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Authors' Contribution

AMAA conceptualized and designed the research study. Meticulously supervised overall experimental planning, ensuring strict compliance with biosafety and ethical guidelines. Contributed to the development of figures and tables, performed the statistical analysis using SAS and Minitab; reviewed the draft of the manuscript, coordinated manuscript formatting in accordance with the target journal's guidelines, and managed the submission process. AHAK performed the laboratory experiments and conducted thorough data collection. Drafted the initial version of the manuscript.

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Morphological Identification and Plant-Based Control of Bed Bugs *Cimex lectularius* in Sana'a City, Yemen

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Abstract:

Bed bugs, *Cimex lectularius*; Hemiptera:Cimicidae, are blood-sucking insects that cause significant public health hazards. This study investigated the detection, morphological identification, and insect control using plant extracts. Adult insects were collected from infested locations in Sana'a City and reared in the laboratory. Using light microscopy, the insects were identified by their morphological characteristics through standard pictorial keys. Ethanolic and acetonic extracts of *Mentha spicata*, *Ocimum basilicum*, and *Cinnamomum cassia* were prepared and evaluated against the adult bed bugs. Different concentrations of 250, 500, 800, 1000, 1500, and 2000 ppm of each plant extract were prepared to evaluate botanical control possibilities. Mortality rates differed considerably among treatments at $p < 0.05$. The *O. basilicum* extracts proved most effective with LC_{50} values of 812.09 ppm for ethanolic and 879.67 ppm for acetonic. *M. spicata* was less active but more consistent, with LC_{50} values of 1064.15 ppm for ethanolic and 1147.59 ppm for acetonic extract, while *C. cassia* was the least active, $LC_{50} > 2000$ ppm. These results show the potential of plant compounds, particularly *O. basilicum*, as sustainable methods for controlling bed bugs. Future research should focus on molecular identification and integrated control methods to enhance sustainable management strategies.

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INTRODUCTION

The bed bug, *Cimex lectularius*, belonging to the order Hemiptera and family Cimicidae, is an ectoparasitic blood-feeder that has been a recurring thorn in the side of human comfort and well-being for centuries. They are flat, wingless, reddish-brown colored insect bugs with piercing-sucking mouthparts enabling them to take mammalian, including human, blood. Bed bugs hide in cracks, reproduce quickly, and resist standard control methods (Doggett *et al.*, 2012; Doggett and Lee, 2023). Although these insects are minute in size and cannot be easily observed with the naked eye, their impact on human health is catastrophic; thus, they are an actual pest that needs to be researched thoroughly to find out their biological traits, habits, and control methods (Chavarria *et al.*, 2020). They are typically not longer than 7 mm and possess a sweet, moldy smell from the discharge of glands. Bed bugs bite at night when the host is in the deepest sleep phase, thus not likely to notice the insect as it feeds (Miller and Polanco, 2018).

They detect hosts through heat, carbon dioxide, and odour sensors. They have desensitized saliva so that the host will not experience the bite. Response to bites varies, with some developing itching welts, while others exhibit extreme reactions. Bed bugs return to their hiding spots after feeding to digest the blood and leave reddish-brown fecal specks (Doggett *et al.*, 2018). An earlier report by Chavarria *et al.* (2020) showed that the most common species of bed bugs in temperate regions like North America and Europe are *C. lectularius*, while other species like *C. hemipterus* and *Leptocimex boueti* are common in tropical regions.

Doggett *et al.* (2018) identified that the infestations of bed bugs had been increasing over the recent past years, especially in urban centers, due to rising travel and migration. The spread of bed bugs also has extensive economic effects, especially in sectors such as hotels, healthcare facilities, and apartments. The infestations could lead to revenue loss, pest control costs, and loss of reputation (Xie *et al.*, 2019). Infestations have been reported

particularly in urban populated cities and multi-unit dwellings, where the insects infest (Sheppard *et al.*, 2022). Urban bed bug infestations have been a significant public health issue worldwide, including Yemen, over the past few decades. There has been a rise in cases reported for bed bug infestations in public and residential places, which has compromised the health of the population (Doggett *et al.*, 2018; Akhoudi *et al.*, 2023; Amin *et al.*, 2023; Yu *et al.*, 2023). Another of the principal problems in bed bug control is that they are resistant to man-made insecticides like pyrethroids, which have been very difficult and costly to manage (Akhtar *et al.*, 2018; Dang *et al.*, 2022; Yu *et al.*, 2023; Vatandoost, 2024). This prompted scientists to explore the use of botanical insecticides as an eco-friendly and sustainable means.

