

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Analysis of lethal and sublethal doses: Comparative toxicity of three commonly used pesticides on *Blattella germanica*, an important allergen and vector of pathogens

Mozaffar Vahedi ^a, Kourosh Azizi ^b, Amin Hosseinpour ^c, Abbasali Raz ^d, Hadi Aligholi ^e, Mohammad Hoseini ^f, Aboozar Soltani ^{b,*}

ARTICLE INFO

Keywords: German cockroach Toxicity tests Lethal dose 50 Organophosphates Pyrethroids

ABSTRACT

The study investigates the comparative toxicity of three widely used insecticides-fenitrothion, malathion, and deltamethrin-on *Blattella germanica*, a major urban pest.

Using bioassay tests based on World Health Organization (WHO) protocols, we determined the lethal doses 50 (LD $_{50}$) for each insecticide. 2.5 mL of each insecticide in acetone was placed in glass jars. Ten adult male cockroaches were tested per dilution, with three to four replicates. Acetone alone served as the control. After 1 h of exposure, the cockroaches were moved to containers with food and water, and mortality was recorded after 72 h. Probit regression analysis was employed to analyze the mortality rates at various doses, and statistical significance was confirmed for all tested insecticides.

Results showed that malathion had the lowest lethal dose, with an LD_{50} of 4.29 ppm, making it more potent at lower concentrations. Fenitrothion followed with an LD_{50} of 5.11 ppm, while deltamethrin exhibited the highest LD_{50} of 8.56 ppm, indicating lower toxicity at standard concentrations.

The study also emphasized the importance of understanding sublethal doses, which, though not immediately fatal, could induce behavioral and physiological changes leading to pesticide resistance. The findings provide critical insights for pest management strategies, highlighting the need for appropriate dosing to balance efficacy with safety concerns. This research establishes baseline data for future studies on *B. germanica* and its resistance mechanisms, contributing to improved pest control measures with minimal environmental impact.

E-mail addresses: mozaffarvahedi@gmail.com (M. Vahedi), azizik@sums.ac.ir (K. Azizi), aminhoseinpour11@gmail.com (A. Hosseinpour), raz. biotech@gmail.com (A. Raz), aligholi@sums.ac.ir (H. Aligholi), m_hoseini2174@yahoo.com (M. Hoseini), abu2sol@yahoo.com (A. Soltani).

^a Student Research Committee, Department of Medical Entomology and Vector Control, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

^b Research Center for Health Sciences, Institute of Health, Department of Medical Entomology and Vector Control, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

^c Department of Medical Entomology and Vector Control, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

d Malaria and Vector Research Group (MVRG), Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran

e Department of Neuroscience, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

f Research Center for Health Sciences, Institute of Health, Department of Environmental Health, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

^{*} Corresponding author. Kouye Zahra, School of Health, Shiraz, 7153675541, Iran.

1. Introduction

Insects are incredibly diverse and crucial organisms, with cockroaches being one of the most prevalent pests found in households [1]. Cockroaches are well known for their remarkable survival skills and resistance to various insecticides in tough conditions [2]. Belonging to the order Blattodea, which encompasses around 4600 species, cockroaches have remained relatively unchanged in appearance for over 320 million years, making them one of the most primitive winged insects still alive today [3].

Blattella germanica is a small species commonly found in kitchen and bathroom areas. It is typically around 1.1–1.6 cm long and can vary in color from tan to nearly black [4]. It has two dark streaks on its pronotum that run from behind the head to the base of the wings. B. germanica are omnivorous scavengers, feeding on meats, starches, sugars, and fatty foods. In times of food scarcity, they may even consume household items like soap, glue, and toothpaste [5].

B. germanica has three stages of development: egg, nymph, and adult. Its lifespan of around 100 days can vary due to factors such as temperature. These cockroaches have the ability to reproduce continuously, with a female capable of laying nearly 400 eggs throughout her lifetime [6]. The egg stage of a German cockroach begins when a female produces a brown egg casing called an ootheca. These oothecae contain around 31 nymphs and are carried by the females until a couple of days before hatching [7]. The females then deposit the oothecae in a safe location. Nymphs, which are small, wingless insects that cannot reproduce, emerge from these egg casings. Over the course of about 100 days, under ideal conditions, the nymphs go through five to six molts before they reach the adult stage. Adults of *B. germanica* are around 12 mm long, have wings, but they rarely fly and are active mainly during the night, scavenging for food, and hiding during the day [6].

B. germanica is considered as pest due to its association with unclean environments. They have the potential to contaminate food and spread diseases through their excrement and shed skins [8,9]. This pest is known to produce allergens that can trigger asthma [10, 11]. B. germanica is commonly found in places where people live and work, such as restaurants, hotels, hospitals, and food processing facilities [12]. They thrive in warm and humid areas with a steady food supply, making residential and commercial bathrooms and kitchens especially vulnerable to infestation [12,13].

B. germanica can spread diseases by collecting harmful bacteria on its legs while walking through decomposing material. When it comes into contact with food or surfaces that humans often touch, it transfers these pathogens, leading to diseases like Salmonellosis, Staphylococcus and *E. coli* infections, Typhoid fever, Gastroenteritis, and diarrhea [14,15].

The use of insecticides is a common practice in pest control management. Among the most commonly used insecticides are Organophosphates and Pyrethroids [16]. *B. germanica* is one of the most pervasive urban pests worldwide; the susceptibility to these insecticides provides critical information in pest control management. The proper and limited usage of recommended dosages of insecticides can mitigate their harmful effects on non-target organisms while still maintaining effectiveness against targeted pests [17].

