Research Article

Comparative Evaluation of Some Unconventional Non-Chemical Approaches to Eradicate Bed Bugs *Cimex Lectularius* L. (*Hemiptera*: *Cimicidae*)

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Abstract | Bed bugs (Hemiptera: Cimicidae) are notorious and nuisance pests and are hematophagous ectoparasites of humans. Their infestations in human dwellings pose a serious threat to human populations. The extensive use of synthetic insecticides against bed bugs results in environmental contamination and human health hazards. Moreover, these pests have attained resistance to commonly used synthetic insecticides. This study was hence intended to test some safe physical methods to eliminate bed bugs without pesticides. Treatments included the exposure of bed bug adults and/or non-hatched eggs for different time intervals to the microwave oven, automatic washing machine, and to fumigants naphthalene and carbon dioxide. Results showed that exposing infested commodity or clothing for 25 to 30 seconds in an electric microwave oven or washing it for 15 minutes at 60 °C or above water temperature or exposing it to carbon dioxide (CO_2) for at least 48 h can result into 80 to 100% mortality of exposed bed bug adults and non-hatched eggs. Moreover, it was found that naphthalene remained ineffective against bed bugs, exhibiting only 7% maximum mortality till 72 h exposure.

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Introduction

Synthetic pesticides have been an inevitable part of urban pest management, and are the most effective way for combating the majority of household insect pests across the globe (Meftaul *et al.*, 2020). Extensive use of these highly persistent chemicals causes many ecological consequences such as environmental contamination and human health hazards (Zhu *et al.*, 2016; Dang *et al.*, 2017; Dang *et al.*, 2021). Moreover, many studies have demonstrated that multiple resistance is being manifested by these insect pests upon prolonged or repeated treatments of human dwellings and household entities with these pesticides (Dang *et al*., 2021; Yu *et al*., 2025).

Bed bugs (*Hemiptera: Cimicidae*) are notorious and nuisance pests present around the globe (Akhoundi *et al*., 2023). They are hematophagous ectoparasites with humans as primary hosts and have lived with the people for a long time. They are very dangerous, posing a threat to public health, and their prevalence is increasing worldwide (Zorrilla-Vaca *et al*., 2015; Chebbah *et al*., 2021). Bed bug populations in human dwellings have shown an increasing trend for the last few decades (Doggett and Lee, 2023), which has led to many urban health issues associated with these pests (Shum *et al*., 2012). These bugs are a regular problem in cities, and unlike many other ectoparasites that feed on human blood (Sheele *et al*., 2021; Akhoundi *et al*., 2023).

There are two major species of bed bugs. *Cimex lectularius,* or the common bed bug, is found in temperate areas of Asia, Australia, Africa, South America, and moderate climate areas of the Northern hemisphere (Delaunay *et al*., 2011). While *Cimex hemipterus,* or tropical bed bug, is the second most frequent species, which is common in warm and subtropical, and tropical regions of the world. (Doggett and Russell, 2008). Recently, it has been observed that both species exhibit migratory behavior beyond their original geographical habitats (Doggett and Russell, 2008; Chrysler *et al*., 2010 AFPMB-USDD, 2019; Moshaverinia *et al*., 2022).

Apart from darkness and dampness, our bodies release heat and carbon dioxide, which attract these hematophagous insect pests to their hosts (Singh *et al*., 2012). The number of people who are infested with bed bugs is rapidly growing across the globe (Barbalat *et al*., 2025). Furthermore, due to travel, immigration, and insecticide resistance, bed bugs are projected to become a more significant problem in the future (Hwang *et al*., 2005). Bed bugs actively disperse by walking, and they can spread between hotel or apartment floors. Bed bug passive diffusion happens when afflicted clothing, luggage, or furniture is moved (Akhoundi *et al*., 2020). Human responses to bed bug bites could vary. Some people have only small, painless bumps, while others have swollen, raised sores that resemble eruptive nevocytic nevi, *Nevus multiforme* (Shmidt and Levitt, 2012).

