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Research Article
Antagonistic Impact of Lavender and Cranberry Essential Oils Against *Xanthomonas campestris* pv. *vesicatoria*

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Abstract

Background and Objective: Essential oils have emerged as a potential alternative to the synthetic chemicals for the control of pathogenic microorganisms. In the current study, the bactericidal capability of some essential oils was assayed against *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) that is the causal agent of bacterial spot disease of tomato. **Materials and Methods:** The antibacterial activity and minimum inhibitory concentration of the selected oils were calculated. At the same time, the influence of these essential oils on tomato seedling growth and some antioxidant metabolites was investigated in presence of *X. campestris* pv. *vesicatoria*. Moreover, the bioactive compounds of the selected oils were analyzed using Gas Chromatography-Mass Spectrometry. **Results:** Lavender and cranberry essential oils exhibited the maximum antibacterial activity against *Xcv*. The seed germination and growth of oil treated seedlings in presence of *Xcv* were markedly enhanced. At the same time, the ascorbate and reduced glutathione levels of oil treated seedlings was increased compared with the corresponding control. The analysis of lavender and cranberry essential oils demonstrated the existence of various phytochemicals. The major constituents of lavender oil were linalool, linalyl acetate, β-ocimene, geraniol, lavandulol, 1,8-cineole meanwhile α-pinene, β-myrcene, sabinene, β-caryophyllene and δ-cadinene were the most abundant ingredients of cranberry oil. **Conclusion:** The application of lavender and cranberry essential oils demonstrated a marked ameliorative effect on tomato seedlings treated with *Xcv* that was attributed to the presence of a wide array of bioactive compounds. Some of these molecules can suppress bacterial growth while others can induce plant antioxidant system.

Key words: Essential oils, antibacterial, *Xanthomonas campestris* pv. *vesicatoria*, GC-MS, phytochemicals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetables worldwide. Due to its economic importance and the potential health benefits, both the world harvested area and the total production was increased[1]. In the Middle East, tomato was begun to cultivate at the end of the 18th century. In 2008, KSA ranked 5th in the world with about 9 million t of the production[2]. In Egypt, tomato is one of the most important vegetable crops. However, production faces some problems in summer season due to high temperature as insect born viruses prevailing in these months[3]. Other factors including cultivar selection, management practices, diseases and pests can limit tomato production[4]. It has been reported that more than 60 pathogens including bacteria, fungi, viruses and nematodes can cause disease in tomato and can result in severe problems in global tomato production[5].

Bacterial diseases of tomatoes can be some of the most serious and destructive diseases[6]. Bacterial spot considers one of the most destructive diseases of tomato grown in moist warm environments. It is highly destructive both in greenhouses and field grown tomatoes causing 10-50% yield loss in many tomato production areas[7]. The causal agent of the bacterial spot disease is a complex of several *Xanthomonas* species for which classification have been changed several times in the past 30 years[8]. *Xanthomonas campestris* is the causal agent of bacterial spot disease of tomato[9]. *Xanthomonas campestris* invades the plant through stomata and wounds created by windblown soil, insect punctures or any mechanical means.

Increasing use of chemical causes many negative effects such as development of pathogen resistance to the applied agents. Furthermore, the high cost of pesticides and consumer demand for pesticide-free food has led to a search for another substitute products. Biological control is considered as an alternative method of reducing the use of chemical control effect. Also, the lack of resistance of some commercial tomato, cultivars have stimulated a search for biological control agents[10-12]. The use of essential oils for disease control serve as an alternative method for decreasing the use of chemical product in agricultural crops. Essential oils are volatile, natural and complex compounds from secondary metabolites of aromatic plants and characterized by strong odour[13,14]. In recent years, using a large number of antimicrobial essential oils and their constituents have been considered as a reliable method of biological control for tomato disease caused by *Agrobacterium tumefaciens* and *Xanthomonas axonopodis*[15].

