Acute and Sublethal Effects of some Botanical and Chemical Insecticides on The Khapra Beetle, Trogoderma granarium (Coleoptera: Dermestidae) Larvae As Long-Term Storage Protectants

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ABSTRACT

Globally, there are growing concerns about the overuse of synthetic insecticides to their subsequent damage to the environment and mammalian. Therefore, developing new and safe eco-friendly insecticides based on natural products, such as essential oils, is considered a substitute for chemical pesticides in pest control. In the present study, mimicry the storage environment for grain products stored in a small scale by surface treatment method to determining the lethal and effective concentrations for three chemical insecticides (Imidacloprid, Zeta-cypermethrin and Lufenuron) and three essential oils (Citrus bergamia, Citrus limon and Cuminum cyminum) against larvae of Trogoderma granarium at various concentrations and time intervals. Results revealed that the C. bergamia EO exhibited high larvical activity compared to the other two oils, i.e., 30.36% after 24h of exposure at the higher concentration (10 v/v), while after 72h of exposure at the same mortality reached 75.33%. Concerning chemical insecticides, the imidacloprid insecticides at all tested concentrations were the most effective against T. granarium larvae, leading to 40% mortality at the lower concentration (1 v/v) after 24h of exposure, and 96.6% mortality at the higher concentration (10 v/v)24h. post-exposure. Whereupon, C. limon oil had significantly higher LC₅₀ and LC₉₀ values than other oils, indicating that C. bergamia oil was more toxic against T. granarium larvae than to both oils. In contrast, LC₉₀ values of Lufenuron were the highest significantly being about 1.61 and 2.31 times compared to Zeta-cypermethrin and Imidacloprid at the maximum concentration tested after 72h of exposure, respectively. In sum, our research focuses on the potential of selected EOs as a suitable substitute for chemical pesticides against this noxious species.

Keywords: Acute toxicity; Lethality; Trogoderma granarium; Botanical insecticides; Chemical insecticides; Surface treatments

INTRODUCTION

Globally, the storage of grains and their by-products, and food commodities are an important and vital component of food security, whether for domestic consumption or for export abroad, as the quantities of stored grains amount to more than 80% of the total production in many countries (Tadesse and Eticha, 2000). During storage, grain and its products are damaged by more than 20,000 field arthropods, especially insect pests, including 600 species of beetles, 70 species of moths, and about 355 species of mites that cause quantitative and qualitative losses (Pimentel, 2002; Nagral and Kumar, 2012). The amount of these losses varies significantly from one country to another around the world, but generally, these losses increase in developing countries due to poor sanitary conditions, processing, non-hygienic transportation, conventional storage techniques, and poorly maintained storage units (Hubert et al., 2004). It is estimated that about 10-20% of the losses of stored products are due to insect infestations, as these insect injuries affect the quantity and quality of commodities stored (Pedigo and Rice 2014).

One of the most important insect pests classified as an A2 quarantine organism according to European and Mediterranean Plant Protection Organization (EPPO 2011) is the khapra beetle, Trogoderma granarium (Everts) (Coleoptera: Dermestidae). Although the pest originates from India, it has spread in Afrotopical and Palaearctic realms, making it among one of the hundred “world’s worst” invaders (Lowe et al., 2000). This is evident in the European Union countries, which warns of the possibility of this insect species spreading worldwide (EPPO, 2018). The reasons for the wide distribution are that this insect pest has developed its ability to cope with worst conditions, such as its development in a wide range of temperatures (21-40 ºC with an optimum of 35 ºC), its feeding on several foodstuffs (over 96 different commodities), its preference for dry conditions (2% relative humidity (r.h.) and low-moisture food (2% moisture content ), its capability to survive longer without food and its resistance to many pesticides (Kavallieratos and Boukouvala, 2018; Islam et al., 2020).

