2 = a few water-soaked lesions, 3 = many spots with coalescence and slight plant wilting, 4 = severe wilting and defoliation, and 5 = plants dead (28). Forty five days after planting, the seedlings were evaluated, and data shoot fresh weight and root fresh weight were recorded as average milligrams of biomass per plant. The values were reported as averages for the three repetitions±standard errors. The assays were repeated three times with 10 lettuce seeds per pots for each treatment. Inhibition of disease severity as percentage [IDS(%)] was calculated as follows: $IDS(\%) = 100 \times (A_{control} - A_{sample}) / A_{control}$

Where $A_{control}$ is the disease severity of the control (only pathogen application) and A_{sample} is the disease severity of the essential oils, extracts and streptomycin sulfate applications.

RESULTS

Antibacterial activities of the essential oils and extracts against plant pathogenic bacterium on Petri plates. According to the in-vitro test results (Table 1), the essential oils showed a strong antibacterial activity based on the zone of inhibition against pathogen on Petri plates. The inhibasyon zones and MIC values of the essential oils ranged from 29-42 mm and 15.62-125 µl/mL, respectively. However, the extracts showed weak or no antibacterial activity. Their inhibition zones and MIC values ranged from 0-20 mm and 40-90 mg/mL, respectively. Among the extracts, the strongest antibacterial effect was obtained from the acetone extract of *O. onites*. *S. spicigera* hexane and acetone extract and *O. rotundifolium* essential oil and acetone extract had a bactericidal effect against pathogen.

Efficiacy of of the essential oils and extracts on the germination percentage and number of infected seedlings of pepper seeds on Petri plates assays. The results of the percentage of geminated pepper seeds and number of infected seedlings treated with essential oil and extract suspensions were given in Table 2,3.

TABLE 1
Antibacterial activities of the extracts, essential oils and effective component the isolated from plant species against *X. axonopodis* pv. *vesicatoria* strain Xcv 110c

	Extracts									
Plant species	Essential oil		Hexane		Chloroform		Acetone		Methanol	
	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC
S. hortensis	36	31.25	11	70	8	90	11	90	-	-
S. spicigera	29	31.25	11*	70	-	-	9*	90	-	-
O. onites	34	15.62	15	40	14	60	20	40	-	-
O. rotundifolium	42*	125	-	-	-	-	8*	90	-	-
Thymol	41	20.00	-	-	-	-	-	-	-	-
Carvacrol	27	15.63	-	-	-	-	-	-	-	-

IZD: Inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; MIC: Minimal inhibitory concentration as $\mu L mL^{-1}$ for the essential oil and in $mg\ mL^{-1}$ for the extracts; *: Bactericidal effect was observed; -: Not active

TABLE 2
Number of the infected seedling and percentage of the germinated pepper seeds coated with *X. axanopodis* pv. *vesicatoria* strain Xcv 110c and than treated with *S. spicigera* and *O. rotindufolium* essential oils on Petri plate assays

		S. spicigera essential oil		*O. rotindufolium* essential oil	
Treatments	Dose	Germinated seeds (%)	Number of the infected seedling	Germinated seeds (%)	Number of the infected seedling
Essential oil					
	62.50 µL/mL	56.7 a	8.30 b	40.0 a	6.67 bc
	31.25 µL/mL	60.0 a	6.15 b	53.3 ab	4.37 b
	15.62 µL/mL	66.7 ab	9.26 b	66.7 bc	7.0 bc
Controls					
Streptomycin sulfate	500 µg/mL	73.3 ab	8.78 b	73.3 cd	8.18 c
Only pathogen	-	63.3 a	9.73 b	63.3 bc	10.0 c
No pathogen	-	83.3 b	0.0 a	83.3 d	0.0 a

TABLE 3
Number of the infected seedling and percentage of the germinated pepper seeds coated with *X. axanopodis* pv. *vesicatoria* strain Xcv 110c and than treated with *O. onites* hexane, acetone and chloroform extracts on Petri plate assays