Lavender oil is an essential oil obtained by distillation from the spikes of the flowers. Lavender oils are distinguished in two forms. The first form is lavender flower, a colorless and insoluble in water. The second form is lavender spike oil, a distillate from the herb *Lavandula latifolia*. Like all essential oils, it is not a pure compound; it is a complex mixture of phytochemicals[16]. Also, cranberry seed oil is an essential oil where it is equally high in omega-3 and omega-6 fatty acids, primarily used in the cosmetic industry[17]. The aim of the current study was to evaluate the antibacterial activity of some plant essential oils against phytopathogen *Xanthomonas campestris* pv. *vesicatoria* (Xcv). Then the influence of the selected oils on seed germination and seedling growth of tomato in presence and in absence of *Xcv* were investigated.

MATERIALS AND METHODS

Study area and sampling site: The study was carried out in Biology Department, Faculty of Science, Jazan University, Saudi Arabia between October, 2018-September, 2019.

Bacterial isolates and culture conditions: The phytopathogenic bacterial strain *Xanthomonas campestris* pv. *vesicatoria (Xcv*) was purchased from MIRCEN, Faculty of Agriculture, Ain-Shams University, Egypt. Cultures were routinely grown aerobically on nutrient broth medium using shaking incubator at 30°C for 24 h. Appropriate aseptic techniques and standard operating procedures were followed during this study.

Essential oils: Thirteen essential oils used in the current study (Fenugreek, lemon, cinnamon, basil, onion, garlic, nagarmotha, jasmine, camphor, lavender, mint, cranberry and black seed) were purchased from specialty retailer shops in Saudi Arabia. The oils were 100% pure with no added synthetic compounds.

Antibacterial effect of the essential oils: The antibacterial impact of the essential oils against *Xcv* was investigated. The tested oils were dissolved in 5% dimethyl sulfoxide. About 9 mL of nutrient broth containing 1% (v/v) of oils were inoculated with 1 mL of mid-log phased cultures of the fresh culture of each bacterial strain (10^7 CFU mL^{-1}). After incubation at 30°C for 24 h, the bacterial optical density was measured spectrophotometrically at 600 nm. At the same time, protein content of bacteria exposed to essential oils was estimated using the Bicinchoninic Acid (BCA) assay kit (Sigma-Aldrich BCA-1, B9643) with bovine serum albumin as a standard. The developed purple color was measured spectrophotometrically[18] at 562 nm.

Determination of minimum inhibitory concentration of the essential oils: The essential oils which exhibited the maximum antimicrobial activity were selected for determining the Minimum Inhibitory Concentration (MIC) using broth dilution method[19]. A bacterial colony was sampled with a loop and inoculated in 25 mL nutrient broth and incubated at 30°C for 24 h. The cultures were diluted with fresh medium to 10^7 CFU mL^{-1}. The tested oils (2.5, 5.0, 7.5, 10.0 and 20.0 μL mL^{-1}) were mixed with bacterial suspension and incubated at 30°C. After 24 h, the bacterial optical density was measured at 600 nm. The MIC was considered as the lowest concentration that prevented the visible growth. The experiments were carried out in three replicates.