Plant derivatives, especially essential oils, have traditionally been used as bio-insecticides and new insecticide research has shed light on the importance of...
phytol derivatives as an active ingredient in order to avoid some negative properties that associate with conventional insecticides (Islam et al., 2016; Awadalla et al., 2017; Hashem et al., 2018). Essential oils (EOs) derived from the Rutaceae and Apiaceae families are highly effective alternatives to synthetic insecticides and highly applied to protecting stored grains (Boukhatem et al., 2014; Bhumi et al., 2017). The toxic action of essential oils on insects is due to negative actions on the nervous system, metamorphosis, natural growth, regulation of oxygen consumption and rate of carbon dioxide released (FAO, 2010; Brari and Thakur, 2015).

Surface treatment is an effective way to grain protectants and controls several stored grain pests. Since *T. granarium* larvae are more than adults in the population size and are more voracious in feeding, so pre-treatment of exterior surfaces and interior of empty storage facilities, cracks and crevices is closely related to the survival and development of this pest (Barak, 1991; Kavallieratos et al., 2017). The use of chemical pesticides for contact toxicity is the most widely used method around the world (Zettel and Arthur, 2000), but the excessive use of conventional insecticides is considered an issue of concern due to their negative effects on environmental and health levels and non-target organisms. Moreover, frequent exposure of *T. granarium* to chemical insecticides may produce tolerant offspring leading to increase the development of resistant populations (Ghimire et al., 2017). Therefore, the attempt to explore safer compounds on mammals and the environment and at the same time effective on stored grain pests has become an urgent necessity.

The purpose of this research therefore is to imitative a small-scale stored-product habitat environment, since the internal surfaces of Petri dishes were treated with various concentrations (1, 2.5, 5, 10 %, v/v) of the three EOs, namely *C. limon*, *C. bergamia* and *C. cyminum*, in addition to the three chemical insecticides, namely Imidacloprid, Zeta-cypermethrin and Lufenuron against *T. granarium* larvae to evaluating the mortality rates and lethality concentrations over selected time intervals (24, 48 and 72h).

**MATERIALS AND METHODS**

**Insects**

All experiments conducted by using small larvae of *T. granarium* (usually 2-4 mm size and beginning of the fourth larval instar) that were reared at Economic Entomology Department, Faculty of Agriculture, Mansoura University, Egypt, at 30 °C, 65% r.h. and complete darkness. These insects have been reared for more than four generations without exposure to any chemical pesticides or imperfect conditions.

**Formulations tested (FT)**

The following three insecticidal formulations were tested: Imidacloprid, Zeta-cypermethrin and Lufenuron. On the other hand, three essential oils were used as follows; Bergamot (*Citrus bergamia*), Lemon (*Citrus limon*) and Cumin (*Cuminum cyminum*); that belong to Rutaceae and Apiaceae plants. The essential oils were kindly provided by Stored Product Pests Research Department, Plant Protection Research Institute, Sakha, Kafr El-Sheikh, Egypt.

**Bioassays**

The toxicity bioassays of the FT were determined against *T. granarium* larvae by a contact toxicity procedure (Kanda et al., 2017). The essential oils and chemical insecticidal were solved in acetone to obtain the required concentrations (1, 2.5, 5 10%, v/v). The trials were performed in a completely randomized block design, with three replicates and three subreplicates. Sterile disposable Petri dish (8 cm diam. × 1.5 cm high, with a surface area of 50.27 cm²) with ventilation were considered to assess the contact toxicity of the FT. The internal bottoms of the dishes were treated with 0.5 ml of the FT, as a fine fog, that involved the required concentrations corresponding to each dose, and left to allow evaporation of the solvent. Additional dishes were sprayed with acetone only as previously described as controls. Subsequently, ten larvae of *T. granarium* were released in each dish and placed in incubators (ST 5 COM F/S, Pol-Eko-Aparatura, Wodzislaw Sl’aski, Poland) set at 30 °C, 65% r.h. and complete darkness. After 24, 48, 72 and 96 h of exposure, the number of dead larvae (non-moving insects) was recorded by gently touching their bodies, in each dish, with a brush to detect any movement.

