A preliminary Study on Antimicrobial Activities of Essential oils and Egyptian Pollen Extract against Some Phytopathogenic Fungi and bacteria


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Abstract

Antimicrobial activity of some essential oils and Egyptian pollen extract were investigated against 10 microbes, 5 fungal strains (Fusarium oxysporium, Rhizoctonia solani, Botrytis fabae, Alternaria solani and Aspergillus flavus) and 5 bacterial isolates (Erwinia amylovora, Erwinia carotovora, Agrobacterium tumefaciens, Pantoea agglomerans and Pseudomonas lachrymans). In vitro tests of plant essential oils of Clove, Eucalyptus, Lemon grass, Sweet fennel and pollen extract showed inhibitory action against fungal strains at different concentrations (0.2, 0.4 and 0.8%) compared with the antifungal pesticide Mycozan and the antibiotic Garamycin. All tested oils and Egyptian pollen extract except sweet fennel showed inhibitory action against tested bacterial isolates, in vitro. Also, Clove and Eucalyptus oils were more effective than Lemon grass, Sweet fennel and Pollen extract against growth of bacterial isolates.

Key words: Plant essential oils, pollen extract, antimicrobial, phytopathogenic fungi and bacteria

Essential oils are highly volatile substances synthesized and stored in glandular trichomes of odiferous plants. The term essential oil refers not only to the complex oils isolated from the plant, but also to their constituent compounds. Volatile components of essential oils are lipophilic molecules that volatilize at low temperatures (Cavanagh, 2007).

Furthermore, the use of many synthetic fungicides that have various degrees of persistence in crop protection has now been cautioned due to their carcinogenicity, teratogenicity and other residual toxicities. Several of the synthetic fungicides are reported to cause adverse effects on treated soil ecosystems because of their non–biodegradable nature (Pandey and Dubey, 1992).

The search for antimicrobial agents has continued to be concentrated on lower plants, fungi and bacteria. Less research has focused on higher plants although identified plant compounds such as berberine, emetine, quinine and sanguinarine still find specialized uses. Secondary metabolities from higher plants serve as defense agents against invading microorganisms. Some screening has yielded additional promising results. A few of these have proceeded to give active antimicrobial compounds like polygodial, anethole, safrole methykeugenol and cryptolepine (Taniguchi and Kubo, 1993).

Several strategies were used for biological control of soil plant pathogens through using of organic soil amendments (Barakat et al., 1983 and Ibrahim, 1990); intercropping (Arie
et al., 1987); cross-protection (Husain et al., 1986); plant extracts (Assadi and Behroozin, 1987); and essential oil (Abo-El Seoud et al., 2005) as well as microbial interaction (Sivan and Chet, 1992).

Under natural conditions, essential oil of Eucalyptus is also known to provide allelopathic property to the tree (Kohli, 1990). Additionally, the presence of essential oil provides defense advantage to Eucalyptus leaves against herbivory and attack by harmful insects (Brooker and Kleinig, 2006). Eucalyptus oil has been placed under RAS (Generally Regarded as Safe) category by Food and Drug Authority of USA and classified as non-toxic (USEPA, 1993).

Several studies have examined the effect of compounds isolated from oils on fungi to search for natural fungicides and a number of these oil constituents have shown to be inhibitory (Chao et al., 2000). In general, the response to the different essential oils depends on the fungal species tested, and ranges from a lack of inhibition (resistance) to various degrees of susceptibility (Pattnaik et al., 1996).

Svoboda and Hampson (1999) reported that volatile oils of many plants are known to have antimicrobial activity (Piccaglia et al., 1993). This activity could act as chemical defense against plant pathogenic diseases. Deans and Ritchie (1987) examined 50 plant volatile oils for their antibacterial against 25 genera of bacteria, using an agar diffusion technique. Volatile oils exhibited various reductions in growth of microorganisms, depending on the oil concentration and chemical composition.