Plant materials, treatments and growth parameters: The cultivated tomato seeds (*Solanum lycopersicum* L.; Solanaceae) were surface sterilized by immersion in ethyl alcohol (70%) for 2 min followed by rinsing 3 times with sterilized deionized water. Surface sterilized seeds were soaked in lavender and cranberry oils, separately for 24 h and then kept on a sterilized filter paper for 2 h. Treated seeds were subjected to germination experiment in Petri-dishes containing sterilized filter papers moistened by 10 mL sterilized dH_2O. Plates were kept in dark at 25°C and the percentage of seed germination was calculated. Moreover, the influence of lavender and cranberry oils on the growth of tomato seedlings in absence and in presence of *Xcv* was evaluated. A loopful of the O/N activated bacterial strain was inoculated into nutrient broth medium then incubated at 30°C in a rotary shaking incubator at 150 rpm. After 24 h of incubation, cultures were subjected to centrifugation at 10,000 rpm for 10 min and bacterial pellets were re-suspended in sterile distilled water at cell number 10^7 CFU mL^{-1}. Then 10 mL of the bacterial suspension was added to the surface of the 15 cm diameter pots, 10 seeds per pot. Triplicates of each treatment were done and all pots were kept at 25°C for 9 days. The treatments of tomato seedlings were carried out as following; H1: Untreated seeds, H2: Seeds treated with cranberry oil, H3: Seeds treated with lavender oil, H4: Seeds in presence of *Xcv*, H5: Seeds treated with lavender oil in presence of *Xcv*, H6: Seeds treated with cranberry oil in presence of *Xcv*. The length of seedlings, fresh weight and dry weight of seedlings were determined according to Essa *et al.*[20].

Estimation of ascorbic acid: The concentration of ascorbic acid in tomato seedlings was estimated using 2,4-Dinitrophenylhydrazine method according to Roe and Kuether[21]. About 1 g of plant samples was homogenized with 4% TCA. The supernatant obtained after centrifugation at 4000 rpm for 10 min was treated with few drops of bromine water until the solution became colored. Then few drops of thiourea were added to it to remove the excess bromine and thus the clear solution was obtained. Then 2,4-Dinitrophenylhydrazine solution was added. After incubation for 30 min at room temperature, the samples were read at 540 nm and the levels of ascorbic acid in the samples were determined as mg ascorbate/g leaf.

Estimation of reduced glutathione: The estimation of the levels of reduced glutathione was done using the method proposed by Moron *et al.*[22]. Reduced glutathione (GSH) is measured by its reaction with 5,5-Dithiobis (2-nitrobenzoic acid) to give a yellow colored product that absorbs at 412 nm. The values of the reduced glutathione are explicit as nmol of GSH g^{-1} tissue.

Gas Chromatography Mass Spectrometry (GC-MS) analysis of essential oils: To identify the compounds in the essential oils, Gas Chromatography Mass Spectrometry (GC-MS) system model 7890 (Regional Centre for Food and Feed, ARC) was used according to the recommendations of the Essa and Fathy[23]. An Hp5MS fused silica capillary column (Hewlett Packard, 30 m, 0.25 mm i.d., 0.25 μm film thickness, cross-linked to 5% phenyl methyl siloxane stationary phase) was used. The entire system was controlled by MS ChemStation software (Hewlett Packard, version A.01. 01). Electron impact mass spectra were recorded at 70 eV. Ultra-high purity helium (99%) was used as the carrier gas at a flow rate of 1 mL min^{-1}. The injection volume was 1 μL and all the injections were performed in a splitless mode. Injector temperatures were 250°C. Temperature program was used: 60°C (2 min), 30°C min^{-1}, 170°C (5 min), 7°C min^{-1} and 250°C (10 min). The isolated compounds were identified by their retention indices determined in reference to a series of n-alkanes and verified by comparing their mass spectra with the GC-MS spectral library (Wiley 8 and FFNSC 1.2 libraries).

Statistical analysis: The data presented in the current study are the mean value of at least three replicates. Using MS Excel 2007, the standard errors were calculated for all values.

