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Original Article

Susceptibility Status of Several Field-Collected German Cockroaches (*Blattella germanica*) to a Pyrethroid Insecticide and Molecular Detection of Knockdown Resistance (*kdr*)

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Abstract

Background: High frequency of insecticide used to control German cockroaches may lead to insecticide resistance development. We aimed to compare the level of insecticide resistance and heterogeneity in insecticide resistance of field-collected German cockroaches in eight selected zones of Mashhad City, Razavi Khorasan Province, Iran.

Methods: The present study was conducted from October 2019 to May 2021. Adult and nymphs of German cockroaches were collected from infested restaurants or hotels in eight zones of Mashhad City and then colonized in an insectarium. The cockroaches were subjected to bioassay against cypermethrin insecticide. In addition, the genomic DNA of each cockroach population was analyzed in the region where the *kdr* mutations reside in the German cockroach.

Results: The LT_{50} values against cypermethrin in the susceptible strain (SS) and eight field-collected strains so varied from 17.52 to 95.36 min. The resistance ratio of the strains was also different in response to the insecticide. The similarity of multiple sequence alignments at the amino acid level was 97.5%-100%. An exon 20 transversion mutation and a nonsynonymous substitution were found. The L1014F substitution was detected in 83.4% of the cockroach samples.

Conclusion: The field-collected strains were resistant to cypermethrin at different levels. Furthermore, the molecular study confirms the heterogeneity in the level of resistance among eight strains. Therefore, eight strains might have a different history of insecticide treatment.

Keywords: German cockroach; Field strains; Insecticide resistance; Heterogeneity

Introduction

It is widely acknowledged that cockroaches have been present on the Earth for a minimum of 250 million years and are considered to be among the planet's earliest inhabitants. These insects are frequently found in human-populated environments such as hospitals, hotels, and homes, particularly in tropical and subtropical regions (1). The German cockroach (*Blattella germanica*) species is particularly suited for living in indoor environments, such as homes, hospitals, kitchens, bakeries, and restaurants, due to its adaptability to various conditions and its ability to reproduce readily in these settings (2). Nevertheless, infestations of German cockroaches are most prevalent in Iran (3). *B. germanica* (L.) (Blattodea: Blattellidae) is a common indoor pest in the world and it can act as potential vector of medically important pathogens (4-6) The feces, saliva, eggs, and shed cuticles of German cockroaches are known to contain proteins that cause allergies, such as asthma(7, 8). It is also a vector of many bacterial and viral illnesses (9, 10).

"Given the detrimental effects on human and societal health, it is imperative to continuously monitor and regulate populations of German Cockroaches. Control strategies encompass preventative measures as well as non-chemical and chemical approaches (11). Chemical control is frequently employed, incorporating the application of liquid, gel, and solid formulations (12)."

Worldwide, cockroaches have developed resistance to many classes of insecticides, including pyrethroids, organochlorine, organophosphate, and carbamate and thus it makes it more difficult to control them (13-15). German Cockroaches exhibit resistance to 43 active ingredients (16). Pyrethroids and synergized pyrethrins, which are frequently utilized in residual sprays, aerosols, and fogs, have been frequently identified as being ineffective due to the development of resistance (17). Consequently, managing resistance is a vital aspect of any integrated pest management (IPM) strategy for cockroaches. Wild populations of German Cockroaches are commonly resistant to pyrethroids (18). Numerous studies from various regions globally have reported the prevalence of pyrethroid resistance in German Cockroaches (19). A high reproductive rate and three to four generations per year of the German cockroach contribute to the rapid evolution of insecticide resistance (20). Additionally, over-the-counter (OTC) pyrethroid-based aerosols, many of which have been shown to be ineffective or even harmful, are still widely used by restaurant owners and the general public as a do-it-yourself (DIY) method of controlling German Cockroaches. This use can contribute to the exacerbation of insecticide resistance in German Cockroaches (21).

The resistance to insecticides can be classified as behavioral and physiological forms (22, 23). Both behavioral and physiological resistance forms are often found among insect pests (24), and both forms have been reported in *B. germanica* (14, 25). In addition, metabolic detoxification decreased penetration, and target site insensitivity, are other mechanisms of resistance (5, 26). Although using chemical insecticides is the most common way to control cockroaches (27), the German cockroach has developed resistance to almost all insecticide classes (28) and mixed functional oxidases play an important role in insecticide resistance to pyrethroids (29-31).

