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### 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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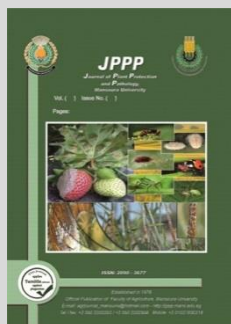
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#### ABSTRACT

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 larvicidal 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 (1v/v) after 24h. of exposure, and 96.66% mortality at the higher concentration (10 v/v) 72h. post-exposure. Whereupon, *C. limon* oil had significantly higher LC<sub>50</sub> and LC<sub>99</sub> values than other oils, indicating that *C. bergamia* oil was more toxic against *T. granarium* larvae than to both oils. In contrast, LC<sub>99</sub> 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; Nagpal 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 Afrotropical 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 °C with an optimum of 35 °C), 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

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phytoderivatives 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 (Zettler 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. cuminum*, 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 cuminum*); 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 cm<sup>2</sup>) 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, Wodzisław Śląski, 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.

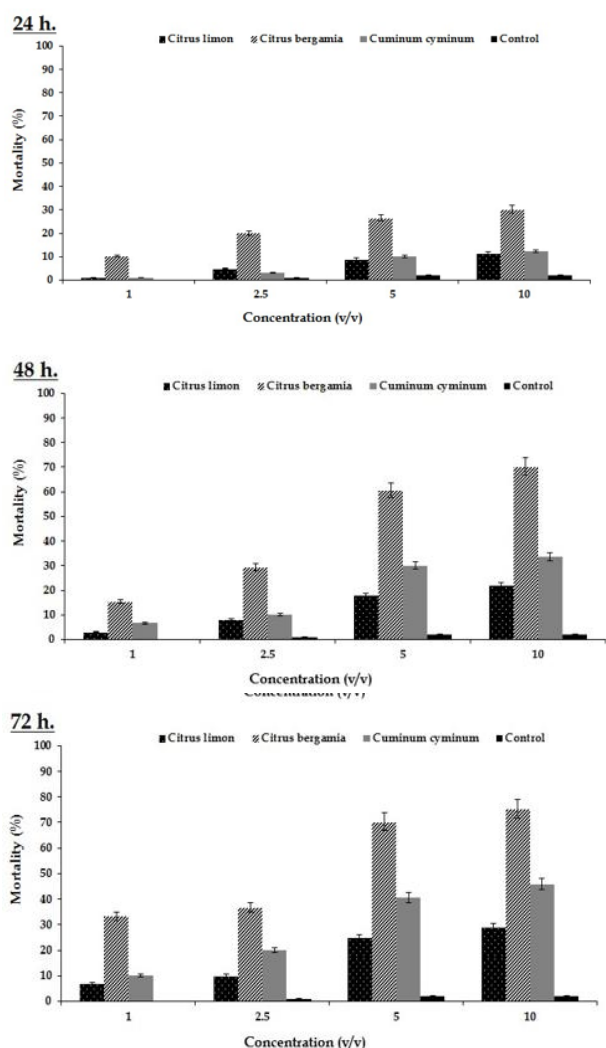
### Data analysis

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 (LC<sub>50</sub>, LC<sub>90</sub>) 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

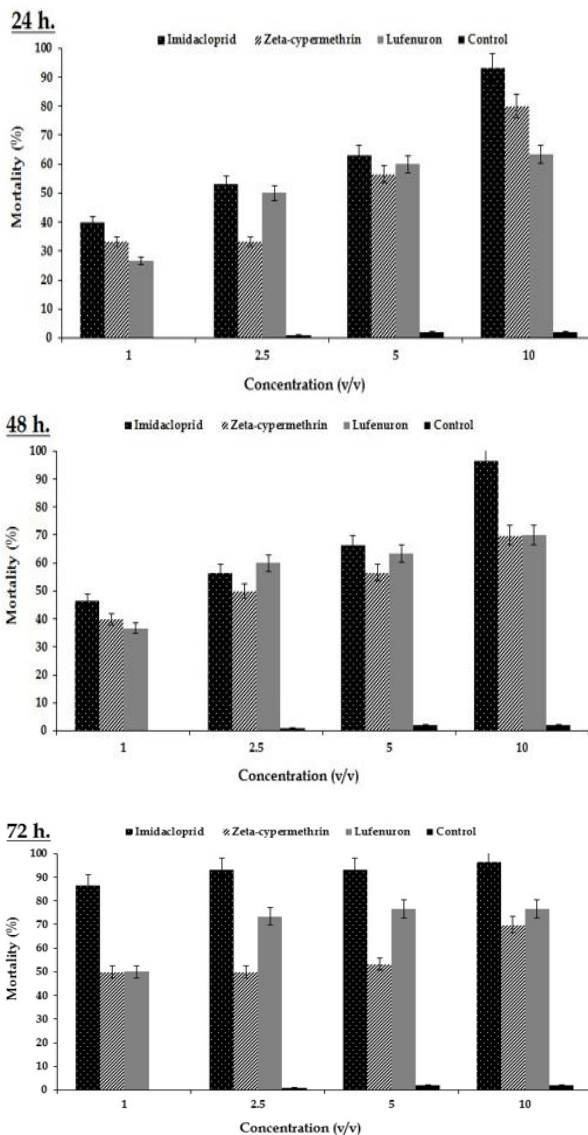
The contact toxicity of the test oils and other insecticides was variable and species-specific. Mortality was significantly affected among exposure intervals and various concentrations for essential oils and insecticides (Figs. 1 and 2). Regarding the bioassays for essential oils, the *C. bergamia* EO 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 postexposure. 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).