Some studies have demonstrated the effectiveness of essential oils and plant-derived neem extracts, basil, mint, garlic, and eucalyptus, among others, in managing *C. lectularius* as new bed bug control agents in laboratory and field testing (Gaire *et al.*, 2019; Salem and El-Sayed, 2019; Iqbal and Ashraf, 2019; Ahmed and Khalid, 2020; Aghaei Afshar *et al.*, 2023).

A search of the literature and online databases could not find any published works on controlling bed bugs using plant extracts in Yemen. Therefore, the present study attempts to fill this seemingly gaping knowledge gap by investigating the potential for applying plant extracts in the control of bed bugs in accordance with the Yemeni context. The study aimed to conduct the morphological identification and Plant-Based Control of bed bugs in Sana'a City, Yemen.

MATERIALS AND METHODS

Study site

This study was conducted in Amman Al Assemah, Sana'a, the capital city of Yemen, where it is located at an elevation of 2150 m above sea level, GPS coordinates 15.356695° N, 44.200218° E. Winter is cold, while summer

is moderate. Five of the selected sites were utilized to conduct infestation surveys: Al-Tahreer, Madhbah, Haddah, Al Dairy, and Bab-

Al-Yemen (Figure 1). The sampling sites were chosen based on population density as well as the potential for infestation by bed bugs.

