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The Efficacy of some Insecticides and Essential Oil-Based Products against The Bed Bug, *Cimex lectularius*

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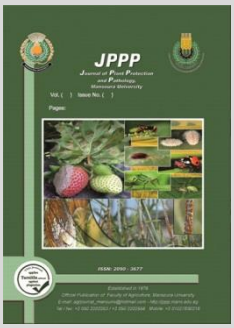

ABSTRACT

The bed bug, *Cimex lectularius* L., is a hard pest to control. The occurrence of insecticidal resistance amongst bed bug populations and concerns over human-insecticidal exposure has motivated the development of alternative bed bug control measurements. Therefore, the present study aimed to evaluate the efficacy of five essential oil products (Cinnamon oil, Clove oil, Garlic oil, Peppermint oil, and Thyme oil) compared to two pyrethroid products (Deltamethrin and Cypermethrin), and one organophosphorus (Diazinon) against field strain of bed bug (*Cimex lectularius* L.). The obtained results indicated that among the five essential oils, the Clove oil, and Cinnamon oil showed high efficacy against bed bug. Likewise, Deltamethrin exhibited the highest effectiveness among the three insecticides within 90 min after treatment. Also, Diazinon gave the maximum mortality against bed bug amongst the three insecticides tested after 24 hours of treatment. Accordingly, the present study suggested that using natural essential oils could be effective method, somewhat easier, safe and less expensive for controlling bed bugs.

Keywords: Bedbug, Clove oil, insecticide, essential oil, control

INTRODUCTION

The bed bug, *Cimex lectularius* is one of the most important urban pests, which have a worldwide distribution and was found living among all developed human populations (Hwang *et al.* 2005) .The bed bug an obligate blood-feeding ectoparasite that favorably feeds on humans, which can cause blood loss, skin irritations, depression, and nervousness in their human hosts (Reinhardt *et al.* 2009, Pritchard and Hwang 2009, and Goddard *et al.* 2012). Moreover, all phases of the insect require a blood meal to survive (molt for the nymphs, and reproduce for the adults).

The traditional control of bed bug mainly depends on the usage of synthetic chemical insecticides, which are necessary for both short-term and long-term bed bug management (Cooper and Harlan 2004). Pyrethroids are the most used compounds to control bed bugs and other indoor pests (Sutherland *et al.* 2015 and Lee *et al.* 2018).

Pyrethroids have been extensively used against bed bugs, because these compounds are less toxic to mammalians and have fast knockdown activity (Dang *et al.* 2015). Also, organophosphates are commonly used alongside pyrethroids for bed bug control (Berenji *et al.* 2019). However, using pyrethroids had many problems, such as toxic residues and widespread development of resistance, and undesirable effects on nontarget organisms (Sutherland *et al.* 2015 and Lee *et al.* 2018). Therefore, it is critical to seek for new materials to control bed bugs in an environmentally safe way. Previous studies suggested that plant essential oils and their constituents can be alternative sources for insect control, not just because their selectivity, but also because they can biodegrade to nontoxic products and have no negative effects on nontarget organisms and the environment (Isman, 2006, Jiang *et al.* 2012 and Liu *et al.* 2012). Effective natural products may be valuable tools to control bed bugs. Also, there is evidence supporting the efficacy of these products for bed bug control. (Chaieb *et al.* 2007, Kafle and Shih 2013 and Singh *et al.* 2013).

Accordingly, the objective of this study was to evaluate the activity of five essential oils (Cinnamon, Clove, Garlic, Peppermint, and Thyme) compared to two pyrethroid products (Deltamethrin and Cypermethrin), and one organophosphorus (Diazinon) against the bed bug, *Cimex lectularius*.

MATERIALS AND METHODS

Culture of insects

The bed bug, *Cimex lectularius* was collected from beds in a worker residence hall at Marsa Matrouh Governorate, (northwest of Egypt) during May until October 2018. All insects were kept at temperature of 27 ± 2°C, 60 ±10 RH %, and 12 L-12 hrs. photoperiod. They were feed on defibrinated rabbit blood (Rockland Immunochemical, Inc., Limerick, Pennsylvania, USA.) once every week.