		Hexane extract		Acetone extract		Chloroform extract	
Treatments	Dose	Germinated seeds (%)	Number of the infected seedling	Germinated seeds (%)	Number of the infected seedling	Germinated seeds (%)	Number of the infected seedling
Extracts							
	20 mg/mL	0.6 a	10.0 e	63.3 a	8.94 c	10 a	10.0 c
	15 mg/mL	0.6 a	10.0 e	63.3 a	7.89 bc	100 b	8.66 b
	10 mg/mL	10.0 a	0.92 b	66.6 a	8.00 c	100 b	9.66 c
	5 mg/mL	53.3 b	3.75 c	66.6 a	9.50 cd	100 b	9.66 c
Controls							
Streptomycin sulfate	500 µg/mL	93.3 d	6.46 d	56.6 a	6.46 b	100 b	8.33 b
Only pathogen	-	76.6 c	10.0 e	63.3 a	10.0 d	100 b	10.0 c
No pathogen	-	90.0 d	0.0 a	90.0 b	0.0 a	100 b	0.0 a

As shown in Table 2, none of the concentrations of *S. spicigera* essential oil had a toxic effect on the pepper seed germination according to only pathogen application. But, the number of the infected seedlings was not reduced in these concentrations as compared to both streptomycin sulfate and only pathogen applications. According to the results of *O. rotindufolium* essential oil, some of the concentrations (31.25 and 62.50 µL/mL) had toxic effect on the pepper seed germination, but the concentration of the oil with 15.62 µL/mL had not toxic as compared to only the pathogen application. This application caused a decrease on the number of the infected seedlings with *X. axanopodis* pv. *vesicatoria.* Furthermore, this decrease was statistically significant compared to both streptomycin sulfate and only pathogen applications.

As shown in Table 3, *O. onites* hexane extract had a toxic effect on the pepper seed germination, but all concentration of the acetone extract and chloroform extracts (except 20 mg/mL) had no toxicity. Furthermore hexane extract with 5 and 10 mg/mL of the concentration; acetone extract with 10, 15 and 20 mg/mL of the concentration; chloroform extract with15 mg/mL of the concentration caused a decrease on the number of the infected seedlings with the pathogen as compared to only the pathogen application.

Efficiacy of the essential oils and extracts on the percentage of germinated pepper seed, disease severity, shoot fresh weight and root fresh weight on pot assays. The results showed the effect of *S. spicigera* and *O. rotindufolium* essential oils, and *O. onites* hexane, acetone and chloroform extracts on the percentage of germinated pepper seed, disease severity, shoot fresh weight and root fresh weight on pot assays for *X. a.* pv. *vesicatoria* were given in Tables 4-8.

As shown in Table 4, all of the applied concentrations of *S. spicigera* essential oil significantly provided the higher germination rate than that of the only pathogen, no pathogen and streptomycin sulfate applications. Furthermore, these applications significantly reduced disease severity as compared to both streptomycin sulfate and only pathogen applications. The maximum inhibition of the disease severity (75%) was obtained from the concentrations of the essential oil application with 62.5 µL/mL. The highest plant height, root fresh weight and shoot fresh weight were obtained from streptomycin sulfate, the concentrations of the oil with 15.62 and 15.62 µL/mL, respectively. Furthermore, the positive effects of 15.62 µL/mL concentration application on root fresh weight and shoot fresh weight were statistically significant as the controls.

As shown in Table 5, all of the concentrations of *O. rotindufolium* essential oil caused the high germination rate and reduced disease severity as compared to only pathogen applications. Furthermore, some of these positive effects were statistically significant compared to only pathogen applications. There was no significant difference in disease severity among 125 and 250 µL/mL concentrations of the essential oil in comparison to the no pathogen application. The maximum inhibition of the disease severity (68.11%) was obtained from both 125 and 250 µL/mL concentrations of the oil. The maximum plant height was obtained from streptomycin sulfate. The root fresh weight and shoot fresh weight were obtained from 125 µL/mL concentrations of the oil. Furthermore, the effect on shoot fresh weight was statistically significant as compared to only pathogen applications.

As shown in Table 6; there was not any significant effect of *O. onites* hexane extract on germination of pepper seeds as compared to both no pathogen and only pathogen applications. The lowest disease severity and the maximum inhibition of the disease severity in the extract applications were obtained from that of 10 mg/mL concentration. The maximum plant height, plant root fresh weight and shoot fresh weight were obtained from only pathogen application. The extract applications had not statistically significant activity on growth parameters.

As shown in Table 7, *O. onites* acetone applications caused the low germination rate. But, all of the concentration of the oil significantly reduced disease severity as compared to both streptomycin sulfate and only pathogen applications. However, there are not any significant differences among all of the essential oil applications in comparison to the no pathogen application. Maximum inhibition of the disease severity (77.4%) was obtained from 5 mg/mL concentration of *O. onites* acetone extract. The maximum plant height, root fresh weight and shoot fresh weight were obtained from 10 mg/mL concentrations of the extract. But, all of these positive effects on vegetative growth of plants were not statistically significant compared to only pathogen or no pathogen application.

