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مقارنة بين المكافحة الكيميائية والبيولوجية لحشرة Cimex lectularius

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Comparative of chemical and biological control on Cimex lectularius

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الملخص:

يعتبر بق الفراش (Cimex lectularius) من الطفيليات البشرية ذات الأهمية الاقتصادية والطبية على مستوى العالم، إضافةً إلى المبيدات الحشرية الاصطناعية، فإن الزيوت الأساسية (العطرية) المشتقة من النباتات تستخدم كمريقة بديلة لمكافحة بق الفراش. كان الغرض من هذه الدراسة هو تقديم بيانات شاملة عن فعالية وسمية وآلية عمل الزيوت العطرية المكافحة بق الفراش. كان الغرض من هذه الدراسة هو تقديم بيانات شاملة عن فعالية وسمية وآلية عمل مؤسسات) الموبوءة المواش. كان الغرض من هذه الدراسة هو تقديم بيانات شاملة عن فعالية وسمية وآلية عمل الزيوت العطرية و المبيد الحشري Diazino على بق الفراش. تم جمع بق الفراش من العديد من (شقق، منازل، مؤسسات) الموبوءة الواقعة في مدينة بنغازي، ليبيا تكونت التجربة من المبيد الكيميائي ديازينون بتركيز (2، 1، 5.0) مؤسسات) الموبوءة الواقعة في مدينة بنغازي، ليبيا تكونت التجربة من المبيد الكيميائي ديازينون بتركيز (2، 1، 5.0) مركرات. أظهرت النتائج أن زيادة تركيز الزيت العطري نزرين (00، 75، 50، 25، 210، 0 مجم مل-1)، مع ثلاث مكررات. أظهرت النتائج أن زيادة تركيز الزيت العطري لنبات النيم إلى (100، 25، 25، 25، 210، 0 مجم مل-1)، مع ثلاث مكررات. أظهرت الذي إلى زيادة تركيز الزيت العطري لنبات النيم إلى (100 مبكرولتر) وزيادة مدة التعرض حتى معامية وفيات 100% لبق الفراش، في حين لم تسجل معاملة ألشاهد أي وفيات لبقراش، كما أن زيادة تركيز الديازينون إلى (2 ميكرولتر) وزيادة مدة التعرض حتى ألمالية وفيات 100% لبق الفراش، في حين لم تسجل معاملة ألشاهد أي وفيات لبق الفراش، كما أن زيادة تركيز الديازينون إلى (2 ميكرولتر) وزيادة مدة التعرض حتى 100% لبق الفراش، في حين لم تسجل معاملة ألما هذى إلى زيادة نسبة الوفيات حيث سجل معاملية وفيات الفراش، في حين لم تسجل معاملة ألم الشرائي في حين لم تسجل معاملية أدى إلى زيادي العرض م 100% لبق الغرائي في حين لي تسجل ميام تسجل معاملية المانية ألى زيادة منابة الفراش، في حين لم تسجل مالي أدى إلى زيادي ألى زيادة منه الوفيات العرض م الفراش معار في وفيات لبق وفيات لبق وفيات لبق وفيات لبق وفيات الفراش، كمن النيم ولمين والمي زيادي ألى زيان الغري ووليات الم ووليات المالي ووليات الفراش. كمم مالي ألمرائي ما لم تي ووليات الفراش في مي ألمري والميزين الفراش في مالمري والميزي ووليات الفراي ووليان الفري ووليات الفري والمي

الكلمات المفتاحية: بق الفراش، المبيدات الحشرية، Diazinon ، زيت النيم.

Abstract:

Bed bugs (*Cimex lectularius* L.) are major medical and economic parasites around the world, and managing them necessitates a variety of control strategies. To control bed bugs, plant-derived essential oil extracts are utilized instead of mechanical treatments and synthetic insecticides. The goal of this study was to provide detailed information on the efficacy, toxicity, and mechanism of essential oils and the insecticidal chemicals found in bed bugs. Bed bugs were collected from a variety of afflicted residences (buildings) in Benghazi, Libya, including houses, apartments and institutions. The experiment consisted of chemical pesticide Diazinon at concentration (2, 1, 0.5, 0.25, 0.125, 0 ml/l) and essential oil at concentration (100, 75, 50, 25, 12.5, 0 mg ml⁻¹), with three replicates. The results showed that whereas the control treatment showed no bed insect mortality, a