Essential oils and chemicals compounds

Five essential oils [Cinnamon (*Cinnamomum verum*), Clove (*Syzygium aromaticum*), Garlic (*Allium sativum*), Peppermint (*Mentha piperita*), and Thyme (*Thymus vulgaris*)], were tested during this study. These oils were supplied by the Faculty of Pharmacy, Alexandria University, Egypt.

The commercial formulations of insecticides which used in bioassays during this study were pyrethroids [GRAP

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(Deltamethrin, 2.5% EC, Chema, Egypt)] and [CHLORO-PLUS, (Cypermethrin 5% EC), KZ, Egypt] and organophosphate [DIAZINOX, (Diazinon 60% EC), KZ, Egypt)].

Filter paper contact test

To determine the effective concentrations of essential oils for the mortality of bed bugs, many doses were tested (LC_{50}). The filter paper contact test was done as described by Fletcher and Axtell (1993). Briefly, two ml of essential oils dissolved in acetone were extended on filter paper discs which kept inside 5.5 cm clean Petri-dish. The treated filter papers were air-dried for 30 minutes for solvent evaporation under room temperature then released adult bed bug inside the dishes. However, for control treatment, only acetone solution was sprayed on filter papers.

Likewise, to determine the activity of insecticides, several concentrations dissolved in water were extended on filter paper discs which kept inside the Petri-dish. The treated filter papers were air-dried for 30 minutes for solvent evaporation under room temperature then released adult bed bug. However, for control treatment, only water was sprayed on filter papers. The Petri dishes were kept at temperature of 27 ± 2°C, 60 ± 10 RH %, and 12 L-12 hrs. photoperiod to record the number of died bed bug after treatments. Five replicates of 10 adult bed bugs were conducted for each concentration (Table 2) and control treatments. The trials were replicated three times. One-way analysis of variance was used to compare mean values among different concentrations of each insecticide. Tuckey's test was used to separate the means and P-value < 0.05 was considered as statistically significant. All analyses were performed with JMP version 11 (SAS Institute 2012).

RESULTS AND DISCUSSION

The efficacy of Cinnamon oil, Clove oil, Garlic oil, Peppermint oil, and Thyme oil against bed bug, *Cimex lectularius* are presented in Table (1). As shown the highest efficiency was recorded for Clove oil. This oil showed a lethal concentration (LC_{50}) of 2.84 mg/ml after 48 hours and 1.98 mg/ml after 72 hours of treatment. While, the lowest efficiency was observed with Thyme oil (LC_{50} of 10.12 mg/ml after 48 hours, and 8.82 mg/ml after 72 hours of treatment, respectively, Table 1). Likewise, the LC_{50} values after 48 hours of treatment for Cinnamon oil, Garlic oil, and Peppermint oil were 4.67, 5.88, and 7.44 mg/ ml, respectively. However, the LC_{50} values after 72 hours of treatment were 3.79, 4.89, and 6.99 mg/ml, respectively (Table 1). Some studies have focused on the use of essential oils from plants as potential bioactive agents against bed bug (Hussain *et al.* 2006, Tripathi *et al.* 2009, Vinayaka *et al.* 2009 and Abbasia *et al.* 2010). Among them, Thymol (Pandey *et al.* 2009, Phillips *et al.* 2010), and Clove oil (Cornelius *et al.* 1997 and Ngoh *et al.* 1998) proved to be effective, as direct sprays, against insects of urban plants and medical importance. Consequently, the present study explained the insecticide effect of essential oils for controlling bed bugs.