Several genetic mutations at specific locations in the genome of the German cockroach enable normal channel function in the presence of insecticides, thereby leading to reduced susceptibility (32). Resistance to pyrethroids, which modulate sodium channels, has been linked to multiple mutations in the para-homologous sodium channel known as knockdown resistance (*kdr*) or *kdr*-type mutations (26).

The knowck-down resistant (*kdr*) strains of German cockroaches carry such a mutation (TTG to TTC [L993F]) that occurs close to the center of transmembrane helix S6 of domain II of the para-type sodium channel gene. The change in amino acids caused by this change is from phenylalanine to leucine (33).

Every year, more than 30 million religious tourists visit Mashhad City. As a result, tourist traffic persuades hotels and restaurants to be more vulnerable to pest infestations, such as cockroaches. In addition, chemical insecticides are widely used in hotels and restaurants in Mashhad City, but not uniformly (interview with Mashhad's Health Authorities). To determine the spatial distribution of the German cockroach's resistance to insecticides in Mashhad City, this study was undertaken. The findings are expected to aid in the development of more accurate systems for monitoring resistance in urban areas.

Materials and Methods

Sample collecting sites

The study was conducted from October 2019 to May 2021in the holy city of Mashhad (36.2972° N, 59.6067° E), in northeastern Iran. The city has a large number of hotels and restaurants, especially in its central areas. German cockroach was collected by hand catch and trap from infested restaurants or hotels in eight of the 13 city municipal zones (Fig. 1). According to interviews with managers of restaurants and pest control services, cypermethrin, permethrin, chlorpyrifos, and imidacloprid are the most common insecticides used for cockroach control in the city.

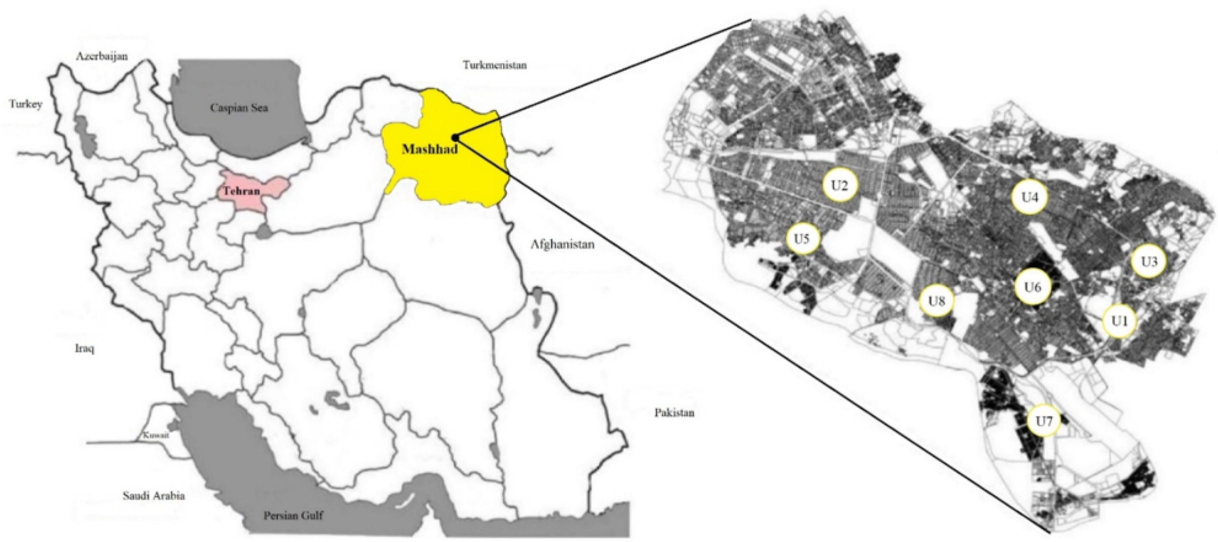

Fig. 1: Map of collection sites of German cockroaches from the metropolis of Mashhad City located in northeastern Iran.

Ethics approval

This research was approved by the ethics committee of the Tehran University of Medical Sciences
(IR.TUMS.SPH.REC.1398.315).