higher concentration of neem (*Azadirachta indica*) essential oil up to 100 μ ml⁻¹ and longer durations up to 72 h enhanced the mortality percentage, recording 100% bed bug death. Furthermore, increasing the Diazinon concentration to 2μ ml⁻¹ and prolonging the time to 72 hours led to a 100% bed bug mortality rate, whereas the control treatment showed no mortality. The diazinon insecticide and the essential oil of neem were found to be toxic to bed bugs at lethal dosages (LD₅₀, LD₉₀) after 24, 48, 72 hours of exposure; however, the control group did not experience any mortality.

Keywords: Bed bug, *Cimex lectularius*, pesticides, Diazinon, essential oils, *Azadirachta indica*.

Introduction:

Bed bugs are members of the genus Cimex and family Cimicidae. Twenty-three species in the latter category sucking blood form bats, birds and humans (Akhoundi *et al.*, 2020). Some organisms will occasionally consume humans when their usual host is absent. From egg to adult, bed bugs go through five phases of life. Over the course of their lives, females lay 200–500 egg or around five eggs every day. In 4-12 days, the eggs mature into the first instar nymph. Before moulting to the following stage, each nymphal stage consumes a blood meal (Reinhardt and Siva-Jothy, 2007). Blood is the only food source for both sexes at every stage. They have flattened bodies that give them an oval form, and by adulthood, they are around 5 mm long. The adult forms are reddish-brown, whereas the early life stages are tiny and bright yellow. Adults can live for six to twelve months, and they can go up to 140 days without eating, depending on the species (Reinhardt and Siva-Jothy, 2007).

Bed bugs feed on people of all ages, genders, and socioeconomic backgrounds. In addition to causing significant psychological discomfort, bed bugs' bloodsucking behavior can cause a range of clinical symptoms, from mild responses to severe allergies or blisters (Akhoundi et al., 2023). The common bed bug (Cimex lectularius) has experienced a global upsurge in the past 20 years. The health of people is negatively impacted by the allergies that indoor pests cause. Curiously, though, no evaluation of their effects on indoor microbial ecosystems has been carried out. In affordable and senior housing, bed bug infestations can cause serious issues. They discharge a great deal of liquid waste into the home, which may alter the microbial population's composition (Kakumanu et al., 2020). These days, bed bug infestations are prevalent not only in homes and hotels but also in public and private settings like senior living communities, healthcare facilities, and public transportation. Due to their widespread distribution, these pests will be challenging to manage and eradicate completely (Penn and Hu, 2020). Bed bugs are a significant worldwide human parasite in terms of both health and economy. They feed on human blood, and in addition to escalating mental health conditions, their bites can result in rashes, allergies, itching, and sleep disturbances (Eddy and Jones, 2011). According to the U.S. Environmental Protection Agency (EPA) and the U.S. Centers for Disease Control and Prevention (CDC), bed bugs are a major health risk (CDC, 2010). Over the past eighteen years, bed bugs have made a comeback and are continuing to spread.

According to Ashbrook *et al.* (2017), one of the main causes of their recurrence is insecticide resistance, which has been made worse by the improper use of synthetic

pesticides with comparable mechanisms of action. Because of the possible negative effects of repeated exposure, the use of synthetic pesticides inside buildings or indoor spaces raises concerns for public health (Yang, 2017). Proven strategies for handling bed bugs include integrated pest management, or IPM. This plan, according to Bennett et al. (2016), calls for the use of a variety of non-chemical control measures like heat treatments, mattress encasements, and removal of infested furniture in addition to the application of both synthetic and essential oil-based insecticides. Furthermore advised are resident education programs and active and passive bed bug trapping techniques. In 2010, the Centers for Disease Control and Prevention and the U.S. Environmental Protection Agency classified bed bugs as a pest of public health concern, demonstrating the broad consensus among public health experts regarding the significance of bed bugs as a public health concern. This insight showed that workable solutions were required to address the problem of bed bug infestations and protect the public's health (Kaylor et al., 2015). Bed bugs are a major annoyance in urban areas. Due to their presence, homeowners and apartment tenants frequently face physical, psychological, and financial difficulties. The feeding status of bed bugs, which is closely related to mass, mounting, and metabolism, has not received much attention despite being the focus of multiple pesticide bioassays (DeVries et al., 2015).

