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Research Paper / Araştırma Makalesi

Reduction of Tissue Maceration in Potatoes by Rose Essential Oil

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ABSTRACT

Erwinia carotovora is a phytopathogen which causes soft-rot disease in a wide variety of economically important plants. *E. carotovora* is known to produce a range of exoenzymes that enhance its ability to damage the host tissue and cause disease. A cell to cell communication mechanism called quorum sensing which is mediated by small signalling molecules regulates exoenzymes (cellulase, pectinase and protease) and carbapenem production in *E. carotovora.* Thus the exploration of new strategies to manipulate this communication pathway for the prevention of *E. carotovora* infections is valuable. In this study, the inhibitory effects of the rose, orange, lavender, clove, cinnamon, black pepper and cumin oils on the production of the exoenzymes (cellulase, pectinase and protease) and carbapenem in the *Erwinia carotovora subsp. carotovora* ATCC 39048 were investigated. And potato tissue maceration was also tested in the presence of oils. Rose and lavender essential oils markedly inhibited the production of pectinases by 38.7 and 9.7%, cellulases by 36.6 and 31.7% and proteases by 29 and 16.1%, carbapenem by 61.9 and 54%, and maceration of potatoes by 61.4 and 30.7% in the *E. carotovora* respectively without affecting the growth of cells. Although several studies have reported antibacterial effects of rose and lavender essential oils, there is no report describing their antivirulence potential. To the best of our knowledge, this is the first report on the rose and lavender essential oils with potential antivirulence components against soft rot caused by *E. carotovora.*

Keywords: *Erwinia carotovora,* Quorum sensing, Rose essential oil

Patateslerde Yumuşak Çürüklüğün Gülyağıyla Azaltılması

ÖZ

Erwinia carotovora ekonomik açıdan önemli pek çok bitkide yumuşak çürüklük hastalığına neden olan bir fitopatojendir. *E. carotovora*'nın konak dokulara zarar vermesini sağlayan ve hastalığa neden olan bir dizi ekzoenzim ürettiği bilinmektedir. *E. carotovora*'da ekzoenzim (selülaz, pektinaz ve proteaz) ve karbapenem üretimi, küçük sinyal moleküllerinin aracı olduğu çevreyi algılama sistemi adı verilen hücreler arası iletişim mekanizması tarafından düzenlenir. Bu nedenle *E. carotovora* enfeksiyonlarının önlenmesi için, bu iletişim yolunun manipülasyonunu sağlayacak yeni stratejilerin araştırılması değerlidir. Bu çalışmada, gül, portakal, lavanta, karanfil, tarçın, karabiber ve kimyon yağlarının *E. carotovora*'da, ekzoenzim (selülaz, pektinaz ve proteaz) ve karbapenem üretimine inhibitör etkileri araştırılmıştır. Ayrıca patateste yumuşak çürüklük miktarı, yağların varlığında test edilmiştir. Gül ve lavanta uçucu yağları, bakteriyel hücre büyümesini etkilemeksizin, *E. carotovora*'da pektinaz üretimini sırasıyla %38.7 ve 9.7, selülaz üretimini %36.6 ve 31.7 ve proteaz üretimini %29 ve 16.1, karbapenem üretimini %61.9 ve 54 ve patates yumuşak çürüklüğünü %61.4 ve 30.7 oranlarında önemli ölçüde inhibe etmiştir. Gül ve lavanta uçucu yağlarının antibakteriyel etkileri çeşitli çalışmalarla rapor edilmiş olsa da, virülensi engelleyici potansiyelini açıklayan herhangi bir çalışma bulunmamaktadır. Bildiğimiz kadarıyla bu çalışma, gül ve lavanta uçucu yağlarının bileşenlerinin, *E. carotovora*'nın neden olduğu yumuşak çürüklük hastalığına karşı potansiyeli hakkındaki ilk raporudur.