Fenitrothion, malathion, and deltamethrin are widely used insecticides in agriculture and public health management. Fenitrothion and malathion are organophosphate insecticides that act on the nervous system of insects. They are primarily used in agriculture to control a wide range of pests and in public health to manage cockroaches, flies, and mosquitoes. Deltamethrin, on the other hand, is a synthetic pyrethroid insecticide with broad-spectrum insecticidal activity. These pesticides exhibit high efficacy and are utilized at low concentrations, making them cost-effective solutions for pest management. Nevertheless, the use of these insecticides has raised concerns about their toxicity to non-target organisms and the environment. Furthermore, the rapid development of resistance to various insecticides in most pests and disease vectors is another serious concern in the field of ecotoxicology [18,19].

It is becoming more evident that the decrease in the population of insects may be due to the sublethal rather than lethal effects of pesticides. Studies show that neurotoxic pesticides like pyrethroids can impact the behavior and physiology of insects. It is believed that this could be linked to the recent decline in insect populations, especially in polluted areas [20].

Considering the absence of comprehensive studies regarding the calculation of lethal and sublethal concentrations of widely used pesticides in German cockroaches, this study aims to address this scientific gap with rigorous laboratory implementation. The results of this research can provide comprehensive baseline information about the dose-response analysis of routine pesticides on an important health pest.

2. Materials and methods

2.1. Rearing protocol

B. germanica was carefully reared in cylindrical plastic containers measuring 23 cm in diameter and 30 cm in height within the Shiraz University of Medical Sciences, Insectarium of Medical Entomology Department. These containers provided optimal conditions, maintaining a temperature of 27 °C and a humidity of 60 %, with a 12-h light and 12-h dark cycle. The cockroaches were fed a diet of rat pellets rich in crude protein, fat, fiber, ash, calcium, and phosphorus. Water was readily available to them through a plastic bird water dispenser. Corrugated cardboard was provided to ensure their comfort and provide hiding places. The cages were cleaned and changed every two weeks to maintain a clean and pathogen-free environment.

A layer of Vaseline (petroleum jelly) was applied to the top edge of the breeding box as a barrier to prevent the cockroaches from escaping. Newly hatched nymphs were carefully collected and placed in a separate cage to maintain a population of the same age, ensuring they were simultaneously reared. This process was continued for several generations to provide a large population of the same age.

2.2. Chemicals

The technical grade of three commonly used insecticides including, deltamethrin 98.5 % ([(S)-cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate), fenitrothion 95 % (dimethoxy-(3-methyl-4-nitrophenoxy)-sulfanylidene-lambda5-phosphane) and malathion 95 % (diethyl 2-dimethoxyphosphinothioylsulfanylbutanedioate) was kindly provided to us by MOSHKFAM FARS CHEMICAL CO (MFCTM) to be used in this research.

2.3. Bioassay tests (WHO glass jar test)

World Health Organization (WHO) standard jar tests were used to evaluate the lethal doses of cockroaches to different insecticides. Serial dilutions of each insecticide were prepared in ultra-pure Merck™ acetone (CAS number 67-64-1) (Table 1). Each dilution was prepared in a separate glass vial and tested in a glass jar (diameter, 15 cm; height, 15 cm).

For the bioassay test, 2.5 mL of the prepared concentration of each dissolved insecticide in analytical-grade acetone was placed inside the glass jars. The jars were then rotated under a chemical fume hood until the acetone evaporated, leaving a thin layer of insecticide coating on the inner surfaces of the jars. To prevent the cockroaches from escaping, the inner rim of the jars was smeared with petroleum jelly.

To immobilize the cockroaches for easier handling, we exposed them to CO_2 gas for 15 s. Four tests were conducted; we used 10 adult male cockroaches for each test, and 3–4 replicates were performed for each dilution (Fig. 1A and B). Acetone without insecticide was used alongside each test to establish a control group. After 1 h contact period, the cockroaches were transferred into a separate plastic container and were provided with food and water. The number of dead cockroaches was then recorded after 72 h.

2.4. Data analysis

All tested insecticide dilutions were imported into the SPSS software version 26, and regression lines were drawn in probit mode (Figs. 2–4). This model is typically used to analyze binary response variables and can be particularly useful in understanding how the probability of an event (such as pest mortality) changes as a function of the pesticide dose. Also, 50 % end-point mortality (LD_{50}) reached after 24h for each pesticide was calculated using probit analysis. In addition, to increase the accuracy of data analysis related to bioassay WHO glass jar tests, we also analyzed the data using the specialized probit analysis software [21].

3. Results

All parameters estimate, standard error and P value for each insecticide are shown in Table 2 and all results are statistically significant (P value < 0.0001). Based on the regression line shown in Table 3, lethal doses were estimated for each insecticide, ranging from 1 to 99. These data provide an approximation of the expected dose required to cause lethality for each insecticide within the given dosage.

The lethal dose of fenitrothion ranges from 1.55 to 16.88 ppm at LD_1 and LD_{99} respectively. Its LD_{50} value is 5.11 ppm, indicating that 0.47 mg/m² is required to affect effectively half of the *B. germanica* population. Malathion starts at a lower lethal concentration of 0.61 ppm for LD_1 , escalating to 30.19 ppm at LD_{99} . Moreover, the LD_{50} of malathion was calculated 4.29 ppm (0.25 mg/m²) for the tested population. Deltamethrin begins with a higher lethal dose of 1.58 ppm at LD_1 , going up to 46.43 ppm at LD_{99} . Its LD_{50} was recorded 8.56 ppm (0.53 mg/m²) (Table 3).