Goddard and Deshazo (2009) claim that the common bed bug has afflicted people for thousands of years. These flat, oval-shaped, reddish-brown insects have no wings and can grow up to 5 mm in length. They resemble tiny ticks or cockroaches. During the day, bed bugs hide in the crevices in floors, walls, furniture, and beds (Pritchard and Hwang, 2009). They have a lengthy history in human cultures that can be found all over the globe. Indeed, one of the most prevalent ectoparasites affecting humans is the bed bug, and its presence has long posed a significant threat to public health (Shum, 2012). It is now essential to use integrated pest management (IPM) techniques to reduce bed bug infestations. Before a pest control company arrives, infested items and areas should be carefully inspected, isolated, and treated. Applying heat, cold, or pesticide treatments alone or in combination may be necessary for this (Potter, 2008; Lewis *et al.*, 2009). Globally, bed bugs are a serious parasite that affects people. Essential oil-based insecticidal chemicals have been suggested as a component of integrated pest management (IPM) strategies (Gaire *et al.*, 2020).

Being positively thigmotactic insects, bed bugs prefer direct contact with surfaces and will often hide in cracks, crevices, and other awkward places. It's common knowledge that bed bugs can readily pierce cardboard, which is why many bed bug traps on the market today are made of thin cardboard pieces (Doggett *et al.*, 2018). Hemiptera: Cimicidae, which includes the bed bug, is a serious indoor pest that needs to be managed with integrated pest management (IPM) employing a variety of techniques (Aak *et al.*, 2023). Bed bugs are blood-feeding insects classified as major public health pests because of their propensity to feed on human blood, produce unpleasant bite reactions, and negatively impact the mental well-being of those who are bitten (Doggett *et al.*, 2012). Over the past 20 years, there has been a worldwide resurgence of the common bed bug species, *Cimex lectularius* L., and the tropical species, *Cimex hemipterus* (F.) (Doggett and Lee, 2012). Chemical control is a popular method for dealing with bed bug infestations. The effectiveness of widely used pesticide treatments against local bed bug populations and the degree of insecticide resistance that has developed in these pests are still unknown, but (Yu *et al.*, 2023). Over the past 20 years, bed bugs which are widely distributed throughout the world have grown to be a serious public health concern, particularly in heavily populated urban areas. Chemical treatment of bed bug infestations is frequently required, which not only puts those impacted at risk of poisoning but also results in costs, losses, inconvenience, and psychological suffering (Laborde-Castérot *et al.*, 2024).

Eliminating bed bugs, or *Cimex lectularius* L., is a difficult pest to deal with. Concerns about human exposure to pesticides and the rise in insecticidal resistance in bed bug populations have fueled the development of alternative bed bug control methods (Elbanoby, 2019). According to Doggett *et al.* (2018), bed bugs pose serious health and economic problems for cities. Using insecticides is one of the main strategies used to control this pest (Lee *et al.*, 2018). However, bed bugs may become resistant to pyrethroids, neonicotinoids, and pyrroles, among other classes of insecticides, as a result of repeated pesticide use (Ashbrook *et al.*, 2017; Caceres *et al.*, 2019).

The rise in pesticide resistance and inadequate pest control techniques that are unable to handle resistant bed bugs are the root causes of this issue (Dang *et al.*, 2017). Reactions to bed bug bites can cause blister-like swelling or skin irritations in addition to general discomfort. Partie *et al.* (2015) state that psychological stress, which can result in anxiety and insomnia, is the primary risk associated with bed bugs in addition to the discomfort caused by bites. One well-known parasite that affects humans is the common bed bug. The Environmental Protection Agency has classified bed bugs as a serious threat to public health that needs to be controlled in order to avoid infestations and protect public health (Dejene, 2024).

The public is finding that effective "green" pest management products are increasingly important. Essential oils are among the botanical pesticides that are deemed safe due to their low toxicity to both humans and animals (Politi, 2017). Essential oils derived from plants are becoming a competitive substitute for conventional pesticides (Regnault-Roger *et al.*, 2012).