Cockroach populations

Adults and nymphs of German cockroaches were collected from infested hotels and restaurants and then transferred to an insectary. The Laboratory strain has been reared in the laboratory without exposure to insecticides since 1975. To establish colonies of the collected German cockroaches, the insect populations were maintained in glass jars at a temperature of 27±2 ºC, 60±10% RH. A constant supply of bread and water was provided to the cockroaches.

Insecticide resistance bioassays

The WHO glass jar Bioassay method was conducted to determine cypermethrin resistance in German cockroaches as recommended by the previous studies (34, 35). Cypermethrin (92%, technical grade) cis: trans 60:40 (Cyanamid Agro, India) was used in a range of time to determine the median lethal time (LT_{50}) and 90% lethal time (LT_{90}) values for each population of German cockroaches. In summary, the bioassays were conducted as follows: Initially, the laboratory population of cockroaches was exposed to a serial dilution of cypermethrin to determine the diagnostic dose of the insecticide (the dose that kills more than 95% of German cockroaches from a susceptible population within 30 mins contact), following the WHO recommendations (36). Based on the estimated diagnostic dose of cypermethrin (100 mg/m^2), LT_{50} and LT_{90} of all cockroach populations were determined at 5 times in the time interval of 6 mins to 140 mins. Cypermethrin at a concentration of 100 mg/m^2 was coated onto the inside of glass jars, and then ten males from each population of different zones of the study areas were exposed separately to the insecticide to determine the median lethal time (LT_{50}) and 90% lethal time (LT_{90}). Control jars were treated with acetone alone. Four replicates were performed for each test.

Statistical analysis

Data from the replicates were pooled, and the time exposure mortality was assessed by probit analysis (37), using the SPSS package on an IBM computer. The resistance ratios were calculated by dividing the 50% response value (LT_{50}) of the field strain by the 50% response value of the laboratory strain (SS). Time exposures to insecticide (log10) are plotted against percentage mortality (probit). A graph pad prism program was used to create this chart.

DNA extraction and PCR

A YTA genomic DNA extraction mini kit (Yekta Tajhiz Azma, Tehran, Iran) was used to extract genomic DNA from the thorax of each cockroach. Each sample was homogenized in 200 l TG1 buffer by grinding with a micro pestle in liquid nitrogen. Following the addition of 20 l proteinase K, the mixture was incubated at 60°C for 1–2 h(s). The rest of the DNA extraction process followed the manufacturer's instructions. DNA was resuspended in 100 µl of elution buffer (5 mM Tris/HCl, pH 8.5) or ddH2 O (pH 7.5–9.0) and kept at −20°C until use. The *kdr* -related mutations fragment was amplified in a molecular laboratory using German cockroach-specific primers (KUF and KUR) (38).

The PCR was performed at 95 ℃ for 5 min, followed by 30 cycles at 95℃ for 1 min, 62℃ for 1 min, 72 ℃ for 1 min, followed by an extension step at 72℃ for 10 mins. Consequently, PCR products were sequenced in an ABI377 automatic sequencer in both directions.

Bioinformatics Analysis

Bioinformatics software Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/blast/), Chromas version 2.3.1, and Clustal Omega (39) were used to analyze the sequences. The final sequences were aligned with the vgsc representative haplotype sequences in the GenBank using Molecular Evolutionary Genetics Analysis version 7.0 (MEGA7)(40). The genotype frequencies were calculated by dividing the number of German cockroaches of each genotype (RR, RS, and SS) by the total number of German cockroaches.

Results

According to our observations, organophosphates and pyrethroids were the most commonly used insecticides at collection sites (Table 1). Insecticide application periods were varied in the collection sites. The floors of all the places were covered by non-absorbent surfaces such as ceramic and stone tiles, whereas the walls of some places were covered with absorbent surfaces such as plaster (Table 1). No information was given regarding how often the places were cleaned or sprayed with insecticides after insecticide spraying.

Insecticide resistance bioassays

The results of the bioassay indicated that the resistance ratios (RR) of the cockroach populations at LT_{50} varied among eight populations (Table 2). The highest RR was found in the U6 (5.44-fold) population followed by U1 (4.80-fold) and U4 (4.09-fold); the lowest RRs were found in U8 (1.84-fold) population, U7 (1.88-f old), U2 (1.95-fold), U4 (2.03-fold), and U5 (2.11-fold). In addition, probit analysis of lethal times of cypermethrin at the discriminating dose (100 mg/m^2) for the nine collected populations of German cockroaches in different was varied (Fig. 2).

