Original Article

Testing the Sensitivity of the Tropical Bed Bug *Cimex hemipterus* (Hemiptera: Cimicidae) to Deltamethrin, Phoxim and Propetamphos in Eastern Iran

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Abstract

Background: Bedbugs are nocturnal ectoparasites that have made a comeback after 20 years and have become one of the main challenges for pest control methods worldwide. Monitoring chemical pesticide resistance is crucial for identifying the best bed bugs management strategies to effectively manage arthropods. This study aims to assess the susceptibility of *Cimex hemipterus* (Hemiptera: Cimicidae) collected from different parts of Khorasan-Razavi Province, (northeast of Iran) to deltamethrin (pyrethroid), phoxim (organothiophosphate) and propetamphos (phosphoramidate).
Methods: This study was conducted from Dec 2020 to May 2021. The efficacy of three insecticides (deltamethrin, phoxim, and propetamphos) on adult *C.hemipterus* was assessed using a bioassay method recommended by the World Health Organization (WHO),. Concentrations of deltamethrin used were 10, 20, 40, 80, 160, 320, 640, 1280, and 2560 ppm, while concentrations of phoxim and propetamphos were 10, 40, 160, and 320 ppm. The bed bugs were continuously exposed to the insecticide for 24 hours, and mortality was assessed at regular intervals during the observation period. The concentration-response data were subjected to POLO-PC software and data were analyzed by the one-way and two-way ANOVA procedures.
Results: The lethal concentration fifty values of deltamethrin, phoxim and propetamphos on the examined bed bugs were 0.551, 0.148 and 0.237 ppm, respectively. Insecticide effects of phoxim were significantly higher ($P \leq 0.05$) compared to each of either deltamethrin and propetamphos agents.
Conclusions: The insecticide effects of phoxim against bed bugs were significantly higher compared to each of either deltamethrin or propetamphosinsecticides.

Keywords: Organothiophosphate; *Cimex hemipterus*; Insecticide resistance; LC_{50}; Khorasan Razavi

Introduction

Bedbugs (Hemiptera: Cimicidae) are nocturnal ectoparasites that feed on human blood. *Cimex hemipterus* is primarily found in tropical and subtropical areas, whereas *C. lectularius* is found mainly in temperate regions. Unfortunately, bed bugs have made a comeback around the world in recent decades. Several causes contribute to the global revival of bed bugs, including increased global travel, frequent interchange of used products, and pesticide resistance (1, 2).

Chemical insecticides have been the foundation of bed bug management for many years, successfully reducing their population. However, over the past 20 years, the comeback of bed bugs (*C. lectularius* and *C. hemipterus*) has emerged as one of the major issues with pest control methods all over the world (3). This

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rebound is partly because of the extensive and liberal use of chemical insecticides, which leads to the development of insecticide resistance (4).

Due to this strategy, pesticide resistance is growing and bed bug infestations are once again on the rise (1,2). The insecticide resistance of bed bugs, which has been documented frequently in many countries, is reportedly caused by broad-spectrum insecticides such as pyrethroids, neonicotinoids, carbamates, organochlorines, and organophosphates (1). Due to their low mammalian toxicity, effectiveness and affordability, pyrethroid insecticides like permethrin and deltamethrin are the most often used class of insecticides for the control of insect pests. As a result, they have a significant impact on pesticide resistance in both species, particularly in the accumulation of knockdown resistance (kdr) mutations (6, 7) as well as other resistance mechanisms including metabolic resistance and cuticular penetration resistance. The latter two mechanisms have also been reported to incur resistance against different classes of insecticides including the commonly used organophosphates and neonicotinoids (8). Monitoring chemical pesticide resistance is crucial for identifying the best management strategies to effectively manage bed bug infestations. While there is no information on the effectiveness of phoximn (organothiophosphate) and propetamphos (phosphoramidate) on *C. hemipterus* as well as *C. lectularius*, the two insecticides have been effectively used for the control of other insect health pests (9).

Because it is currently unknown if tropical bed bugs in northeastern Iran are resistant to these regularly used insecticides, the current study used the bioassay method to assess the level of resistance of *C. hemipterus* collected from various cities in the Khorasan Razavi Province towards phoxim and propetamphos.

Materials and Methods

This study was done from December 2020 to May 2021.

