

# Sublethal Effects of Lambda-Cyhalothrin, Imidacloprid, and Indoxacarb on Some of the Behavioral and Physiological Patterns of German Cockroach (Dictyoptera: Blattellidae)

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**Abstract.** German cockroach, *Blattella germanica* (L.), is a common household pets and a serious global public health risk. This study aimed to investigate the sublethal effects of lambda-cyhalothrin, imidacloprid, and indoxacarb on aggregation behavior, sexual communication, feeding efficiency, bodyweight, and nutritional indices of *B. germanica*. However, the study showed that the LD<sub>10</sub> and LD<sub>25</sub> doses of these insecticides impaired aggregation behavior and sexual communication of *B. germanica*; In addition, the data analyses of Excess Proportion Index (EPI), indicated that the aggregation response and sexual communication of adult cockroaches were decreased due to its exposure to LD<sub>10</sub> and LD<sub>25</sub> compared to the control treatment (LD<sub>0</sub>). Although the cockroaches' food intake increased, their bodyweight decreased after being exposed to LD<sub>10</sub> and LD<sub>25</sub> compared with the control treatment. Moreover, a negative impact of LD<sub>10</sub> and LD<sub>25</sub> was observed on some nutritional indices e.g. approximate digestibility (AD), relative growth rate (RGR), and efficiency of conversion of digested food (ECD). On the other hand, the LD<sub>10</sub> and LD<sub>25</sub> have positively affected on other nutritional indices, including efficient conversion of ingested food (ECI) and relative consumption rate (RCR) when compared with control group. This study showed that the sublethal effects of lambda-cyhalothrin, imidacloprid, and indoxacarb employed to control insect pests widely, have considerable effects on the insect's behavioral and physiological patterns.

**Keywords:** *Blattella germanica*; insecticides; behavioral effects; physiological effects; sublethal effects.

## 1. Introduction

The German cockroach, *Blattella germanica* is a global insect pest found in public places, for instance, homes, hotels, and apartments [1]. These roaches are active at night, but a day, they hide in dark areas such as cracks and crevices in walls [2]. Due to their omnivore feeding habits and random release of fecal materials, the roaches are famous agents for accommodating and mechanical transmission of various medically important microorganisms [3-5]. German cockroach might cause allergic reactions and asthma attacks [6].



The first generation of pyrethroids developed in the 1960s, included bioresmethrin, resmethrin, tetramethrin, and bioallethrin. And the second-generation including beta-cyfluthrin, lambda-cyhalothrin, and fenvalerate was developed by Rothamsted Research scientists in 1974 [7]. Different formulations of these insecticides have been applied to control agricultural and urban insects for over 45 years.

25% of the insecticide world market belongs to pyrethroids. Lambda-cyhalothrin has been widely used to control a variety of insect pests, including medically important insects [7] by effecting on channels of voltage-gated sodium as the primary target [8].

Imidacloprid is the best-known representative of the neonicotinoid insecticides. Neonicotinoids join and bind to postsynaptic nicotinic acetylcholine receptors of the insect's central nervous system. This induces a neuronal hyper-excitation, which can lead to the insect's death within a few minutes [9].

Indoxacarb an insecticide affects neuronal sodium channels of insects and blocks them, applied to control various agricultural and urban insect pests [10].

The residues of insecticides, after being sprayed, might have sublethal effects on insects and other organisms [11], resulting in behavioral and/or physiological alteration influencing pest management [2]. Although the sublethal dose does not occur immediately death, it impairs an insect's ability to carry out other normal functions. Accordingly, entomologists have categorized the sublethal effects on arthropods into behavioral and physiological.

Behavioral impacts might involve stimulation of reduction in oviposition behavior, alterations in the number of feeding and foraging events, inappropriate migration and dispersal, aggregation and attraction, and other behavior disorders [12-14]. The aggregation pheromone of *B. germanica* is released from rectal pad cells and excreted with the fecal material [15], which can result in aggregation other cockroaches [16]. The components contact sex pheromone produced by virgin females stimulates a courtship in males of *B. germanica* [17]. Employing behavioral assays with olfactometer devices indicated that the sex pheromone of *B. germanica* is usually produced by virgin adult females, and stimulates behavioral responses in adult males [18].