As shown in Table 8, all of the applied concentrations of *O. onites* chloroform extract significantly caused the lower germination rate than that of the no pathogen application. The lowest disease severity and maximum inhibition of the disease severity were seen at 15 mg/mL concentration of the extract. The highest plant height, root fresh weight and shoot fresh weight were obtained from only the pathogen application. However, there are not any significant differences among all of the applications.

TABLE 4

The effects of *S. spicigera* essential oil on the germination of pepper seeds and disease severity caused by *X. axanopodis* pv. *vesicatoria* strain Xcv 110c on pots.

Treatments	Dose (µL/mL)	PGS (%)	DS	IDS (%)	PH (mm)	RFW (g/plant)	SFW (g/plant)
Essential oil	62.5	40.0 c	1.00±0.00 a	75.00	18.17±0.42 a	6.73±1.13 a	1.98±0.37 ab
	31.25	53.3 cd	1.83±0.16 b	54.25	19.33±0.64 ab	9.74±0.80 bc	2.86±0.24 bc
	15.62	60.0 d	3.17±0.16 c	20.75	19.66±0.73 ab	11.60±0.14 c	3.52±0.05 c
Streptomycin sulfate	500 µg/mL	16.7 a	1.33±0.21 a	66.75	24.00±1.67 b	5.00±0.61 a	1.46±0.27 a
Control (only pathogen)	-	30.0 b	4.00±0.25 d	-	21.53±2.37 ab	5.49±1.44 a	1.55±0.47 a
Control (no pathogen)	-	33.3 b	1.00±0.00 a	-	20.41±2.49 ab	6.96±1.12 ab	2.17±0.38 ab

PGS: Percentage of germinated pepper seeds; DS: Disease severity; IDS: Inhibition of disease severity according to control; PH: Plant height (PH); RFW: Root fresh weight; SFW: Shoot fresh weight on pots. Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test

TABLE 5

The effects of *O. rotindufolium* essential oil on the germination of pepper seeds and disease severity caused by *X. axanopodis* pv. *vesicatoria* strain Xcv 110c on pots.

Treatments	Dose (µL/mL)	PGS (%)	DS	IDS (%)	PH (mm)	RFW (g/plant)	SFW (g/plant)
Essential oil	250	40.0 b-d	1.17±0.16 a	68.11	19.80±0.69 ab	6.01±0.87 ab	1.97±0.37 ab
	125	53.3 d	1.17±0.16 a	68.11	19.73±0.51 ab	8.34±0.28 b	2.86±0.06 b
	62.50	50.0 cd	2.17±0.16 b	40.87	18.55±0.74 a	7.79±0.61 ab	2.63±0.11 ab
Streptomycin sulfate	500 µg/mL	16.7 a	1.33±0.21 a	63.76	24.00±1.67 b	5.00±0.61 a	1.46±0.27 a
Control (only pathogen)	-	30.0 ab	3.67±0.33 c	-	21.53±2.37 ab	5.49±1.44 ab	1.55±0.47 a
Control (no pathogen)	-	33.3 a-c	1.00±0.00 a	-	22.41±2.49 ab	6.96±1.12 ab	2.17±0.38 ab

PGS: Percentage of germinated pepper seeds; DS: Disease severity; IDS: Inhibition of disease severity according to the control; PH: Plant height (PH); RFW: Root fresh weight; SFW: Shoot fresh weight on pots. Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test

TABLE 6

The effects of *O. onites* hexane extract on the germination of pepper seeds and disease severity caused by *X. axanopodis* pv. *vesicatoria* strain Xcv 110c on pots.

