Antibacterial and Biochemical Activities of Phenylpropanes and Monoterpenes on Phytopathogenic Bacteria
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ABSTRACT
In the present study, six monoterpenes and two phenylpropanes were evaluated for their antibacterial effect against three phytopathogenic bacteria, Agrobacterium tumefaciens, Ralstonia solanacearum and Erwinia carotovora var. carotovora. The inhibitory effects of these compounds on polygalacturonase and dehydrogenases activities were also tested. The results revealed that trans-cinnamaldehyde, (-)-citronellal, (-)-terpinen-4-ol had the highest antibacterial activity against A. tumefaciens. Their minimum inhibitory concentration (MIC) values were 1000, 1500 and 1500 mg/l, respectively. Similarly, trans-cinnamaldehyde (MIC = 2000 mg/l), and (-)-citronellal (MIC = 2000 mg/l) were the highest activity compounds against E. carotovora var. carotovora. Moreover, (-)-citronellal caused the greatest antibacterial effect against R. solanacearum with MIC value of 1000 mg/l. Further, trans-cinnamaldehyde showed the highest inhibitory effects on polygalacturonase and dehydrogenases activities of A. tumefaciens, while (-)-citronellal represented the most potent effect of inhibition on polygalacturonase and dehydrogenases activities of E. carotovora var. carotovora and R. solanacearum.

Keywords: Phytopathogenic bacteria; minimum inhibitory concentration; monoterpenes; phenylpropanes; polygalacturonase; dehydrogenases

INTRODUCTION
Plant pathogenic bacteria are responsible for huge economic losses in agriculture by decreasing the yields and marketing values of crops (Obradovic et al., 2008). Agrobacterium tumefaciens, Erwinia carotovora subsp. carotovora and Ralstonia solanacearum are soil borne bacteria that cause crown gall, soft rot and lethal wilt, respectively. These three bacteria are among the most common plant pathogenic bacteria worldwide. They attack numerous plant families including fruits, vegetables and flowers, and cause devastating loss in the production of infected crops (Hayward, 1991; Wright 1998; Wang et al., 2000).

The control of plant diseases caused by phytopathogenic bacteria is mainly focusing in the continuous use of synthetic chemicals. However, the use of synthetic chemicals emerges several environmental and health problems such as pollution of environment components, food toxic residues and development of resistance (Vidaver 2002; Montesinos and Bardaji 2008; Yuliar et al., 2015). Resistance of many bacterial strains to commonly used antibiotics becomes evidence and decreases their efficacy in the management of plant diseases (Sundin and Wang 2018).

The increasing awareness of the drawbacks of synthetic chemicals and the increasing demand on safe products for controlling plant diseases encourage the search for new alternatives for plant disease control. Plant materials, such as plant extracts, essential oils and plant secondary metabolites are among the most promising alternatives for controlling plant diseases (Isman, 2008).

Monoterpenes and phenylpropanes are two classes of plant secondary metabolites with low molecular weights and boiling points. These compounds are commonly present as the major constituents of plant essential oils. Monoterpenes and phenylpropanes have been described to display wide spectrum of biological effects against agricultural pests, such as fungicidal, insecticidal and herbicidal activities (Grodnitzky and Coats, 2002; Singh et al., 2002; Wuryatmo et al. 2003; Cheng et al., 2008; Ahuja et al. 2015; Saaid et al., 2018). However, the antibacterial activity of monoterpenes and phenylpropanes against plant pathogenic bacteria are poorly studied. Monoterpenes and phenylpropanes have been reported to possess inhibitory effect on the growth of A. tumefaciens and E. carotovora var. carotovora (El-Zemity et al., 2008; Abdel Rasoul et al., 2012), E. amylovora (Sato et al., 2007; Scortichini and Rossi 2008) and Xanthomonas campestris pv. phaseoli var. fuscans (Cantore et al., 2009).