Rhizoctonia solani is one of the causal agents of diseases associated with roots and tubers of different crops, causing significant yield losses (Carling et al., 1989). The incidence of this pathogen increases cost of these commodities by use of agrochemicals (fungicides) needed for its control. The use of some of these fungicides induces genetic resistance in fungal populations and causes environmental damage (Hernández et al., 2008; Cooke et al., 2003; Leroux, 2003). The potential use of plant extracts with antifungal properties for control of phytopathogens has been demonstrated at laboratory, greenhouse and at field level (Bergeron et al., 1995).

Most bactericides have harmful effects on the environments so, natural substances attracted wide interest. Some plant oils and plant extracts used as antibacterial agents in controlling plant disease (Hassanein and Desheesh, 1998; Qiao et al., 2001; Vannest and Boyd, 2002 and Mahmoud et al., 2004) with less effect on environments. The antimicrobial activity of these natural substances is attributed mainly to the toxicity of its major compounds (Daferera et al., 2003). In Egypt, some plant oils and plant extracts showed promising activity against some plant pathogenic bacteria, for example E. amylovora and A. tumefaciens (Hassanein et al., 1998 and Mahmoud et al., 2004). In this study we aimed to evaluate the antibacterial and anti-fungal activity of some essential oils and Egyptian pollen extract against five isolates of different plant pathogenic bacteria and fungi, in vitro.

1. MATERIAL AND METHODS

1.1. Source of phytopathogenic microorganisms:
Five phytopathogenic bacterial isolates of *Erwinia amylovora*, *Erwinia carotovora*, *Agrobacterium tumefaciens*, *Pantoea agglomerans* and *Pseudomonas lachrymans* were obtained from Bacterial Disease Department, Plant pathology institute A.R.C. Gizza. *Fusarium oxysporium*, *Botrytis fabae*, *Aspergillus niger*, *Alternaria solani* and *Rhizoctonia solani* were obtained from Plant Pathology department, Faculty of Agriculture, Mansoura University.

1.2. Isolation of plant essential oils.

The plant essential oils were obtained by steam distillation of volatile oils method *Chakrabarty (2003)*. The pollen grains extract was prepared using 70% methanol, according to *Wagner et al (1996)*.

### Table 1. List of plant extracts used in antimicrobial test

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Latin Name</th>
<th>Family</th>
<th>Part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clove</td>
<td><em>Syzygium aromaticum</em></td>
<td>Myrtaceae</td>
<td>flower buds</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td><em>Eucalyptus globulus</em></td>
<td>Myrtaceae</td>
<td>leaves</td>
</tr>
<tr>
<td>Lemon grass</td>
<td><em>Cymbopogon citratus</em></td>
<td>Poaceae (Graminae)</td>
<td>leaves</td>
</tr>
<tr>
<td>Sweet Fennel</td>
<td><em>Foeniculum vulgare var. dulce</em></td>
<td>Apiaceae(Umbelliferae)</td>
<td>Fruits</td>
</tr>
<tr>
<td>Pollen extract</td>
<td>Bee Hives</td>
<td>Mixed</td>
<td>pollen</td>
</tr>
</tbody>
</table>

1.3. Sensitivity of phytopathogenic bacteria and fungi to essential oils and Egyptian pollen extract:

Four plant essential oils (Clove, Eucalyptus, Lemon grass and Sweet fennel) and Egyptian pollen extract were screened for antibacterial and antifungal activity against phytopathogenic bacteria by agar well diffusion methods (*Bobbaralal et al., 2009*). 50 µl of tested essential oil was filled in well made with sterile cork borer of size 6.0 mm at the center of the plate containing King’s B agar medium (*King et al., 1954*) previously seeded with phytopathogenic bacterial isolate. The flask which containing 250 ml of sterilized king’s B agar medium or potato dextrose agar inoculated with 10 ml of 24 hours old culture of each bacterial isolate grown on King’s broth medium. The inoculated medium was poured into Petri dishes. After solidification, the wells were made on inoculated agar plates by using cork borer. After treatment with plant essential oils the plates were incubated at 28º C for 72 hours. Five plates of each phytopathogenic isolate used as replicates for each essential oil. Sterilized water filled in wells of five plates of each phytopathogenic isolate used as a control. Antibacterial and antifungal activity of Egyptian pollen extract was evaluated by the same method. After incubation time, the antibacterial activity was evaluated by measuring zones of inhibition of phytopathogenic bacteria and fungi surrounding the essential oils and Egyptian pollen extract.