Treatments	Dose (mg/mL)	PGS (%)	DS	IDS (%)	PH (mm)	RFW (g/plant)	SFW (g/plant)
Hexane extract	20	73.3 b	1.66±0.81 bc	50.15	5.73±0.35 ^{ns}	0.03±0.01 bc	0.21±0.02 ^{ns}
	15	70.0 ab	2.00±0.89 bc	39.93	5.52±0.73	0.02±0.00 ab	0.20±0.04
	10	70.0 ab	1.33±0.51 b	60.06	6.12±0.26	0.01±0.00 a	0.19±0.04
	5	80.0 b	2.33±0.51 c	30.03	5.79±0.47	0.02±0.00 ab	0.25±0.01
Streptomycin sulfate	500 µg/mL	56.6 a	2.33±0.51 c	30.03	6.20±1.22	0.02±0.00 ab	0.28±0.11
Control (only pathogen)	-	83.3 b	3.33±0.81 d	-	6.78±1.05	0.04±0.01 c	0.32±0.05
Control (no pathogen)	-	76.6 b	1.00±0.00 a	-	6.23±1.28	0.02±0.00 abc	0.26±0.10

PGS: Percentage of germinated pepper seeds; DS: Disease severity; IDS: Inhibition of disease severity according to control; PH: Plant height (PH); RFW: Root fresh weight; SFW: Shoot fresh weight on pots; ^{ns}: Not significant; Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test

TABLE 7

The effects of *O. onites* acetone extract on the germination of pepper seeds and disease severity caused by *X. axanopodis* pv. *vesicatoria* strain Xcv 110c on pots.

Treatments	Dose (mg/mL)	PGS (%)	DS	IDS (%)	PH (mm)	RFW (g/plant)	SFW (g/plant)
Acetone extract	20	30.0 a	1.33±0.51 a	60.06	6.00±0.61 ab	0.02±0.01 ^{ns}	0.23±0.03 ^{ns}
	15	60.0 d	1.33±0.51 a	60.06	5.60±0.87 a	0.01±0.00	0.21±0.05
	10	43.3 b	1.33±0.51 a	60.06	7.61±0.62 b	0.08±0.08	0.33±0.04
	5	43.3 b	1.00±0.00 a	69.96	6.40±0.44 ab	0.01±0.00	0.30±0.03
Streptomycin sulfate	500 µg/mL	50.0 c	2.33±0.51 b	30.03	6.20±1.22 ab	0.02±0.02	0.27±0.11
Control (only pathogen)	-	63.3 de	3.33±0.81 c	-	5.99±0.31 ab	0.03±0.00	0.31±0.04
Control (no pathogen)	-	76.6 e	1.00±0.00 a	-	6.23±1.28 ab	0.02±0.00	0.26±0.10

PGS: Percentage of germinated pepper seeds; DS: Disease severity; IDS: Inhibition of disease severity according to control; PH: Plant height (PH); RFW: Root fresh weight; SFW: Shoot fresh weight on pots; ^{ns}: Not significant; Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test

TABLE 8

The effects of *O. onites* chloroform extract on the germination of pepper seeds and disease severity caused by *X. axanopodis* pv. *vesicatoria* strain Xcv 110c on pots.

Treatments	Dose (mg/mL)	PGS (%)	DS	IDS (%)	PH (mm)	RFW (g/plant)	SFW (g/plant)
Chloroform extract	20	36.6 abc	1.66±0.81 bc	50.15	5.93±0.55 ^{ns}	0.03±0.10 ^{ns}	0.19±0.02 ^{ns}
	15	26.6 a	1.50±0.54 b	54.95	5.72±0.19	0.02±0.00	0.19±0.02
	10	43.3 bc	2.66±0.51 de	20.12	5.46±0.58	0.02±0.00	0.16±0.12
	5	60.0 d	3.66±0.51 f	ND	6.25±1.20	0.02±0.00	0.26±0.05
Streptomycin sulfate	500 µg/mL	50.0 c	2.33±0.51 cd	30.03	6.20±1.22	0.02±0.00	0.27±0.11
Control (only pathogen)	-	63.3 d	3.33±0.81 e	-	6.78±1.05	0.04±0.00	0.32±0.05
Control (no pathogen)	-	76.6 e	1.00±0.00 a	-	6.23±1.28	0.02±0.00	0.26±0.09

PGS: Percentage of germinated pepper seeds; DS: Disease severity; IDS: Inhibition of disease severity according to control; PH: Plant height (PH); RFW: Root fresh weight; SFW: Shoot fresh weight on pots; ^{ns}: Not significant; Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test

DISCUSSION

Bacterial spot disease of pepper, caused by *X. axonopodis* pv. v*esicatoria,* is a serious problem in the processing pepper fields in the eastern Mediterranean region of Turkey [27]. Contaminated seeds by the pathogen are considered contaminated seeds as the primary inoculum source of the disease and have a significant role in disease outbreak in this region [28]. Use of pathogen-free seeds and/or seed treatments can be recommended for effective management of the disease.