Table 1: Characteristics of the collection sites of German cockroaches from different sites in Mashhad City, Iran, 2020

Code	*Type of residence*	*Collection sites*	*floor covering*	*Cover of wall*	*Insecticide spraying period*	*Type of insecticide used*	*Cockroach Infestation*
U1	Hotel	Kitchen & dining hall	Ceramic tiles	Stone tiles	Irregular	Organophosphate & Pyrethroid	High
U2	Hotel	Kitchen	Ceramic tiles	Ceramic tiles	Irregular	Pyrethroid	Moderate
U3	Restaurant	Kitchen	Stone tiles	Plaster wall	Biannual	Organophosphate & Pyrethroid	Moderate
U4	Delivery kitchen	Kitchen	Ceramic tiles	Plaster wall	Irregular	Organophosphate & Pyrethroid	Moderate
U5	Restaurant	Kitchen	Ceramic tiles	Stone tiles	Irregular	Organophosphate Pyrethroid & Neonicotinoid	Moderate
U6	Hotel	Kitchen & dining hall	Ceramic tiles	Plaster wall	Annually	Organophosphate & pyrethroid	High
U7	Restaurant	Kitchen	Ceramic tiles	Plaster wall	No operation	-	Very low
U8	Restaurant	Kitchen	Ceramic tiles	Stone tiles	Irregular	Organophosphate Pyrethroid & Neonichotinoid	Moderate

In comparison with the laboratory population (LP) the populations collected from the south (U7) and southeast of Mashhad City (U3 & U1) showed high resistance to the cypermethrin insecticide.

Table 2: The results of probit analysis on the bioassay results against cypermethrin performed on adults of German cockroaches from eight different areas of Mashhad Municipality and the laboratory population, Iran

Population	*n*	X^2 *(DF)*	LT_{50}	LT_{90}	RR^a
LP	217	10.19(3)	17.52	28.60	1.00
U1	207	1.22(3)	84.16	125.6	4.80
U2	208	6.40(3)	34.26	53.84	1.95
U3	214	1.38(3)	71.8	101.5	4.09
U4	213	2.29(3)	35.6	54.65	2.03
U5	211	1.99(3)	37.07	53.97	2.11
U6	215	0.10(3)	95.36	124.62	5.44
U7	208	10.19(3)	33.02	51.64	1.88
U8	210	2.75(3)	32.28	51.77	1.84

LP is the laboratory population; populations U1- U8 are the field-collected population. Df= Degrees of freedom, LT= Lethal Time, X2= chi-square. aRR (resistance ratio) = LT_{50} of field-collected population / LT_{50} of the laboratory population. n=number of cockroaches