Table 1. The lethal effect of essential oils against the bed bug, *C. lectularius*

Essential oils	Time (h)	LC_{50} (mg/ml)	95% Confidence limits
Clove	48	2.84	2.6 – 3.06
	72	1.98	1.75 – 2.07
Cinnamon	48	4.67	4.21 – 5.32
	72	3.79	3.44 – 4.19
Garlic	48	5.88	5.43 – 6.98
	72	4.89	4.53 – 5.98
Peppermint	48	7.44	6.11 – 8.21
	72	6.99	6.53 – 7.98
Thyme	48	10.12	9.89 – 10.88
	72	8.82	8.56 – 9.27

Our results showed that Clove oil was the most effective essential oils against the bed bug. Similar findings were demonstrated in several studies (Chaieb *et al.* 2007 and Kafle and Shih 2013). In these studies, Clove essential oil has been widely studied for its insecticidal and repellent activities against many species of pests. Furthermore, Singh *et al*. (2014) reported that Bed Bug Patrol, a conventional insecticide exempt product consisting of 0.003% Clove oil, 1% Peppermint oil, and 1.3% sodium lauryl sulfate, was resulted in an over 90% mortality of bed bugs (*C. lectularius*). The previous finding might be in partial agreement with the results of the present study. In addition, Zhu *et al.*(2001) indicated that Clove bud oil killed 100% of Formosan termites (*C. formosanus*) in two days at 50μg/cm^2. Also, Park and Shin (2005) found that Clove oil produced 100% mortality in Japanese termites (*Reticulitermes speratus* Kolbe) at 0.5 μL/L of air. Clove oil toxicity varies among different insects because of different insect susceptibilities, variability in the concentration of eugenol and other active substances in the clove oil, and feeding behaviors

As shown in Table (2), the three traditional insecticides showed vital activity against the bed bug. Furthermore, the activity of Deltamethrin was the highest amongst the three insecticides within 90 min after exposure. Additionally, mortality of the bed bug was higher (73.0% at 1 mg/ml, $P < 0.05$) compared to 85.7% at 4 mg/ml, and 95.6% at 6 mg/ml of Deltamethrin (Table 2). The same trend was found after 24 hours of exposure, when the bed bug mortality was higher (97.3%, $P < 0.05$) at 6 mg/ml of Deltamethrin as compared to the other concentrations (Table 2). Likewise, mortality of the bed bug was higher ($P < 0.05$) at 6 mg/ml of Cypermethrin after 90 min, and 24 hours of exposure (Table 2). However, Diazinon had no effect during the first 90 min of treatment. But it showed the maximum mortality (100%, $P < 0.05$) within 24 hours of treatment at 0.09 mg/ml (Table 2). Most of the studied natural insecticides failed to cause high mortality to bed bugs as a direct spray (Singh *et al*. 2014). Therefore, homeowners often use insecticides when they find pests at home (Horton *et al*. 2011). Moreover, using insecticides namely, pyrethroids, pyrroles, and neonicotinoids are the most common compounds for bed bug control (Sutherland *et al.* 2015 and Lee *et al.* 2018). The previous studies indicate that the commercial insecticide products that containing *d -d -t* cyphenothrin, permethrin, *d* -phenothrin or fenitrothion have the ability to kill the bed bug (Okamoto *et al.* 2010). These finding might be in agreement with the results of the present study.

Table 2. The lethal effect (Mean ± SEM) of insecticides against the bed bug, *C. lectularis*

Insecticides	Conc. (mg/ml)	% mortality after 90min	%mortality after 24 hours
Deltamethrin	1	73.0 ± 1.68^{c}	87.0 ± 1.56^{b}
	4	85.7 ± 1.07^{b}	91.2 ± 1.23^{ab}
	6	95.6 ± 1.72^{a}	97.3 ± 1.78^{a}
Cypermethrin	1	63.0 ± 1.58^{c}	70.2 ± 1.35^{c}
	4	79.0 ± 1.21^{b}	81.0 ± 1.23^{b}
	6	89.3 ± 1.06^{a}	90.2 ± 1.82^{a}
Diazinon	0.008	0.0	84.9 ± 1.23^{c}
	0.05	0.0	93.5 ± 0.62^{b}
	0.09	0.0	100.0 ± 0.15^{a}
Control	0	0.0	4.7

^{a,b,c} **Means in the same column of the same variable with different superscript significantly differ at (P < 0.05).**