Although bed bugs cause itching, irritation, and sleeplessness because of their bites, and some people would experience allergic reactions, these bugs are not usually considered as vectors of human diseases, such as ticks and mosquitoes. However, the existence of bed bug colonies in regions where Chagas disease is widespread has raised many questions about the ability of these insects to transmit *Trypanosoma cruzi*, the pathogen that causes the sickness (Salazar *et al*., 2015). Moreover, some significant infections have been experimentally shown to be passively transmitted by bed bugs, although epidemiological evidence is lacking (Cleary and Buchanan, 2004; Ter Poorten and Prose, 2005; Lai *et al*., 2016).

Because of the above-mentioned serious adverse effects of bed bugs on human and animal health and due to their growing resistance to synthetic insecticides, this study aimed to test the effectiveness of some non-chemical decontamination methods that can eliminate bed bug infestations from human dwellings. For instance, treating bed bugs-infested laundry with a washing machine and exposing it to the microwave radiations, carbon dioxide and naphthalene could be effective practical solutions combating bed bug infestations (Naylor and Boase, 2010; Wang *et al*., 2014; Martinsons, 2023).

Materials and Methods

Experimental protocols

Adult specimens of common bed bug species *C. lectularius* and their eggs were collected from a heavily infested residence (Figure 1) and were placed in sterilized plastic containers.

Figure 1: *The extent of the infestation caused by the bed bugs in laundry and clothing from where bed bug population samples were taken for the study.*

For microwave exposure, a commercial microwave oven (Gorenje® model MO17E1S; set to the maximum with a nominal power of 700 W) was used. The bed bugs or their eggs were placed either in a glass Petri plate (Ø 10 cm) covered with a fine gauze cotton cloth, or on a small piece of cotton clothing. Ten adult individuals or five non-hatched eggs of bed bugs were placed in each experimental sample, and these were exposed to microwaves for different time intervals ranging from 10 to 30 seconds.

For washing machine exposure, ten adult individuals or five non-hatched eggs of bed bugs were placed in a 15 mL plastic laboratory tube with a rotating cap, which had some small holes drilled with a heated fine needle for aeration of the tube. The sample tube was placed in an automatic washing machine (Samsung® model WW70J328KW with a nominal power of 2000-2400W), and was exposed for 15 minutes to a washing cycle with a water temperature set to 20, 40, 60 or 95 °C.

For naphthalene exposure, phenyl naphthalene ($C_{10}H_8$) tablets (weight 3.67 g each), sold in pest control stores, were used to evaluate their effectiveness in eliminating bed bugs at different stages of their life cycle. One tablet was placed in a black plastic (nylon) bag, and the cotton clothes were placed inside the nylon bag. In the middle of the clothes, there was a plastic box containing 10 insects of different ages. The top of the box had a clip that allowed gas or air exchange with the insects and the entry of naphthalene vapors for periods of 12, 24, 36, 48, 60 and 72 h.

In case of treatment with carbon dioxide gas, a modified procedure was applied as described in Quarles (2015). The clothes were placed inside black plastic bags, and a vacuum cleaner was used to remove the air from the middle of the clothes. Then, a metal cylinder containing compressed CO_2 was connected to each bag to fill it with carbon dioxide gas. Each experimental treatment was repeated thrice.

Mortality of the exposed bed bug adults or non-hatched eggs was confirmed using a 10X magnifying hand lens or under a binocular microscope. The percentages of killed adult bed bugs or their non-hatched eggs were calculated with a formula defined by Henderson and Tilton, (1955).

$$Corrected\ mortality\ (\%) = \left(1 - \frac{n\ in\ Co\ before\ treatment \times n\ in\ T\ after\ treatment}{n\ in\ Co\ after\ treatment \times n\ in\ T\ before\ treatment}\right) \times 100$$

Where the term "*n*" specifies the total number of insects exposed, "T" signifies the treatments, and "Co" indicates the control treatments.