4. Discussion

The bioassay analysis of fenitrothion ($LD_{50} = 5.11$ ppm) showed a steady increase from LD_1 to LD_{50} , suggesting a predictable and consistent dose-response relationship. This indicates a degree of predictability in its effectiveness and potential environmental impact. Our results demonstrated that malathion with an LD_{50} of 4.29 ppm (0.25 mg/m²) was slightly more toxic than fenitrothion, suggesting higher potency at lower concentrations. The slope of the dose-response curve from LD_1 to LD_{50} is less steep than fenitrothion,

Table 1
Serial dilutions of deltamethrin, fenitrothion and malathion used in WHO standard jar bioassay tests.

Deltamethrin		Fenitrothion		Malathion	
ppm	mg/m ²	ppm	mg/m ²	ppm	mg/m ²
50	3.0864	50	3.0864	15	0.92592
25	1.5432	25	1.5432	10	0.61728
18.75	1.157408	12.5	0.7716	7.5	0.46296
12.5	0.7716	6.25	0.3858	5	0.30864
6.25	0.3858	5	0.30864	2.5	0.15432
4.69	0.289504	4.16	0.2567	1.25	0.07716
3.12	0.1929	3.57	0.2202	0.625	0.03858
1.56	0.09645	3.12	0.1929	0.312	0.01929

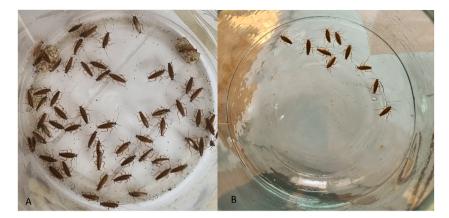


Fig. 1. A: The same-aged population of male B. germanica, separated for WHO jar bioassay. B: Ten male cockroaches were used in each test.

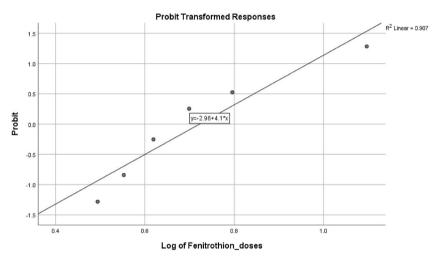


Fig. 2. Regression line and dose-response curve for fenitrothion.

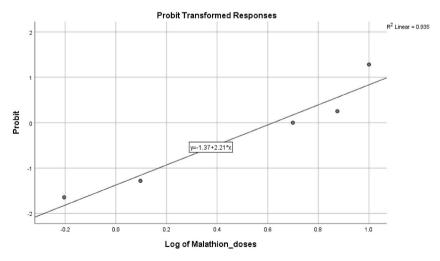


Fig. 3. Regression line and dose-response curve for malathion.

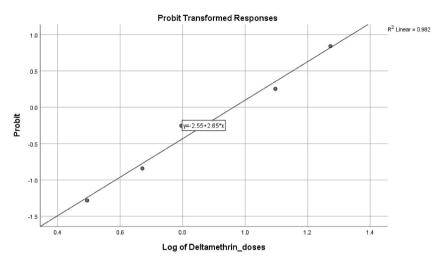


Fig. 4. Regression line and dose-response curve for deltamethrin.

Table 2Probit analysis of mortality (at 24h after treatment) of *B. germanica* treated with three insecticides.

Parameter		Estimate	Std. Error	Z	Sig.	95 % Confidence Interval	
						Lower Bound	Upper Bound
PROBIT ^a	Fenitrothion doses	4.481	0.724	6.193	0.000	3.063	5.900
	Intercept	-3.174	0.502	-6.320	0.000	-3.676	-2.672
PROBIT ^a	Malathion doses	2.499	0.354	7.056	0.000	1.805	3.193
	Intercept	-1.537	0.263	-5.837	0.000	-1.801	-1.274
PROBIT ^a	Deltamethrin doses	3.141	0.439	7.154	0.000	2.281	4.002
	Intercept	-2.936	0.425	-6.900	0.000	-3.361	-2.510

^a PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm). The estimates represent the change in the log odds of the response variable (binary outcome) associated with a one-unit increase in the respective pesticide doses. The intercepts represent the estimated log odds when the pesticide dose is zero. The Z-values and significance levels indicate the statistical significance of each estimate. All three pesticides are highly significant in this case, as indicated by very low p-values (p = 0.000). The 95 % confidence intervals provide a range of values within which we can be reasonably confident that the true parameter lies.

indicating a more sensitive reaction at lower doses, which could imply a higher risk of non-target effects at these lower concentrations. The LD_{50} of deltamethrin was recorded at 8.56 ppm (0.53 mg/m²), the highest among the three tested pesticides, suggesting lower toxicity per unit and a need for higher concentrations for effectiveness. The gradual increase in concentration from LD_1 to LD_{50} indicates a lower initial toxicity, which might make it a safer option regarding immediate environmental impact. These results are crucial for understanding the efficacy and predictability of each insecticide.

Understanding the sublethal doses of pesticides on insects is crucial for studying the molecular, biochemical, and behavioral changes that occur in insects [22]. Sublethal doses refer to doses that do not cause immediate death in the cockroach population. However, these doses can still initiate significant changes in the behavior and biology of cockroaches, potentially leading to the development of pesticide resistance.

Numerous studies have recently focused on the impact of sublethal doses of insecticides on various insect species [23–25], including the German cockroach [26]. Researchers can gain insights into the mechanisms underlying these changes by determining the specific concentrations and doses of pesticides that have sublethal effects. This knowledge is essential as it allows us to investigate the long-term effects of continuous exposure to low pesticide doses.

