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Induction of insecticide tolerance in German cockroach (Dictyoptera: Blattellidae) due to sublethal doses of imidacloprid, indoxacarb, and lambda-cyhalothrin

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ABSTRACT

The German cockroach is a global urban pest that causes serious public health problems. The induced levels of insecticide tolerance in the first-generation strains of German cockroach from previous topical treatment of laboratory colonies with LD_{10} or LD_{25} doses of imidacloprid, indoxacarb, or lambda-cyhalothrin four times with seven-day interval between each were investigated. Our results showed that the resistant ratios (RR_{50}) at LD_{50}, cytochrome P450 content, and glutathione S-transferase (GSTs) activity in adult cockroaches increased in the first generation when compared to the parental (field) and susceptible strains (SS). Therefore, cockroaches treated with insecticidal sublethal dose are likely to have more insecticide resistance in comparison to untreated ones.

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Blattella germanica; insecticide resistance induction; sublethal dose; imidacloprid; indoxacarb; lambda-cyhalothrin

Introduction

The German cockroach, *Blattella germanica* (L.), is a common household pest living in close association with humans around the world (Tang et al. 2019). Cockroach is a serious risk to public health as they are able to 1) mechanically or physically transmit many human pathogenic microorganisms and parasites, such as bacteria (Jalil et al. 2012), medically important fungi (Haghi et al. 2014), and parasitic worms (Salehzadeh et al. 2007), and 2) cause allergic reactions and asthma among people (Mueller et al. 2015).

Generally, pyrethroid, neonicotinoid, and oxadiazine insecticides are effective and have lower mammalian toxicity compared with that of other groups of insecticides (Dalefield 2017), hence widely used. Frequent application of insecticides has resulted in resistance to many insecticide groups in the German cockroach (Chang et al. 2010). Although frequently intended to rapidly eliminate a targeted pest species, over time, insecticide molecules degrade to sublethal levels in the outside environment, thereby exhibiting less lethal toxicity to insects and other organisms (Kreutzweiser et al. 2008; Edwards 2013). Physiological and behavioral changes resulting from this issue may affect pest management (Wang et al. 2004). Prolonged exposure to sublethal doses of pesticides may lead to the development of pesticide resistance (Hardin et al. 1995; Gressel 2011; Guedes et al. 2016, 2017).

Resistance evolution by pests, including the German cockroach, is the current challenge in insect control and has prompted the use of alternative management tactics to reduce the economic losses. The first case of insecticide resistance in German cockroach was resistance to organochlorines (DDT) in Texas, the United States (Heal et al. 1953), followed by organophosphates (Grayson 1965), carbamates (McDonald and Cochran 1968), pyrethroids (Lee et al. 1996), neonicotinoids (Wen et al. 2009), and oxadiazines (Ko et al. 2016). Many new records have been reported at several locations worldwide, including Iran. Insecticide resistance in the German cockroach has been recorded in some Iranian cities such as Sari (Enayati and Motevali 2007), Kermanshah (Limoee et al. 2011), and Shiraz (Moemenbellah-Fard et al. 2013).

Several mechanisms have been studied in relation to insecticide resistance in German cockroaches. Valles et al. (2000) found reduced insecticide penetration in resistant strains. The changes in amino acids responsible for insecticide binding at its site of action caused the insecticide to be less or even ineffective (Dong 1997). Moreover, one of the most

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important resistance mechanisms in insect pests is the increase in detoxifying enzymes, such as cytochrome P450s, esterases, oxidases, and glutathione S-transferases (GST) (Enayati and Motevali 2007 Limoee et al. 2011; Kasai et al. 2014, Lin et al. 2014).

The objectives of the present study were (1) to determine the susceptibility of the first generation of German cockroaches to indoxacarb, imidacloprid, and lambda-cyhalothrin, where the parental strains were topically treated with LD_{10} or LD_{25} of indoxacarb, imidacloprid, and lambda-cyhalothrin and (2) to determine the total content and activity of the detoxifying enzymes, P450s and GSTs in the first-generation colonies.