Anahtar Kelimeler: *Erwinia carotovora,* Çevreyi algılama, Gül yağı

INTRODUCTION

Erwinia carotovora, a Gram-negative phytopathogen causes soft-rot disease in potatoes, and other economically important plants such as members of the Brassica family [1-4]. Virulence of this bacterium depends on the production of plant cell wall-degrading enzymes such as pectinase, cellulase and protease [5]. In *E. carotovora*, exoenzyme production has been found to be under quorum sensing (QS) control [6]. In addition to these virulence factors, regulation of production of the secondary metabolite, 1-carbapen-2-em-3 carboxylic acid (carbapenem), in *E. carotovora* ATCC 39048 is also subject to control by QS [6, 7]. The critical role of QS system in regulating virulence of *E. carotovora*, makes it an attractive target for blocking or interfering of post-harvest *E. carotovora*-related infections.

Utilization of essential oils (EOs) to control plant pathogens has a potential interest due to their antimicrobial and anti-quorum sensing activities against food borne pathogens [8-10]. Additionally unlike antibiotics, QS inhibitors could prevent pathogenesis without killing the pathogen and, reduce the risk of increasing antibiotic resistance.

Hence in this study we showed that essential oils having antibacterial properties can reduce production of pectinase, cellulase, protease and carbapenem in *E. carotovora.* This may provide an alternative strategy for plant protection against plant pathogen *E. carotovora.*

MATERIALS and METHODS

Seven kinds of natural oils rose (Sebat), orange (Karden), lavender (Mecitefendi), clove (Karden), cinnamon (Karden), black pepper (Ecodab) and cumin (Talya) oils obtained from markets were used in this study. Oils were diluted with ethanol, filter-sterilized using 0.45µm (pore size) filters and stored at +4°C until use.

Bacterial Strains and Media

Erwinia carotovora subsp. carotovora ATCC 39048 and β-lactam super sensitive *E. coli* (ESS) strain were obtained from the Department of Biology, Süleyman Demirel University, Isparta. In this study, Luria Bertani (LB) broth (Difco) or agar medium was used. And the medium was supplemented with antibiotics where appropriate.

Determination of Minimum Inhibitory Concentrations (MIC) using Broth Dilution

Minimum inhibitory concentrations (MIC) of oils were determined according to Broth Dilution Assay. Bacterial strains were grown in Mueller-Hinton (MH) broth and suspended in MH broth to give a final density of 10^5CFU/mL. Two fold dilutions ranging from 4-0.0156% (v/v) of the oils were prepared in the test tubes, including one growth control (MH broth) and one sterility control (MH broth + test oil). Test tubes were incubated at 30°C for 48 h. The MIC values were determined as the lowest concentration of oil preventing visible growth of microorganisms.

Estimation of Cellulase, Pectinase and Protease Production

Cellulase and pectinase activities were detected on assay plates as published previously [11]. Protease assays were performed using a method adapted from [12] with or without cinnamon oil (0.0025%: v/v), rose and clove essential oil (0.005%: v/v), orange, lavender, black pepper and cumin oil (0.001%: v/v).

Carbapenem Plate Assays

Carbapenem production assay was carried out as described previously [13], using the β-lactam super sensitive *E. coli* (ESS) strain. Cultures of *E. carotovora* ATCC 39048 were grown in the presence of cinnamon oil (0.0025%), rose essential oil (0.005%), orange, lavender, clove, black pepper and cumin oil (0.001%) and pelleted by centrifugation. Aliquots (100 µL) of filter-sterilized culture supernatants were applied to wells cut into an LB agar plate seeded with a lawn of *E. coli* ESS and incubated overnight at 30°C. Carbapenem production was indicated by a clear zone around the test strain where *E. coli* ESS did not grow.