In the present study, the commercial insecticide products, Deltamethrin, and Cypermethrin were killed the bed bug during the first 90 min after treatment. However, after 24-h exposure to three insecticides, collected mortality data revealed that Diazinon was the most toxic to exposed bed bugs (100% mortality at 0.09 mg/ml). These results were in agreement with the results of Berenji *et al*. (2019), who indicated that Diazinon was the most toxic against bed bugs (LC_{50} = 1,337.40 ppm) after 24-h of treatment as compared to Malathion, and λ-cyhalothrin. Likewise, Steelman *et al.* (2008) exposed three different field-collected bed bug populations to Diazinon using adult vial test method. After 24-h exposure, the LC_{50} values for Diazinon in three bed bug populations were 9.8, 28.3 and 561.5 ppm. The similarity between Diazinon results of the present study with those obtained in other study indicated that our bed bugs population was resistant to Diazinon. Furthermore, the improper, extensive, and repeated usage of these insecticides to control bed bugs can lead to behavioral and/or physiological resistance (Marangi *et al.* 2009, Dang *et al.* 2017).

Traditionally, chemical insecticide especially organophosphates and synthetic pyrethroids are used against bed bugs, but recently application of these commercial insecticides does not give a satisfactory control (Berenji *et al*. 2019). The bed bug control failure can be attributed to many reasons, such as poor technical skills, wrong pesticide selection, inadequate dosing of applied insecticide, and developing resistance (Tawatsin *et al.* 2011). Too, essential oil-based pesticides and detergents are attractive to both manufacturers and consumers (Singh *et al.* 2014). For manufacturers, it is comparatively easier and less expensive to market products that are generally regarded as safe and for consumers, essential oil-based pesticides and detergents are perceived as safer to use (Singh *et al*. 2014).

Consequently, in conclusion, the present study suggested that using natural essential oils of Cinnamon, Clove, Garlic, Peppermint, and Thyme might be an alternative method for controlling bed bugs. However, additional studies are warranted to determine the field efficacy of these natural essential oils and how they can be combined into a bed bug controlling program.

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فاعلية بعض المبيدات الحشرية و منتجات الزيوت الأساسية ضد بق الفراش (*Cimex lectularius*)

منى ابراهيم البانوبي

قسم وقاية النبات ، كلية الزراعة ، جامعة دمنهور

حشرة بق الفراش (*Cimex lectularius* L) من الآفات التي يصعب السيطرة عليها ونظرا لظهورصفه المقاومة للمبيدات الحشرية بين مجاميع حشرة بق الفراش والمخاوف من التعرض المكثف للمبيدات الحشرية فقد حفز ذلك تطوير مواد بديلة لمكافحة حشرات بق الفراش. لذلك كان الهدف من الدراسة الحالية هو تقييم فعالية خمسة من منتجات الزيوت الأساسية (زيت القرفة وزيت القرنفل وزيت الثوم وزيت النعناع وزيت الزعتر) مقارنة بمنتجين من البيروثرويد (الدلتا ميثرين والسيبرمثرين) ومنتج من الفسفور العضوي (الديازينون) ضد السلالة الحقليه من بق الفراش. حيث أشارت النتائج المتحصل عليها إلى أنه من بين الزيوت الأساسية الخمسة المستخدمة كان كل من زيت القرنفل وزيت القرفة الاكثر فعالية ضد حشرة بق الفراش. وبالمثل كان الدلتا ميثرين هو الاعلى فعالية بين المبيدات الحشرية ضد حشرة بق الفراش خلال 90 دقيقة من المعاملة. بينما أظهر الديازينون فاعلية كبيرة من خلال نسبة الموت للحشرات والتى وصلت لـ 100% بعد 24 ساعة من المعاملة. ووفقا لذلك تقترح الدراسة الحالية أن استخدام الزيوت العطرية الطبيعية يمكن أن يكون وسيلة فعالة وأسهل إلى حد ما وآمنة وأقل تكلفة للسيطرة على بق الفراش.