Materials and methods

Cockroaches and rearing conditions

Two German cockroach strains were tested in this study: (1) using a vacuum apparatus similar to the one designed by Wright (1966), the field strain was originally collected from infested apartments and houses in the central part of Mashhad, Iran, in the summer of 2017. These places had frequently been treated with pyrethroids insecticides (local information); (2) the susceptible strain (SS) was freely provided by the School of Public Health, Tehran University of Medical Sciences and has been maintained in the laboratory since 1975 without insecticide exposure. The *B. germanica* colonies were established in plastic cages (30 × 30 × 30 cm) and supplied with food and water *ad libitum* as described by Piquett and Fales (1952) at the Toxicology Lab, Plant Protection Department, Ferdowsi University of Mashhad, Iran. The rearing conditions consisted of 27 ± 2 °C, 70 ± 5% relative humidity (RH), and 12:12 (L:D) photoperiod. Adults from the fourth and fifth generations were employed in the bioassays.

Insecticides and chemicals

Technical grade imidacloprid (97.00%), indoxacarb (96.2%), and lambda-cyhalothrin (97.31%) were a gift from Kavosh Kimia Kerman Co., Ltd (Kerman, Iran). Carbon monoxide gas (CO, 99.95% purity) was purchased from Faran Sanat Co. (Tehran, Iran). Acetone, ethanol, isopropanol, Tris HC1 buffer, phosphate-buffered saline (PBS), glycerol, dithiothreitol (DTT), safranin, ethylenediaminetetraacetic acid (EDTA), 1-chloro-2, 4-dinitrobenzene (CDNB), glutathione (GSH), phenylmethylsulfonyl fluoride (PMSF), sodium dithionite ($Na_2S_2O_4$), and other chemicals, which had high analytical grades (> 95% quality), were obtained from Kian Chemistry Co. (Mashhad, Iran).

Bioassay

LD Determination

Toxicity assays to specify the susceptibility of field and susceptible strains (600 males and 600 females each insecticide) to indoxacarb, imidacloprid, and lambda-cyhalothrin were performed with a range of concentrations for each insecticide (Table 1). Technical grade formulations of each insecticide were serially diluted in acetone. Selected cockroaches were anesthetized using CO_2 (Sherman and Hayakawa 1961). Using a repeating micropipette (Hamilton Company, Reno, NV), one microliter of each insecticide dose (or 1 µl of acetone alone as control) was topically applied to the ventral portion of each insect between the metacoxae according to the method described by Ko et al. (2016). A total of 45 cockroaches were topically treated with each dose. Three replications per dose (15 insects in each replication) were maintained in plastic containers (21 × 12 × 7 cm) that were

Table 1. Range of applied concentrations for each insecticide (indoxacarb, imidacloprid, and lambda-cyhalothrin) to the field and susceptible strains of *Blatella germanica* using topical application method.

Insecticides AI[a]	Susceptible strain Dose / ppm	Field strain Dose / ppm	After treated with LD doses / ppm LD10	LD25
Imidacloprid (97.00%)	30	50	120	140
	70	106	258	297
	160	224	549	627
	368	473	1172	1325
	850	1000	2500	2800
Indoxacarb (96.2%)	10	15	35	50
	24	35	78	106
	73	82	173	224
	136	192	383	473
	325	450	850	1000
lambda-cyhalothrin (97.31%)	15	20	30	40
	33	45	67	87
	73	100	158	190
	160	224	352	413
	350	500	800	900

[a]Active Ingredient

properly sealed with fine nylon mesh fabric cloth at the top for ventilation and provided with food and water *ad libitum*. Following 72 h, mortality was checked and corrected using Abbott's formula (Abbot); afterwards, the data were pooled and analysed to specify LD indices, including LD_{10} and LD25 for each insecticide using a standard probit analysis (Le Ora 1987).

Topical application of LD10 and LD25 on B. germainca adults

Once LD values were determined, virgin males and females (3–6 days old) of the field strain were anesthetised using CO_2; they were then separately treated through delivering 1 µl of LD_{10} or LD_{25} of lambda-cyhalothrin, imidacloprid, indoxacarb, or 1 µl acetone in the control group using topical application method. In the same group, four applications were employed with a seven-day interval between each. Six replications [three replications for males and three for females (20 insects in each replication)] with 120 individuals (60 males and 60 females) per dose were used. Cockroaches of each replication were placed into a small plastic container (21 × 12 × 7 cm).