One aspect of concern is the potential accumulation of insecticides over time in insects exposed to sublethal doses. By understanding the different sublethal concentrations and doses, we can evaluate the effects of long-term pesticide exposure. This information is crucial for assessing the ecological impact and developing effective pest management strategies to minimize resistance development and potential harm to other beneficial organisms [23–25,27,28].

A recent study showed that exposure to sublethal doses of pyrethroids decreased the total hemocyte and differential hemocyte count in both male and female adult American cockroaches (*Periplaneta americana*). Additionally, these insecticides caused morphological changes in the hemocytes [29].

Another study investigates the induction of insecticide tolerance in *B. germanica* due to sublethal doses of imidacloprid, indoxacarb, and lambda-cyhalothrin. The study found that sublethal doses of these insecticides can induce insecticide tolerance in cockroaches. They also found that the resistant cockroaches had increased levels of detoxifying enzymes and reduced levels of acetylcholinesterase [30].

Table 3Different lethal dose concentration of fenitrothion, malathion and deltamethrin for *B. germanica*.

Lethal Doses	Fenitrothion		Malathion		Deltamethrin	
	Estimate (ppm)	mg/m ²	Estimate (ppm)	mg/m ²	Estimate (ppm)	mg/m ²
LD ₁	1.55	0.09	0.61	0.03	1.58	0.10
LD_2	1.78	0.11	0.76	0.04	1.92	0.12
LD_3	1.94	0.12	0.88	0.04	2.18	0.13
LD_4	2.08	0.13	0.99	0.05	2.40	0.15
LD_5	2.19	0.14	1.08	0.06	2.59	0.16
LD_6	2.30	0.15	1.16	0.06	2.76	0.17
LD ₇	2.39	0.16	1.24	0.07	2.93	0.18
LD_8	2.48	0.17	1.32	0.07	3.08	0.19
LD ₉	2.56	0.18	1.39	0.07	3.23	0.20
LD_{10}	2.64	0.19	1.46	0.08	3.37	0.21
LD ₁₅	3.00	0.22	1.80	0.10	4.03	0.25
LD_{20}	3.31	0.26	2.11	0.12	4.64	0.29
LD ₂₅	3.61	0.29	2.43	0.14	5.24	0.32
LD_{30}	3.90	0.32	2.76	0.16	5.84	0.36
LD ₃₅	4.19	0.36	3.10	0.18	6.46	0.40
LD_{40}	4.48	0.39	3.46	0.20	7.12	0.44
LD ₄₅	4.79	0.43	3.86	0.23	7.81	0.48
LD_{50}	5.11	0.47	4.29	0.25	8.56	0.53
LD ₅₅	5.45	0.51	4.76	0.29	9.37	0.58
LD ₆₀	5.82	0.56	5.30	0.32	10.29	0.64
LD ₆₅	6.23	0.62	5.92	0.36	11.32	0.70
LD ₇₀	6.69	0.68	6.65	0.41	12.53	0.78
LD ₇₅	7.22	0.76	7.55	0.47	13.97	0.87
LD_{80}	7.87	0.86	8.68	0.55	15.78	0.98
LD ₈₅	8.70	0.98	10.23	0.66	18.18	1.13
LD ₉₀	9.87	1.17	12.56	0.83	21.72	1.36
LD ₉₁	10.17	1.22	13.20	0.88	22.68	1.42
LD ₉₂	10.51	1.28	13.93	0.93	23.76	1.49
LD_{93}	10.90	1.35	14.79	0.99	25.02	1.57
LD ₉₄	11.36	1.43	15.80	1.07	26.50	1.66
LD_{95}	11.89	1.52	17.04	1.16	28.29	1.77
LD ₉₆	12.56	1.64	18.62	1.28	30.55	1.91
LD ₉₇	13.43	1.80	20.77	1.44	33.58	2.11
LD ₉₈	14.67	2.04	24.02	1.69	38.08	2.39
LD99	16.88	2.48	30.19	2.17	46.43	2.92

Jankowska et al. evaluated the biochemical, behavioral, and physiological toxicity of an extremely low (sublethal) dose of bendiocarb insecticide in the *P. americana*. The study found that bendiocarb caused a decrease in heart rate, an increase in gas exchange, and changes in grooming behavior. The study also found that bendiocarb increased the sensitivity of cockroaches to effective doses of the same insecticide [31].

It has been reported that the cellular and physiological changes induced by a sublethal dose of imidacloprid in the American cockroach, *P.americana*. The study found that imidacloprid caused a decrease in the activity of the nicotinic acetylcholine alpha2 subunit (nAChR-alpha2). Also found that this decrease in nAChR-alpha2 activity was correlated with an increase in imidacloprid sensitivity [32].

Moreover, the influence of sublethal effects of imidacloprid, indoxacarb, and lambda-cyhalothrin on life history traits of *B. germanica* (Blattodea: Ectobiidae) was examined by other researchers. They found that sublethal doses of these insecticides can decrease the longevity of adult cockroaches. The study also found that sublethal doses of these insecticides can increase the longevity of nymph cockroaches. The study also found that sublethal doses of these insecticides can decrease the fecundity of cockroaches [33].

In order to better summarize and compare the results obtained in the present study with those of studies conducted in this field in different parts of the world, detailed information is presented in Table 4.