**Data analysis**

The mortality of control sample was low (<5%), therefore no correction was considered necessary. The resulting data from the bioassays were subjected to analysis of variance (ANOVA), with concentration and exposure time as main effects while mortality was the response variable. The interaction of the main effects was also derived from the analysis. Mean were separated by the Tukey’s post hoc test at 0.05 probability. Probit analysis of mortality vs. concentration using Ldp Line program was performed and lethal concentrations (LC50, LC90) and their corresponding 95% confidence intervals (95% CI) were estimated. LC’s were deemed to be significantly different when the 95% CI’s did not overlap. All analyses were conducted using SigmaPlot 14.0 software.

**Results**

The contact toxicity of the test oils and other insecticides was variable and species-specific. Mortality was significantly affected among exposure intervals and various concentrations for essential oils and insecticides (Figs. 1 and 2). Regarding the bioassays for essential oils, the *C. bergamia* EO exhibited high larvacidal activity, i.e., 30.36% after 24h of exposure at the higher concentration (10 v/v), while after 72h of exposure at the same, mortality reached 75.33% (Fig. 1). The EO of *C. limon* was the least effective against *T. granarium* larvae, causing 11.65, 22.22, and 29% at the higher concentration after 72h of exposure. Furthermore, larvae mortality elevated at 10 (v/v) reaching 12.21, 33.66 and 45.86% after 24, 48 and 72h of exposure, respectively (Fig. 1). Concerning insecticides toxicity (Fig. 2), the imidacloprid insecticides at all tested concentrations were the most effective against *T. granarium* larvae, leading to 40% mortality at the lower concentration (1 v/v) after 24h. of exposure, and 96.66% mortality at the higher concentration (10 v/v) 72h. post-exposure. The Lufenuron insecticide achieved moderate adult mortality at all tested concentrations, not exceeding 76.6% after 72h of exposure. However, after 24 h of exposure at 10% (v/v), larvae mortality was 63.33%, while it reached 70% after 48h of exposure. The Zeta-cypermethrin caused the lowest mortality compared to other insecticides, i.e., 50% at 1% (v/v) 48 and 72h post-exposure. Although the same insecticide at the higher concentration (10%, v/v) caused low mortality on *T.
granarium larvae that exposed for 48 and 72h, it killed 70% of the beetles in the same times (Fig. 2).

Based on the aforementioned mean ± SE percentage mortality of EOs, and chemical insecticides, LC50, LC99 values and their confidence limits were illustrated in Tables (1 and 2). The χ² values for goodness-of-fit did not significantly differ, emphasizing a good fit of probit model to our data. Likewise, the slope values showed the consistency and homogeneity of the insect population to varying degrees, but they are generally considered acceptable. There was a significant difference between the lethality concentrations of EOs and chemical insecticides against T. granarium larvae. In the case of tested essential oils, the LC50 and LC99 values were 1.47 and 3.91 (% v/v), respectively, when T. granarium larvae were exposed for 72 h to C. limon oil. The corresponding LC50 and LC99 values were 0.38 and 1.97 (% v/v), respectively, when larvae treated with C. bergamia oil for 72 h. While, LC50 and LC99 values were 0.97 and 2.99 (% v/v), respectively, when larvae were treated with C. cyminum oil for 72 h (Table 1). Overall, C. limon oil had significantly higher LC50 and LC99 values than other oils, indicating that C. bergamia oil was more toxic to T. granarium larvae than both other oils. Regarding chemical insecticides toxicity, based on LC50 values, Imidacloprid was the most active being about 1.16 and 1.22 times more toxic than Zeta-cypermethrin and Lufenuron at 10 (% v/v) and 72h post-exposure, respectively. In contrast, LC99 values of Lufenuron were the highest significantly being about 1.61 and 2.31 times compared to Zeta-cypermethrin and Imidacloprid at the maximum concentration tested after 72h of exposure, respectively (Table 2). Indeed, the LC50 and LC99 values denoted that T. granarium larvae had the least susceptibility to Imidacloprid and Lufenuron insecticides, respectively. Generally, the mortality rates of insects mainly depended on the concentration of tested compounds and the time of exposure. For all formulations, the largest mortality was obtained at the highest concentration and the longest period of exposure.
Table 1. Probit regression estimates and concentrations required for 50 and 99% mortality for Trogoderma granarium larvae depend on mortality data after 24, 48, 72 h exposure to acute contact toxicity at various concentrations of three essential oils.