2. RESULTS AND DISCUSSION
In the present study, antibacterial activity of some essential oils (Clove, Eucalyptus, Lemon grass, Sweet fennel) and Egyptian pollen extract were investigated against five bacterial isolates of different plant pathogenic bacteria (*E. amylovora*, *E. carotovora*, *A. tumefaciens*, *P. agglomerans* and *P. lachrymans*), *in vitro*. Data in Table (2) & plate (1) indicate that all tested oils and Egyptian pollen extract except sweet fennel showed inhibitory action against tested isolates. Generally, *E. amylovora* and *A. tumefaciens* were more sensitive to effective oils (Clove, Eucalyptus and Lemon grass) where inhibition zone diameters ranged from 19.4 to 35.4 mm and 16.2 to 34.2 mm, respectively compared with *E. carotovora*, *P. agglomerans* and *P. lachrymans* where inhibition zone diameters ranged from 15.8 to 26.8, 7.4 to 12.8 mm and 14.2 to 17.2 mm respectively. Our data agree with some plant oils have antibacterial activity against plant bacterial pathogens (Isman, 2000 and Qiao et al., 2001) and some have no effect (Elgayyar et al., 2001). Also our data showed that pollen extract was effective against *E. amylovora*, *A. tumefaciens* and *P. lachrymans* where diameters of inhibition zones are 8.4, 12.8 and 7.4, respectively, and no effect was observed against *E. corotovora* and *P. agglomerans*.

In conclusion, Clove and Eucalyptus oils were the most effective against plant pathogenic bacteria. The mechanism of inhibitory action of natural plant oils may due to toxicity of its major compounds or presence of terpenes and phenols (Isman, 2000 and Daferera et al., 2003).

**Table 2. Effect of essential oils and Egyptian pollen extract on growth of phytopathogenic bacteria, *in vitro***

<table>
<thead>
<tr>
<th>Plant Material</th>
<th>Tested bacteria</th>
<th>Diameter of inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. amylovora</em></td>
<td><em>E. carotovora</em></td>
</tr>
<tr>
<td>Clove oil</td>
<td>35.4</td>
<td>26.8</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td>22.6</td>
<td>17.4</td>
</tr>
<tr>
<td>Lemon grass oil</td>
<td>19.4</td>
<td>15.8</td>
</tr>
<tr>
<td>Sweet fennel oil</td>
<td>00.0</td>
<td>00.0</td>
</tr>
<tr>
<td>Pollen extract</td>
<td>8.4</td>
<td>00.0</td>
</tr>
<tr>
<td>Control</td>
<td>00.0</td>
<td>00.0</td>
</tr>
</tbody>
</table>

Data are means of three replications.
Table 3. Effect of essential oils and Egyptian pollen extract on growth of phytopathogenic fungi, *in vitro*

<table>
<thead>
<tr>
<th>Plant Material</th>
<th>Tested fungi</th>
<th>Diameter of inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Fusarium oxysporium</em></td>
<td><em>Botrytis fabae</em></td>
</tr>
<tr>
<td>Clove oil</td>
<td>72.3</td>
<td>62</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td>38.6</td>
<td>28</td>
</tr>
<tr>
<td>Lemon grass oil</td>
<td>30.3</td>
<td>21.6</td>
</tr>
<tr>
<td>Sweet fennel oil</td>
<td>31.7</td>
<td>24.7</td>
</tr>
<tr>
<td>Pollen extract</td>
<td>44.7</td>
<td>38.6</td>
</tr>
<tr>
<td>Control</td>
<td>00.0</td>
<td>00.0</td>
</tr>
</tbody>
</table>

Plate 1. Results of the inhibition zone of tested essential oils and Egyptian pollen extract against *tested bacteria*
Diameters of the inhibition zones expressed in millimeter including the disc (6mm) listed in Tables (2 and 3) that, clove eucalyptus essential oils had highest antifungal activity more than lemongrass and sweet fennel essential oil. Also, pollen extract showed high antifungal activity against all tested fungi especially *F. oxysporium* and *botrytis fabae* 44.7 and 37.6 mm zones diameters respectively.