RESULTS

In the current study, the antibacterial capability of several plant essential oils was screened against the phytopathogen *Xcv*. Data in Fig. 1 showed that the maximum antibacterial

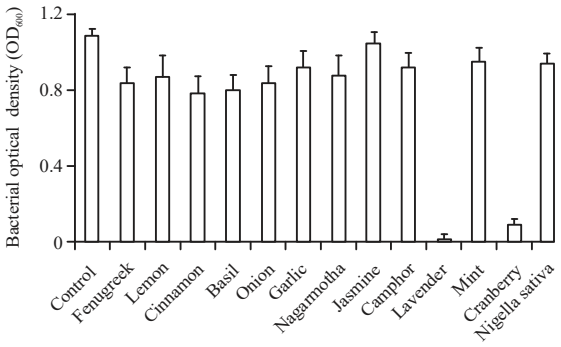

Fig. 1: Antibacterial impact of some essential oils against *Xanthomonas campestris* pv. *vesicatoria*

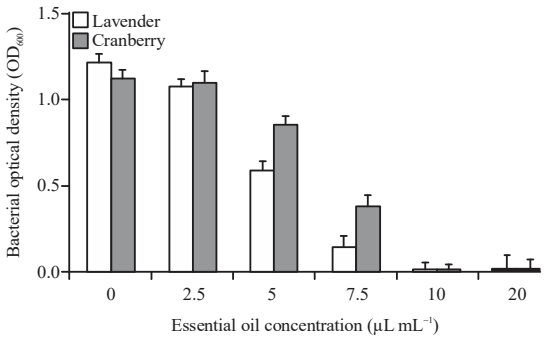

Fig. 2: Effect of lavender and cranberry oil concentration on the growth of *Xanthomonas campestris* pv. *vesicatoria*

Table 1: Effect of essential oils on protein content of *Xanthomonas campestris* pv. *vesicatoria* after incubation at 30°C for 24 h

Essential oil	Protein content (µg mL^{-1})
Fenugreek	126±09
Lemon	123±11
Cinnamon	118±14
Basil	122±10
Onion	125±16
Garlic	134±12
Nagarmotha	126±08
Jasmine	176±13
Camphor	133±11
Lavender	017±12
Mint	141±16
Cranberry	028±15
Black seed	138±12

Experiment was carried out in triplicate and values are means of replicates±standard error

activity was recorded by lavender and cranberry essential oils where the bacterial growth expressed as optical density was sharply decreased. The bacterial growth was assayed by measuring the culture optical density (OD_{600}) after 24 h. The experiment was carried out in triplicate and values are means of replicates±standard error.

At the same time, results in Table 1 demonstrated a severe reduction in the protein content of bacteria treated with lavender (17 µg mL^{-1}) and cranberry (28 µg mL^{-1}). The total protein content in absence of essential oils was 189 µg mL^{-1}. Data in Fig. 2 demonstrated the minimum inhibitory concentration influence of lavender and cranberry essential oils against *Xcv*. The maximum bacterial inhibition was recorded at 10.0 µL mL^{-1} for both oils. Lower oil concentrations showed an inhibition of *Xcv* at varying levels. Bacterial growth was assayed by measuring the culture optical density (OD_{600}) after 24 h. The experiment was carried out in triplicate and values are means of replicates±standard error.

Table 2: Effect of lavender and cranberry essential oils on tomato seed germination in presence and absence of the phytopathogen *Xanthomonas campestris* pv. *vesicatoria*

	Seed germination (%)		
Treatments	2 days	4 days	6 days
H1	33.4	83.5	100.0
H2	26.7	80.2	100.0
H3	20.1	73.4	100.0
H4	13.3	33.4	55.4
H5	20.1	53.4	81.3
H6	20.1	46.7	63.7

H1: Untreated seeds, H2: Seeds treated with cranberry oil, H3: Seeds treated with lavender oil, H4: Seeds in presence of *Xcv*, H5: Seeds treated with lavender oil in presence of *Xcv*, H6: Seeds treated with cranberry oil in presence of *Xcv*. Experiment was carried out in triplicate and values are means of replicates±standard error

The obtained results (Table 2) showed a non-significant impact of the tested oils on seed germination process compared with untreated seeds. In presence of *Xcv*, the percentage of seed germination was markedly reduced for oil untreated seeds (55.4%). Meanwhile oil treated seeds recorded an improvement of seed germination with lavender oil (81.3%) and cranberry oil (63.7%).