Therefore, the aim of this study was to evaluate the antibacterial efficacy of six monoterpenes and two phenylpropanes against three plants pathogenic bacteria A. tumefaciens, E. carotovora var. carotovora and R. solanacearum. Also, the inhibitory effects of these compounds on the activity of two extracellular enzymes polygalacturonase and dehydrogenases were studied in order to understand their possible mechanism of action.

MATERIALS AND METHODS
Chemicals
Two phenylpropanes and six monoterpenes were selected to study their antibacterial and biochemical effects. Tested compounds were (-)-citronellal (95%), p-cymene (99%), (-)-menthone (90%), α-pinene (98%), α-terpinene (85%), (-)-terpinen-4-ol (95%), trans-cinnamaldehyde (99%) and eugenol (99%). These compounds were bought from Sigma Aldrich Chemical Co. (Steinheim, Germany). Figure 1. shows the chemical structures of tested monoterpenes and phenylpropanes.

Test bacteria
Bacterial strains of Agrobacterium tumefaciens (Erwin Frink Smith & Town.) (Family: Rhizobiaceae; Class: Alpha Proteobacteria), Erwinia carotovora var. carotovora (Erwin Frink Smith) (Family: Enterobacteriaceae; Class: Gamma Proteobacteria) and Ralstonia solanacearum (Erwin Frink Smith) (Family: Burkholderiaceae; Class: Betaproteobacteria), were obtained from Laboratory of Microbiology, Department of Plant Pathology, Alexandria University. Nutrient agar medium (NA) which prepared by mixing peptone (10 g), meat extract (5 g), sodium chloride (2.5 g) and agar (10 g) in one liter of distilled water was used for maintaining bacterial strains.
**Figure 1. Chemical structure of monoterpenes and phenylpropenes.**

**Minimum inhibitory concentration (MIC) assay**

The minimum inhibitory concentrations (MICs) of the tested phenylpropenes and monoterpenes on the three bacterial strains were determined by using agar dilution method (ESCMID 2000). Stock solutions of tested compounds were first prepared in acetone. A series of concentrations of each compound were prepared by adding different volumes of the prepared solutions to molten NA to give a series of concentrations ranged between 10 and 10000 mg/l. Then the media were poured into Petri dishes.

The petri dishes were left for solidifications and 2 µl of bacterial cultures (approximately 10⁸ CFU/ml) was spotted on the surface of agar using 2 µl standard loops. Three spots were made per each plate. The inoculum spots were left to dry. The plates were incubated at 37°C for 24 h. The lowest concentration of each compound in which no visible growth of bacterial strain in the plate was observed and taken as MIC value.

**Dehydrogenases activity assay**

These compounds have been estimated on dehydrogenases activity of E. carotovora var. carotovora, A. tumefaciens, and R. solanacerium by using a methylene blue technique (Schoenhard, 1962). The phenylpropenes and monoterpenes were evaluated at 10, 50, 100, 500 and 1000 mg/l. Inhibition percentage (I %) of dehydrogenases activity was calculated from the following equation:

\[
I\% = ((T - C)/ T_{max} - C) \times 100
\]

T: Is known as the time of reduction (min) for 90% of methylene blue in the treatment, T_{max} : Is known as the maximum time of reduction (min) for the 90% methylene blue recorded in treatments, C: Is known as the time of reduction (min) for 90% of methylene blue in control treatment. Values of IC_{50}, concentration causing 50% of enzyme inhibition, of tested compounds were calculated using Probit analysis (Finney, 1971).