Exposure to a very low concentration of insecticides significantly impacted both the behavior and physiology of insects, making them more resistant to the same insecticide when given effective doses or induction if cross-resistance to other insecticides. Similar effects may occur in other organisms, including humans [34]. Therefore, it is important to consider the potential effects of trace pesticide residues in assessing public health risks. Furthermore, understanding lethal and sublethal doses of commonly used pesticides and their effects on insects can inform the selection and application of appropriate insecticides and dosages for more effective pests and disease vectors control programs.

Insecticides are crucial in managing disease vectors like mosquitoes, ticks, and other vectors that transmit important diseases such as Malaria, Dengue fever, Encephalitis, Rift Valley fever Zika, Chikungunya, Leishmaniasis, and Crimean-Congo hemorrhagic fever [35–37]. Understanding the sublethal doses of insecticides allows us to determine the amount required to incapacitate these vectors, impeding their ability to reproduce or transmit diseases. Moreover, knowledge of the effects on insects allows for selecting insecticides with minimal ecological impact, reducing the risk of harming beneficial insects, pollinators, and other non-target organisms [34].

Heliyon 10 (2024) e40601

 Table 4

 Comparison of different lethal doses for two main medical important cockroaches in different locations and years.

1957 USA Blattella germanica Topical Application Application Application Para-Oxon U.5 μ.	Year	Location	Target Insects	Bioassay	Pesticide	LD_{50} (ppm or mg/m ²)	LD_{95} (ppm or mg/m ²)	LD_1 (ppm or mg/m ²)
Para	1957	USA	Blattella germanica	Topical	Diptercx	30 μg/g	NR	NR
				Application	Phosdrin	7 μg/g	NR	NR
					Para-Oxon	0.75 μg/g	NR	NR
Part					Diethyl 2,4,5-Trichlorophenyl Phosphate	60 μg/g	NR	NR
Disopropylinorophosplame (DFP) 160 μg/g NR					O,O-Diethyl 0-5-(3-Methy Lpyrazolyl) Phosphate Pyrazoxon	70 μg/g	NR	NR
Disopropyfisorophosplante (DFP) 160 μg/g NR NR NR NR NR NR NR N					O-Ethyl O'P-Nitrophenyl Phenylphosphonate	2 μg/g	NR	NR
O,O-Diethyl S(Isopropylthio) Methyl Phosphorodithioate (Am. Cyanamid) 15 μg/g NR NR NR					Diisopropylfiuorophosplmte (DFP)		NR	NR
Methyl Parathion 12 μg/g NR NR NR					O,O-Diethyl S-(Isopropylthio) Methyl Phosphorodithioate (Am. Cyanamid		NR	NR
Malathion Mal					12008)			
Mipafox Mip					Methyl Parathion)	2 μg/g	NR	NR
Net					Malathion	120 μg/g	NR	NR
Permethrin Pe					Mipafox		NR	NR
1					Octamethyl Pyrophosphoramide (Schradan)	1000 μg/g	NR	NR
Application Fipronil Color Poletametrin Color Col					0-2-(Ethoxy)Ethyl 0, O-Diethyl Phosphate	45 μg/g	NR	NR
2010 Singapore Blattella germanica Application	2006	Iran	Blattella germanica	Topical	Permethrin	0.43 μg/individual	NR	NR
Application β- Cyfluthrin ρτοροχιτ β- Cyfluthrin ρτοροχιτ β- Cyfluthrin ρτοροχιτ β- Cyfluthrin ρτοροχιτ β- Cyfluthrin β			-	Application	Fipronil	0.96 ng/individual	NR	NR
Let all the proposition of the pr	2010	Singapore	Blattella germanica	Topical	Deltamethrin	0.046 μg/g	0.866 μg/g	NR
Propour Chlorytrifos Chlorytr				Application	β- Cyfluthrin	0.024 μg/g	0.452 μg/g	NR
Standard Glass Jar Jection Jarian Jari					Propoxur			NR
Standard Glass Jar Jection Jarian Jari					Chlorpyrifos	0.867 μg/g	16.328 μg/g	NR
Carbaryl Carbaryl Carbaryl Carbaryl Carbaryl Carbaryl Methamphetamine Salat mg/kg NR NR NR	2011	Iran	Blattella germanica	Standard Glass Jar	Cyfluthrin			NR
Deltamethrin Delt	2016	Iran	Blattella germanica	Standard Glass Jar	Bendiocarb	38.44 mg/m^2	NR	NR
August Standard Glass Jar Alpha-Cypermethrin Deltamethrin			_		Carbaryl	280.87 mg/m ²	NR	NR
2021Turkey americanaPeriplaneta americanaStandard Glass Jar DeltamethrinAlpha-Cypermethrin Deltamethrin0.1NRNR2021IranBlattella germanicaStandard Glass Jar Standard Glass Jar DeltamethrinFenitrothion1.292.76NR2022Malaysia americanaPeriplaneta americanaStandard Glass Jar OletamethrinDeltamethrin0.63.4NR2023IndiaBlattella germanica Blattella germanicaStandard Glass Jar Standard Glass Jar DeltamethrinDeltamethrin0.54NR2023IndiaBlattella germanicaStandard Glass Jar Imidacloprid Clothianidin7.711NRThis StudyIranBlattella germanicaStandard Glass JarFenitrothion5.11 (0.47)11.89 (1.52)1.55 (0.09)	2020	USA		Injection	Methamphetamine	832.1 mg/kg	NR	NR
Americana Deltamethrin Deltame	2021	Turkev		Standard Glass Jar	Alpha-Cypermethrin	0.3	NR	NR
Lambda-Cyhalothrin permethrin 1.1 NR NR 2021 Iran Blattella germanica Standard Glass Jar Periptoneta Standard Glass Jar Deltamethrin 0.6 3.4 NR 2022 Malaysia Periplaneta Standard Glass Jar Deltamethrin 0.6 3.4 NR 2023 India Blattella germanica Standard Glass Jar Deltamethrin 0.5 4 NR 2023 India Blattella germanica Standard Glass Jar Deltamethrin 0.5 NR This Study Iran Blattella germanica Standard Glass Jar Standard Glass Jar Deltamethrin 0.5 NR Imidacloprid Clothianidin 0.5 NR Fenitrothion 5.11 (0.47) 11.89 (1.52) 1.55 (0.09)		,	*					
Permethrin 1.1 NR NR 2021 Iran Blattella germanica Standard Glass Jar Standard Glass Jar Deltamethrin 1.29 2.76 NR 2022 Malaysia Periplaneta Standard Glass Jar Jarianetriana americana 2023 India Blattella germanica Standard Glass Jar Jarianetriana 2023 India Blattella germanica Standard Glass Jar Jarianetriana This Study Iran Blattella germanica Standard Glass Jar Jarianetriana Standard Glass Jar Jarianetriana Imidacloprid Jarianetriana Clothianidin 2.5 5 5 NR Fenitrothion 1.1 NR N								
2021IranBlattella germanica 2022Standard Glass Jar Malaysia Periplaneta americanaFenitrothion1.292.76NR2023IndiaBlattella germanica IndiaStandard Glass Jar Standard Glass Jar DeltamethrinDeltamethrin0.63.4NR2023IndiaBlattella germanicaStandard Glass Jar Standard Glass Jar ClothianidinDeltamethrin0.54NRThis StudyIranBlattella germanicaStandard Glass Jar ClothianidinImidacloprid Clothianidin2.55NRThis StudyIranBlattella germanicaStandard Glass Jar FenitrothionFenitrothion5.11 (0.47)11.89 (1.52)1.55 (0.09)					·			
2022MalaysiaPeriplaneta americanaStandard Glass Jar JamericanaDeltamethrin0.63.4NR2023IndiaBlattella germanica IndiaStandard Glass Jar Standard Glass Jar Imidacloprid ClothianidinDeltamethrin Imidacloprid Clothianidin0.54NRThis StudyIranBlattella germanicaStandard Glass Jar Imidacloprid Clothianidin2.55NRThis StudyIranBlattella germanicaStandard Glass Jar FenitrothionFenitrothion5.11 (0.47)11.89 (1.52)1.55 (0.09)	2021	Iran	Blattella germanica	Standard Glass Jar				
mericana Blattella germanica Standard Glass Jar Deltamethrin 2023 India Blattella germanica Standard Glass Jar Imidacloprid Clothianidin This Study Iran Blattella germanica Standard Glass Jar Fenitrothion Deltamethrin Delta								
Blattella germanica Standard Glass Jar Deltamethrin 0.5 4 NR 2023 India Blattella germanica Standard Glass Jar Imidacloprid 7.7 11 NR This Study Iran Blattella germanica Standard Glass Jar Fenitrothion 5.11 (0.47) 11.89 (1.52) 1.55 (0.09)			*					
2023 India Blattella germanica Standard Glass Jar Imidacloprid 7.7 11 NR This Study Iran Blattella germanica Standard Glass Jar Fenitrothion 5.11 (0.47) 11.89 (1.52) 1.55 (0.09)				Standard Glass Jar	Deltamethrin	0.5	4	NR
Clothianidin 2.5 5 NR This Study Iran Blattella germanica Standard Glass Jar Fenitrothion 5.11 (0.47) 11.89 (1.52) 1.55 (0.09)	2023	India	•					
This Study Iran Blattella germanica Standard Glass Jar Fenitrothion 5.11 (0.47) 11.89 (1.52) 1.55 (0.09)								
	This Study	Iran	Blattella germanica	Standard Glass Jar				
		-			Malathion	4.29 (0.25)	17.04 (1.16)	0.61 (0.03)
Deltamethrin 8.56 (0.53) 28.29 (1.77) 1.58 (0.10)								