Although in our case we did not use the control groups due to a very limited time of exposure, during which obviously the bed bug individuals or exposed insects in the control (non-treated) groups would have suffered no loss.

Statistical analysis

Statistical package Web Agri Stat Package (WASP) (http://ccari.icar.gov.in/wasp/index.php) was used for the statistical analysis of the experimental data. The experimental design was completely randomized (CRD). One-way analysis of variance (ANOVA) was used separately for each treatment, and treatment means were compared using the least significant difference (LSD) post-hoc test at a standard level of significance (α = 0.05).

Results and Discussion

Samples containing 10 adults or non-hatched eggs of bed bugs (*C. lectularius*) were placed individually in a glass Petri dish and were heated individually in a 700 W microwave oven for different time intervals (*i.e.* for 10, 13, 15, 18, 20, 23, 25, 28 and 30 seconds, respectively). Similar experiments were performed with adult bed bugs placed on 2 kg pieces of cotton clothing and was heated in the microwave oven for same above-mentioned time periods. Each treatment was replicated independently three times and the average mortality percentages of exposed insects are shown in Figure 2 and Figure 3.

According to the results, the average mortality of the exposed bed bug adults was directly proportional to the exposure time to microwaves. Maximum mortality (80 – 100%) was achieved from 24 to 30 seconds of exposure in the case of the Petri-plate (Figure 2A), while this mortality was exhibited at 28 to 30 seconds in the case of exposing bed bug adults in cotton clothing (Figure 2B).

A similar trend of mortality was observed in the case of non-hatched eggs of bed bugs (Figure 3). The average mortality of the exposed non-hatched eggs was directly proportional to the exposure time to microwaves. Maximum mortality of exposed eggs (100%) was achieved at 25 seconds of exposure in the case of the Petri-plate (Figure 3A), while 100% eggs' mortality was recorded at 28 seconds in the case of exposing bed bug eggs in cotton clothing (Figure 3B).

Another type of experiment was performed with adult bed bugs (ten per sample) or their eggs (five per sample) placed in a 15 mL plastic laboratory tube with fine holes, which was closed and put in an automatic washing machine, and was exposed for 15 minutes to washing programs with a water temperature set to 20, 40, 60 or 95 °C. The average results of these experiments are shown in Figure 4. Results of this experiment with washing machine indicate that there was no mortality at 20°C, while it increased gradually for 40 to 60 °C and maximum mortality (*i.e.* 100%) was achieved for both bed bug adults and non-hatched eggs while treating with a water set at temperature of 60 °C (Figure 4A and Figure 4B).