The influence of lavender and cranberry essential oils on the growth of tomato seedlings was evaluated in presence and in absence of *Xcv*. The obtained data (Fig. 3, 4) showed that seedling length was reduced in presence of *Xcv* (5.8 cm) while oil treated seedlings recorded a less length retardation with lavender and cranberry compared to untreated seedlings (Fig. 4a). At the same time, results in Fig. 4b showed an increase in fresh weight of tomato seedlings treated with lavender (1.01 g) and cranberry (0.97 g) in absence of *Xcv*. In presence of the phytopathogen, a significant reduction of fresh weight of tomato seedlings was observed while seedlings fresh weight was enhanced with lavender (0.69 g) and cranberry (0.57 g) compared with the corresponding control.

Fig. 3: Effect of oil treatment on tomato seedling after 9 days in presence and in absence of *Xanthomonas campestris* pv. *vesicatoria*

H1: Untreated seeds, H2: Seeds treated with cranberry oil, H3: Seeds treated with lavender oil, H4: Seeds in presence of *Xcv*, H5: Seeds treated with lavender oil in presence of *Xcv*, H6: Seeds treated with cranberry oil in presence of *Xcv*

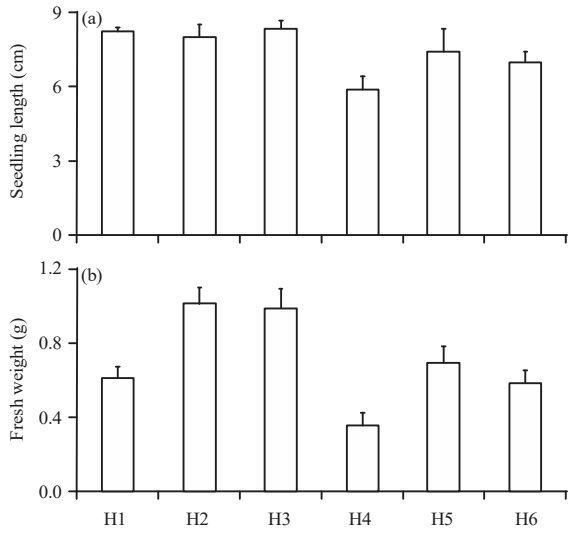

Fig. 4(a-b): Effect of oil treatment on the (a) Length and (b) Fresh weight of tomato seedlings after 9 days in presence and in absence of *Xanthomonas campestris* pv. *vesicatoria*

H1: Untreated seeds, H2: Seeds treated with cranberry oil, H3: Seeds treated with lavender oil, H4: Seeds in presence of *Xcv*, H5: Seeds treated with lavender oil in presence of *Xcv*, H6: Seeds treated with cranberry oil in presence of *Xcv*. Experiment was carried out in triplicate and values are means of replicates±standard error.

Data in Fig. 5a-b demonstrated the contents of some non-enzymatic antioxidant metabolites in tomato seedlings treated with lavender and cranberry oils in absence and in

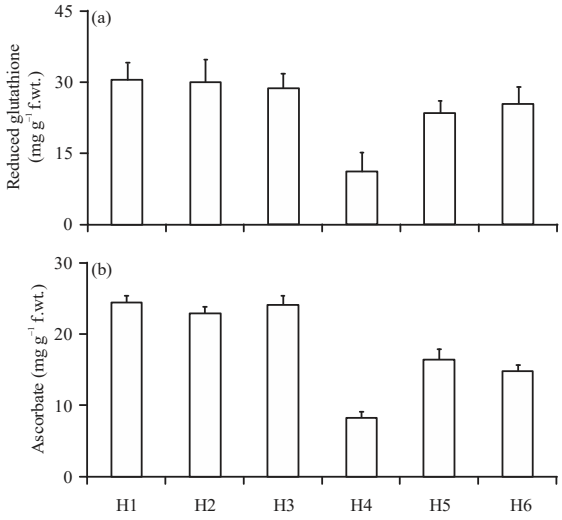

Fig. 5(a-b): Effect of oil treatment on antioxidant metabolites accumulated (a) Reduced glutathione and (b) Ascorbate in tomato seedling after 9 days in presence and in absence of *Xanthomonas campestris* pv. *vesicatoria*