**Fig. 1. Mortality of *Trogoderma granarium* larvae by three essential oils at different concentrations in surface contact bioassays.**

Based on the aforementioned mean  $\pm$  SE percentage mortality of EOs, and chemical insecticides, LC<sub>50</sub>, LC<sub>99</sub> values and their confidence limits were illustrated in Tables (1 and 2). The  $\chi^2$  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 LC<sub>50</sub> and LC<sub>99</sub> 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 LC<sub>50</sub> and LC<sub>99</sub> values were 0.38 and 1.97 (%v/v), respectively, when larvae treated with *C. bergamia* oil for 72 h. While, LC<sub>50</sub> and LC<sub>99</sub> 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 LC<sub>50</sub> and LC<sub>99</sub> values than other oils,



**Fig. 2. Mortality of *Trogoderma granarium* larvae by three traditional insecticides at different concentrations in surface contact bioassays.**

indicating that *C. bergamia* oil was more toxic to *T. granarium* larvae than both other oils. Regarding chemical insecticides toxicity, based on LC<sub>50</sub> 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, LC<sub>99</sub> 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 LC<sub>50</sub> and LC<sub>99</sub> 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.**

Essential oil	ET <sup>a</sup> (h)	Mean ± SE		LC (95% CI) (v/v)		R <sup>2</sup> Linear	χ <sup>2b</sup>
		Intercept	Slope	LC <sub>50</sub>	LC <sub>99</sub>		
<i>Citrus limon</i>	24	-1.95±0.19	0.71±0.28	2.35 (1.62-5.86)	5.15 (3.32-14.21)	0.707	0.13
	48	-1.66±0.16	2.25±0.59	1.68 (2.1-1.34)	4.04 (5.05-3.23)	0.797	5.13
	72	-1.42±0.14	1.02±0.22	1.47 (1.17-1.88)	3.91 (3.13-4.88)	0.823	2.12
<i>Citrus bergamia</i>	24	-1.11±0.13	0.73±0.19	1.65 (1.19-3.41)	5.14 (3.41-12.09)	0.738	0.71
	48	-0.93±0.12	1.61±0.19	0.56 (0.45-0.71)	2.11 (1.69-2.64)	0.846	3.29
	72	-0.51±0.11	1.26±0.18	0.38 (0.31-0.48)	1.97 (1.57-2.46)	0.812	8.37
<i>Cuminum cyminum</i>	24	-2.05±0.21	1.15±0.33	2.11 (1.69-2.64)	4.49 (3.59-5.61)	0.786	1.29
	48	-1.41±0.14	1.21±0.22	1.27 (1.01-1.59)	3.36 (2.69-4.21)	0.798	3.89
	72	-1.12±0.12	1.24±0.19	0.97 (0.77- 1.21)	2.99 (2.39-3.74)	0.797	2.29

<sup>a</sup>ET = Exposure times are in hours

<sup>b</sup> if χ<sup>2</sup> 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**