Essential oil compounds do not require full EPA registration because of their low risk (Federal Insecticides, Fungicides, and Rodenticides Act-FIFRA, 40 CFR 152.25) (US., 2013). Regnault-Roger *et al.* (2012) state that there are certain disadvantages to employing essential oils for pest control. Some of these are: (i) a short residual life that requires frequent applications; (ii) high volatility that may result in unpleasant odors for nearby residents; and (iii) a lack of field efficacy data for a variety of insect pest species.

Thus, the primary goal of this study was to examine the relationship between the essential oil of *Azadirachta indica* and the toxicity of the synthetic pesticide diazinon.

2. Experimental design and measurements:

2.1 Bed bug collection

Following standard protocol, bedbugs were collected from several infected residences (buildings) (apartments, homes, and institutions) at a private laboratory in the city of Benghazi, Libya. The insects used in this study were housed in 240 ml glass rearing jars with 90 mm filter paper circles and cardboard harborages folded into the

shape of a fan (Whatman no. 1). To stop insects from escaping, a nylon mesh covering with 90 μ m perforations was placed over the rearing jar entrance (Pereira, 2009). Once a week, artificial feeding was used to achieve engorgement (Montes *et al.*, 2002). All bed bug populations were kept in a temperature chamber with 25 ± 1°C, 45 ± 10% relative humidity, and a photoperiod of 12:12 (L:D) hours. Before bed bugs were fully fed, three to seven days were spent conducting all of the bioassays. Fed using an artificial (in vitro) feeding system once a week on expired human plasma and red blood cells (Feldlaufer *et al.*, 2010).

2.2 Application of insecticide

As directed on the product labels, a chemical insecticide (Diazinon) was applied to the treated material's whole surface. All items (fabric pieces, wooden furniture, and primed wood) were weighed both before and after pesticide treatment. In order to spray surfaces, concentrated insecticide formulations were diluted to the proper label rate using the aerosol-based Preval Sprayer (Nakoma Products, LLC., Bridgeview, IL, USA).

2.3 Preparation of Insecticides

The following concentrations of diazinon were prepared by diluting it with acetone: 100, 75, 50, 25, 12, 50, and control. Using this methodology, 1.2 ml of the intended insecticide concentration was impregnated onto a filter paper (Whatman No. 1, 11-cm diameter, China) without any runoff. Bioassays were carried out with this technique. Paper filters that had been soaked in acetone were used as a control. The control and impregnated filter papers were dried in a fume hood. Randomly selected adult bed bugs of mixed sexes were placed in a 10-cm inverted petri dish with filter paper impregnated on it. Then a lid was put on top. The plates were incubated at 25°C and 80% RH for 24, 48, and 72 hours. Finally, a dissecting microscope was used to assess bed bug mortality. An insect was considered dead if it remained motionless when prodded with an entomological pin. There were five copies of each concentration.

2.4 Essential oil

Using glass Petri dishes $(94 \times 3 \times 20 \text{ mm})$ with a bottom divided into equal quarters by two perpendicular ridges, the test compounds' initial efficacy against bed bugs was evaluated (Felsen and Weil, 1951). Filter paper (Whatman No. 1) segments in the shape of pies, each cut to the appropriate quadrant size, filled three of the four quadrants.

Adult bed bugs (n¹/433–77 per dish) were placed in three quadrants, and a cotton ball containing 0.3 ml of test compound was placed over the glass bottom of one quadrant. Parafilm TM (Bemis Company, Inc., Oshkosh, WI) was used to position, seal, and cover the Petri dish cover. After treatment, the dishes were left in their natural habitat, and the rates of death were recorded 24, 48, and 72 hours later. **Feldlaufer** *et al.* (**2013**) classified bed bugs as dead, living, or morbid-moribund. There were two replications carried out. Because the treated cotton ball would not be in close proximity to bed bugs, able to use these Petri plates to do an initial screening of chemicals for fumigation activity.