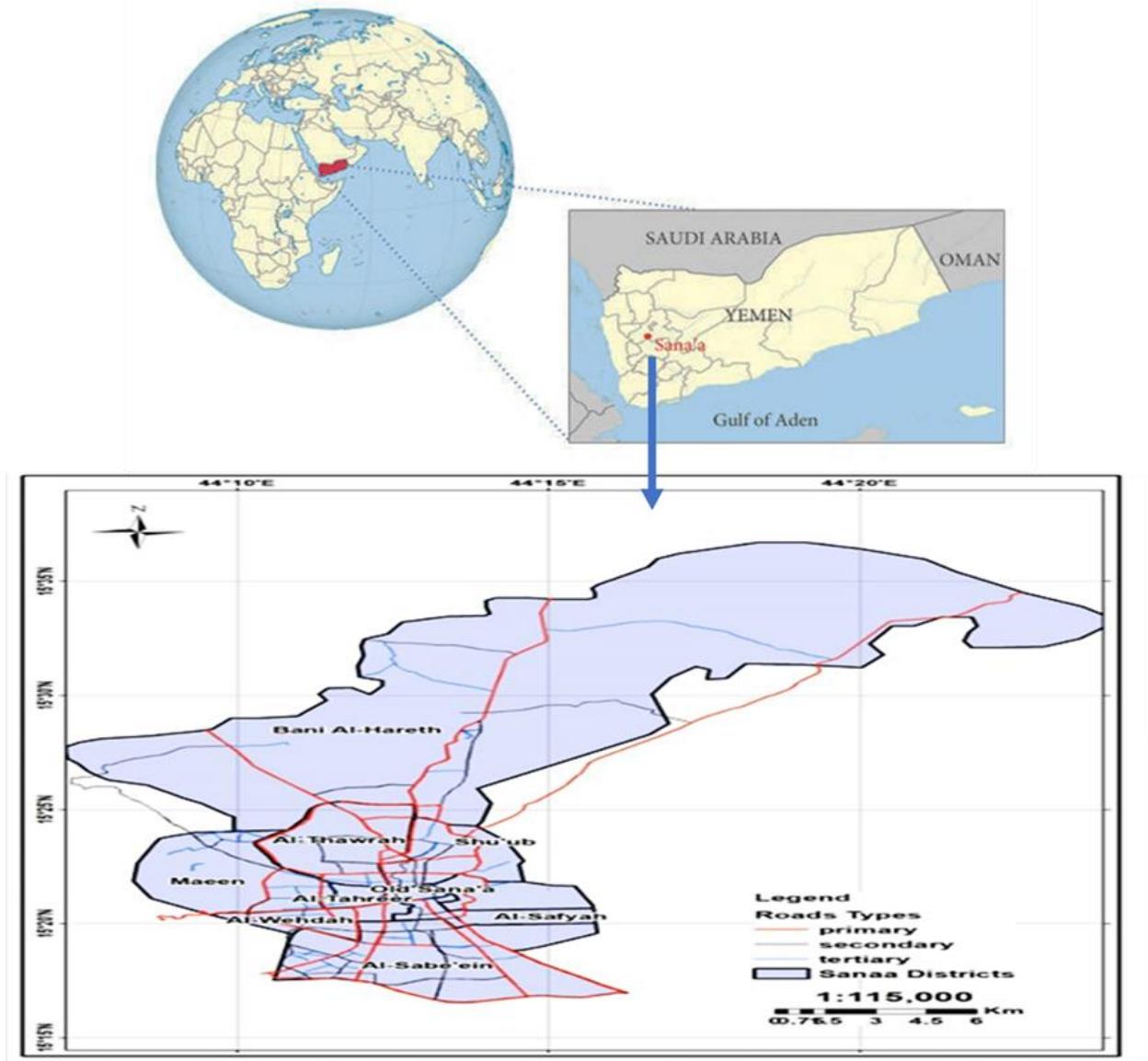


Fig. 1. Map of Sana'a City showing study areas and bed bug sampling sites.

Map 1: Map obtained from the Journal of Tropical Medicine. This work is protected by copyright, with terms and conditions applied. DOI: 10.1155/2022/5976640

Map 2: Yemen Remote Sensing and GIS Centre, Sana'a University.

Bed bugs collection

A total of one hundred bed bugs were collected from five selected locations, Sana'a City from

June to August 2024. Collection was achieved through sticky traps and manual collection as outlined by Doggett et al. (2018). The samples were obtained from visual inspection of general

hiding sites, such as cracks, bedding seams, furniture, wall crevices, and headboard regions. The inspection incorporated both sleeping quarters and residential units. Specimens obtained were categorized into two groups; some were reared in the Medical Entomology laboratory Conditions, while the rest were preserved in 70% ethanol alcohol for identification.

Bed bug rearing

Bed bug rearing was conducted in Sana'a University, Faculty of Sciences, Medical Entomology laboratory, Biological Science Department, according to Yu et al. (2023) and Feldlaufe et al. (2014), with minor modifications.

Morphological identification of collected bed bugs

A light microscope was used to examine the bed bugs' features. External features such as body form, size, color, middle coxa with beak, pronotum, and antennal segments were examined to identify them. Microscopic slides of all life stages (eggs, nymphs, and adults) were also made for morphological examination. Because the species of bed bugs was not apparent initially, a taxonomic key was used for identification (Pratt and Stojanovich, 1967).

Plant collection and extraction

Mentha spicata and *Ocimum basilicum* (Lamiaceae) were collected from Sana'a City, whereas *Cinnamomum verum* was purchased from a local market. Dr. Hassan M. H. Ibrahim, a professor of Plant Taxonomy and Flora at the Department of Biological Sciences, Faculty of Science, Sana'a University, Yemen, identified and confirmed the plants. The fresh leaves of the medicinal plants, *M. spicata* and *O. basilicum*, and the bark of *C. verum* were washed with dechlorinated water. The plant material was air-dried in shaded and airy rooms at room temperature of 26 ± 2 °C. Extractions were later made to obtain bioactive compounds based on the standard protocol described by Eidi et al. (2005) with slight modifications.

Biological effects of plant extracts on bed bug adults

The biological activity of plant extracts against bed bugs was demonstrated according to Yu et al. (2023) and Krüger et al. (2021). Stock solutions of crude extracts from all the plants were prepared by mixing 1 mL of extract with 99 mL of distilled water containing 0.3% dimethyl sulfoxide (DMSO) to facilitate complete solubility. From these stock solutions, different concentrations of 250, 500, 800, 1000, 1500, and 2000 ppm of each plant extract were prepared using distilled water and sprayed onto filter papers by a hand sprayer. Adult insects were exposed to a range of concentrations of the plant extracts (250-2000 ppm). The negative control was distilled water containing 0.3% dimethyl sulfoxide (DMSO), whereas insecticide Torspan 480 EC was used as the positive control. Each treatment was replicated four times.

Statistical analysis

The statistical analysis for this study was conducted using a completely randomized design (CRD). The recorded mortality percentages at different concentrations were appropriately corrected. The collected data were statistically analyzed utilizing variance analysis (ANOVA) and the least significant difference (LSD) test, with a significance level of $P \leq 0.05$ for mean comparisons using Minitab Statistical Software (Release 13.30). The lethal concentrations (LC_{50} and LC_{95}) were calculated using probit analysis, as described by Finney (1972).