Potato Tissue Maceration

To evaluate the effectiveness of the selected oils in reducing soft rot infection in storage, potato tissue maceration assay was performed as described in [14]. Potato tubers obtained from a local supermarket were immersed in 10% hypochlorite solution for 10 min then washed with distilled water and allowed to air dried at room temperature. 100 microliters of *E. carotovora* ATCC 39048 culture (10^8 cfu/mL) treated oils (cinnamon oil (0.0025%), rose essential oil (0.005%), orange, lavender, clove, black pepper and cumin oil (0.001%) was injected on upper surface of potato. Than infected tubers wrapped with tissue paper and cling film to prevent dehydration and incubated in a moist chamber at 30°C for 48 h. After 3 days, the soft, macerated tissue surrounding each injection site was carefully scraped out using a spatula and weighed. Each experiment contained 5 internal replicates.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The percentage composition of essential oil was determined and identified by GC-MS. Analysis was carried out on a Shimadzu GC-MS QP 5050 (Kyoto, Japan) gas chromatograph-mass spectrometer system equipped with a CP WAX 52 CB capillary column (50 m *0.32 mm ID, df :1.2 lm- Varian) and helium (99.999 %) was used as carrier gas. The mass spectrometer was run in the electron impact mode at 70 eV. The enjection volume was 1 µL. GC temperature program; the initial temperature of the oven was 60°C, which is increased from 2°C in this case to 220°C in this minute and left at

220°C for 20 minutes. Detector and injector temperature was 250°C.

RESULTS and DISCUSSION

Determination of Minimum Inhibitory Concentrations (MIC) of Oils against *E. carotovora* ATCC 39048 and β-lactam Super Sensitive *E. coli* (ESS) Strain Broth Dilution Method

MICs of the oils were determined against *E. carotovora* ATCC 39048 and *Escherichia coli* ESS strain. The rose, cinnamon, lavender and clove essential oils exhibited concentration-dependent inhibition of growth. MIC values for *E. coli* ESS strain was higher (i.e., 4.5 mg/mL) than that for *E. carotovora* ATCC 39048 (Table 1). Cumin, black pepper and orange oils did not exhibited any antibacterial activity against *E. carotovora* ATCC 39048 and *E. coli* ESS strain in tested concentrations.

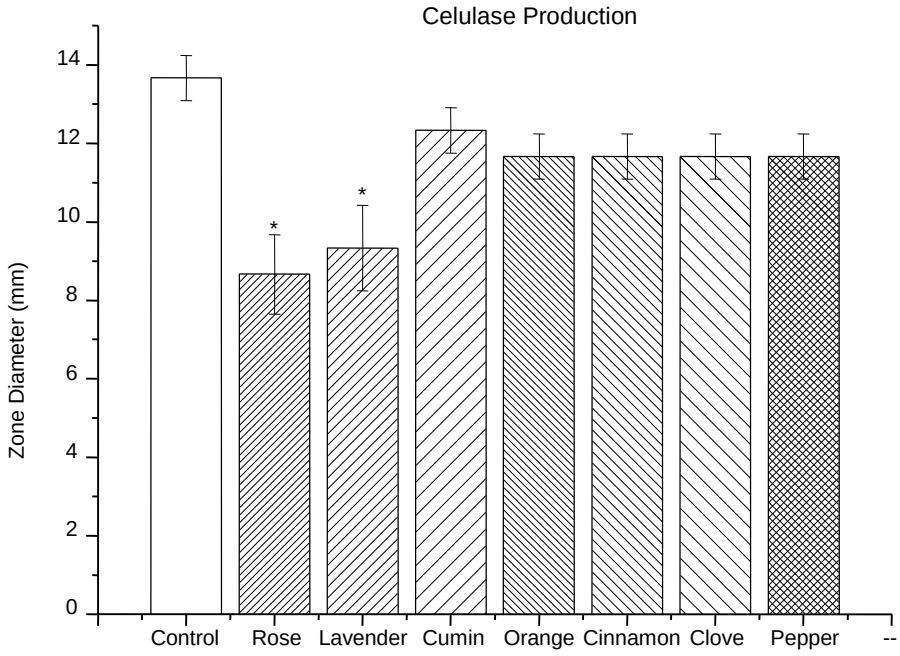
Cellulase, Pectinase and Protease Production of *E. carotovora* ATCC 39048 in the Presence of Oils