For this study, adult bed bugs were carefully separated using forceps and placed in Petri dishes. To immobilize the bed bugs, carbon dioxide (CO_2) was employed as an

anesthetic agent. For the treatment group, a solution containing varying concentrations of the plant extract (1 g/l to 0.053 g/l) was carefully applied to the dorsal abdomens of the insects using a 100 μ l micropipette. The solution was prepared using acetone, methanol, ethanol, or distilled water extraction. Conversely, the control group received an application of 100 μ l of either methanol, ethanol by itself, distilled water, or acetone. Following treatment, 20 ml clear glass vials containing bed bugs were filled with paper strips made from standard 92 multipurpose papers. These vials were then placed in a growth chamber so that the effects of the treatment could be monitored. After treatment, the insects' mortality rates were measured at 1, 2, and 3 days to see if the plant extract was successful in lowering the number of bed bugs. A bed bug was considered dead if, upon being pricked with a needle, no part of its body moved.

2.5 Statically analysis:

The data were analyzed statically by SAS program (SAS, 1999). Duncan test was used at significant level of 5% for comparison of means.

3. Results:

3.1 Chemical insecticide

The findings in Table (1) and Fig. (1) demonstrated that raising the Diazinon concentration to 2 ml⁻¹ and extending the exposure times to 72 hours resulted in a 100% bed bug mortality rate, followed by a 94.4% bed bug mortality rate from 1 ml⁻¹, while the control treatment showed no bed bug mortality. Table (3) displays the fatal dose (LD₅₀, LD₉₀) values of diazinon insecticides against the bed bug, *Cimex lectularius*. Diazinon was harmful to exposed bed bugs (LC₅₀ = 0.17 (0.13–0.21 ppm) and (LC₉₀ = 0.65 (0.49–1.02)), according to mortality data taken after 24, 48, and 72 hours of exposure to the insecticide. The control group did not experience any mortality, as shown in Table (3).

Table (1): Mortality (%) of bed bugs (*Cimex lectularius*) exposed to Diazinon at different concentrations.

Diazinon	24h		48h		72h		Mortality (%)	
$(\mu \text{ ml}^{-1})$	Live	Dead	Live	Dead	Live	Dead	\pm SD	
2	5	15	0	20	0	20	$100 (\pm 2.345)^{a}$	
1	9	11	1	19	1	19	94.4 (±1.169) ^a	
0.5	13	7	10	10	6	14	$66.7 (\pm 1.602)^{a,b}$	
0.25	11	9	8	12	8	12	$38.9 (\pm 1.329)^{b,c}$	
0.125	13	7	13	7	13	7	$27.7 (\pm 0.548)^{c,d}$	
Control	20	0	20	0	20	0	-	

Values followed by the same lowercase letters (a–d) do not exhibit significant differences by the Student-Newman-Keuls test ($p \le 0.05$). The data correspond to the mean of two assays performed at different times.

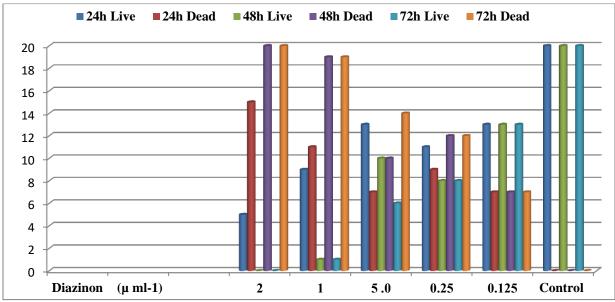


Fig. (1): Mortality (%) of bed bugs (*Cimex lectularius*) exposed to Diazinon at different concentrations.

3.2 Essential oil

Table (2) shows that increasing the concentration of neem (*Azadirachta indica*) essential oil up to (100 ml^{-1}) and increasing the periods up to 72 h increased the mortality percentage of bed bugs, with 100% mortality recorded at (75 ml⁻¹) and 85.00% at (75 ml⁻¹). The control treatment did not result in any mortality of bed bugs. Table (3) shows the fatal doses (LD₅₀, LD₉₀) of neem essential oil (*Azadirachta indica*) against the bed insect *Cimex lectularius*. After 24, 48, 72 hours of exposure to neem essential oil (*Azadirachta indica*), collected mortality data revealed that neem essential oil was toxic to exposed bed bugs (LC₅₀ = 15.85 (12.61-19.44) and (LC₉₀= 55.44 (41.89-84.21), and no mortality was observed in the control group, as shown in Table (3).