RESULTS

Morphological identification

A total of one hundred bed bugs were collected from five infested locations selected for this study. It was not initially known what type of bed bugs were present in Sana'a. All samples were identified as *C. lectularius* based on their external morphology, using morphological standard keys (Figure 2).

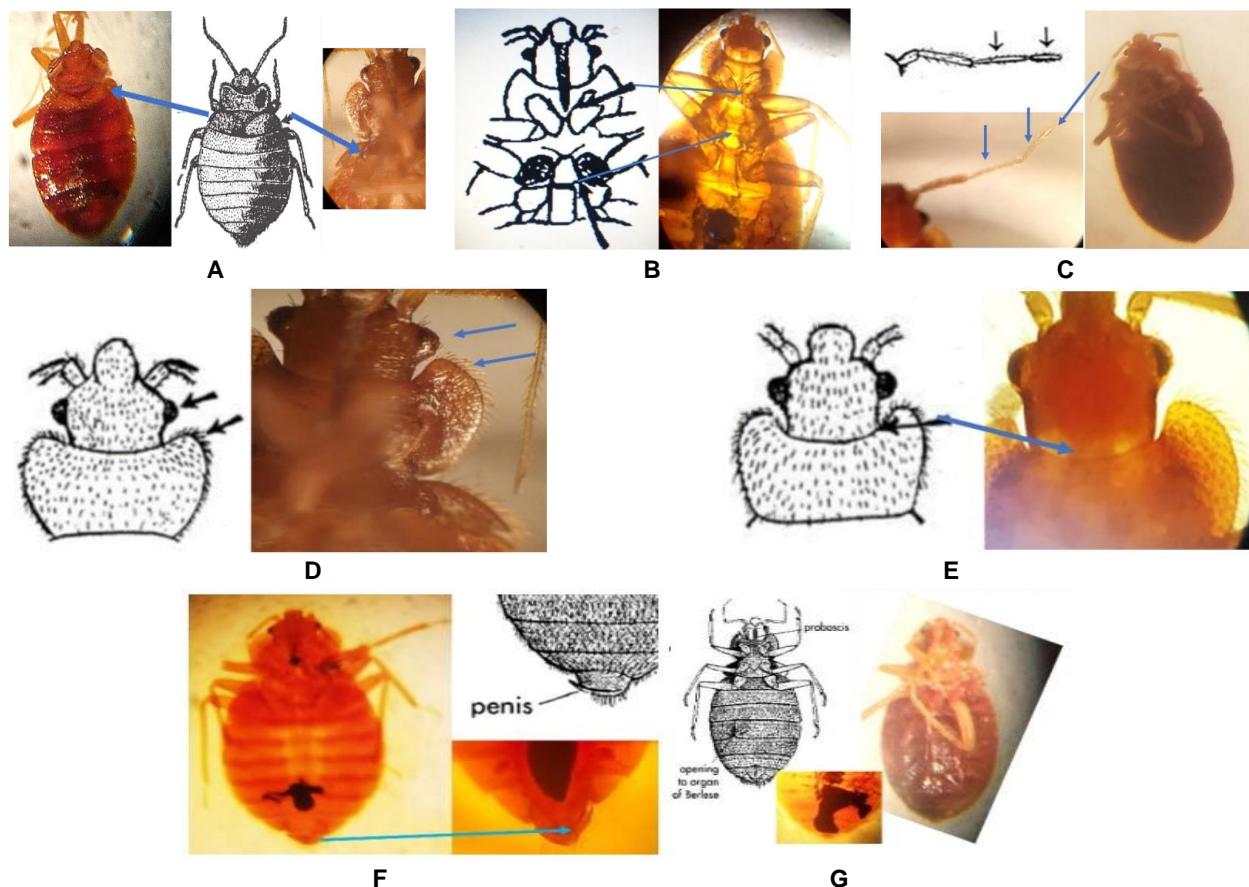


Fig. 2. Morphological identification of *Cimex lectularius*: **A.** Wings reduced; Body broadly oval. Family: Cimicidae, **B.** Middle coxae widely separated, **C.** 4th antennal segment, shorter than 3rd segment, **D.** Fringe hairs on the pronotum are shorter than the width of the eye, **E.** Anter margin of the pronotum deeply excavated, **F.** Male bed bug, **G.** Female bed bug.