Insects

The bed bugs were collected from different infested houses in Mashhad (Elevation: 995 m, weather: 21 °C, wind E at 8 km/h, 35% humidity), Kashmar (Elevation: 1,063 m, weather: 25 °C, wind E at 14 km/h, 15% humidity), and Neyshabur (Elevation: 1,250 m, weather: 21 °C, wind E at 8 km/h, 30% humidity) located in Khorasan Razavi Province, northeast of Iran (36.2605°N, 59.6168°E). The field-collected colonies were transferred separately to the plastic vials and transported to the parasitology laboratory of Mashhad University of Medical Sciences. Bed bugs were identified at the species level using a stereomicroscope (ZEISS, Stemi 305, Germany) based on the morphological features. *Cimex hemipterus* can be distinguished from *C. lectularius* based on the presence or absence of the tibial pad, that this characteristic is not of *C. hemipterus*. In addition, in *C. hemipterus* the pronotum is less than two and a half times as wide as long at the middle (10). Adult specimens are distinguished according to the thorax morphology characteristics of the nymphs. Live adults were kept in an insectarium and used for insecticide resistance by the bioassay method.

Insecticides

Three technical-grade insecticides including deltamethrin (98.5%, Anhui Chizou Sincerity Chemical Co., China), phoxim (82%, NingboSunjoy Agroscience Co., China) and propetamphos (97%, Indogulf Cropsciences Ltd, India) were employed. Acetone (Merck, Cat. No. 1000141000, Germany) was used to prepare the desired concentrations from technical-grade insecticides. Acetone-diluted deltamethrin was prepared at concentrations of 10, 20, 40, 80, 160, 320, 640, 1280 and 2560 ppm. Prepared concentrations of phoxim and propetamphos were 10, 40, 160 and 320 ppm. For each concentration, 10 adult *C. hemipterus* (mixed sex) were exposed to the insecticide-impregnate filter paper. Similarly, control bed bugs were tested based on filter paper treated with acetone only. All steps were repeated two times (two replicates for each concentration), because of limitations in sample size.

Bioassays

The susceptibility of collecting bed bugs was determined using insecticide-impregnated filter papers, following the bioassay method recommended by the World Health Organization (WHO-SOP: 14 January 2022) for each insecticide concentration. Papers were dried in a fume hood for 12 h. The control group received the same dose of acetone only.

Bed bugs were transferred to a 10 cm Petri dish lined with impregnated filter paper. The bed bugs were exposed continuously on the insecticide-impregnated papers for 24 h at 25 °C and 80% RH in an incubator (INCO2-108SS). After 24 h, the bed bugs were then transferred to a clean Petri dish and kept under observation for another 24 h. The bed bug mortality was assessed at regular intervals during the observation period. The bed bug was considered dead if it had no sign of movement when it was agitated with an entomological pin.

Statistical Analysis

The percentage of mortality in each treatment was calculated and analyzed daily. The concentration-response data were subjected to POLO-PC software (2).

Mean comparison was performed by using one-way and two-way ANOVA to determine the significant difference between the control populations and the effect of insecticide on field colonies of bedbugs. Resistance ratios (RR) were calculated by dividing the LC_{50} value of the resistant strains by the corresponding value of the susceptible strains. The classification of resistance followed: ≤ 1 time: no resistance; > 1 to ≤ 5 times: low resistance; > 5 to ≤ 10 times: moderate resistance; > 10 to ≤ 50 times: high resistance; >50 times: very high resistance (8).

The data was submitted to SPSS software (V.22.0 SPSS Inc.) and LC_{50} values of different insecticides against examined bed bugs were calculated by Probit test. This software was used to calculate mean percentage mortality and its standard deviation.

Results

After a 24 h exposure to the three insecticides, deltamethrin, phoxim and propetamphos had lethal concentration 50 (LC_{50}) values of 0.551, 0.148 and 0.237 ppm, respectively, as shown in Table 1. Additionally, Lethal concentration 90 (LC_{90}) values of these pesticides were 1.424, 1.091 and 6.358 ppm, respectively. Phoxim showed greater lethality, than deltamethrin and propetamphos, as it required lower concentration of insecticides to achieve a similar effect on the bed bugs. Effects phoxim was significantly higher ($P \leq 0.05$) compared to each of either deltamethrin or propetamphos agents. The field-collected population of northeastern Iran was found completely resistant to propetamphos with final mortality ranging between 10–20% in the highest concentration.

Table 1. LC_{50} and LC_{90} values were determined using a bioassay method for deltamethrin, phoxim, and propetamphos against *C.hemipterus* collected from different cities in the Khorasan Razavi Province from December 2020 to May 2021

Insecticide	LC_{50}	LC_{90}	Slope± SE	RR	df
Deltamethrin	0.551	1.424	3.104±1.023	22	0.25
Phoxim	0.148	1.091	1.478±0.434	20	1.40
Propetamphos	0.237	6.358	0.898±0.383	60	1.88

SE: Standard error
RR: relative risk

Discussion

Based on the results of the in vitro study and the levels of LC_{50} and LC_{90}, it has been determined that phoxim demonstrated higher potential lethality effects. These effects were significantly higher compared to either deltamethrin or propetamphos agents. *Cimex lectularius* used to be widespread in Iran, but now it has been replaced by *C. hemipterus* as the predominant species (11). In our previous study, we showed that diazinon, λ-cyhalothrin, and malathion were not effective against *C. lectularius* populations in northeastern Iran (12), however, this study demonstrated that bed bugs are susceptible to phoxim.