 Table (2): Mortality (%) of bed bugs (*Cimex lectularius*) exposed to the essential oil of neem (*Azadirachta indica*) at different concentrations

Essential oil	24h		48h		72h		Mortality (%)	
$(\mathrm{mg} \mathrm{ml}^{-1})$	Live	Dead	Live	Dead	Live	Dead	± SD	
100	8	12	2	18	0	20	100 (±2.041)a	
75	5	15	3	17	3	17	85 (±1.722)a	
50	11	9	8	12	8	12	60 (±1.975)a,b	
25	16	4	12	08	10	10	50 (±1.633a,b	
12.5	19	1	18	02	17	03	15 (±0.894)b,c	
Control	20	0	20	0	20	0	-	

Values followed by the same lowercase letters (a–d) do not exhibit significant differences by the Student-Newman-Keuls test ($p \le 0.05$). The data correspond to the mean of two assays performed at different times.

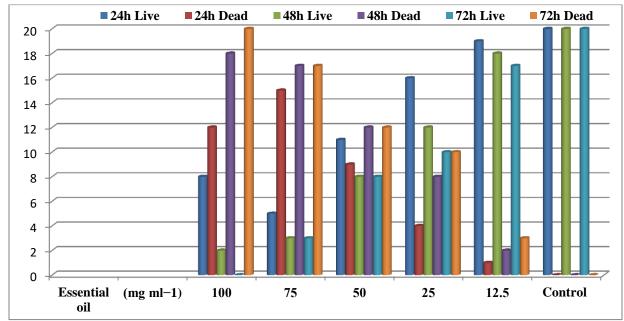


Fig. (2): Mortality rate of bed bugs (*Cimex lectularius*) exposed to the essential oil of neem (*Azadirachta indica*) at different concentrations

 Table (3): LC₅₀ and LC₉₀ values of Diazinon and essential oil of essential oil of neem (Azadirachta indica) on bed bug Cimex lectularius

Sample	LC50 mg ml ⁻¹ (LCL–UCL)a	$\frac{LC_{90} \text{ mg ml}^{-1}}{(LCL-UCL)a}$	Standard error	Chi- square	p value	Rb
Diazinon	0.17(0.13–0.21)	0.65 (0.49–1.02)	0.02	1.276	0.734	0.734
Essential oil	15.85 (12.61–19.44)	55.44 (41.89–84.21)	1.75	1.448	0.694	0.820

4. Discussion:

In the past, pesticides were primarily used to control bed bug infestations. In chemical pesticides, particularly organophosphates and synthetic pyrethroids, have historically been used to control bed bugs. Still, the degree of control provided by the most recent treatments with these commercial pesticides has proven inadequate. Many factors, such as a lack of technical expertise, poor pesticide selection, insufficient insecticide application dosage, and the emergence of resistance, can be blamed for the failure of bed bug treatment (Tawatsin *et al.*, 2011).

Comparing exposed bed bugs to the compound's LC_{50} reported by Fletcher *et al.* (1993), the exposed bed bugs demonstrated insensitivity to malathion at 10,000 percent. This implies that, at least in this particular population, the compound's effectiveness against *C. lectularius* has been lost. Because of their quick knockdown time and low toxicity to mammals, pyrethroids have been used extensively in the fight against bed bugs (Dang *et al.*, 2015). Misuse, overuse, and repeated application of these pesticides in an attempt to eradicate bed bugs may result in physiological and/or behavioral resistance (Dang *et al.*, 2017a). The results of this traditional toxicity bioassay showed that the *C. lectularius* population under investigation was resistant to diazinon, λ -cyhalothrin, and

malathion. Molecular and biochemical testing should be used to identify any potential resistance pathways. The aforementioned assays are highly sensitive in verifying resistance and its particular mechanisms, but they necessitate costly reagents and apparatus (Dang *et al.*, 2017b).

Despite having an RR50 value of 1.4, Leong *et al.* (2020) discovered that only 53% of bed bugs treated with Tandem insecticide perished. Dang *et al.* (2017) found that target site insensitivity (kdr-type mutation) and the overexpression of detoxifying enzymes are the main mechanisms causing pyrethroid resistance in bed bugs. Dang *et al.* (2015) suggest that the heterozygous kdr mutation in bed bugs could impact the knockdown response and result in low mortality.