For each insecticide, two colonies were generated from the first generation based on the LD_{10} or LD_{25} dose: (1) for imidacloprid, QF1M and ZF1M, (2) for indoxacarb, QF1N and ZF1N, and (3) for lambda-cyhalothrin, QF1L and ZF1L, which were used in subsequent bioassay experiments.

Resistance ratio assays

To assess the resistance ratio (RR) of QF1M, ZF1M, QF1N, ZF1N, QF1L, ZF1L, and field strains, 20 individuals per replication, three replications per dose, and five doses each insecticide yielding > 0 and < 100 mortality were utilized as previously described in topical bioassays. The treated cockroaches were placed into plastic containers as described above. Food and water were provided *ad libitum* and maintained under optimum rearing conditions. After 72 h, mortality was recorded, and the cockroaches that did not move were considered as dead. Data on mortality from the three replicates were corrected using Abbott's formula and then pooled and analysed using standard probit analysis. If the LD_{50} values of non-overlap within the 95% confidence interval (CI) limits of the lethal dose ratio did not contain one, they were considered as significantly different (Robertson et al. 2017).

The resistance ratio (RR) of cockroaches against each insecticide was computed using the formula:

$$RR = \frac{LD50\ RS}{LD50\ SS}$$

In which **RS** is the resistant strain (**F1** from field strain), and **SS** is the susceptible strain (**SS**).

Cytochrome P450 preparation

Ten females per replication and three replications per dose (LD_{10} and LD_{25}) were used. The cockroaches were chilled at −20 °C for 2 to 3 min and immediately washed out once with ethanol 70%; next, they were washed three times with dH_2O and dissected out in ice-cold buffer comprising 1.15% KCl by cutting and removing the head, thorax, legs, and wings.

PMSF (1 mM) was added to the remaining abdomens and then homogenized for 30 sec using a Teflon-pestle homogenizer and a small glass mortar with 5 ml of homogenization buffer (100 mM TrisHC1 pH 7.5, 1.15% KC1, 20% glycerol, 1 mM EDTA, and 0.2 mM DDT). The suspension was filtered using three layers of cheesecloth and centrifuged at 10,000 g for 25 min at 4 °C. The supernatant was filtered through two layers of cheesecloth and recentrifuged at 105,000 g for 1 h at 4 °C using a Beckman optima L-90k ultracentrifuge (BECKMAN, USA). The sediment was suspended in 1 ml of resuspension buffer (0.1 M PBS, pH 7.4, containing 0.1 mM DTT, 1 mM PMSF, 20% (v/v) glycerol, and 1 mM EDTA) (Scott and Lee 1993, 1993b). The Bradford protein assay (Bradford 1976) was utilized to determine the protein concentration in the final suspension of each replication using the bovine serum as a reference and diluted it to 2 mg protein.ml^{-1}. The resulting suspensions were stored at −80 °C until use.

Cytochrome P450 contents were estimated via measuring the difference spectrum of dithionite-reduced carbon monoxide (CO) according to the method proposed by Omura and Sato (1964).

The P450 source was put into both reference and sample cuvettes and the baseline was recorded using a spectrophotometer instrument ultraviolet/visible-near infrared (UV/Vis-NIR from Researchers of Nanotechnology Co. Iran). In the fume hood, the sample content was saturated with CO by delivering approximately 30 to 60 bubbles in 30 sec. To enhance the reduction of dithionite, 1 mg of sodium dithionite was added to the sample and the reference cells. Two cuvettes (reference and sample) were