**Polygalacturonase activity assay**

The phenylpropenes and monoterpenes were evaluated at 10, 50, 100, 500 and 1000 mg/l. Inhibition percentage (I %) of polygalacturonase activity was calculated according to (Ayers et al., 1966; Nasuno and Starr, 1966)

\[
I\% = ((A_{control} - A_{treatment})/A_{control}) \times 100
\]

I %: Is known as the inhibition of polygalacturonase activity
A: Is known as the absorbance

**Statistical analysis**

Probit analysis was carried out to determine IC_{50} values, 95% confidence limits and other statistical parameters of tested compounds on both enzymes (Finney, 1971) using SPSS v21.0 software program (Chicago, USA).
RESULTS AND DISCUSSION

Results

Antibacterial effect of monoterpenes and phenylpropenes

The eight compounds were assayed for in vitro antibacterial activity against three plants pathogenic bacteria A. tumefaciens, E. carotovora var. carotovora and R. solanacearum. Table 1 shows the values of MIC for the tested compounds on the three bacterial strains. It was clear that phenylpropenes and monoterpenes possessed variable levels of antibacterial activity.

Table 1. Minimum inhibitory concentration (MIC) of monoterpenes and phenylpropenes on plant pathogenic bacteria

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (mg/l)</th>
<th>Agrobacterium tumefaciens</th>
<th>Erwinia carotovora var. carotovora</th>
<th>Ralstonia solanacearum</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Citronellal</td>
<td>1000</td>
<td>2000</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>5000</td>
<td>&gt;6000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>(+)-Menthone</td>
<td>4000</td>
<td>6000</td>
<td>4000</td>
<td>4000</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>2000</td>
<td>3000</td>
<td>3000</td>
<td>3000</td>
</tr>
<tr>
<td>α-Terpinine</td>
<td>1500</td>
<td>2500</td>
<td>2000</td>
<td>2000</td>
</tr>
</tbody>
</table>

Generally, A. tumefaciens was more susceptible than E. carotovora var. carotovora and R. solanacearum to the monoterpenes and phenylpropenes. trans-Cinnamaldehyde was the most active compound against A. tumefaciens and E. carotovora var. carotovora with MIC values of 1000 and 2000 mg/l, respectively. α-Pinene and p-cymene were the less active compounds against A. tumefaciens and E. carotovora var. carotovora. In addition, (+)-citronellal showed the highest antibacterial activity against R. solanacearum (MIC = 1000 mg/l).

Eugenol, (+)-menthone, α-terpinene and (+)-terpinen-4-ol had a moderate effect of inhibition on the dehydrogenases activity of the three plant pathogenic bacteria.

Effect of monoterpenes and phenylpropenes on dehydrogenases activity

These results cleared that (+)-citronellal caused the highest inhibition of enzyme dehydrogenases activity of E. carotovora var. carotovora and R. solanacearum with IC50 values of 125.10 and 83.41 mg/l, respectively (Tables 3 and 4). In contrary, p-cymene exhibited the lowest inhibitory effect on the dehydrogenases activity of A. tumefaciens and E. carotovora var. carotovora. Moreover, trans-cinnamaldehyde caused the highest inhibitory effects on dehydrogenases of A. tumefaciens (IC50 = 75.18 mg/l).

Eugenol, (+)-menthone, α-terpinene and (+)-terpinen-4-ol had a moderate effect of inhibition on the dehydrogenases activity of the three plant pathogenic bacteria.

Effect of monoterpenes and phenylpropenes on polyglacturonase activity

The results of the inhibitory effect of the tested compounds on polyglacturonase of the three bacteria detected that all of the tested compounds caused pronounced inhibition of the enzyme (Tables 2, 3 and 4). (+)-Citronellal revealed the highest inhibition on polyglacturonase activity of E. carotovora var. carotovora (IC50 = 13.99 mg/l) and R. solanacearum (IC50 = 15.69 mg/l) respectively, while trans-cinnamaldehyde was the most potent inhibitor on polyglacturonase from A. tumefaciens (IC50 = 11.49 mg/l). p-Cymene showed the lowest inhibition of polyglacturonase from A. tumefaciens and R. solanacearum, while (+)-terpinen-4-ol was the less active on the enzyme from E. carotovora var. carotovora.