In recent years, the use of synthetic pesticides in plant disease protection programs around the world has resulted in disturbances of the environment, pest resurgences, and pest resistance to pesticides and lethal effect to non target organisms in the agro-ecosystems in addition to direct toxicity to users [29,30,31]. It is also known that many plant pathogenic bacteria have acquired resistance to synthetic pesticides [32]. Therefore, considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms. One of the alternative control methods of the disease is use of effective plant extracts as seed treatments in conventional and organic agriculture.

Many publications have been previously documented on the antimicrobial activity of the essential oil or extracts against plant pathogenic bacteria *Xanthomonas* sp.

[8,19,20,21,22,23,28,31,33]. However, there are few reports on using of plant essential oils and extracts as seed treatments for fungal pathogens and bacterial pathogens under in vivo conditions. It was stated that that seed treatment with essential oil and extract of *Cleistocalyx operculatus* effectively inhibited *Xanthomonas* spp [31], and bacterial spot disease severity on tomato and pepper seedlings was reduced between 77 and 96% at ratios by *Alium sativum* and *Eucalyptus* sp. extracts, respectively [28]. Similar results have also reported in some studies [34,35].

The plant essential oils and extracts of *S. hortensis*, *S. spicigera*, *O. onites* and *O. rotindufolium* have been recognized as having antibacterial effects, but their efficacy as seed disinfectants on plant pathogenic bacteria has not been studied. The present study showed that the essential oils and/or extracts of *S. hortensis*, *S. spicigera*, *O. onites* and *O. rotindufolium* possess antibacterial activities against all the tested plant pathogenic bacterium on Petri plate and pot assays. These results are in agreement with the previous literature reported on these species [3,5,6,7,8,9,10,11,13,14,15,16,17,36,37,38]. We think that this is related to main compounds of the extracts and essential oils obtained from tested plant species, in which found to be rich monoterpene phenols, especially carvacrol and thymol. It is known that phenolic compounds such as carvacrol and/or thymol are one of the main components of the *S. hortensis* [5,38], *S. spicigera* [4], *O. onites* [36] and *O. rotundifolium* [39].

In the Petri plate assays, especially the hight concentrations of the essential oils and/or extracts showed a toxic effect on the pepper seed germination. Genarally, the number of the infected seedlings were not reduced as compared to both streptomycin sulfate and no pathogen applications. We think that this is related to high relative in Petri plate. Because, some of these concentration of the essential oils or extracts significantly reduced disease severity as compared to both streptomycin sulfate and only pathogen applications.

Many plant essential oils, extracts and their pure compounds are considered to play a role in host defense mechanisms against plant pathogens. It is stated that biologically active compounds present in plant products act as elicitors and induce resistance in host plants resulting in reduction of disease development [39,40,41]. In addition, peroxidases have diverse functions in plant life such as defense against pathogen, cross-linking of cell wall components, formation of lignin and suberin, auxin catabolism, and antioxidant defense. Because of the antioxidant properties found in *S. hortensis*, it is important to characterize the peroxidases present in this labiatae [7].

In this study, some of the concentrations of the essential oil or the extracts caused a positive effect on the plant growth parameters. Therefore, this increase can be related to host defense mechanisms in plants against plant pathogens. The mechanism of the action of the essential oil is probably related to the outer membrane disintegrating properties of thymol and carvacrol [42]. Our results also confirmed that the potent antibacterial effect of the essential oils and extracts of tested plant species is probably related to its carvacrol and thymol constituents. In the literature, some investigations suggest that these compounds penetrate inside the cell, where they interfere with cellular metabolism [11,43,44]. Other studies also indicated that they disturb the structure of the cellular membrane and react with the active sites of enzymes or act as an H^+ carrier, depleting adenosine triphosphate pool [45,46].

Application of the plant essential oils and extracts as a seed disinfectant is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptances of this technology by farmers. In this study, some concentrations of the essential oils and extracts were not toxic to the pepper seeds, and could be used as potential disinfection agents against *X. axonopodis* pv. v*esicatoria*. In conclusion, our results show that some of the concentraitions of the essential oils or the extracts of *S. hortensis*, *S. spicigera*, *O. onites*

and *O. rotindufolium* can be used as a seed disinfectant and as potential control agents for management of bacterial disease. Further studies on the combined effects of many local plant essential oils, extracts and their components as seed disinfectant are in progress in our model systems.

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