Many publications have documented the antimicrobial activity of clove (*Velluti et al., 2003*) and mint (*Abo-El-Seoud et al., 2005*) oils against different microbial species. Moreover, *Velluti et al., (2003)* studies the effect of cinnamon, clove, orgegano, palmarose, and lemongreaa oils and they found that the 5 essential oils inhibited the growth of *Fusarium proliferatum*. Clove oil was the most effective in decreasing growth of *F.proliferatum*. Also, *Jobling, (2000)* tested the antifungal activity of a range of plant essential oils against *Botrytis cinerea* and showed that plant essential oils from *Thymus zygis, Eugenia caryophyllus* and *Cinnamomum zeyophyllata* prevented the growth of *Botrytis*
Eucalyptus oil has been known for hundreds of years as antibacterial, antifungicidal and antiseptic in nature (Brooker and Kleinig, 2006).

Abo-El-Seoud et al. (2005) tested the antimicrobial activity of some plant essential oils fennel, peppermint, caraway, eucalyptus, geranium and lemon against some plant pathogenic fungi (Fusarium oxysporum, Alternaria alternata, Botrytis cinerea and Penicillium digitatum). They found that fennel, peppermint and caraway were efficient in controlling all the tested pathogenic fungi.

The previous results were also in agreement with that of Antonov et al., (1997) and Walter et al., (2001) as well as that of Elkaffash and Al-Menofy (2003) who reported that clove oil (Eugenia caryophyllus) suppressed the growth of F.oxysporum, R.solani, and Verticillium dahliae. This high antimicrobial activity probably related to Eugenol as a major compound (Velluti et al., 2003).

Also, Garcia and Lawas (1990) studied the antifungal activity of the crude water extract of 127 plant species against R.solani and they found that garlic extract (Allium sativum) was the most effective one. Moreover, Hammad and Youssef (1994) studied the antifungal activity of the essential oils of Allium sativum and Cuminum cyminum against Aspergillus flavus, A.ochraceus, A.niger and Penicillium chrysogenum. They found that garlic essential oil prevented growth of all tested fungi, while cumin oil had little activity against the tested fungi. Svoboda and Hampson (1999) reported that essential oils of many plants are known to have antimicrobial activity and this activity could act as chemical defense against plant pathogenic diseases.
3. REFERENCES


النشاط المضاد للميكروبات للزيوت العطرية مستخلص حبوب اللقاح المصرية تم دراسته على عشيرة من الميكروبات منهم خمسة سلالات من الفطريات وهم (الفيوزاريم اوكسي سيرور، رايوزكوتنيا سولاني، البوتريتيس فاني، الالترناريا سولاني والاسبرجلس فلافوس) وخمسة عزلات من البكتيريا وهم (اروينيا اميلفورا، اروينيا كاروتوفورا، ارووباكيريم توميفانس، بانثوبيا اجلومبرانس و سيديموناس لاريمانس). اوضحت الاختبارات المعملية باستخدام تركيزات مختلفة (0.2, 0.4 and 0.8%) من الزيوت الأساسية النباتية من الفرنفل، الكافور، حشيشة الليمون، الشم الاملود ومستخلص حبوب اللقاح ان الزيوت الأساسية المختارة ومستخلص حبوب اللقاح لها القدرة على تثبيط نمو السلالات الفطرية بالمقارنة بمبيد الفطريات الميكوزان و المضادات الحيوى الجاراسيين. جميع الزيوت المختبرة ومستخلص حبوب اللقاح ماعدا زيت الشم الحلو، اظهرت قدرة على تثبيط نمو العزلات البكتيرية المختبرة في المعمل. كما أثبتت النتائج أيضا ان زيوت كلا من الفرنفل والكافور كانت الأكثر كفاءة في قدرتها على تثبيط نمو العزلات البكتيرية.