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>ET (h)</th>
<th>Mean ± SE</th>
<th>LC50 (95% CI) (v/v)</th>
<th>R² Linear</th>
<th>χ²b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus limon</td>
<td>24</td>
<td>-1.95±0.19</td>
<td>0.71±0.28</td>
<td>2.35 (1.62-5.86)</td>
<td>5.15 (3.32-14.21)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>-1.66±0.16</td>
<td>2.55±0.59</td>
<td>1.68 (2.1-1.34)</td>
<td>4.04 (5.05-2.3)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>-1.42±0.14</td>
<td>1.02±0.22</td>
<td>1.47 (1.17-1.88)</td>
<td>3.91 (3.13-4.88)</td>
</tr>
<tr>
<td>Citrus bergamia</td>
<td>24</td>
<td>-1.11±0.13</td>
<td>0.73±0.19</td>
<td>1.65 (1.19-3.41)</td>
<td>5.14 (3.41-12.09)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>-0.93±0.12</td>
<td>1.61±0.19</td>
<td>0.56 (0.45-0.71)</td>
<td>2.11 (1.69-2.64)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>-0.51±0.11</td>
<td>1.26±0.18</td>
<td>0.38 (0.31-0.48)</td>
<td>1.97 (1.57-2.46)</td>
</tr>
<tr>
<td>Cuminum cyminum</td>
<td>24</td>
<td>-2.05±0.21</td>
<td>1.15±0.33</td>
<td>2.11 (1.69-2.64)</td>
<td>4.49 (3.59-5.61)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>-1.41±0.14</td>
<td>1.21±0.22</td>
<td>1.27 (1.01-1.59)</td>
<td>3.36 (2.69-4.21)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>-1.12±0.12</td>
<td>1.24±0.19</td>
<td>0.97 (0.77-1.21)</td>
<td>2.99 (2.39-3.74)</td>
</tr>
</tbody>
</table>

1ET = Exposure times are in hours
2If χ² values for goodness-of-fit did not significantly differ (P > 0.05), implying a good fit of probit model to data.

Table 2. Probit regression estimates and concentrations required for 50 and 99% mortality for Trogoderma granarium larvae depend on mortality data after 24, 48, 72 h exposure to acute contact toxicity at various concentrations of three traditional insecticides.

<table>
<thead>
<tr>
<th>Insecticides name</th>
<th>ET (h)</th>
<th>Mean ± SE</th>
<th>LC50 (95% CI) (v/v)</th>
<th>R² Linear</th>
<th>χ²b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>24</td>
<td>-0.45±0.11</td>
<td>1.21±0.15</td>
<td>1.55 (0.65-1.66)</td>
<td>1.61 (1.01-8.13)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>-0.35±0.11</td>
<td>1.23±0.16</td>
<td>0.24 (0.15-0.37)</td>
<td>1.51 (1.26-1.88)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>-1.14±0.15</td>
<td>0.46±0.12</td>
<td>0.18 (0.08-0.25)</td>
<td>1.37 (1.15-1.74)</td>
</tr>
<tr>
<td>Zeta-cypermethrin</td>
<td>24</td>
<td>-0.66±0.11</td>
<td>0.98±0.13</td>
<td>0.44 (0.34-5.25)</td>
<td>4.06 (2.67-9.71)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>-0.23±0.11</td>
<td>0.45±0.15</td>
<td>0.31 (0.25-0.39)</td>
<td>3.51 (2.81-4.39)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>-0.13±0.12</td>
<td>0.36±0.12</td>
<td>0.21 (0.16-0.26)</td>
<td>1.97 (1.64-2.52)</td>
</tr>
<tr>
<td>Lufenuron</td>
<td>24</td>
<td>-0.36±0.17</td>
<td>0.57±0.13</td>
<td>0.58 (0.46-0.72)</td>
<td>4.21 (3.37-7.26)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>-0.17±0.14</td>
<td>0.56±0.13</td>
<td>0.46 (0.37-0.58)</td>
<td>3.43 (2.74-4.29)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>-0.28±0.12</td>
<td>0.48±0.13</td>
<td>0.22 (0.18-0.27)</td>
<td>3.16 (2.53-3.95)</td>
</tr>
</tbody>
</table>

1ET = Exposure times are in hours
2If χ² values for goodness-of-fit did not significantly differ (P > 0.05), implying a good fit of probit model to data.