Insecticide resistance is a significant challenge in pest control. Understanding the lethal and sublethal insecticide doses helps implement resistance management strategies. By rotating or alternating different classes or modes of action, we can prevent or delay the development of resistance in targeted pest populations.

Based on the results of our study, malathion's lower LD₅₀ suggests it may be more effective in smaller quantities but poses greater environmental and health risks. Deltamethrin, with a higher LD₅₀, may be safer for ecosystems but less effective at lower concentrations. On the other hand, fenitrothion offers a balance between efficacy and safety. Deltamethrin might be the preferred choice in sensitive environments due to its lower toxicity at usual operational concentrations. Conversely, malathion's higher potency could make it suitable for intense infestations, despite the increased environmental risk. The choice between these pesticides should consider the specific infestation scenario, environmental sensitivity, and the desired balance between immediate effectiveness and long-term safety [38]. Overreliance on a single pesticide can lead to resistance; given their varying toxicities, rotational use of these pesticides could be a strategic approach to manage resistance in *B. germanica*. Understanding the lethal doses of these pesticides is essential for effective pest control, ensuring that the chosen pesticide is used to maximize efficacy while minimizing risks to human health and the environment [34,38]. The data provides a foundation for developing guidelines and safety measures for pesticide use, especially in residential or environmentally sensitive areas.