Table 2. The effect of inhibition monoterpenes and phenylpropenes on polyglacturonase and dehydrogenases activities of Agrobacterium tumefaciens

<table>
<thead>
<tr>
<th>Compound</th>
<th>Polyglacturonase</th>
<th>Dehydrogenases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50a (mg/l)</td>
<td>Slope SE</td>
</tr>
<tr>
<td>trans-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>11.49 (6.29-17.67)</td>
<td>0.94±0.11</td>
</tr>
<tr>
<td>(-)-Citronellal</td>
<td>13.36 (8.10-19.40)</td>
<td>0.66±0.11</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>12.15-167.11</td>
<td>0.92±0.09</td>
</tr>
<tr>
<td>Eugenol</td>
<td>19.40 (11.46-28.78)</td>
<td>0.88±0.10</td>
</tr>
<tr>
<td>(+)-Menthone</td>
<td>20.55 (11.79-30.99)</td>
<td>0.82±0.09</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>27.83 (14.67-44.15)</td>
<td>0.66±0.09</td>
</tr>
<tr>
<td>α-Terpinene</td>
<td>53.62 (17.01-115.82)</td>
<td>0.97±0.09</td>
</tr>
<tr>
<td>(+)-Terpinen-4-ol</td>
<td>52.01 (36.46-70.66)</td>
<td>0.93±0.09</td>
</tr>
</tbody>
</table>

The inhibitory concentration of 50% from enzyme.

Slope of the concentration inhibition regression line ± standard error (SE).

Intercept of the regression line ±SE.

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The current research summarizes the antibacterial activities of phenolic monoterpenes, such as carvacrol, eugenol and thymol, against different microorganisms. It has been observed that the presence of phenolic components in the oils increased their antibacterial potential against different microorganisms (Penalver et al., 2005). Similarly, (El-Zemity and Ahmed, 2008) reported the effect of inhibition of 12 monoterpenes on the growth of *E. carotovora var. carotovora* and *A. tumefaciens*. They found that myrcene and thymol were the most potent antibacterial compounds, and these two compounds strongly inhibited polyglacturonase and dehydrogenases. The results of the current study also indicated that the tested phenylpropenes and monoterpenes were more effective against *A. tumefaciens* and *R. solanacearum* than *E. carotovora var. carotovora*. These findings pointed out that the activity of monoterpenes and phenylpropenes may differ with the bacterial species under investigation.

### Discussion

The results of the current study also indicated that the tested phenylpropenes and monoterpenes were more effective against *A. tumefaciens* and *R. solanacearum* than *E. carotovora var. carotovora*. These findings pointed out that the activity of monoterpenes and phenylpropenes may differ with the bacterial species under investigation.
It is known that the essential oils and their major constituents, monoterpenes and phenylpropanes, may cause their antimicrobial activity by elevating leaking and permeability of cell membranes (Lambert et al., 2001; Oussalah et al., 2006). This may lead to loss of ions, reducing in membrane potential, and interruption of the proton pump (Di Pasqua et al., 2006; Turina et al., 2006). Essential oils and their major constituents, monoterpenes and phenylpropanes, may also inhibit protective enzymes and consecutively inhibit various vital biochemical pathways (Xing et al., 2012). They may cross the cell wall and the cytoplasmic membrane and damage the structure of different fatty acids, polysaccharides and phospholipids layers (Longbottom et al., 2004). They may also coalesce in the cytoplasm and damage proteins and lipids (Burt, 2004). The results of the biochemical studies of the current study revealed that the tested monoterpenes and phenylpropanes caused significant inhibition of polygalacturonase and dehydrogenases of the three tested bacterial strains. Strong inhibition was observed on polygalacturonase activity.

In conclusion, the tested monoterpenes and phenylpropanes showed variable levels of antibacterial activity against the three plant pathogenic bacteria with *trans*-cinnamaldehyde, (−)-citronellal, (−)-menthol, eugenol and (−)-terpinen-4-ol being the most active compounds. These compounds also caused potent inhibition of polygalacturonase. These results indicated that monoterpenes and phenylpropanes could be possible candidates for biocontrol of plant pathogenic bacteria.

REFERENCES


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