Bioassay of plant extracts on bed bugs

As shown in Tables 1,2, and Figures 3-8, the *O. basilicum* extract was the most potent suppression of bed bugs among the extracts tested, with an activity comparable to insecticide Torspan at 2000 ppm, with LC₅₀ values of 812.09 ppm for ethanolic and 879.67 ppm for acetonic, but it took a longer time to be effective (2days). *M. spicata* extract had good repelling and controlling activity against bed bugs with LC₅₀ values of 1064.15 ppm for ethanolic and 1147.59 ppm for acetonic extract; it was less toxic than basil. On the other hand, at the higher concentration of 2000 ppm, *M. spicata* was moderately but less general in causing bed bug

mortality. *C. cassia* was less effective for controlling bed bugs when used as a repellent rather than as a killing factor. Its efficacy in control was significantly lower compared to *O. basilicum* and *M. spicata*, with LC₅₀ values of 2036.259 ppm and 2164.11 ppm for ethanolic and acetonic, respectively. The *O. basilicum* at 2000 ppm had the same efficacy for controlling bed bugs as that witnessed with the extract of *O. basilicum* and the manufactured insecticide Torspan. Nonetheless, the onset of activity was less quick than that of the insecticide. On the other hand, mint proved to be moderately effective but less toxic compared to basil. Cinnamon showed greatly reduced action in

terms of both repellency and toxicity. The *O. basilicum* extracts proved most effective, and *M. spicata* was less active but more consistent,

while *C. cassia* was the least active, $LC_{50} > 2000$ ppm.

Table 1. Biological effects of plant extracts against the adult bed bug *C. lectularis*.

Extracted Plant	Concentration (ppm)	Biological effects	
		Acetonic extract Mean \pm SE	Ethanoic extract Mean \pm SE
<i>Ocimum basilicum</i>	250	10 \pm 0.00	10 \pm 0.0
	500	20 \pm 0.41	25 \pm 0.29
	800	40 \pm 10.41	45 \pm 0.48
	1000	60 \pm 0.41	63 \pm 0.48
	1500	80 \pm 0.71	85 \pm 0.50
	2000	100 \pm 0.00	100 \pm 0.00
	Control	0.0	0.0
<i>Mentha spicata</i>	250	0 \pm 0.00	0.0 \pm 0.00
	500	8 \pm 0.25	10 \pm 0.00
	800	20 \pm 0.41	23 \pm 0.25
	1000	60 \pm 0.41	65 \pm 0.25
	1500	75 \pm 0.29	80.5 \pm 0.41
	2000	83 \pm 0.25	90 \pm 0.41
	Control	0.0	0.0
<i>Cinnamomum verum</i>	250	0.0 \pm 0.0	0.0 \pm 0.00
	500	0.0 \pm 0.41	3.0 \pm 0.25
	800	5 \pm 0.41	8 \pm 0.25
	1000	18 \pm 0.41	23 \pm 0.25
	1500	28 \pm 0.71	33 \pm 0.25
	2000	40 \pm 0.00	45 \pm 0.29
	Control	0	0
Positive control (Trospan)	100		100

Note: Nil mortality in control; *Significant at $P < 0.05$ level.

Table 2. Statistical parameters of plant extracts against *C. lectularis*.

Insect	Statistical parameters	<i>C. lectularis</i> Plant extract	
		Acetonic extract	Ethanoic extract
<i>Ocimum basilicum</i>	LC_{50}	879.67	812.09
	LCL - UCL	769.92 - 981.83	705.01 - 910.01
	LC_{95}	1892.80	1793.400
	LCL - UCL	1637.59 - 2332.76	1551.28 - 2208.43
	Slop	1.91	1.84
<i>Mentha spicata</i>	LC_{50}	1147.59	1064.15
	LCL - UCL	1021.51 - 1271.02	949.76 - 1172.65
	LC_{95}	2296.74	2040.02
	LCL - UCL	1996.32 - 2809.51	1803.57 - 2418.97
	Slop	2.11	2.24
<i>Cinnamomum verum</i>	LC_{50}	2164.11	2036.259
	LCL - UCL	1844.90 - 2864.86	1734.68 - 2645.76
	LC_{95}	4051.76	4172.32
	LCL - UCL	3010.59 - 7616.58	3068.20 - 7763.85
	Slop	1.91	2.04

LCL: Lower Control Limit, UCL: Upper Control Limit, LC_{50} : lethal concentration 50, LC_{95} : lethal concentration 95

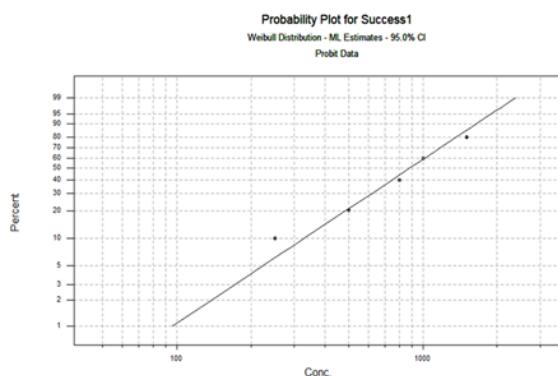


Fig.3. The acetonic *O. asilicum* extract concentrations and their correlation to the mortality rate on *C. lectularius* adults.