This study is the first to determine sublethal doses of conventional pesticides for the German cockroach, providing key insights into both lethal and sublethal effects on this significant urban pest. Unlike previous research that primarily focuses on lethal outcomes, it examines how various insecticides affect immediate mortality as well as long-term resistance mechanisms and behavioral changes. The findings address a critical knowledge gap by offering baseline data on sublethal pesticide effects, which is vital for understanding the influence of continuous low-dose exposure on insect behavior, physiology, and resistance. This research establishes a foundation for future studies on pest management and resistance development.

5. Conclusions

In summary, understanding the lethal and sublethal doses of pesticides on *B. germanica* is crucial for comprehending the molecular, biochemical, and behavioral changes in these pests and assessing the long-term effects of pesticide exposure. This knowledge is essential for developing sustainable approaches to pest control. The detailed comparative analysis of fenitrothion, malathion, and deltamethrin against *B. germanica* reveals significant differences in their toxicities, efficacy, potency, and reliability and implications for their use in pest control. These insights are invaluable for informed decision-making in pest control management, balancing the need for effective control with environmental and health considerations, ensuring both effectiveness and safety.

Enhancing research on the long-term effects of continuous low-dose pesticide exposure is crucial for uncovering the complicated molecular and biochemical changes that contribute to the development of insecticide resistance. This resistance presents a significant challenge in the realm of urban pest control, particularly when it comes to managing populations of cockroaches. Understanding these changes is essential, as it will provide valuable insights into how pests adapt to chemical treatments, ultimately informing more effective strategies for pest management in urban environments.

CRediT authorship contribution statement

Mozaffar Vahedi: Writing – original draft, Resources, Methodology, Investigation, Formal analysis. Kourosh Azizi: Writing – original draft, Validation, Investigation. Amin Hosseinpour: Methodology, Data curation. Abbasali Raz: Writing – review & editing, Methodology, Hadi Aligholi: Writing – original draft, Methodology, Conceptualization. Mohammad Hoseini: Methodology, Investigation. Abbozar Soltani: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data used to support the findings of this study are available from the corresponding author upon request.

Code availability

Not applicable.

Ethical approval

The study was approved by Iran National Committee for Ethics in Biomedical Research (IR.SUMS.SCHEANUT.REC.1400.009).

Funding

This study was an approved research project (Grant No: 23478-04-01-99) funded by the Shiraz University of Medical Sciences.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Aboozar Soltani reports financial support was provided by Shiraz University of Medical Sciences. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are thankful for the assistance of vice-chancellorship for research and technology at Shiraz University of Medical Sciences (SUMS). This study was an approved research project (Grant No: 23478) funded by the SUMS.