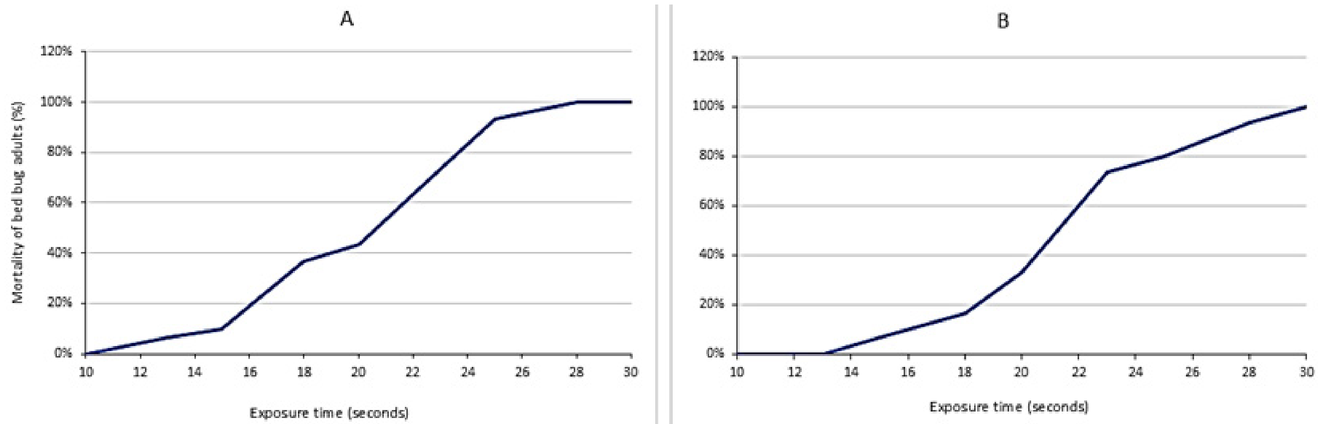

Figure 2: *Percent mortality of bed bug adults when exposed in petri-plates (A) and within a clothing piece (B) to microwaves in an electric oven for different time intervals.*

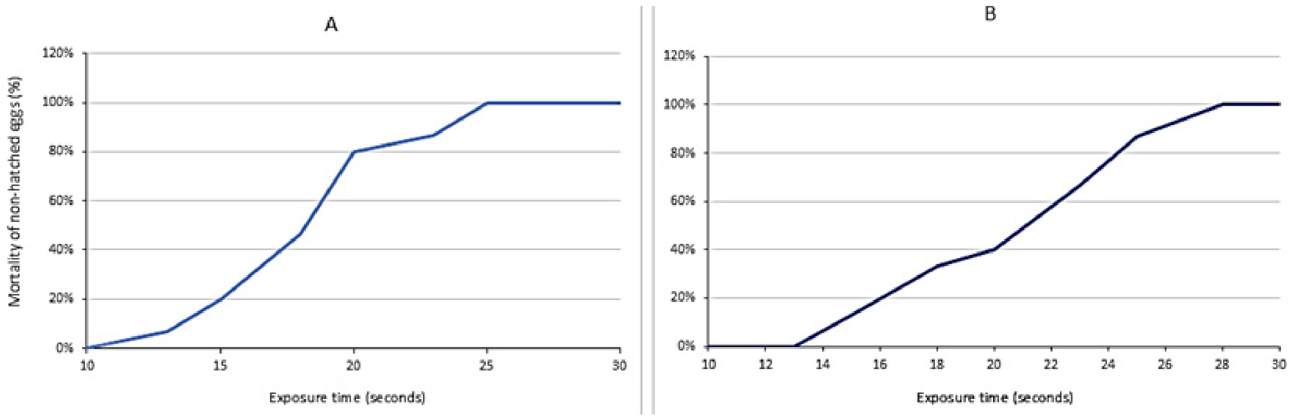

Figure 3: *Percent mortality of non-hatched eggs of bed bugs when exposed in Petri-plates (A) and within a clothing piece (B) to microwaves in an electric oven for different time intervals.*