Insecticides name	ET <sup>a</sup> (h)	Mean ± SE		LC (95% CI) (v/v)		R <sup>2</sup> Linear	χ <sup>2b</sup>
		Intercept	Slope	LC <sub>50</sub>	LC <sub>99</sub>		
Imidacloprid	24	-0.45±0.11	1.21±0.15	1.55 (0.65-1.66)	1.61 (1.01-8.13)	0.982	2.85
	48	-0.35±0.11	1.23±0.16	0.24 (0.15-0.37)	1.51 (1.26-1.88)	0.965	6.26
	72	-1.14±0.15	0.46±0.21	0.18 (0.08-0.25)	1.37 (1.15-1.74)	0.838	1.62
Zeta-cypermethrin	24	-0.66±0.11	0.98±0.13	0.44 (0.34-5.25)	4.06 (2.67-9.71)	0.971	5.15
	48	-0.23±0.11	0.45±0.15	0.31 (0.25-0.39)	3.51 (2.81-4.39)	0.785	6.43
	72	-0.13±0.12	0.38±0.12	0.21 (0.16-0.26)	1.97 (1.64-2.52)	0.918	1.68
Lufenuron	24	-0.36±0.17	0.57±0.13	0.58 (0.46-0.72)	4.21 (3.37-5.26)	0.531	12.61
	48	-0.17±0.14	0.56±0.13	0.46 (0.37-0.58)	3.43 (2.74-4.29)	0.677	5.52
	72	-0.28±0.12	0.48±0.13	0.22 (0.18-0.27)	3.16 (2.53-3.95)	0.251	8.49

<sup>a</sup>ET = Exposure times are in hours

<sup>b</sup> if χ<sup>2</sup> 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 27.30% mortality, followed by 25.65% for *C. reticulata*, 22.36% by *C. sinensis* and 20.0% by *C. paradise*. 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. reticulata* 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 cyfuthrin.

Heterogeneous responses in mortality rates of *T. granarium* larvae were observed in our study, which was evident in slope, χ<sup>2</sup> (chi-square) and R<sup>2</sup> 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 LC<sub>50</sub> 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 LC<sub>99</sub> 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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## التأثيرات الحادة وشبه المميتة لبعض المبيدات الحشرية النباتية والكيميائية على يرقات خنفساء الخابرا كواقيات طويلة الأجل للمنتجات المخزونة

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على الصعيد العالمي، هناك مخاوف متزايدة بشأن الإفراط في استخدام المبيدات الحشرية المصنعة بسبب تأثيراتها السلبية على البيئة المحيطة والصحة العامة. لذلك كان لزاما علينا تطوير مبيدات حشرية جديدة تكون آمنة وصديقة للبيئة اعتمادا على منتجات ذات أصل نباتي. مثل الزيوت النباتية التي تعتبر بديلا للمبيدات الحشرية ذات الأصل الكيميائي في مكافحة الآفات الحشرية. لذلك صممت هذه الدراسة لمحاكاة البيئة اللازمة لحفظ المنتجات المخزونة على نطاق صغير من خلال معالجة أسطح هذه البيئات لتحديد التركيزات المميتة ومعدل الموت لثلاثة من المبيدات الحشرية من أصل كيميائي والمختلفة في طريقة تأثيرها والمجموعات الكيميائية التي تنتمي لها وهي مبيد الإيميداكلوبرايد ومبيد الزيتا سايبيرميثرين ومبيد اللوفينورون ومقارنتها بثلاثة زيوت نباتية ذات أصل النباتي وهي زيت البرجموت وزيت الليمون وزيت الكمون على يرقات حشرة خنفساء الخابرا (خنفساء الصعيد) بتركيزات مختلفة وفترات تعريض متنوعة. أوضحت نتائج الدراسة ان زيت البرجموت كان الاعلى تأثيرا وفاعلية في أبادة اليرقات مقارنة بالزيوت الأخرى حيث كانت نسبة الموت 30.36% بعد تعريضها لمدة 24 ساعة على تركيز 10% حجم/حجم فيما زادت نسب الموت الى 75.33% عند مرور 72 ساعة من التعريض لزيت البرجموت. وفيما يتعلق بتأثيرات المبيدات الحشرية الكيميائية فكان مبيد الإيميداكلوبرايد هو الأكثر فاعلية ضد يرقات خنفساء الخابرا مما أدى الى موت 40% من الحشرات بعد مرور 24 ساعة عند تركيز 1% حجم/حجم كما أدى الى موت 96.66% من الحشرات عند تركيز 10% حجم/حجم بعد مرور 72 ساعة. باختصار، يلقي بحثنا الضوء عن إمكانية اختيار الزيوت النباتية كبديل مناسب لمبيدات الآفات الحشرية ذات الأصل الكيميائي ضد هذه الأنواع الحشرية الضارة.