covered with parafilm, and the cuvette content was mixed to dissolve sodium dithionite by inverting and re-inverting for 10 to 15 times (without shaking vigorously). To decrease the reduction time (from 20 min to ~3 to 5 min), 1.5 µM safranin was added to both the reference and sample cuvettes (Sandhu et al. 1994). The cuvettes were returned to the same place in the spectrophotometer device, and the wavelengths between 400 and 500 nm were recorded several times over a few minutes (5–10 min). The resulting spectra were printed out from the spectrophotometer instrument. Wave absorption was set to 420, 450, and 490, and P450 concentration was then calculated by the formulas shown below (Guengerich et al. 2009):

$$\frac{(\Delta A_{450} - \Delta A_{490})}{0.091} = nmol\ of\ P_{450}\ per\ ml$$

when there was a difference between the baseline spectrum in the absorbance at 450 and 490 nm, we used the correction equation as below:

$$\frac{\left[\left(A_{450} - A_{490}\right)_{observed} - \left(A_{450} - A_{490}\right)_{baseline}\right]}{0.091} = nmol\ P_{450}\ of\ per\ ml$$

when the P450 had denatured forms, the following equation had been used to estimate cytochrome P450 content:

$$nmol\ of\ P450\ per\ ml\left(\text{from first formula}\right)\times\left(-0.041\right) = \left(\Delta A_{420} - A_{490}\right) theoretical$$

$$\frac{\left[\begin{array}{l}\left(\Delta A_{420} - A_{490}\right)_{obnserved} - \left(A_{450} - A_{490}\right)_{theoretical} \\ -\left(\Delta A_{420} - A_{490}\right)_{baseline}\end{array}\right]}{0.110} = nmol\ of\ P450\ per\ ml$$

In which **ΔA450**= absorbance at 450 nm, **ΔA490**= absorbance at 490 nm, **0.091**= extinction coefficient at 450 nm.

All spectrophotometric procedures were conducted at 20 to 25 °C.

The differences in P450 concentrations among F1 (QF1M, ZF1M, QF1N, ZF1N, QF1L, and ZF1L), field, and SS strains were estimated by one-way analysis of variance (ANOVA); furthermore, the means of treatments were compared by running a post-hoc Tukey test at 95% CI. The significance level was $P \leq 0.05$. The Minitab™ 17 computer software was used to analyse all listed data (MINITAB Inc., State College, PA, USA).

GST preparation

The same method described above was employed to prepare the protein samples for GST activity. Ten females of the F1 strain per replication were homogenized using a small glass mortar and a Teflon-pestle homogenizer for 30 sec in 5 ml of ice-cold 0.1 M sodium phosphate buffer with a pH of 7.5. The resulting suspension was filtered through three layers of cheesecloth and centrifuged at 10,000 g for 15 min at 4 °C. The supernatant solution was re-filtered through glass wool and recentrifuged at 105,000 g at 4 °C for 1 h using the Beckman optima L-90k ultracentrifuge (BECKMAN, USA) (Qin et al. 2013). The resulting supernatant was considered as the source of the GSTs. Following dilution, the final protein concentration was 2 mg.ml^{-1} using Bradford protein assay and bovine serum albumin (BSA) as a standard. The sample was stored at −80 °C until use.

The activity of the GST enzyme toward 1-chloro-2,4-dinitrobenzene (CDNB) was examined in 96-well polystyrene plates. Therefore, 0.25% Polysorbate-20 was used to wash the plate wells prior to use (Habig et al. 1974) with 100 µl of the mixture A, which contained 10 µl of the final supernatant, 8 mM GSH, and 100 mM PBS; 15% glycerol at pH 8.0 was loaded into the wells and incubated at room temperature for 3 min. Furthermore, 200 µl of the mixture B (100 mM PBS, 1 mM CDNB, 15% glycerol) was added to the mixture A and shaken for 5 sec. The optical density (OD) was measured at 340 nm every 1 min for 5 min using a microplate reader, stat fax 2100 (Awareness Technology, USA). The activity of GST was calculated according to the following equation:

$$GST\ Activity = \frac{\Delta ODA\ 340\, min^{-1} \times Reaction\ Volume}{0.0096\, \mu ml^{-1} cm^{-1} \times 1000\, ml \times 0.2893\, cm \times V} \times D$$

In which **V**= the sample volume added to well (ml), **0.0096** = the extinction coefficient for CDNB conjugate at 340 nm, and **D** = the dilution factor of the original sample.