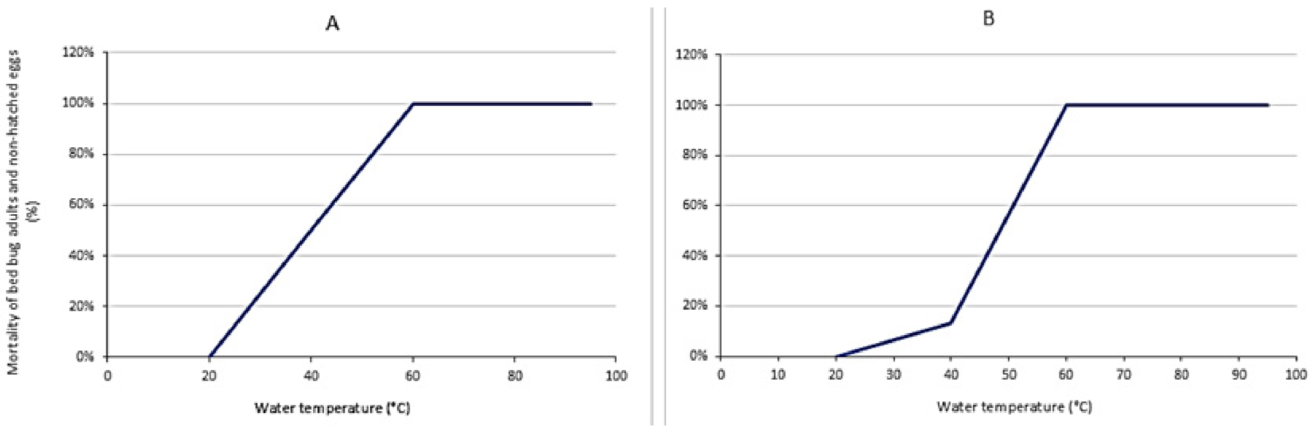

Figure 4: *Percent mortality of bed bug adults (A) and non-hatched eggs (B) when exposed within a drilled plastic tube in a washing machine for 15 minutes at different temperature regimes.*

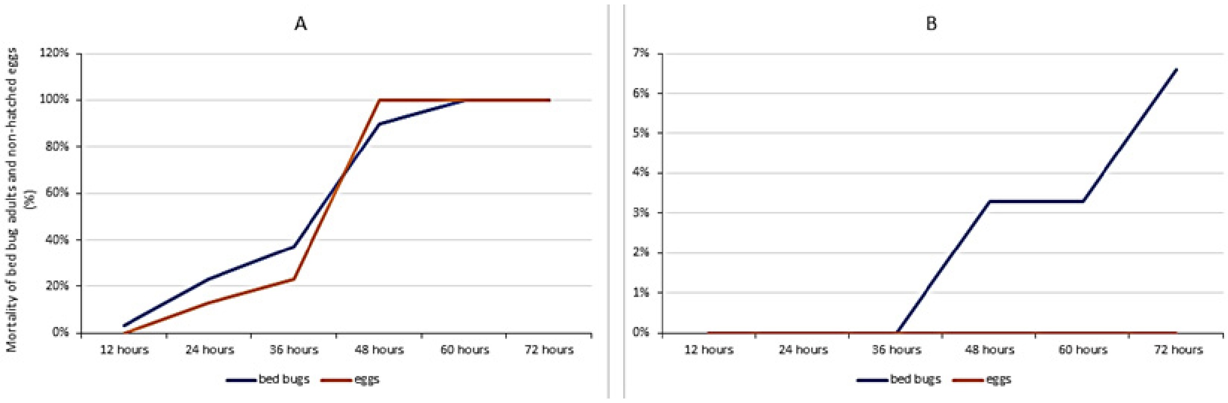

Figure 5: *Percent mortality of bed bug adults and non-hatched eggs when exposed to carbon dioxide* CO_2 *(A) and naphthalene* $C_{10}H_8$ *(B) for different time intervals.*

For exposure of bed bug adults and non-hatched eggs to carbon dioxide and naphthalene, these were treated separately and exposed to these gases at different exposure times (*i.e.,* at 12, 24, 36, 48, 60 and 72 h). The results of this experiment are shown in Figure 5. Results showed a differential response of both fumigants on bed bug adults and their non-hatched eggs. Fumigation by carbon dioxide appeared to be more effective and lethal to both bed bug adults and eggs (Figure 5A), exhibiting maximum (100%) mortality of exposed individuals at 48 h of exposure. While, naphthalene exposure showed no effect on bed bug eggs, while it showed only 7% maximum mortality recorded at maximum (72 h) exposure time (Figure 5B).

Bed bugs are the most notorious pests of human dwellings and pose a serious threat to human health all over the world. These tiny hematophagous pests have attained considerable resistance to the most commonly used synthetic pesticides, including DDT and pyrethroids (Dang *et al*., 2021; Moshaverinia *et al*., 2022). This situation necessitates searching for alternate non-insecticidal approaches to eliminate or get rid of bed bug infestations from human dwellings. To this end, this study evaluated some conventional and non-conventional approaches against bed bugs. This study showed the advantages and disadvantages of employing physical bed bug decontamination methods that are available at houses, hotels, apartments, and common residences. We have demonstrated that the use of high temperatures in an automatic washing machine, ranging from 60 °C to 95 °C, is sufficient to kill different life stages (both adults and eggs) of the bed bugs, including the death of eggs and their inability to hatch. Additionally, increased exposure time led to an increased mortality of exposed insect individuals. The mortality rates were higher for longer time exposures compared to the shorter ones. The exposure times were carefully set in order to acquire the best possible results without causing any damage to the clothes or other treated materials.