H1: Untreated seeds, H2: Seeds treated with cranberry oil, H3: Seeds treated with lavender oil, H4: Seeds in presence of *Xcv*, H5: Seeds treated with lavender oil in presence of *Xcv*, H6: Seeds treated with cranberry oil in presence of *Xcv*. Experiment was carried out in triplicate and values are means of replicates±standard error

presence of the phytopathogen *Xcv*. The obtained results showed that in the absence of the pathogen the ascorbate (AA) levels in oil treated seedlings were relatively concomitant to the corresponding control. In case of seedling infected with *Xcv*, AA concentration was sharply reduced (8.24 mg g^{-1} f.wt.) while oil treated seedlings demonstrated a clear increase of AA with lavender (16.45 mg g^{-1} f.wt.) and cranberry (14.74 mg g^{-1} f.wt) compared with untreated seedlings (Fig. 5b). Regarding reduced glutathione (GSH) level, in absence of *Xcv*, the level of GSH in oil treated seedlings was parallel to corresponding control. In presence *Xcv*, GSH level was clearly diminished (11.32 mg g^{-1} f.wt.) while in oil treated seedlings it was markedly increased with lavender and cranberry with comparison to the untreated seedlings (Fig. 5a).

GC-MS analysis of lavender and cranberry essential oils (Table 3) demonstrated the existence of various bioactive compounds at different concentrations. The major constituents of lavender oil were linalool (29.8%), linalyl acetate (18.8%), β-ocimene (5.4%), geraniol (4.7%), lavandulol (4.5%) and 1,8-cineole (3.7%). At the same time, α-pinene (24.5%), β-myrcene (11.6%), sabinene (6.5%), β-caryophyllene (4.9%) and δ-cadinene (2.1%) were the most abundant compounds in cranberry essential oil.

Table 3: Chemical composition of lavender and cranberry essential oils using GC-MS analysis

Components	RI	Concentration (%) Lavender	Cranberry
α-thujene	0921	-	0.7
α-pinene	0938	1.5	24.5
Camphene	0955	-	0.3
Sabinene	0981	-	6.5
β-pinene	0987	-	2.9
β-myrcene	0993	-	11.6
α-terpinene	1010	-	0.6
P-cymene	1028	-	1.9
α-limonene	1030	0.1	-
γ-terpinene	1041	0.6	-
β-ocimene	1048	5.4	0.1
γ-terpinene	1055	0.6	1.7
α-terpinolene	1077	0.2	1.4
1-octen-3-ol, acetate	1095	0.6	-
Terpinen-4-ol	1127	2.1	2.4
Lavandulol	1149	4.5	-
α-terpineol	1188	1.5	0.4
1-8-cineole	1225	3.7	0.3
Geraniol	1236	4.7	-
Linalyl acetate	1244	18.8	-
α-copaene	1366	-	0.7
β-caryophyllene	1389	2.9	4.9
α-santalene	1410	0.2	-
α-humulene	1423	-	1.7
β-farnesene	1456	3. 5	-
γ-cadinene	1509	0.1	-
δ-cadinene	1539	-	2.1
Linalool	1545	29.8	-

DISCUSSION

The current study highlighted the antibacterial activity of lavender and cranberry essential oils against *Xcv*. In past years there has been a growing concern in producing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to the screening of antimicrobial activity of plant essential oils that have been used for long decades in traditional medicine. These results coincided with those of Hossain *et al*.[24] and Man *et al*.[25] who revealed that lavender essential oil has a potential bactericidal activity against various pathogenic bacteria. Similarly, the antibacterial property of cranberry has been reported by Diarra *et al*.[26] and Lian *et al*.[27].