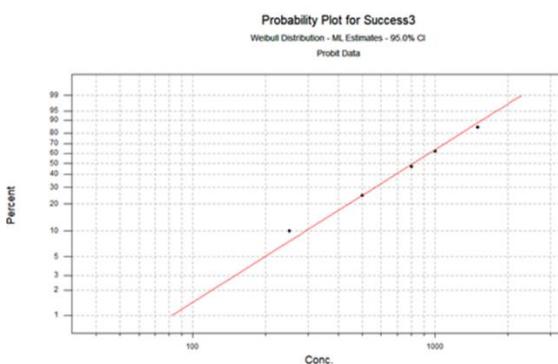


Fig. 4. The ethanolic *O. basilicum* extract concentrations and their correlation to the mortality rate against *C. lectularius* adults.

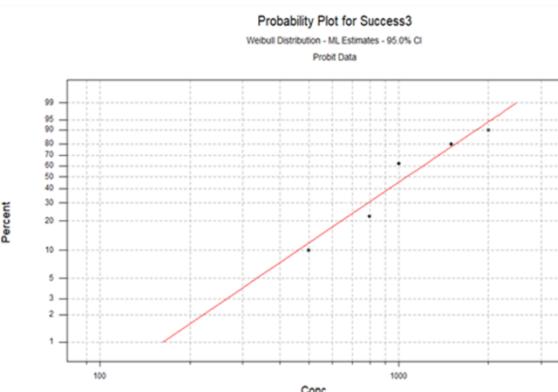


Fig.5. The ethanolic *M. spicata* extract concentrations and their correlation to the mortality rate against *C. lectularius* adults.

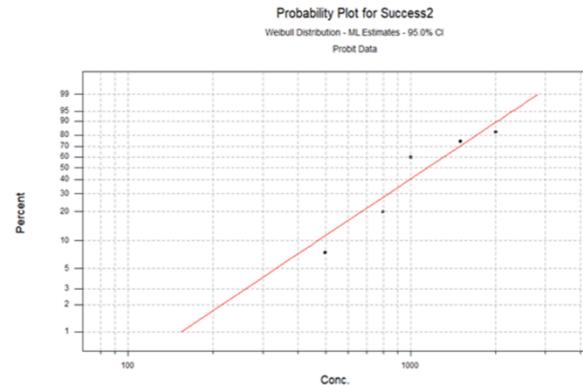


Fig. 6. The acetonic *M. spicata* extract concentrations and their correlation to the mortality rate on *C. lectularius* adults.

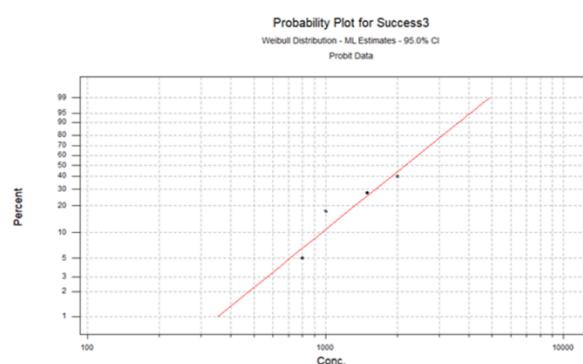


Fig. 7. The acetonic *C. verum* bark extract concentrations and their correlation to the mortality rate on *C. lectularius* adults.

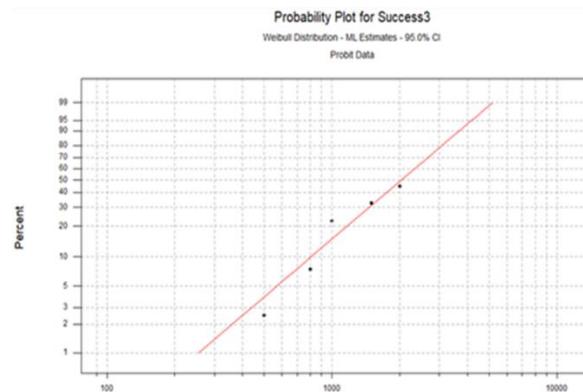


Fig. 8. The ethanolic *C. verum* bark extract concentrations and their correlation to the mortality rate against *C. lectularius* adults.