Discussion

To the best of our knowledge, this study is considered one of the rare studies that have dealt with insecticidal action of essential oils, including two essential oils from the same family and genus, but with different species. Our results indicated that similarity in the family and genus of origin essential oil, not necessarily an indicator or a measure of similarity and compatibility in the effect of vegetable oil against insect pests (Islam et al., 2020). In the same context, Sagheer et al. (2013) tested the effect of essential oils from four citrus species against T. granarium larvae, at a concentration of 8%. Among them, C. aurantium essential oil was highly effective against T. granarium larvae than other oils and achieved 72.30% mortality, followed by 25.65% for C. reticulate; 22.36% by C. sinensis and 20.0% by C. paradisi. In another assessment, the essential oils extracted from peel of different citrus species were evaluated at a concentration of 8% against T. granarium larvae (Zia et al., 2013). They found that the oil of C. paradisi caused 21.67% mortality in larvae of T. granarium, and after 120 h of exposure, oils of C. sinensis, C. grandis and C. reticulate caused 5%, 1.67% and 3.33% mortalities in larvae, while the other oils did not kill insects at 8% concentration even after 72 h exposure. Hence, the results of our research are highly consistent with previous studies.

The chemical insecticides used in our study are diverse in the way they affect insects; i.e. zeta-cypermethrin is a synthetic pyrethroid consisting of a mixture of stereoisomers and is a contact insecticide that also works through stomach contact and impact on sodium channel (Soderlund, 2012); and imidacloprid is a systemic active, called the neonicotinoids, that acts as an insect neurotoxin through extremely fast-acting (Stenersen, 2004). These interpretations of the different ways of doing the chemical insecticides confirmed the compatibility of our results with previous research because it proved that the systemic insecticide represented in imidacloprid is the most effective on insects when treated surface compared with other insecticide groups. Our results show that the application of the chemical insecticides as surface treatment, can provide efficient control against T. granarium larvae. Arthur et al. (2018) found that the IGRs, pyriproxyfen and methoprene were less effective against larvae and adults of T. granarium than deltamethrin, pyrethroids and cyfluthrin.

Heterogeneous responses in mortality rates of T. granarium larvae were observed in our study, which was evident in slope, χ² (chi-square) and R² linear values. This imply a poor fit of the probit model to mortality data. The heterogeneity could be related to sex, size and age of larvae tested. Although, a very small amount of the EOs and chemical insecticides was sprayed in Petri dishes, there could be an unequal distribution of particles on inside surfaces of Petri dishes. Thus, some larvae might have the ability to escape contact with powder particles through occupied the areas with little or no particles (Le Patourel et al., 1989; Malia et al., 2016a, b). This could explain this heterogeneity. Another possible explanation is the differences in dehydration among individuals may interpret this gained heterogeneity (Malia et al., 2016b). However, several pesticide studies on stored-product insects have
been confirmed this heterogeneity response (Sehgal et al., 2013; Subramanyam et al., 2014; Tadesse et al., 2019).

In conclusion, our results revealed that the LC₅₀ of imidacloprid (as the best toxic pesticide) was 2.11 times lower than that of C. bergamia oil (as the best toxic oil), while the LC₅₀ of the same pesticide was 1.44 times less than the same oil at the highest concentration and 72h post-exposure. These minor differences from an environmental and health perspective drive us to replace chemical pesticides with increased concentrations of essential oils or to include the use of essential oil-based biopesticides in the Integrated Pest Management programs (IPM). Therefore, there is an ongoing need to assess the lethality concentrations of many chemical pesticides and compare them with the lethality concentrations of different essential oils for their use as protectors of stored products.

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