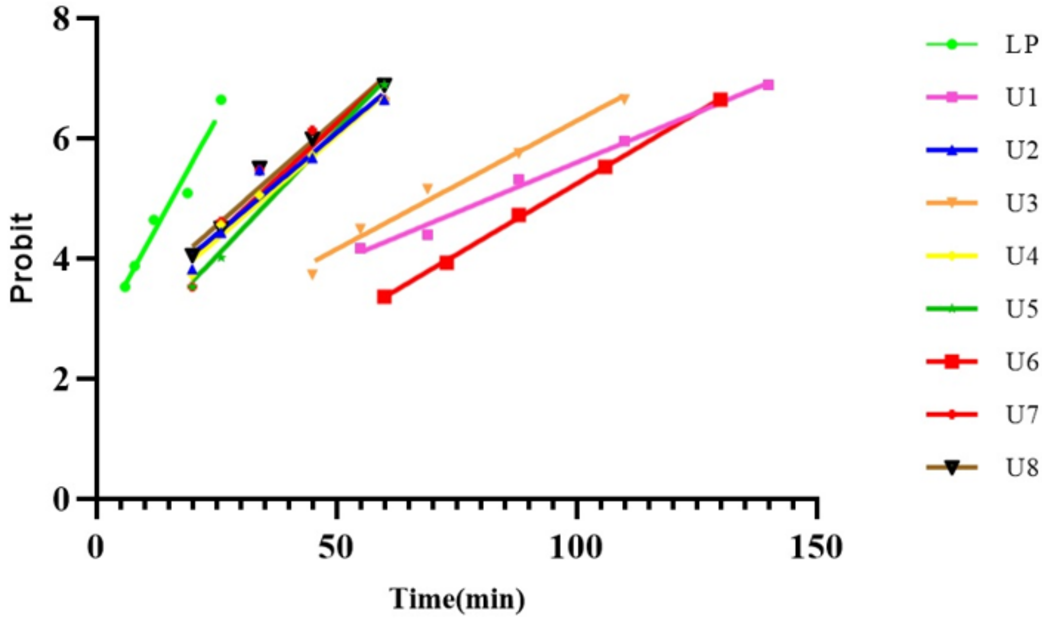

Fig. 2: Probit analysis of lethal times of cypermethrin at the discriminating dose (100mg/m²) for the nine populations of German cockroach collected from different sites of Mashhad City. LP is the laboratory population; populations U1- U8 are the field-collected population

Polymerase chain reaction (PCR)

A 229-bp fragment of the *KDR* gene of the nine cockroach populations was amplified and 27 PCR products (3 from each population) were selected randomly for sequencing. Out of 229 sequenced nucleotides, 120 nucleotides were found in exon 20 (GC rich), while the remaining 109 were in intron 20 (AT rich) of *B. germanica*.

In the multiple sequence alignment, all sequences of the current study showed 99.56% to 100% similarity. A single mutation at position 117 (G/C) caused the differences. Sequence similarity between the results of the current study and *kdr*-susceptible (GenBank ID: PYGN01002366) and resistant sequences (GenBank ID: KC731438) was 98.69%-99.13%. This was the result of three mismatches in 24 (T/C), 27 (A/G), and 117 (G/C) positions.

Among four un-substitute sequences, one (4.1% was homozygote) and three remaining's (12.5%) were heterozygote haplotypes. Multiple sequence alignments in amino acid level showed 97.5%-100%. In exon 20, there was one transversion mutation (G to C), with a no synonymous substitution (L1014F). L1014F substitution obtained in 83.4% of sequenced samples collected from Mashhad. The 16.6%, of samples (U2, U6, U7, and U8) had susceptible (Fig. 3). Sequences of all 27 sequences have been submitted to GenBank, and can be found in the National Center for Biotechnology Information (NCBI) under accession numbers MZ091498-MZ091524.

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#PYGN01002366    MIVFRVLCGE WIESMWDCML VGDWSCIPFF LATVVIGNLV  [40]
#KC731438        .......... .......... .......... ........F.  [40]
MZ091498/RR      .......... .......... .......... ........F.  [40]
MZ091499/SS      .......... .......... .......... ..........  [40]
MZ091500/RR      .......... .......... .......... ........F.  [40]
MZ091501/RS      .......... .......... .......... ..........  [40]
MZ091502/RR      .......... .......... .......... ........F.  [40]
MZ091503/RR      .......... .......... .......... ........F.  [40]
MZ091504/RS      .......... .......... .......... ..........  [40]
MZ091505/RR      .......... .......... .......... ........F.  [40]
MZ091506/RR      .......... .......... .......... ........F.  [40]
MZ091507/RS      .......... .......... .......... ..........  [40]
MZ091508/RS      .......... .......... .......... ..........  [40]
MZ091509/SS      .......... .......... .......... ..........  [40]
MZ091510/RR      .......... .......... .......... ........F.  [40]
MZ091511/RR      .......... .......... .......... ........F.  [40]
MZ091512/RR      .......... .......... .......... ........F.  [40]
MZ091513/RR      .......... .......... .......... ........F.  [40]
MZ091514/RR      .......... .......... .......... ........F.  [40]
MZ091515/RR      .......... .......... .......... ........F.  [40]
MZ091516/RR      .......... .......... .......... ........F.  [40]
MZ091517/RS      .......... .......... .......... ..........  [40]
MZ091518/RR      .......... .......... .......... ........F.  [40]
MZ091519/RR      .......... .......... .......... ........F.  [40]
MZ091520/RR      .......... .......... .......... ........F.  [40]
MZ091521/RR      .......... .......... .......... ........F.  [40]
MZ091522/RR      .......... .......... .......... ........F.  [40]
MZ091523/RR      .......... .......... .......... ........F.  [40]
MZ091524/RR      .......... .......... .......... ........F.  [40]
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Fig. 3: Alignment comparison of a part of the amino acid sequences of exon 20 of the sodium channel gene of the studied German cockroach. The highlighted amino acid related to *kdr* resistance shows that the amino acid phenylalanine has been replaced by leucine

Discussion

In Mashhad City due to over tourism, has to spray insecticides all over its hotels and restaurants to keep them free of pests. We identified heterogeneity in insecticide resistance among the eight cockroach populations. Several studies in Iran have revealed that German cockroaches were insecticide resistant, mainly commercial pesticides (41, 42). It appears that hotels and restaurants use different types of insecticides at different rates and for varying periods at the site where the samples were collected (Table 1), which may lead to variations in the susceptibility of cockroaches to insecticide.