On the other hand, insecticidal sublethal exposure could have physiological impacts on arthropods, perhaps by affecting their nutritional indices, bodyweight, longevity, fecundity, growth development, sex ratio, and metabolic-enzyme induction [12, 19].

Therefore, this study was carried out to investigate the sublethal effects of lambda-cyhalothrin, imidacloprid, and indoxacarb which are widely applied to control various insect pests either in the agricultural fields or in urban areas, on aggregation behavior, sexual communication, feeding efficiency, bodyweight, and nutritional indices of German cockroach, as a medically important insect.

## 2. Materials and Methods

### 2.1. Insects

Adult cockroaches from laboratory colony, which were collected from infested apartments and houses in the central part of Mashhad, Iran, were tested, using a vacuum apparatus (insect aspirator) similar to the one designed by Wright [20] and established in plastic cages (30×30×30cm). The samples were supplied with food and water ad libitum [21]. The rearing condition was approximately 12h light/12h dark, 27±2°C, and 60-65% relative humidity (RH).

### 2.2. Chemicals

Lambda-cyhalothrin (97.31 %), Imidacloprid (97.00 %), and indoxacarb (96.2 %) were provided from Kavosh Kimia Kerman Co., Ltd (Kerman, Iran). Acetone solvent (99.95%), and other chemicals, which had high analytical grade >95% quality were purchased from Vangerman Co., Ltd (Mashhad-Iran). All insecticides tested in this study were of technical grade.

### 2.3. Determination of LD10 and LD25

Toxicity assays to determine the susceptibility of cockroaches to indoxacarb, imidacloprid, and lambda-cyhalothrin were performed with a range of concentrations for each insecticide.

Technical grade formulations of each insecticide were serially diluted in acetone. Selected cockroaches (600 males and 600 females each insecticide) were anesthetized using CO<sub>2</sub> [22]. One microliter of each insecticide dose (or 1  $\mu$ L of acetone alone as control) was topically applied using a repeating micropipette (Hamilton Company, Reno, NV) to the ventral portion of each insect between the metacoxae [23]. A total of 45 cockroaches were treated with each dose. Three replications per dose (15 individuals each) were maintained in plastic containers (21  $\times$  12  $\times$  7 cm) that were properly sealed with fine nylon mesh fabric cloth at the top for ventilation and provided with food and water ad libitum. After 72hr, mortality was checked and corrected using Abbott's formula [24], and the data were then pooled and analyzed to determine various LD indices, including LD<sub>10</sub> and LD<sub>25</sub> for each insecticide using a standard probit analysis [25].

Each microliter of LD<sub>10</sub>s of indoxacarb, imidacloprid, and lambda-cyhalothrin contains (30.92, 81.70, and 41.62), and LD<sub>25</sub>s contain (56.71, 141.63, and 72.72) micrograms active ingredient of insecticide respectively.