DISCUSSION

According to external taxonomic keys such as body form, coloration, and body size, the dominant species in Sana'a City was determined to be *Cimex lectularius*. These results concur with existing authoritative keys and description of *C. lectularius* oval, a dorsoventrally flattened brown to mahogany coloured bug that dark red/purplish-red coloured when fed, and emphasize pronotal features to differentiate at the species level (Al-Sanabani, 2014; Kalangi *et al.*, 2017; Akhoudi *et al.*, 2023; Jadidoleslami *et al.*, 2024), supporting the validity of our morphological identification key. Conversely, the results presented in this analysis conform with those reported by Usinger (1966), who stated that *C. lectularius* has an oval, concave body shape and brown color that darkens to deep red when it feeds on blood. This was used to validate the methods applied to identify.

The present study revealed that the *Ocimum basilicum*, *Mentha spicata*, and *Cinnamomum cassia* plant extracts exhibited varying levels of toxicity to the adult *Cimex lectularius*. *O. basilicum*, among the species tested, proved to be the most bioactive, with 100% mortality being noted at the concentration of 2000 ppm, as indicated by lower LC₅₀ values for the ethanolic (812.09 ppm) and acetonic (879.67 ppm) extracts. This observation has found support in the literature on the insecticidal and repellent activity of *Ocimum spp.* oils and extracts against hematophagous arthropods (Alimi *et al.*, 2022). The efficacy of *O. basilicum* is probably linked to its characteristic monoterpenoid/phenylpropanoid profile, which contains active compounds, especially linalool, estragole, and eugenol, which are frequently dominant constituents of basil chemotypes, affecting the insect's nervous system and the role of volatile organic compounds in bed bug management (Gaire *et al.*, 2019; González-Morales *et al.*, 2021; Oladipupo *et al.*, 2022; Akhoudi *et al.*, 2023; Azizah *et al.*, 2023). Additionally, studies have documented neuroactive actions

(acetylcholinesterase inhibition; modulation of GABAergic and octopaminergic signaling; and fumigant toxicity) in insects (Mossa, 2016). Such multimodal neurotoxicity is a recognized strength of botanical insecticides (Isman, 2020).

The second most effective plant in this study was *M. spicata*, with LC₅₀ values of 1064.15 ppm (ethanolic extract) and 1147.59 ppm (acetonic extract). *M. spicata* showed moderate effectiveness, which increased with the concentration, and its effect was stronger using the ethanolic extract, suggesting that the active compounds are more soluble in alcohol. Although less potent than *O. basilicum*, spearmint extracts still caused significant mortality, aligning with reports that *Mentha spp.* exhibit insecticidal, fumigant, and growth-inhibitory activities against multiple pest taxa, including mosquitoes and stored-product beetles (Singh *et al.*, 2018; Yang *et al.*, 2021; Wu *et al.*, 2023; Mondal *et al.*, 2024). This trend was also supported by Tripathi *et al.* (2009), who confirmed the repellent and lethal effects of mint oils. The bioactivity of *M. spicata* is congruent with its well-known "carvone chemotype" (carvone often ~50–80% with limonene as a common secondary constituent), and both carvone and limonene have been tied to respiratory and nervous-system disruption and to strong fumigant effects, often via interference with cholinergic and other neuronal targets (Shahbazi, 2015; Singh *et al.*, 2018; Yang, *et al.*, 2021). Recent mechanistic and formulation-focused studies have further underscored that spearmint oil and carvone can produce appreciable toxicity as fumigants or in nanoemulsions, while also highlighting volatility and stability as practical constraints for field use (Wu *et al.*, 2023; Remesh *et al.*, 2023; Mondal *et al.*, 2024).

In contrast, *C. cassia* exhibited minimal activity, especially at lower concentrations, and its effect was mostly repellent rather than lethal against *C. lectularius* in our assays (LC₅₀ > 2000 ppm). This is consistent with the study of Singh *et al.*

(2012), which indicated that cinnamon is more commonly used as a natural insect repellent.