To study the impact of oils on exoenzyme production of *E. carotovora* ATCC 39048, the activity of the major virulence determinants (cellulase, pectinase and protease) were measured in the presence of oils. As shown in Figures 1A, B and C, rose essential oil (0.005% v/v) showed statistically significant (p<0.05) decrease in the production of cellulase (36.6%), pectinase (38.1%) and protease (29%).

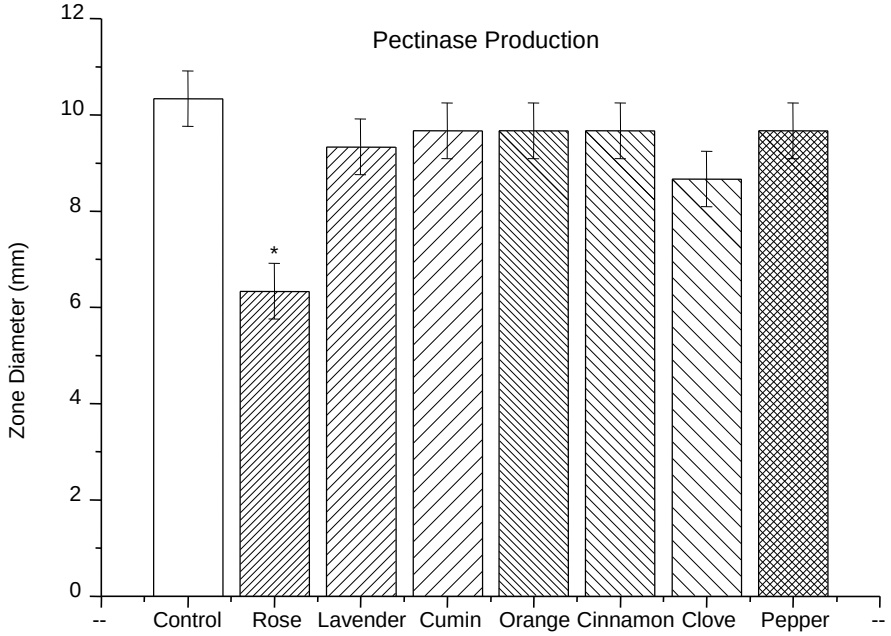
Table 1. Minimum inhibitory concentrations of oils for *E. carotovora* ATCC 39048 and *Escherichia coli* β-lactam super sensitive (ESS) strain.

Oils	Minimum inhibitory concentrations (volume:volume %)	
	E. carotovora	*E. coli*
Rose	0.06	0.25
Lavender	0.25	2
Cinnamon	0.03	0.125
Clove	0.125	0.25
Cumin	>4*	>4*
Blackpepper	>4*	>4*
Orange	>4*	>4*

*no antibacterial effect.



a)



