Fumigant activity of leaf essential oil from *Myrtus communis* L. against the Khapra Beetle

Ghaleb TAYOUB*, Amer ABU ALNASER, Iyad GHANEM

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, Damascus, PO Box 6091, Syria

*Corresponding Author, Fax: 00963-11-6112289, Tel: 00963-11-2132580

Article History: Received 29th February 2012, Revised 19th March 2012, Accepted 20th March 2012.

Abstract: The fumigant toxicity of leaf essential oil from *Myrtus communis* (L.) was assessed against khapra beetle *Trogoderma granarium* Everts. The essential oil was active against different life stages of khapra beetle *T. granarium*. Adult stage was the most sensitive of all developmental stages to essential oil vapours. Whereas, larvae were the most tolerant with 94% and 100% mortality obtained after exposure of larvae to 562.5 μl/l air for 24 and 48h respectively. At 48h exposure period, \( LC_{50} \) and \( LC_{90} \) were 221 μl/l air and 487 μl/l air respectively. The results suggest a possibility for using leaf essential oil of the myrtle (*M. communis*) as an insecticide against *Trogoderma granarium* Everts in grain stores.

Keywords: *Myrtus communis*; Essential oil vapours; *Trogoderma granarium*; Fumigant toxicity.

Introduction

Khapra beetle *Trogoderma granarium* Everts. (Coleoptera: Dermestidae), is the most serious pest in stored products throughout the world. It is a major threat to stored wheat, and has been considered as one of the 100 most invasive pests in the world (Lowe et al. 2000).

Development rates and survival of various stages of the khapra beetle vary considerably depending upon temperature, light, moisture, season, and host species. Khapra beetle may have one to nine or more generations per year as a result of high humidity which has a depressing effect on population buildup (Ramzan and Chahal 1986). At favorable temperatures, eggs, pupae, and adults each take about a week to develop, while the larval stage may last for a month and it may survive for several years under diapause condition (Burges 1962). The insect is present in Syria and the prevailing climatic conditions in the area are conducive to serious outbreaks (Ghanem and Shamma 2007).

Fumigation is an essential tool for control of insect pests in stored products. Currently, phosphine and methyl bromide are the two common fumigants used for stored product protection (Rajendran and Sriranjini 2008). Insect resistance to phosphine is a global issue now, and control failures have been reported in some countries (Tayler 1989; Collins et al. 2002; Rajendran and Sriranjini 2008). Methyl bromide has been declared an ozone depleting substance and is being phased out completely (Rajendran and Sriranjini 2008).

Therefore, there is a growing interest in alternative strategies including development of chemical substitutes, the use of ionizing radiation and exploitation of controlled atmosphere (MBTOC 2002; Aoki et al. 1977; Libby and Black 1978). Interest has been shown in the potential of essential oils of plants as fumigants for the control of stored product pests since it is believed that natural compounds from plants sources may be less toxic to mammals (though this is not always true, degrade rapidly and available in abundance (Rajendran and Sriranjini 2008).

*Myrtus communis* L. (Myrtaceae), a perennial shrub is widely distributed in the Mediterranean area, and has been traditionally used as an antiseptic and disinfectant drug. The leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils (Baytop 1999; Romani et al. 1999). The essential oil is obtained from leaves and mainly used in the treatment of lung disorders (Gauthier...
et al. 1989) and has been found to possess antibacterial (Chevolleau et al. 1993; Hayder et al. 2003), anti candida, anti-inflammatory, antihemorrhagic (Ghannadi and Dezfuly 2011), antilouse (Lauk et al. 1996), antimutagenic and antioxidant activities (Mimica-Dukia et al. 2010; Romani et al. 2004). The myrtle essential oil showed toxicity after 24h of fumigation of adults Mediterranean flour moth Ephesia kuehniella Zeller, the Indian meal moth Plodia interpunctella Hübner, and the bean weevil Acanthoscelides obtectus Say (Ayvaz et al. 2010). A recent study by, Zayzafoon et al. (2011) has elucidated the chemical composition of the essential oil extracted from M. communis leaves in Syria.

In the present study, we report the fumigant toxicity effect of essential oil extracted from leaves M. communis on the khapra beetle (T. granarium Everts).

Materials and methods

Insects

A culture of T. granarium insects was reared in the lab in 3 liter glass jars covered with a piece of muslin and placed in an incubator in continuous darkness at 37±1 ºC. Larvae were isolated using a sieve that allowed their separation from wheat grains.

Plant materials

Leaves of M. communis, were harvested at the flowering stage in May-June 2009 from Wat-Alkhan (Lattakia - Syria). Collection was made from three individual plants growing wild. For each individual leaf collected. Voucher specimens have been deposited in the laboratory of the plant biotechnology department at the Atomic Energy Commission of Syria (AECS).

Essential oil extraction

Samples were initially air-dried for 6 days at room temperature until they were crisp, and then powdered. Oil samples were obtained by hydro-distillation for 3h using a Clevenger-type apparatus (Clevenger 1928). Oil yields (1.68 % w/w) were then estimated on the basis of the dry weight of the plant material. Hydro-distilled mass was about 100 g DW (Tayoub et al. 2006).