Cinnamon bark oils and cinnamaldehyde are frequently potent against mites and other arthropods (e.g., tick and dust-mite models), showing acaricidal/repellent activity in previous studies (Lee *et al.*, 2024). In contrast, other researchers have reported a higher efficacy of cinnamon oil, particularly as a repellent or in certain contact bioassays, suggesting that activity may depend on chemotype, formulation, and assay design (Elbanoby *et al.*, 2019; Krüger *et al.*, 2021). The comparatively low activity observed in bed bugs suggests either species-specific tolerance, insufficient recovery of active aldehydes/phenolics in the tested extracts, or both. Solvent polarity and extraction parameters can substantially alter the yield and spectrum of recovered phytochemicals, which may partly explain the inter-study variability in cinnamon efficacy across arthropod targets (Pavela, 2009; Lee *et al.*, 2024). The extraction solvent modestly influenced the performance in our experiments, with ethanolic extracts being slightly more effective than acetonic extracts for both *O. basilicum* and *M. spicata*. This is consistent with evidence that solvent polarity governs the recovery of polar phenolics and certain oxygenated monoterpenes that often drive bioactivity in Lamiaceae; several reviews and extraction studies note meaningful solvent-driven differences in downstream insecticidal potency and composition (Pavela, 2009; Moradi-Sadr *et al.*, 2023). While the precise constituent drivers were not profiled here, a reasonable inference is that ethanol co-extracted a broader set of oxygenated volatiles and phenolics that enhanced the activity. These findings add to the growing body of evidence that botanicals can contribute to integrated bed bug control, especially where pyrethroid resistance limits the utility of conventional neurotoxicants.

Bed bug populations worldwide show widespread pyrethroid resistance and, in some locales, reduced susceptibility to neonicotinoids, underscoring the value of alternative chemistries and multi-target modes of action (Yu *et al.*, 2023; Lilly *et al.*, 2017). Prior bed bug studies of essential-oil-based products show that while

some formulations can achieve high direct-spray mortality or substantial population reductions under managed field conditions, complete elimination is uncommon without integration with other tactics, and the performance is formulation-dependent. Volatility, short residual life, and the propensity of bed bugs to remain concealed further challenge botanical actives when used alone (Feldlaufer and Ulrich, 2015; González-Morales *et al.*, 2021). Therefore, we recommend that future investigations should focus on chemically characterizing the active fractions of *O. basilicum* and *M. spicata* extracts from this study using GC-MS to link specific constituents and ratios to bioactivity, develop optimized delivery systems such as micro/nanoemulsions and slow-release matrices. To mitigate volatility and enhance residual effects, evaluate synergistic combinations (botanical–botanical and botanical–synthetic), given demonstrated synergy between essential-oil constituents and pyrethroids via metabolic inhibition (Gaire *et al.*, 2021), and test promising formulations in semi-field and field contexts reflecting realistic harborages and behavioral ecology of *C. lectularius* (Feldlaufer and Ulrich, 2015; González-Morales *et al.*, 2021).

Overall, the present results highlight that not all plant-derived extracts exert comparable effects against *C. lectularius*, and their efficacy largely depends on the nature of the bioactive metabolites, their mode of action, and the efficiency of extraction methods. Importantly, these findings indicate that *O. basilicum* holds strong promise as a candidate for the development of eco-friendly bed bug control strategies, whereas *C. cassia* may be more useful as a repellent or in combination with other extracts to achieve synergistic effects.

CONCLUSION

This work presents the first recorded evidence of infestation by *Cimex lectularius* and morphological proof in Sana'a City in Yemen. This study has also established the prospective efficacy of the extracts of *O. basilicum* as prospective plant biocontrol agents. The remarkable efficacy of basil compared with the

poor efficacy of *C. cassia* underscores the important function of phytochemical composition in determining insecticidal potential. These findings support the integration of botanical extracts into eco-friendly management strategies to control bed bugs; however, further molecular characterizations and farm assessments are needed to verify their long-lasting efficacy and application in community-based health pest management programs. Generally, the highest activities were recorded in *O. basilicum* extract in the current investigation, whereas in the case of *M. spicata* and *C. cassia*, low efficacy was recorded to kill adult bed bugs. The findings indicate the critical role of botanical species, chemotypes, and extraction procedures in formulating effective, environmentally friendly botanical products to control bed bugs. The findings again emphasize the need for integrated strategies to control pests, awareness campaigns at community levels and elsewhere, and additional investigations to mitigate the health and social consequences of Yemeni bed bugs in the coming time.

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CONFLICT OF INTEREST

The authors hereby declare that they have no conflict of interest.

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