Insecticides are the main agents for killing the pests worldwide. Combating bed bugs in human dwellings is difficult due to various reasons such as resistance development by the insects, inadequate dosing, inaccurate methods, and the type of insecticide used. Various resistance mechanisms include behavioral, metabolic, esterases, and P450s-mediated resistance, GSTs, penetration, kdr, target site insensitivity, and via ABC-transporters, that overexpression of genes in bedbugs (1), such as P450s, esterases, and GSTs, can lead to insecticide resistance through metabolic enzyme production and cuticle thickening. Resistance to phoxim has been reported in common cutworms and cotton bollworms (13, 14).

While in another study fenitrothion and imidacloprid resistance showed an association between cuticle thickness and resistance. but, the association may be tenuous because the resistance mechanism is multifactorial in *C. hemipterus*, and these mechanisms, in the *C. hemipterus* population were, caused by both M 918I and L1014F mutations, which is likely related to cuticle thickness (15), as well as, in bed bugs populations, there are multiple resistance mechanisms against pyrethroids, that seem caused by several point mutations in the voltage-sensitive sodium channel α-subunit gene and increased metabolic detoxification by cytochrome P450, all contributing to knockdown resistance (kdr)(16, 17).

A low level of propetamphos resistance in field populations of Australian blowflies and a high level of resistance in Iranian ticks were detected (18, 19).

In a study in northern China *Lygus pratensis* (Hemiptera: Miridae), using the glass-vial method, was exposed to phoxim and resistance ratios were calculated at 22.34, 12.66 and 8.24 in three experiments (20). BmHR96 is a nuclear receptor whose expression was changed in exposure to phoxim. Moreover, its expression was different in various organs of *C. lectularius* (21). Reports of resistance to phoxim are in scarcity.

Various mechanisms have led to the inactivation of deltamethrin in *C. lectularius* field populations. In Australia, exposure to 2.5 g/L of deltamethrin resulted in the elimination of 85% of *C. lectularius*. but recent strains exhibited a lower mortality rate, indicating gradual development of resistance over time (22). However, synergism studies have demonstrat-

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ed that the use of piperonyl butoxide inhibits deltamethrin resistance in all strains (23).

In vitro expression of the *C. lectularius* AChE gene (p-Ace) revealed that the F348Y mutation showed a wide range of decreased sensitivity to the tested acetylcholinesterase inhibitors. The effect of F348Y a broad range of decreased sensitivities of ClpAY was presented by the ratio of IC_{50}. Thus, the F348Y mutations in the AChE gene were found to be a causative insecticide resistance in bed bugs (24).

High resistance to pyrethroids was observed in both species studied, with the highest resistance ratios found in *C. lectularius* and *C. hemipterus*. Resistance mechanisms against pyrethroids were reported in most locations, except Iran and Thailand, and these indicate note that chemical control options for bed bugs are limited; therefore, a combination of chemical and non-chemical strategies is recommended for bed bug control. Data regarding resistance to the propetamphos is also in paucity and there is no documented prevalence of this type of resistance (25).

Biochemical, molecular, and insecticide bioassay evaluation methods can be used to detect bed bug insecticide resistance and its mechanisms. But it should be noted, that using biochemical and molecular monitoring assays alone without empirical validation of resistance status would be counter-productive (1).

Future bed bug control programs should be based on integrated approaches, and future research should also focus on designing insecticides with new target sites.

Conclusion

Although this study has some limitations such as a narrow study period, a low number of samples, and a regional focus, it demonstrated that phoxim has stronger insecticide effects compared to other insecticides, such as pyrethroids, especially when it comes to bed bugs. It is important to note that there are no insecticide-susceptible *Cimex* species in Iran, so we cannot compare the resistance rate against the field-collected population. In the future, conducting surveys that examine molecular alterations such as gene expression in insects during exposure to insecticides would be more helpful.

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Ethical considerations

The study was approved by the Ethics Committee at Mashhad University of Medical Sciences (Ethicalcode: IR.MUMS.MEDICAL. REC.1399.807).

Conflict of interest statement

The authors declare there is no conflict of interests.

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