Activities of GST were expressed as µM/min/ml protein. The data of GST activities were subjected to one-way ANOVA analysis, and the means of enzyme activity were compared by the post-hoc Tukey test at 95% CI.

Results

Dose toxicity assays and topical application of LD_{10} and LD_{25} on field strain were analysed. The LD50s ranged from 227.17 to 300.86 ppm for imidacloprid, from 92.96 to 131.92 ppm for indoxacarb, and from 112.36 to 159.40 ppm for lambda-cyhalothrin (Table 2). RRs between field and SS strain for these insecticides were 1.35, 1.44, and 1.410, respectively (Table 2).

Resistance bioassay

The analysed data demonstrated that compared to the susceptible strain (SS), the first-generation groups of the collected field strain, previously treated with sublethal doses of imidacloprid, indoxacarb, or lambda-cyhalothrin (i.e., QF1M, ZF1M, QF1N, ZF1N, QF1L, and ZF1L), were resistant to these insecticides. Therefore, the insecticide resistance in these strains was low-intensity (< 5-fold) to imidacloprid, indoxacarb, and lambda-cyhalothrin depending on LD50 values with 95% CI (3.290-, 3.766-, 2.724-, 3.453-, 2.056-, and 2.484-fold, respectively). Additionally, the RR values of field strain towards these insecticides were 1.40-fold compared to the susceptible strain (SS). However, the strains treated with LD25 of these insecticides exhibited resistant levels higher than those treated with LD10 compared to the field or SS strain (Table 2).

Cytochrome P450s and glutathione S-transferees assays

Statistical differences were observed in the total P450 content ($F = 44.55$; $df = 7$; $P < 0.001$) and in the GST activity ($F = 23.47$; $df = 7$; $P < 0.001$) among the strains (Tables 2 and 3). All strains exposed to the LD25 dose (regardless of the insecticide) showed a higher total P450 content when compared to field or SS strains (Table 3). Two of the three strains exposed to LD_{10} had a higher total P450 content as compared to the field strains (Table 3).

Table 2. Toxicity of three insecticides to eight strains of *Blatella germanica* using topical application method.

Insecticide	N[a]	Cockroach strain	Slope ± SE[b]	X^2(df)	Lethal dose (ppm)[c] LD50 (95%CI)	RR50[d]
Imidacloprid	300	QF1M	2.40 ± 0.23	2.047(3)	631.84 (534.37-749.99)	3.290
	300	ZF1M	2.56 ± 0.24	2.203(3)	723.24 (616.26-852.39)	3.766
	300	field	2.54 ± 0.20	2.635(3)	261.01 (227.17-300.86)	1.359
	300	SS	2.09 ± 0.20	2.306(3)	192.02 (158.93-233.40)	1.000
Indoxacarb	300	QF1N	2.08 ± 0.21	2.238(3)	209.49 (173.75-254.36)	2.724
	300	ZF1N	2.54 ± 0.31	2.754(3)	265.60 (214.59-319.80)	3.453
	300	field	2.30 ± 0.22	0.772(3)	111.23 (92.96-131.92)	1.446
	300	SS	2.15 ± 2.14	2.636(3)	76.90 (63.61-93.20)	1.000
Lambda-cyhalothrin	300	QF1L	2.08 ± 0.20	1.857(3)	197.13 (163.13-240.11)	2.056
	300	ZF1L	2.52 ± 0.33	1.351(3)	238.19 (189.48-288.68)	2.484
	300	field	2.50 ± 0.27	1.942(3)	135.20 (112.36-159.40)	1.410
	300	SS	2.43 ± 0.23	2.056(3)	95.88 (81.03-114.20)	1.000

[a]number of insects per insecticide test (60 insect × 5 concentrations).
[b]slope is parameter model.
[c]Lethal dose (*ppm* AI insecticide/g insect) estimated by (mean ± SEM) of insect body per each group QF1M = 0.0482 ± 0.0018, ZF1M = 0.0434 ± 0.0010, QF1N = 0.0464 ± 0.0019, ZF1N = 0.0416 ± 0.0010, QF1L = 0.0402 ± 0.0014, ZF1L = 0.043 ± 0.0015, and field = 0.0517 ± 0.000.0010 and SS = 0.0355 ± 0.0011.
[d]RR resistant ratio at LD_{50} = LD_{50} tested strain ÷ LD_{50} susceptible strain and their 95% confidant interval.