Plant essential oils are known to exhibit a wide range of biological activities as well as they have low mammalian toxicity, less environmental detrimental effects and wide public acceptance[28]. Hence, they form the basis of many applications in food and agriculture industries to control plant pathogenic bacteria. This investigation clarified a clear decline of tomato seed germination and seedlings growth in presence of the phytopathogen *Xcv*. The inhibition of seed germination could be attributed to the capability of *Xcv* to release germination repressive molecules. A similar phenomenon was recorded with Chandrashekar and Umesha[29] who found a clear reduction of tomato seed germination inoculated with the phytopathogen *Xanthomonas perforans*. Also, Chahtane *et al*.[30] demonstrated a marked repression in the germination of *Arabidopsis* seeds in presence of *Pseudomonas aeruginosa*. The growth slow down of tomato seedlings exposed to *Xcv* could be assigned to the capability of the pathogen to release certain phyto-inhibitory metabolites that can decrease seedling growth[31,32].

The ameliorative effect of lavender and cranberry essential oils on tomato seedling under the stress of *Xcv* was assigned to the presence of metabolites with antibacterial or allelopathic properties. Previous studies had highlighted the antibacterial potentiality of lavender essential oil against Gram-positive and Gram-negative bacteria[33-35]. Similarly, the bactericidal activity of cranberry has been recorded against different bacterial strains[36,37]. The obtained results demonstrated a marked reduction of AA and GSH in seedling exposed to *Xcv*, while the levels of these antioxidant metabolites were increased in oil treated seedlings. These results are in harmony with Kuzniak and Sklodowska[38] who reported a significant decline in ascorbate concentrations in leaf peroxisomes of *Lycopersicon esculentum* upon *Botrytis cinerea* infection. The formation of toxic reactive oxygen species in plant cell as a result of pathogen attack leads to a significant damage to cells by initiating the chain of damaging reactions[39]. Antioxidant system including non-enzymatic components such glutathione and ascorbic acid are found to involve in scavenging of the Reactive Oxygen Specie (ROS)[40]. Various bioactive compounds have been identified in lavender and cranberry essential oils. A number of these metabolites have been reported for their significant antimicrobial properties such as linalool, linalyl acetate, β-ocimene, geraniol, lavandulol, 1,8-cineole, α-pinene and β-myrcene[41,42]. At the same time, the antioxidant activities of some the identified phytochemicals such as linalool, sabinene, β-caryophyllene and δ-cadinene have been recorded by Kundu *et al*.[43], Quiroga *et al*.[44] and Torres-Martinez *et al*.[45]. Actually, tomato seedlings treated with lavender and cranberry essential oils demonstrated a clear growth improvement in the presence of the phytopathogen *Xcv*. The ameliorative role of these oils was attributed to the presence of a panel of secondary metabolites. Some of these molecules can kill or inhibit the pathogenic bacteria while others can trigger the plant systemic resistance against phytopathogen[46-48].

CONCLUSION

The present study demonstrated that lavender and cranberry essential oils can antagonize the negative influence of the phytopathogen *Xcv* on seed germination and seedlings growth of tomato. The presence of various phytochemicals with antibacterial and antioxidants potentialities in these oils could be responsible for the suppression of the pathogen in addition to the induction of the plant antioxidant system. Future studies should be directed towards investigating the impact of purified secondary metabolites of these essential oils that could be applied as bioagents against different phytopathogens.

SIGNIFICANCE STATEMENT

This study discovers the antagonistic effect of lavender and cranberry essential oils against the phytopathogen *Xanthomonas campestris* that was assigned to the existence of several phytochemicals. The application of these essential oils as alternatives for synthetic chemicals is considered an environmentally safe approach for integrated management of diseases caused by *Xcv*.

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