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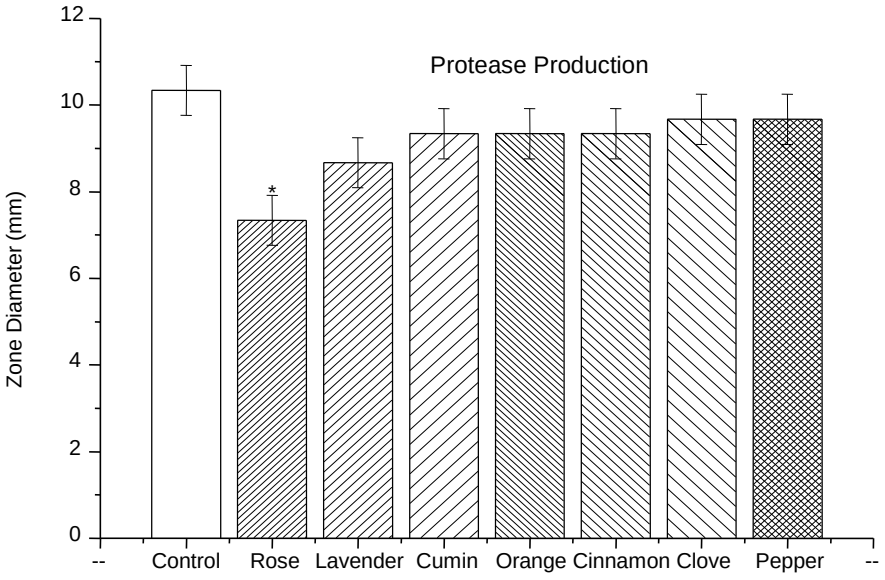

c)

Figure 1. Cellulase (a), pectinase (b) and protease (c) activity of *E. carotovora* ATCC 39048 in the presence of oils. Data shows the means of three replicates vertical bar represents +standard error; Values followed by an asterisk denote significant difference (at $p<0.05$) compared to control.

Carbapenem Plate Assays

To test for the effects of oils on carbapenem production, plate assay was evaluated. Rose essential oil (61.9%), lavender essential oil (54%), clove essential oil (33.3%) and cumin oil (27%) caused statistically significant ($p<0.05$) decrease in the carbapenem production (Figures 2. and 3).

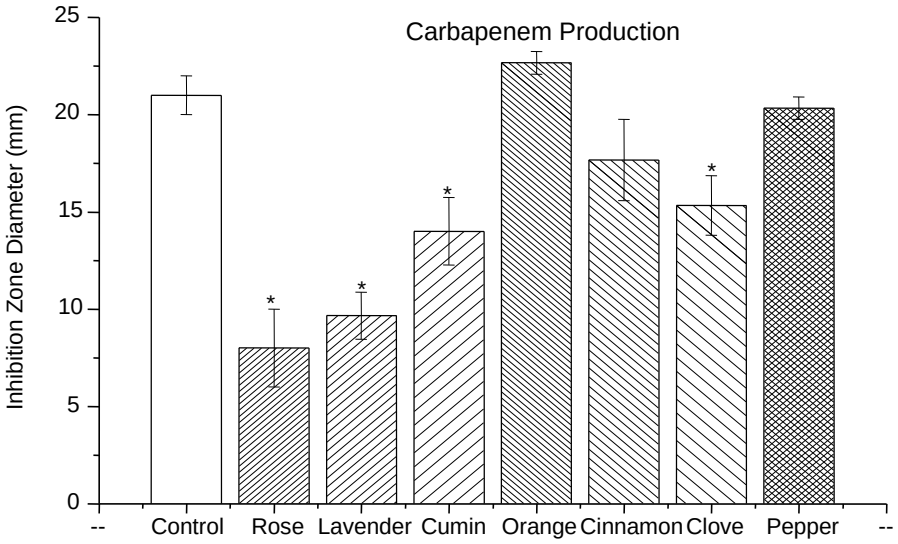

Figure 2. Carbapenem production of *E. carotovora* ATCC 39048 in the presence or absence (control) of oils. Data shows the means of three replicates vertical bar represents +standard error; Values followed by an asterisk denote significant difference (at $p<0.05$) compared to control.