German Cockroaches have become resistant to numerous pesticides due to a multitude of factors. One significant cause is the overutilization and improper application of pesticides. When pesticides are employed excessively or at improper concentrations, cockroaches can rapidly develop resistance to them. Moreover, when pesticides are not cyclically changed, cockroaches may become resistant to multiple chemicals simultaneously. Another contributing factor is the genetic diversity within cockroach populations, where certain individuals may possess genetic mutations conferring resistance to specific pesticides. Lastly, cockroaches may also develop resistance through exposure to low levels of pesticides, a phenomenon referred to as "survival of the fittest," whereby the cockroaches that are resistant to the chemicals survive and reproduce, transmitting their resistance to their progeny. Furthermore, almost all of the field-collected strains were resistant to the tested insecticide at different levels (Table 2). Bioassays as well as molecular studies have confirmed that the collected cockroach populations differ in their susceptibility to insecticide. Wu and Appel investigated the responses of the six field-collected German cockroach strains to five insecticides and found significant differences in resistance among the six strains. The authors suggested that field-collected strains were likely to have had different treatment histories (19).

In Mashhad City, cypermethrin was the most frequently used insecticide against urban pests. Thus, we used cypermethrin as an indicator to demonstrate the effect of using an insecticide periodically on resistance development. Cypermethrin could develop insecticide resistance rapidly in German cockroaches via metabolic mechanisms and contact with their thorax or tarsal pad (43-45). Mixed cypermethrin and PBO were commonly used in the hotels and restaurants of Mashhad. Cypermethrin selection pressure applied to a German cockroach population for three generations led to rapid increases in resistance levels (46).

The relative contributions of metabolic detoxification and target-site mechanisms of resistance are strain-dependent (47, 48).The excessive use of pesticides at high concentrations in Mashhad has led to the development of resistance to cypermethrin among German cockroach strains.

Conclusion

The study revealed a substantial level of insecticide resistance and heterogeneity in German cockroach populations collected from various regions of Mashhad City, with variations observed in the magnitude of resistance and specific mutations detected in the *kdr* gene linked to this resistance. This underscores the necessity for integrated pest management approaches that integrate a blend of diverse control methods to efficiently regulate cockroach populations and mitigate the likelihood of insecticide resistance development.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Competing interests

The authors declare that they have no competing interests.

References

1. Gore JC, Schal C (2007). Cockroach allergen biology and mitigation in the indoor environment. *Annu Rev Entomol*, 52:439-63.
2. Mengoni SL, Alzogaray RA (2018). Deltamethrin-resistant German cockroaches are less sensitive to the insect repellents DEET and IR3535 than non-resistant individuals. *J Econ Entomol*, 111(2):836-43.
3. Shahraki GH, Parhizkar S, Nejad ARS (2013). Cockroach infestation and factors affecting the estimation of cockroach population in urban communities. *Int J Zool,* 2013: 649089.
4. Pridgeon JW, Liu N (2003). Overexpression of the cytochrome c oxidase subunit I gene associated with a pyrethroid resistant strain of German cockroaches, *Blattella germanica* (L.). *Insect Biochem Mol Biol*, 33(10):1043-8.
5. Pridgeon JW, Zhang L, Liu N (2003). Overexpression of CYP4G19 associated with a pyrethroid-resistant strain of the German cockroach, *Blattella germanica* (L.). *Gene*, 314:157–63.
6. Rahimian AA, Hanafi-Bojd AA, Vatandoost H, et al (2019). A review on the insecticide