Table 3. Cytochrome P_{450s} content enzyme in first-generation treated cockroaches with insecticidal sublethal dose i.e., (LD10 or LD25), as well as, in collected cockroaches (NG), and susceptible strain (SS).

Insecticide	Cockroach strain[a]	Mean ± St. Dev. P450 nmol/ml[b]	Theoretical[c]	Observed[d]	Real P450[e]	Final P450 nmol/ml[f]
Imidacloprid	QF1M	1.758 ± 0.000	−0.07209 ± 0.002	−0.05	0.2008 ± 0.000	1.205 ± 0.000 **BC**
	ZF1M	1.6850 ± 0.0634	−0.06908 ± 0.002	−0.05	0.2644 ± 0.023	1.5864 ± 0.1419 **A**
Indoxacarb	QF1N	1.5751 ± 0.0634	−0.06608 ± 0.002	−0.05	0.2234 ± 0.023	1.3407 ± 0.1419 **AB**
	ZF1N	1.9414 ± 0.0634	−0.0796 ± 0.002	−0.05	0.2691 ± 0.023	1.6144 ± 0.1419 **A**
Lambda-cyhalothrin	QF1L	1.7949 ± 0.0634	−0.07359 ± 0	−0.05	0.2145 ± 0.0236	1.2867 ± 0.1419 **AB**
	ZF1L	1.9414 ± 0.0634	−0.0796 ± 0.002	−0.05	0.2691 ± 0.0236	1.6144 ± 0.1419 **A**
Without insecticide	field	1.3919 ± 0.0567	−0.05632 ± 0.002	−0.04	0.15518 ± 0.021	0.9311 ± 0.1269 **C**
Without insecticide	SS	0.6960 ± 0.0567	−0.02778 ± 0.001	−0.02	0.07759 ± 0.021	0.4655 ± 0.1269 **D**

[a]Insect strains that were treated with sublethal doses(i.e,LD10 or LD25) of Imidacloprid (QF1M and ZF1M), Indoxacarb (QF1N and ZF1N), and Lambda-cyhalothrin (QF1L and ZF1L) respectively, as well as, collected strain (NG) and susceptible strain (SS).
[b]P450 = (ΔA450 — ΔA490) / 0.091= nmol of P450 per ml protein.
[c]Theoretical (A420–A490)= P450 x −0.041.
[d]Observed = A420–A490.
[e]Real P450 = (Observed – Theoretical – baseline) / 0.110 = nmol of P450 per ml protein.
[F]Final P450 = Real P450 x Dilution = nmol of P450 per ml protein.
Means followed by the same letters within the same column are not significantly different.

All strains exhibited a higher GST activity than the SS strain (Table 4). The strains exposed to LD25 showed a higher GST activity in comparison to the field strain (Table 4).

Discussion

One of the limitations associated with managing insect pest, such as the German cockroach, is the development of insecticide resistance, which is considered as a serious challenge. The development of insecticide-resistant phenomena is a result of several physiological modifications and biochemical changes in an insect's in *vivo* system. Therefore, the mechanism of insecticide resistance in the German cockroach typically involves the modification of the target site and/or resistant metabolism (metabolic detoxification) (Pridgeon et al. 2002; Chai and Lee 2010). Generally, the physiological changes are genetically transferred from one generation to the next. However, a molecular test on insecticide resistance showed that the responsible gene of insecticide resistance more frequently appeared in the resistant phenotype (Ffrench-Constant 2013).

Field strain cockroaches were collected from infested houses and apartments in the central part of Mashhad, Iran; these insects had been frequently treated with pyrethroids insecticides (local information) and then reared in the Toxicology Lab, Plant Protection Department, Ferdowsi University of Mashhad, Iran (see Materials and Methods). Many of the previous studies showed that topical application was more suitable for susceptibility bioassay studies appropriate (Choo et al. 2000; Ladonni 2001); therefore, the topical application method was used in the present study.