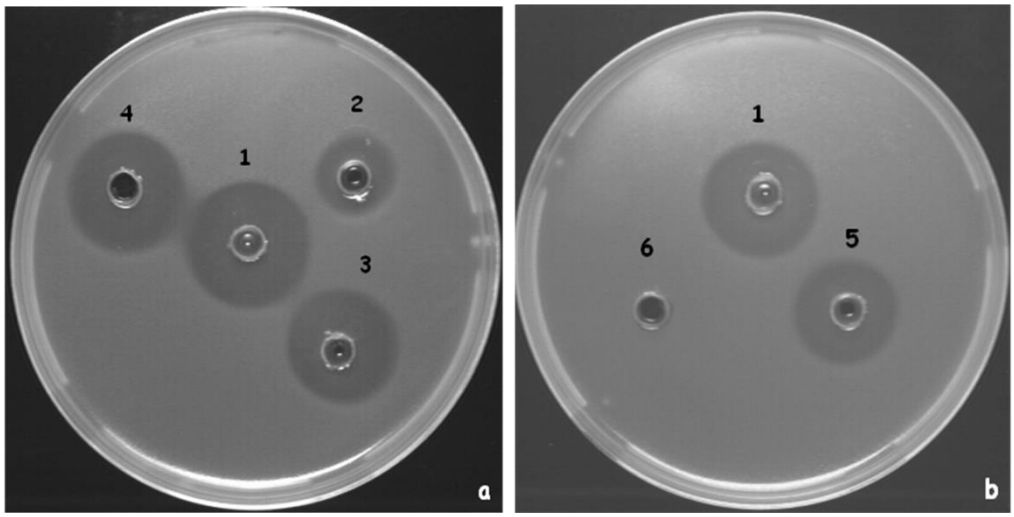

Figure 3. Oils inhibit carbapenem production was assayed using the β-lactam super sensitive *E. coli* (ESS) strain: (1) control, (2) lavender, (3) clove, (4) orange, (5) black pepper and (6) rose oil and incubated overnight at 30°C. The zones of clearing are proportional to the levels of carbapenem secreted by *E. carotovora*. These data are representative of two independent experiments.

Tissue Maceration Assays

Oils were assayed for maceration activity and results showed that tissue maceration were significantly lowered (9.1–61.4%) in treated samples (Figures 4 and 5). Rose essential oil was found to be the most effective to reduce tissue maceration (0.005%) (61.4%) (Figure 5), and the maximum macerated tissue was found in the cinnamon essential oil treated samples (Figure 4).

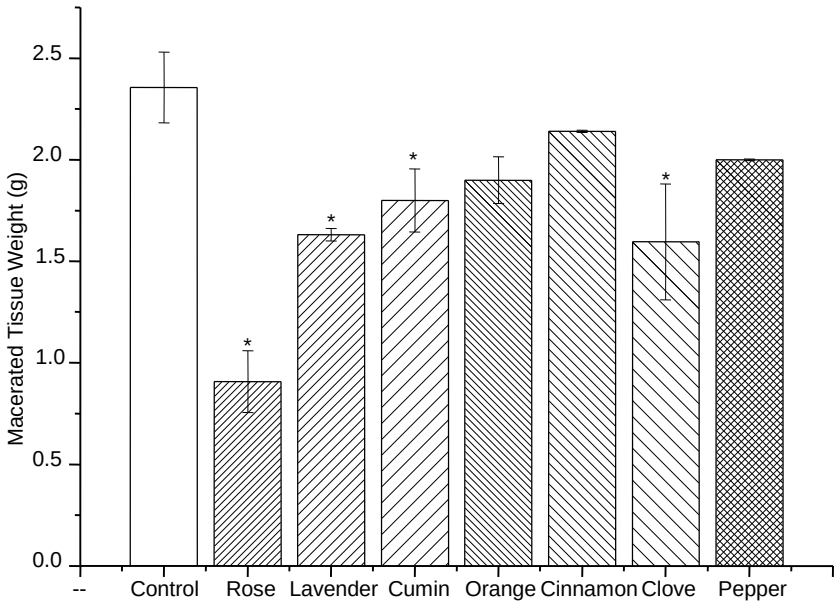

Figure 4. Tissue maceration of *E. carotovora* ATCC 39048 in the presence of oils. Data shows the means of three replicates vertical bar represents +standard error; Values followed by an asterisk denote significant difference (at $p<0.05$) compared to control.