Fumigation bioassay

Fumigation bioassays were conducted by placing insect instars inside glass Petri dishes (9 cm diameter) with wheat provided as source of feed when needed. Petri dishes containing the insects were placed inside other larger glass Petri dishes (11 cm diameter). The essential oil droplets were deposited on the inner surface of the larger Petri dish. The whole system was sealed by parafilm. The volume of the large Petri dish was 160 cm³ air.

Fumigation of larvae

T. granarium larvae were divided into 11 groups, each group consisted of 10 larvae, ten group treated with : 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µl / 160 cm³, and one group as control. All treated and control larvae were incubated at 37 ±1 ºC for 5 days before the number of dead larvae was counted. Each treatment was replicated ten times.

Fumigation of pupae

Newly formed pupae were collected and divided into 6 different groups. Each group consisted of 10 pupae, five groups were fumigated with: 5, 10, 15, 20, and 30 µl / 160 cm³ each, and one group was control. All treated and control pupae were incubated at 37 ±1 ºC. Mortality was determined by adult emergence after 48 h. each treatment was replicated ten times.

Fumigation of eggs

Newly laid eggs were collected and divided into 7 different groups. Each group consisted of 10 eggs, six groups were fumigated with: 1, 2.5, 5, 7.5, 10, and 12.5 µl / 160 cm³ each, and one group was control. All treated and control eggs were incubated at 37±1 ºC, until mortality could be determined by the presence or absence of hatching. Each treatment was replicated ten times.
Fumigation of adults

1 to 5 days old adults collected and divided into 8 groups. Each group consisted of 10 adults, 7 groups fumigated with: 1.5, 2.5, 5, 7.5, 10, 20 and 30 µl / 160 cm$^3$ each, and one group was control. The treated and control adults were incubated at 37±1 ºC; mortality was recorded after 48 h of exposure to essential oil vapour. Each treatment run ten times in duplicate. Mortality data for all treatments were corrected for natural mortality in controls and were subjected to probit analysis to estimate $LC_{50}$ and $LC_{90}$ and slopes were generated (Finney 1971).

Results

Vapour of essential oil of $M. communis$ showed various levels of toxicity at the same exposure period depending on the treated instar of the insect.

Exposure of larvae to the highest dose of 90 µl/160 cm$^3$ air (562.5 µl/l air) resulted in 94% and 100% mortality at 24 h and 48 h-exposure period, respectively. Whereas, the lowest concentration used, i.e. 10 µl/160cm$^3$ air (65.5µl/lair) had no effect after 24 h-exposure period and resulted in only 4% mortality at 48 h-exposure period (Figure 1). There was an evident positive dose effect relationship for the range of doses between the lowest and the highest dose used (Fig1). At 48 h-exposure period, $LC_{50}$ for $M. communis$ essential oil on larvae was 221 µl/l air, whereas the $LC_{90}$ was 487 µl/l air. However, at 24 h, $LC_{50}$ was 307.4 µl/l air and $LC_{90}$ was 645.5 µl/l air (Table 1).

Table 1: Fumigant toxicity of essential oil of $M. communis$ against $T. granarium$ Everts larvae, adults, eggs and pupae.

<table>
<thead>
<tr>
<th>$T. grana$-</th>
<th>exposure</th>
<th>$LC_{50}$</th>
<th>$LC_{90}$</th>
<th>Slope±SE</th>
<th>Fcal</th>
<th>F05</th>
<th>d.f</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rium</td>
<td>time</td>
<td>µl/l air</td>
<td>µl/l air</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae</td>
<td>48h</td>
<td>221</td>
<td>487.8</td>
<td>3.8±0.4</td>
<td>105.1</td>
<td>5.6</td>
<td>8</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>24h</td>
<td>307.4</td>
<td>645.5</td>
<td>4.0±0.4</td>
<td>94.7</td>
<td>5.6</td>
<td>8</td>
<td>11.0</td>
</tr>
<tr>
<td>Adults</td>
<td>48h</td>
<td>25.6</td>
<td>48.2</td>
<td>4.6±0.6</td>
<td>53.8</td>
<td>7.7</td>
<td>5</td>
<td>9.49</td>
</tr>
<tr>
<td></td>
<td>24h</td>
<td>68.4</td>
<td>319.9</td>
<td>1.9±0.2</td>
<td>66.3</td>
<td>6.6</td>
<td>6</td>
<td>11.0</td>
</tr>
<tr>
<td>Eggs</td>
<td>48h</td>
<td>38</td>
<td>67.1</td>
<td>5.2±0.6</td>
<td>46.1</td>
<td>7.7</td>
<td>5</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>24h</td>
<td>54.7</td>
<td>122.6</td>
<td>3.7±0.5</td>
<td>126.1</td>
<td>7.7</td>
<td>5</td>
<td>9.5</td>
</tr>
<tr>
<td>Pupae</td>
<td>48h</td>
<td>83.5</td>
<td>162.1</td>
<td>4.5±0.6</td>
<td>55.98</td>
<td>10.13</td>
<td>4</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>24h</td>
<td>328.6</td>
<td>1090.6</td>
<td>2.5±0.6</td>
<td>19.7</td>
<td>10.13</td>
<td>4</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Units $LC_{50}$ and $LC_{90} = \mu l/l$ air, applied for 24 and 48h at 37 ºC. $d.f = $ Degrees of freedom