Moreover, results of the study suggest that if the garments or household fabrics are to be sterilized or decontaminated from bed bugs, the use of a microwave oven would be a better option because it is a quick and dry sterilization lasting no more than 30 seconds. Our results are in line with the findings of Tyrpak (2016), demonstrating microwave treatment as the best to kill all bed bugs in portable household items. When doing so, one should take into consideration the effect that temperature and time would have on the clothes or stuff being treated, as well as the possible damage to the color or contents which could be caused by utilizing the microwave oven for extended periods of time. When compared to the chemical treatments, such as pesticides, using a washing machine or a microwave oven does not leave any residual negative effect if used appropriately and these decontamination methods are completely harmless (Naylor and Boase, 2010; Martinsons, 2023; Todd, 2023).

Nevertheless, microwave-generated heat acts faster, taking a few seconds versus a few minutes in a fully automatic washing machine versus several days when using CO_2. It is also safer, more efficient, and does not leave any harmful chemicals on the clothes. Additionally, heat is a good way to kill and get rid of all stages of insects, including eggs. This microwave radiation technique has also been shown to be effective against grain insect pests that feed internally within seeds or kernels (Abed *et al*., 2023).

Conclusions and Recommendations

Moreover, this study demonstrated that use of phenyl-naphthalene balls or use of naphthalene fumigation has no significant effect on adult bed bugs and zero effect on their non-hatched eggs. Our results are in contrast to the findings of Darville (1982), who showed a negative impact of naphthalene on different physiological and life-cycle parameters of non-biting midges *Chironomus attenuatus* and *Tanytarsus dissimilis*. Fumigants such as paradichlorobenzene and naphthalene are usually very slow-acting volatiles and exposure to these gases for even for 20 h could not yield good pest control results (Busvine, 1943; Batterman *et al*., 2012). It is, on the other hand, a strong insect repellent (Koganemaru and Miller, 2013).

Due to the fact that physical treatments are readily available in the majority of houses, they can be utilized regularly and at any time until the insects or infestation are effectively eradicated. The objective of decontamination by heat is to raise the temperature of the insect (from egg-larvae to adult) to a level higher than 55–60 °C. A temperature of 48 °C for at least one hour is recommended to achieve a bed bug mortality rate of 100% (Benoit *et al*., 2009). Washing at 60 °C is effective against all stages of development (Naylor and Boase, 2010). Thus, physical methods are highly effective, easy to apply, and may limit the exposure of children and individuals to dangerous pesticidal agents (Koganemaru and Miller, 2013; Sarwar and Salman, 2015; Todd, 2023).

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Novelty Statement

This study has evaluated some non-chemical physical approaches to eliminate bed bugs infestations from household clothing and have demonstrated that exposing infested commodity for 25 to 30 seconds in an electric microwave oven, washing it for 15 minutes at 60 °C or above water temperature or exposing to CO_2 for at least 48 h can eliminate bed bug adults and non-hatched eggs.

Author`s Contribution

Meeri Kadhim Al-Fatlawi: Conceived of research idea, developed the theoretical protocol and performed the statistical analysis of the experimental data, supervised the project, technically proofread the manuscript.
Mohammed Saheb Abed: Developed the theoretical protocol and performed the statistical analysis of the experimental data, performed experiments, wrote the manuscript draft.

Generative AI or AI assisted technology statement
Authors have not used any AI tools or technologies to prepare this manuscript.

Conflict of interest
The authors have no conflict of interest.

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