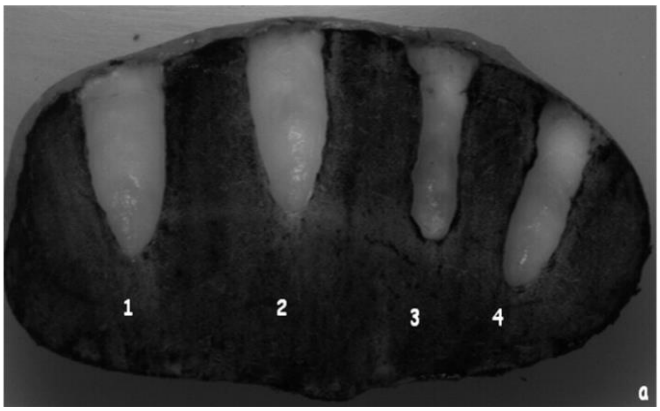

Figure 5. Potato tuber incubated for two days following inoculation with *E. carotovora* ATCC 39048 with or without essential oils. The potato is sliced through the center of inoculation sites and macerated tissue is removed for the assay; *E. carotovora* ATCC 39048 (1-2), *E. carotovora* ATCC 39048 and rose oil 0.005% (3), *E. carotovora* ATCC 39048 and rose oil 0.0025% (4).

GC-MS Analysis of the Essential Oils

GC-MS analysis of the rose and lavender essential oils showed that fifteen and nine components were identified in rose and lavender essential oils, respectively. The major constituents of the rose essential oil were citrenellol (36.2%), nerol (26.5%), geraniol (5.1%) and lavender essential oil were linalool (42.6%), linalyl acetate (40%), camphor (5.25%) and eucalyptol (4.50%). Other components were present in amounts less than 2%.

In recent years, interest in plant extracts and essential oils has increased and numerous studies on the antimicrobial and anti-quorum sensing activity have been reported [15-23]. And using essential oils for controlling post-harvest diseases is an attractive, effective, environment friendly approach and an alternative to pesticides. Because *E. carotovora* uses QS to regulate virulence, strategies designed to interfere with these signaling systems may provide an opportunity to control of soft rot disease.

Although some studies have shown the efficiency of some chemicals and chitosan to control post-harvest diseases such as soft rot [24-26], the anti-quorum sensing effects of rose and lavender essential oils in *E. carotovora* was firstly demonstrated with this study. The potential therapeutic value of rose and lavender essential oils against soft rot disease inhibiting production of the exoenzymes (cellulase, pectinase and protease), carbapenem and potato tissue maceration in

the *Erwinia carotovora subsp. carotovora* ATCC 39048 have been investigated. The GRAS status [27] of essential oils their application to control disease to provide safe food.

It has been reported that the antimicrobial activity of essential oils was most likely due to antimicrobial components present in oil (monoterpenes, sesquiterpenes and aldehydes and alcohols) [28]. Inhibition of QS system can be achieved by either competitive binding of signal-like molecules to cognate receptors, inhibition of reception signal molecules or enzymatic signal degradation [29, 30]. Presently, the exact mechanism underlying inhibitory activity of tested essential oils in the soft rot bacteria *Erwinia carotovora subsp. carotovora* ATCC 39048 is unknown and needs to be further elucidated.

CONCLUSIONS

Our results have shown that rose and lavender essential oils showed significant anti-quorum sensing activity against soft rot bacteria *Erwinia carotovora subsp. carotovora* ATCC 39048 and effectively reduced the bacterial soft rot disease of potato via inhibiting production of the cellulase, pectinase, protease and carbapenem. The rose and lavender essential oils have noticeable anti-QS activities which may make them an alternative to reduce post-harvest soft rot disease of potato.

ACKNOWLEDGEMENTS

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