Adults were more sensitive to the vapour of $M. communis$ essential oil where up to 80% and 100% of exposed adults died following exposure to 30 µl/160 cm$^3$ air for 24 h and 48 h-exposure period, respectively. The lowest concentration of oil vapour (1.5 µl/160cm$^3$ air) to which adult khapra beetle was exposed resulted in only 2% death at 24 and 48 h-exposure period. Adults remained relatively insensitive to the vapour at concentrations less than 5 µl/160 cm$^3$ air, then after the toxic effect of the essential oil became more pronounced reaching 30% and 74% at 24 h and 48 h-exposure period, respectively. At 48 h-exposure period, $LC_{50}$ for $M. communis$ essential oil on adults was 25.6 µl/l air, whereas the $LC_{90}$ was 48.2 µl/l air. However, at 24 h, the $LC_{50}$ was 68.4 µl/l air and the $LC_{90}$ was 319.9 µl/l air (Table 1).

In the case of pupae, exposure to 30 µl/160 cm$^3$ air (187.5 µl/l air) of essential oil vapour resulted in the death of 24% , 98% of pupae at 24 h and 48 h-exposure period, respectively. The lowest concentration of 5 µl/160cm$^3$ air (31.25 µl/l air) to which the pupae were exposed resulted in 5% and 24% mortality at 24 h and 48 h-exposure period, respectively (Fig.1). The $LC_{50}$ of essential oil on pupae was 83.54 µl/l air and 328.6 µl/l air at 48 h and 48 h-exposure period, respectively (Table 1).
Eggs appeared highly sensitive to the toxic effect of *M. communis* essential oil vapour, so at 12.5 µl/160 cm³ air, 68% and 100% of exposed eggs died at 24 h and 48 h exposure period respectively (Fig.1), with LC₅₀ at 48 h and 24 h reaching 38 µl/l air and 54.7 µl/l air, respectively (Table 1).

**Discussion**

Essential oil of the myrtle plant showed a highly toxic effect to all developmental stages of Khapra beetle (*T. granarium*). The toxic effect of the oil was most effective on adults, followed by eggs compared with other insect stages. The toxic effect is almost certainly due to one or more of the components of the essential oil distilled from leaves of *M. communis*, particularly monoterpenes that are found in the oil and from 85.5% of the 95.4% of the total compounds contained in the essential oil (Zayzafoon et al. 2011). It is worth noting that our samples were collected from the same region in Syria.

It is feasible that the essential oil of *M. communis* with its monoterpenoid constituents act against insects as neurotoxins (Ayvaz et al. 2010; Grundy and Still 1985; Enan 2001; Papachristos and Stamopoulos 2004). It was suggested that natural terpenes isolated from essential oils could act as activators of octopaminergic receptors in larvae of *T. granarium* (Kostyukovsky et al. 2002; Shaaya et al. 2007).

A study on essential oil constituents isolated from aromatic plants showed that two natural terpenes termed ZP-51 and SEM-76 isolated and cultivated from unidentified cultivated aromatic plants belonging to Labiatae family have an outstanding fumigant toxicity effect on *T. granarium* larvae, at 1.5 µl/l air they showed 87% and 99% mortality for SEM-76 and ZP-51, respectively (Kostyukovsky et al. 2002). *M. communis* essential oil tested in our present study is less toxic than the above mentioned two compounds but still showed potent toxicity (LC₅₀ =25.6, 38, 83.5 and 221 µl/l air on adults, eggs, pupae and larvae, respectively), considering that it is only a crude oil with no purification of any of its active ingredient was attempted.
Other studies have shown that essential oils and their constituents may potentially be utilized as alternative compounds to synthetic fumigants in current use (Shaaya et al. 1997; Regnault-Roger et al. 1993; Shakarami et al. 2005). The major constituents from aromatic plants, primarily monoterpenes, are of particular interest to industrial market due to their potent biological activities, in addition to their toxicity to insects (Isman 2000; Weinzierl 2000). Monoterpenoids are typically volatile and rather lipophilic compounds, which can rapidly penetrate into insects and interfere with their physiological functions (Lee et al. 2002).

Conclusion

The present study showed that the essential oil of Myrtus communis L. may be used as a fumigant against Khapra beetle. Also, considering that essential oils of myrtle are being used in the pharmaceutical and cosmetic industry (Baytop 1999; Aidi et al. 2007; Boelens and Jimenes 1992; Flamini et al. 2004; Tuberoso et al. 2006), it is thus considered to be less toxic and harmful to humans and environment than conventional insecticides (Isman 2006). Consequently, the possibility of employing these natural fumigants to control stored products insects may warrant further investigation.

Acknowledgement: The authors would like to express their thanks and gratitude to Professor Ibrahim Othman, Director General of Atomic Energy Commission of Syria and Professor Nizar Mir Ali for support and encouragement.

References