References

- [1] R. Castillo-Neyra, et al., Perceptions of problems with household insects: qualitative and quantitative findings from peri-urban communities in arequipa, Peru, Am. J. Trop. Med. Hyg. 109 (6) (2023) 1372–1379, https://doi.org/10.4269/ajtmh.23-0266.
- [2] H. Gul, et al., Fitness costs of resistance to insecticides in insects, Front. Physiol. 14 (2023) 1238111, https://doi.org/10.3389/fphys.2023.1238111.
- [3] J. Guzman, A. Vilcinskas, Bacteria associated with cockroaches: health risk or biotechnological opportunity? Appl. Microbiol. Biotechnol. 104 (2020) 10369–10387, https://doi.org/10.1007/s00253-020-10973-6.
- [4] P. Dhang, Urban Pest Control, CABI, 2018.
- [5] S.A. Kells, Nutritional Status and Feeding Behavior of in Situ Populations of the German Cockroach, Blattella germanica (L.), Purdue University, 1998.
- [6] D.G. Cochran, W.H. Organization, Cockroaches: Their Biology, Distribution and Control, World Health Organization, 1999.
- [7] G. Barson, N. Renn, Hatching from oothecae of the German cockroach (Blattella germanica) under laboratory culture conditions and after premature removal, Entomol. Exp. Appl. 34 (2) (1983) 179–185, https://doi.org/10.1111/j.1570-7458.1983.tb03315.x.
- [8] B. Dingha, et al., Pest control practices for the German cockroach (Blattodea: blattellidae): a survey of rural residents in North Carolina, Fla. Entomol. 96 (3) (2013) 1009–1015, https://doi.org/10.1653/024.096.0339.
- [9] D.D. Biehler, Pests in the City: Flies, Bedbugs, Cockroaches, and Rats, University of Washington Press, 2013.
- [10] D.C. Do, Y. Zhao, P. Gao, Cockroach allergen exposure and risk of asthma, Allergy 71 (4) (2016) 463-474, https://doi.org/10.1111/all.12827.
- [11] L.K. Arruda, et al., Cockroach allergens and asthma, Journal of allergy and clinical immunology 107 (3) (2001) 419–428, https://doi.org/10.1067/mai.2001.112854.
- [12] S. Abudin, M. Martini, N. Nurjazuli, Factors that trigger cockroach density: a literature review, Majalah Kesehatan Indonesia 4 (2) (2023) 71–76, https://doi.org/10.47679/makein.2023155.
- [13] C.-Y. Lee, C. Wang, M. Rust, German cockroach infestations in the world and their social and economic impacts. Biology and Management of the German Cockroach, CSIRO Publishing, Clayton South, Victoria, Australia, 2021, pp. 1–16.
- [14] M. Akter, et al., Antibiogram and MDR pattern of the bacterial isolates from German cockroaches (Blattella germanica L.) at RMCH, rajshahi, Bangladesh, Journal of Scientific Research 15 (2) (2023) 559–570, https://doi.org/10.3329/jsr.v15i2.62557.
- [15] M. Kalantari, et al., First molecular detection of SARS-CoV-2 virus in cockroaches, Biologia (Bratisl) 78 (4) (2023) 1153–1160, https://doi.org/10.1007/s11756-023-01332-7.
- [16] H. Van Den Berg, et al., Global trends in the use of insecticides to control vector-borne diseases, Environmental health perspectives 120 (4) (2012) 577–582, https://doi.org/10.1289/ehp.1104340.
- [17] J.E. Serrão, et al., Side-effects of pesticides on non-target insects in agriculture: a mini-review, Sci. Nat. 109 (2) (2022) 17, https://doi.org/10.1007/s00114-022-01788-8.
- [18] K.S. Chang, et al., Insecticide susceptibility and resistance of Blattella germanica (blattaria: blattellidae) in seoul, Republic of Korea, 2007, Entomol. Res. 39 (4) (2009) 243–247, https://doi.org/10.1111/j.1748-5967.2009.00227.x.
- [19] H. Nasirian, An overview of German cockroach, Blattella germanica, studies conducted in Iran, Pakistan J. Biol. Sci. 13 (22) (2010) 1077, https://doi.org/10.3923/pjbs.2010.1077.1084.
- [20] C.H. Walker, Organic Pollutants: an Ecotoxicological Perspective, CRC press, 2008.
- [21] H. Chi, Computer Program for the Probit analysis. Taichung, Taiwan: National Chung Hsing University, 1997.
- [22] R.N.C. Guedes, N.M.P. Guedes, C.A. Rosi-Denadai, Sub-lethal effects of insecticides on stored-product insects: current knowledge and future needs, Stewart Postharvest Review 7 (3) (2011) 1–5, https://doi.org/10.2212/spr.2011.3.5.
- [23] G. Charpentier, et al., Lethal and sublethal effects of imidacloprid, after chronic exposure, on the insect model Drosophila melanogaster, Environmental science & technology 48 (7) (2014) 4096–4102, https://doi.org/10.1021/es405331c.
- [24] C. Lu, Y.-T. Hung, Q. Cheng, A review of sub-lethal neonicotinoid insecticides exposure and effects on pollinators, Current Pollution Reports 6 (2020) 137–151, https://doi.org/10.1007/s40726-020-00142-8.
- [25] A. Margus, et al., Sublethal pyrethroid insecticide exposure carries positive fitness effects over generations in a pest insect, Sci. Rep. 9 (1) (2019) 11320, https://doi.org/10.1038/s41598-019-47473-1.
- [26] A. Konkala, M.R. Narra, Comparative study on biochemical responses to imidacloprid and clothianidin in cockroaches (Blattella germanica), Physiol. Entomol. 49 (4) (2024) 401–411, https://doi.org/10.1111/phen.12458.
- [27] M.-T. Bartling, et al., Current insights into sublethal effects of pesticides on insects, Int. J. Mol. Sci. 25 (11) (2024) 6007, https://doi.org/10.3390/ijms25116007.
- [28] Å.C. Rodrigues, et al., Sub-lethal toxicity of environmentally relevant concentrations of esfenvalerate to Chironomus riparius, Environmental pollution 207 (2015) 273–279, https://doi.org/10.1016/j.envpol.2015.09.035.
- [29] H.K. Saha, R. Ghosh, Effect of Sublethal Doses of Cypermethrin on the Haemocytes of Periplaneta americana (Dictyoptera: Blattidae). Heritage, 2020, p. 82.
- [30] A. Rajab, G. Moravvej, A. Asoodeh, Induction of insecticide tolerance in German cockroach (Dictyoptera: blattellidae) due to sublethal doses of imidacloprid, indoxacarb, and lambda-cyhalothrin, Int. J. Pest Manag. (2021) 1–9, https://doi.org/10.1080/09670874.2021.1985652.

[31] M. Jankowska, et al., Sublethal biochemical, behavioral, and physiological toxicity of extremely low dose of bendiocarb insecticide in Periplaneta americana (Blattodea: blattidae), Environ. Sci. Pollut. Res. Int. 30 (16) (2023) 47742–47754, https://doi.org/10.1007/s11356-023-25602-8.

- [32] A. Bantz, et al., Exposure to a sublethal dose of imidacloprid induces cellular and physiological changes in Periplaneta americana: involvement of alpha2 nicotinic acetylcholine subunit in imidacloprid sensitivity, Pestic. Biochem. Physiol. 181 (2022) 105014, https://doi.org/10.1016/j.pestbp.2021.105014.
- [33] A.M. Rajab, G. Moravvej, A. Asoodeh, Influence of sublethal effects of imidacloprid, indoxacarb and lambda-cyhalothrin on life history traits of Blattella germanica (Blattodea: Ectobiidae), J. Entomol. Res. 46 (4) (2022) 797–804, https://doi.org/10.5958/0974-4576.2022.00137.2.
- [34] S.M. De França, et al., The Sublethal Effects of Insecticides in Insects, 2017, pp. 23-39.
- [35] P. Soltan-Alinejad, A. Soltani, Vector-borne diseases and tourism in Iran: current issues and recommendations, Trav. Med. Infect. Dis. 43 (2021) 102108, https://doi.org/10.1016/j.tmaid.2021.102108.
- [36] W.H. Organization, A Global Brief on Vector-Borne Diseases, World Health Organization, 2014.
- [37] R. Müller, et al., Vector-borne diseases, Biodiversity and health in the face of climate change (2019) 67-90, https://doi.org/10.1007/978-3-030-02318-8_4.
- [38] M.F. Ahmad, et al., Pesticides Impacts on Human Health and the Environment with Their Mechanisms of Action and Possible Countermeasures, 2024.