resistance of three species of cockroaches (Blattodea: Blattidae) in Iran. *J Econ Entomol*, 112(1): 1–10.
7. Do DC, Zhao Y, Gao P (2016). Cockroach allergen exposure and risk of asthma. *Allergy*, 71(4):463–74.
8. Sookrung N, Chaicumpa W (2010). A revisit to cockroach allergens. *Asian Pac J Allergy Immunol*, 28(2-3):95-106.
9. Menasria T, Moussa F, El-Hamza S, et al (2014). Bacterial load of German cockroach (*Blattella germanica*) found in hospital environment. *Pathog Glob Health*, 108(3):141-7.
10. Solomon F, Belayneh F, Kibru G, et al (2016). Vector potential of *Blattella germanica* (L.) (Dictyoptera: Blattidae) for medically important bacteria at food handling establishments in Jimma town, Southwest Ethiopia. *Biomed Res Int,* 2016: 3490906.
11. Gondhalekar AD, Appel AG, Thomas GM, et al (2021). A review of alternative management tactics employed for the control of various cockroach species (Order: Blattodea) in the USA. *Insects,* 12(6): 550.
12. Zha C, Wang C, Buckley B, et al(2018). Pest prevalence and evaluation of community-wide integrated pest management for reducing cockroach infestations and indoor insecticide residues. *J Econ Entomol*, 111(2):795-802.
13. Hemingway J, Dunbar SJ, Monro AG, et al (1993). Pyrethroid resistance in German cockroaches (Dictyoptera, Blattelidae) - Resistance levels and underlying mechanisms. *J Econ Entomol*, 86(6):1631-8.
14. Wang C, Scharf M, Bennett G (2004). Behavioral and physiological resistance of the German cockroach to Gel Baits (Blattodea: Blattellidae). *J Econ Entomol*, 97:2067–72.
15. Zhu F, Lavine L, O'Neal S, et al (2016). Insecticide resistance and management strategies in urban ecosystems. *Insects*, 7(1):2.
16. Whalon M, Mota-Sanchez D, Hollingworth R (2008). Global Pesticide Resistance in Arthropods. CABI Publishing, Cromwell Press, Trowbridge, UK, pp 169.
17. Lee SH, Choe DH, Rust MK, et al (2022). Reduced susceptibility towards commercial bait insecticides in field German cockroach (Blattodea: Ectobiidae) populations from california. *J Econ Entomol*, 115(1):259-65.
18. Umeda K, Yano T, Hirano M (1988). Pyrethroid-resistance mechanism in German cockroach, Blattella germanica (Orthoptera: Blattellidae). *Appl Entomol Zool*, 23(4):373-80.
19. Wu X, Appel AG (2017). Insecticide resistance of several field-collected German cockroach (Dictyoptera: Blattellidae) strains. *J Econ Entomol,* 110(3):1203–9.
20. Scharf ME, Neal JJ, Bennett GW (1998). Changes of insecticide resistance levels and detoxication enzymes following insecticide selection in the German cockroach, *Blattella germanica* (L.). *Pestic Biochem Physiol*, 59(2):67–79.
21. De Vries ZC, Santangelo RG, Crissman J, et al (2019). Exposure risks and ineffectiveness of total release foggers (TRFs) used for cockroach control in residential settings. *BMC Public Health*, 19(1):96.
22. Georghiou GP (1972). The evolution of resistance to pesticides. *Annu Rev Ecol Syst,* 3(1):133–68.
23. Zalucki MP, Furlong MJ (2017). Behavior as a mechanism of insecticide resistance: evaluation of the evidence. *Curr Opin Insect Sci,* 21:19–25.
24. Sparks TC, Lockwood JA, Byford RL, et al (1989). The role of behavior in insecticide resistance. *Pestic Sci,* 26(4):383–99.
25. Hostetler ME, Brenner RJ (1994). Behavioral and physiological resistance to insecticides in the German cockroach (Dictyoptera: Blattellidae): an experimental reevaluation. *J Econ Entomol*, 87(4):885–93.
26. Dong K (1997). A single amino acid change in the para sodium channel protein is associated with knockdown-resistance (*kdr*) to pyrethroid insecticides in German cockroach. *Insect Biochem Mol Biol*, 27(2):93–100.
27. Zahraei-Ramazani AR, Saghafipour A, Vatandoost H (2018). Control of American cockroach (*Periplaneta americana*) in municipal sewage disposal system, central Iran. *J Arthropod Borne Dis*, 12(2):172-9.
28. Hou W, Xin J, Lu H (2021). Resistance development characteristics of reared German cockroach (Blattodea: Blattellidae) to chlorpyrifos. *Sci Rep,* 11(1):3505.