The mainstay of traditional bed bug control is the use of synthetic chemical pesticides. Cooper and Harlan state that these pesticides are necessary for both short-term and long-term bed bug control. Pyrethroids are the most widely used pesticides for controlling bed bugs and other indoor rodents (Sutherland *et al.*, 2015; Lee *et al.*, 2018). Frequent application of insecticides has led to the development of resistance in many mealy bug populations, and the use of chemicals has been linked to environmental contamination and negative effects on organisms that are not the intended target (Franco *et al.*, 2009, Mansour *et al.*, 2017). The current state of affairs has prompted a surge in the search for environmentally safe methods of managing pests (Lucchi *et al.*, 2019, Franco *et al.*, 2021).

The use of artificial chemical insecticides is the foundation of traditional bed bug control. According to Cooper and Harlan (2004), these pesticides are essential for both temporary and permanent bed bug treatment. Pyrethroids are the most commonly used pesticides for controlling bed bugs and other indoor pests (Sutherland *et al.*, 2015).

Before creating these remedies, though, a few things must be addressed in regards to the use of essential oils or their active components in the management of urban pests. Due to their high volatility, essential oils only make a fleeting impression when used as fumigants or in close proximity to bed bugs (Gaire *et al.*, 2019). Furthermore, some individuals find essential oils' potent scent offensive. Nonetheless, essential oil compositions that is nano-and/or microencapsulated show less volatility, which helps to minimize odor problems and extend residual activity (Oliviera *et al.*, 2017).

Therefore, additional research can examine the use of microencapsulated formulations as carriers for pyrethroid and essential oil containing products. Given that bed bugs also display high levels of resistance to a range of neonicotinoid insecticides, it is imperative to look into whether essential oils or any of their constituents can increase the toxicity of neonicotinoid pesticides in populations of resistant bed bugs (Romero and Anderson, 2016; Caceres *et al.*, 2019).

Because of their low toxicity, botanical pesticides which include essential oils are believed to be safe for use around humans (Politi, 2017). Essential oils derived from plants are becoming a competitive substitute for conventional methods of managing insect pests (Regnault-Roger *et al.*, 2012). Essential oil compounds are exempt from complete EPA registration due to their low risk (Federal Insecticides, Fungicides, and Rodenticides Act-FIFRA, 40 CFR 152.25) (U.S. 2013). Regnault-Roger *et al.* (2012) state that there are certain disadvantages to employing essential oils for pest control. Some of these are: (i) a short residual life that requires frequent applications; (ii) high volatility that may result in unpleasant odors for nearby residents; and (iii) a lack of field efficacy data for a variety of insect pest species.

The disparity between our findings when comparing organic neem seed oil with a commercial product containing cold-pressed neem oil is most likely due to variations in the azadirachtin content of different neem seed oils. Our finding that bed bugs exposed to acetophenone had higher death rates casts doubt on this conclusion. According to the USEPA (2013), acetophenone is used as a food flavoring and fragrance. When exposed to the six most hazardous components of plant essential oils, bed bugs showed symptoms of poisoning such as paralysis, hyperactivity, and leg tremors.

Hyperactivity and leg tremors were reported to be common poisoning symptoms linked to essential oil ingredients by Coats *et al.* (1991). All of these findings suggested that intrinsic toxicity (i.e., distinct target-receptor interactions) rather than volatility alone most likely determined the toxicity of the investigated essential oil components. Further investigation may be necessary to ascertain whether compounds with low evaporation exhibit increased toxicity against bed bugs in long duration bioassays (3–7d), as demonstrated by Feldlaufer and Ulrich (2015), since fumigant toxicity is dependent on the exposure time.

5. Conclusion: The study's findings suggest that diazinon may have anti-bedbug effects. It is also critical to have a "integrated pest management program." Bed bugs are resistant to some conventional insecticide treatments, thus employing these pesticides will likely make bed bug control more difficult. Certain insecticides are wholly restricted, while others are legal for other uses but cannot be used to control bed bugs. It also goes over the baseline toxicity of essential oil components utilized in bed bug IPM. Certain essential oil components influence how bed insects' nervous systems work normally. When taken together, these studies provide the information required to pinpoint the precise binding sites and modes of action of the components contained in essential oils.

6. Conflicted interest: None

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