Based on our results, sublethal doses of imidacloprid, indoxacarb, and lambda-cyhalothrin induced multiple biochemical changes in adults of *B. germanica* when topically treated several times. We also focused on the induction of insecticide tolerance in an insect population following multiple exposures to sublethal doses of insecticide. Additionally, the same mechanism can evolve to other insecticides classes with the same mode of action (Zhu et al. 2016). Therefore, when the resistant bioassays were conducted on the first generation of cockroaches, the adult cockroaches were found to show low resistance to the same insecticide after initially treating the parental strain with the LD_{10} or LD_{25} of those insecticides. As a result, there were significant differences in terms of LD_{50s} among strains (Table 2) compared to the parental strain (field strain) and SS. The foregoing strains had a low-intensity insecticide resistance (< 5-fold) (World Health Organization 2016) to these insecticides depending on the LD_{50} values with 95% CI; however, this shows the impact of an insecticidal sublethal dose (quantity and quality) of several times on the development of insecticide resistance. The RRs at LD_{50} increased after exposure to insecticides in F1 when compared to SS, which is in line with the previous results obtained by Ko et al. (2016) depending on the sublethal dose value that was previously used. In other words, the RR_{50} values of QF1M (3.290), QF1N (2.724), and QF1L (2.056) strains were less than ZF1M (3.766), ZF1N (3.453), and ZF1L (2.484), which is consistent with (Hardin et al. 1995; Gressel 2011; Amarasekare et al. 2016; Guedes et al. 2016, 2017; Ko et al. 2016).

Moreover, the resistant ratios at LD_{50} of field strain toward indoxacarb, lambda-cyhalothrin, and imidacloprid insecticides were approximately 1.4 fold compared to the susceptible strain (SS) (Table 2). Although indoxacarb and imidacloprid insecticides were not used to control German cockroach or other household pests in collection areas. In contrast, pyrethroids that have been applied frequently in control household pests including German cockroach (local information); based on the results, the

Table 4. Glutathione S-transferase activity in first-generation treated cockroaches with insecticidal sublethal dose i.e., (LD10 or LD25), as well as, in field and susceptible strain (SS).

		Mean ± St. Dev.	
GSTs Source[a]	**N**[b]	**ΔA340 nm**[c]	**GST μM/min/ml**[d]
QF1M	4	0.04713 ± 0.00217	84.830 ± 3.91 **BC**
ZF1M	4	0.05417 ± 0.00289	97.500 ± 5.2 **AB**
QF1N	4	0.04829 ± 0.00394	86.920 ± 7.1 **BC**
ZF1N	4	0.05754 ± 0.00117	103.58 ± 3.15 **A**
QF1L	4	0.04967 ± 0.00464	89.400 ± 8.36 **ABC**
ZF1L	4	0.05779 ± 0.00541	104.03 ± 10.49 **A**
field	4	0.04442 ± 0.00302	79.950 ± 5.44 **C**
SS	4	0.03137 ± 0.0016	56.630 ± 3.09 **D**

[a]GST enzyme source from insect strains that were treated with sublethal doses(i.e,LD10 or LD25) of Imidacloprid (QF1M and ZF1M), Indoxacarb (QF1N and ZF1N), and Lambda-cyhalothrin (QF1L and ZF1L) respectively, as well as, field strain and susceptible strain (SS).
[b]Number of replications per each strain
[c]Δ A340 /min = A340 (final read) — A340 (initial read)/ reaction time(min)
[d]GST activity = Δ A340 min^{-1} x 0.036 x D / V = (μmol/min/ml). Means followed by the same letters within the same column are not significantly different.

adults of the field strain were tolerated those insecticides.