29. Bloomquist JR (1996). Ion channels as targets for insecticides. *Annu Rev Entomol,* 41(1):163–90.
30. Busvine JR (1951). Mechanism of resistance to insecticide in houseflies. *Nature,* 168(4266):193–5.
31. Omotayo AI, Ande AT, Oduola AO, et al (2022). Multiple insecticide resistance mechanisms in urban population of *Anopheles coluzzii* (Diptera: culicidae) from Lagos, South-West Nigeria. *Acta Trop,* 227:106291.
32. Scharf ME, Gondhalekar AD (2021). Insecticide resistance: perspectives on evolution, monitoring, mechanisms and management. In: Biology and Management of the German Cockroach, ed, C Wang, CY Lee, M Rust, CSIRO Publishing, Ordibehesht 13, pp 231-56.
33. Liu Z, Valles SM, Dong K (2000). Novel point mutations in the German cockroach para sodium channel gene are associated with knockdown resistance (*kdr*) to pyrethroid insecticides. *Insect Biochem Mol Biol,* 30(10):991–7.
34. Ladonni H (2001). Evaluation of three methods for detecting permethrin resistance in adult and nymphal *Blattella germanica* (Dictyoptera: Blattellidae). *J Econ Entomol*, 94(3):694-7.
35. Nasirian H, Ladonni H, Shayeghi M, et al (2006). Duration of fipronil WHO glass jar method toxicity against susceptible and feral German cockroach strains. *Pakistan J Biol Sci*, 9(10):1955-1959.
36. Fardisi M, Gondhalekar AD, Scharf ME (2017). Development of diagnostic insecticide concentrations and assessment of insecticide susceptibility in German Cockroach (Dictyoptera: Blattellidae) field strains collected from public housing. *J Econ Entomol*, 110(3):1210-1217.
37. Wadley FM (1962). Probit Analysis: a Statistical Treatment of the Sigmoid Response Curve. 2nd Ed. Cambridge Univ. Press, London, UK, pp 318.
38. Gholizadeh S, Nouroozi B, Ladonni H (2014). Molecular detection of knockdown resistance (*kdr*) in *Blattella germanica* (Blattodea: Blattellidae) from northwestern Iran. *J Med Entomol,* 51(5):976–9.
39. Sievers F, Higgins DG (2018). Clustal omega for making accurate alignments of many protein sequences. *Protein Sci,* 27(1):135–45.
40. Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol,* 33 (7):1870–4.
41. Farmani M, Basseri HR, Norouzi B, et al (2019). Ribosomal DNA internal transcribed spacer 2 sequence analysis and phylogenetic comparison of seven cockroach species in northwestern Iran. *BMC Res Notes,* 12(1):53.
42. Ghaderi A, Baniardalani M, Basseri HR (2021). Level of pyrethroid-resistance associated with cytochrome P450 expression in German cockroach *Blattella germanica* (Blattodea: Ectobiidae) in the field collected strains. *J Arthropod Borne Dis*, 15(2):152–61.
43. Nardini L, Christian RN, Coetzer N, et al (2012). Detoxification enzymes associated with insecticide resistance in laboratory strains of *Anopheles arabiensis* of different geographic origin. *Parasit Vectors,* 5(1):113.
44. Valles SM, Dong K, Brenner RJ (2000). Mechanisms responsible for cypermethrin resistance in a strain of German cockroach, *Blattella germanica. Pestic Biochem Physiol,* 66(3):195–205.
45. Zhai J, Robinson WH (1992). Measuring cypermethrin resistance in the German cockroach (Orthoptera: Blattellidae). *J Econ Entomol,* 85(2):348–51.
46. Valles SM (2004). Effects of cypermethrin Sselection on expression of insecticide Resistance Mechanisms in the German Cockroach (Blattaria: Blattellidae). *J Entomol Sci,* 39(1):84–93.
47. Brengues C, Hawkes NJ, Chandre F, et al (2003). Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Med Vet Entomol*, 17(1):87–94.
48. Chang X, Zhong D, Lo E, et al (2016). Landscape genetic structure and evolutionary genetics of insecticide resistance gene mutations in *Anopheles sinensis. Parasit Vectors,* 9:228.