Cross-resistance is an important and common phenomenon in resistant strains of German cockroach; the prolonged exposure to insecticide can develop physiological resistance of insects to the same or other insecticides. Also, elevated tolerance to some insecticides can induction into elevated cross-tolerance to other insecticides that have the same or different modes of action (Hua et al. 2014). Liang et al. (2017) detected continuous providing with fipronil baits to German cockroach raised cross-resistance to indoxacarb. Although beta-cyfluthrin, acetamiprid, indoxacarb, fipronil, lambda-cyhalothrin, and bifenthrin are different insecticides, the field strains of German cockroach displayed differing resistance levels to them (Fardisi et al. 2017). In another study, Hu et al. (2020) found that a high level of cytochrome P450 in resistant strains of German cockroach to deltamethrin played an important role in developing cross-resistance to indoxacarb, imidacloprid, and fipronil.

Several researchers have established that metabolic resistance is a result of cytochrome P450s biochemical reactions in resistant strains. All resistance strains of German cockroach to pyrethroids contain a high level of cytochrome P450 and hydrolases; it is evidence that it has been involved in the biodegradation of insecticides, rendering them ineffective to insects (Wei et al. 2001). On the other hand, the biotransformation studies of indoxacarb indicated that oxadiazine ring-opened metabolite formation is cytochrome P450-dependent; it may play a role in indoxacarb resistance (Gondhalekar et al. 2016)

Furthermore, understanding the roles of enzymes in insecticides resistance is important for adopting an appropriate strategy for insecticide resistance management and enhancing integrated pest management (IPM) against the German cockroach with new application techniques. We used an indirect assay to measure the level and activity of detoxification enzymes according to Hemingway (1998); such methods, however, are considered important in insecticide resistance to estimate the differences in the values of these enzymes in biochemical assays until now.

The results of biochemical assays clearly showed that both cytochrome P450 contents and glutathione-S-transferase (GSTs) activities increased in the field strain compared to the SS. These methods rapidly detected insecticide resistance in German cockroach populations. The present study also focused on detoxification enzymes (P450s and GSTs) that play a major role in insecticide resistance in the German cockroach; in addition, the biochemical assays resulted in a good understanding of the resistant levels in *B. germanica* after comparing the total content or activity of those enzymes to the SS. The ratio of the enzymes was 2.00 and 1.41-foldcompared to SS. The total P450 content and GST activities of the first-generation strains (ZF1N, ZF1L, ZF1M, QF1N, QF1L, and QF1M) also increased compared to parental strain (field strain) and SS. As a result, the ratios of total P450 content were 3.470, 3.470, 3.410, 2.881, 2.765, and 2.591 fold compared to SS strain respectively (Table 2). The high level of GST and ratios were 1.82, 1.83, 1.72, 1.53, 1.57, and 1.49 fold compared to SS strain respectively.

The increase in the total content and activities of P450 and GST enzymes were clearly demonstrated in the development of insecticide resistance in German cockroaches (Scharf et al. 1997; Vontas et al. 2000b; Pridgeon et al. 2002; Habes et al. 2006; Enayati and Motevali 2007; Lin et al. 2014). Many researchers have observed that these enzymes work to detoxify insecticides *in vivo*, reduce their impact, and make insecticides more soluble in water. Finally, these compounds are easily excreted outside the insect's body. Chai and Lee (2010) reported the resistance/tolerance of six insecticides from different groups includes pyrethroids, neonicotinoid, and oxadiazine in *B. germanica*, suggesting the sharing of cytochrome P450 and esterase enzymes to award the cockroach tolerance or resistance to insecticides. Enayati and Motevali (2007) found that the GST, cytochrome P450, and esterases were elevated in the resistant German cockroach compared with the susceptible cockroach.

Insecticides are widely applied for their short-term efficacy against insect pests, but problems of their indirect and sublethal effects have been disregarded. The sublethal effects of insecticides may induce behavioural and physiological changes in cockroaches, causing resistance evolution over time. Accordingly, insecticide-induced resistance is an interesting topic, which might be conducive to elucidating insecticide-induced outbreaks of the German cockroach and other insect species. To determine the essential mechanisms of resistance to insecticides, more research should be done into the sublethal effects of insecticides on the development of insecticide resistance and its pattern of cross-resistance to neurotoxic insecticides.

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