#### 2.4. Sublethal effects of insecticides on aggregation behavior and sexual communication of *Blattella germanica*

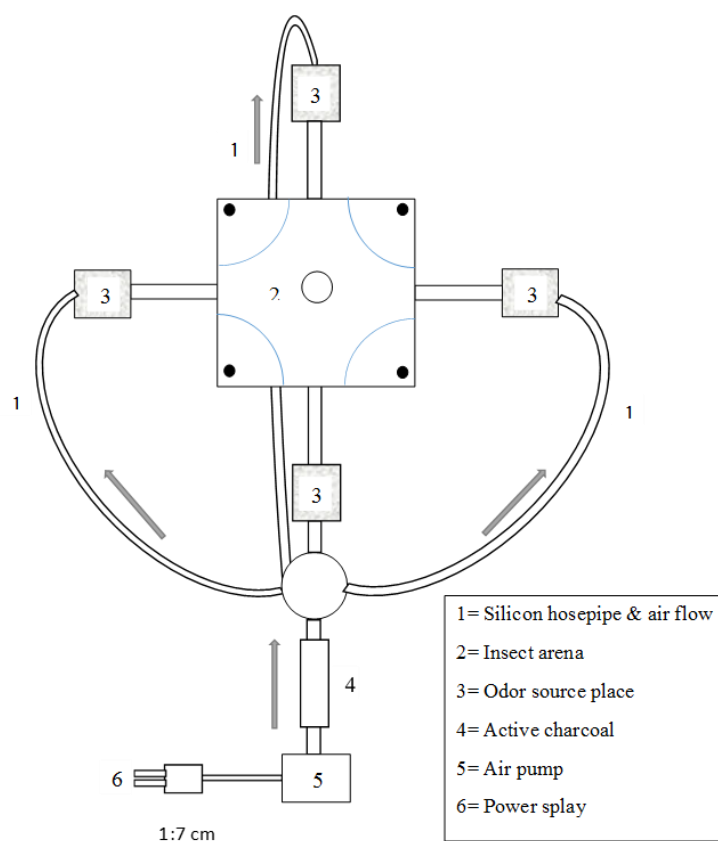
##### 2.4.1. Bioassay

Two groups of virgin *B. germanica* adults (male and female, 50 individuals each) were placed separately in plastic containers (21  $\times$  12  $\times$  7cm). Treatments of LD<sub>10</sub> or LD<sub>25</sub> with acetone for control were carried out topically, four times with seven-day interval between each treatment. The treated roaches were maintained in an incubator at 27 $\pm$  2°C, 60 to 65% RH, and 12h light/12h dark.

Twenty-four hours after the last test, alive and healthy insects were randomly selected. All tests were conducted under in vitro conditions.

##### 2.4.2. "Four-choice" olfactometer

A "four-choice" olfactometer device was utilized to observe aggregation behavior and sex communicative response of male and female cockroaches. It is a square shape, with a central chamber (insect area) and an opening hole at the bottom with a small tower, which is connected to four glass tubes perpendicular to each other that are joint to the source cells and silicon pipe in order to provide aeration by using a mini air pump 1.5-3L/min. The air passes through active charcoal to get purified air (Figure 1).



**Figure 1.** Diagram of “four-choice” olfactometer device.

The “four-choice” olfactometer device cleaned with alcohol, followed by continual aeration for 20 min to remove other odors and placed on a clean table.

The mixture of exuviae and feces produced by German cockroaches were used as an aggregation pheromone source and control cells were odorless. They placed in opposite cells, as described previously by Adams et al. [26]. In the same way, untreated virgin females as sex pheromone sources were placed in treatment cells in the next test.

Adult males, females for aggregation study, or only males for attraction study were released individually into the opening hole, and their response was observed for 5 min. The selection of treatment cells or control cells (odorless) by sample insects was assumed a positive or negative choice. If the sample selected neither of the cells, it would not be considered as a choice.

The direction taken by male or female samples in the olfactometer device was transformed into an excess proportion index (EPI) [27] by using the following equation:

$$EPI = (NR - NC) / (NR + NC)$$

Where NR is the number of roaches choosing the test cells and NC is the number of roaches choosing the control cells.

EPI ranges between (+1) to (-1), indicate the insect choice polarity, and positive numbers indicate appositive response of the approach. The data were listed on percent roaches’ aggregation and sexual communication for each material against control (odorless).

## 2.5. Sublethal effects of insecticides on feeding efficiency, bodyweight, and nutritional physiology of *Blattella germanica* adults

### 2.5.1. Bioassay

Two groups of adult *B. germanica* (100 males and 100 females) were treated separately with LD<sub>10</sub> or LD<sub>25</sub> of lambda-cyhalothrin, imidacloprid, and indoxacarb or only acetone on control group through

topical application for four times with 7-day intervals. Then, all samples were left to starve for 24 hours. Afterwards, alive and apparently healthy insects were randomly chosen, and placed in plastic containers (21×12×7cm) that were described previously. These containers were maintained in an incubator at 27± 2°C, 65-70% RH, and 12h light/12h dark.

### 2.5.2. Artificial Diets

Each replication was provided with 0.25g of artificial diet and water ad libitum (male or female). All experimental insects were weighed before and after exposure to LD<sub>10</sub> or LD<sub>25</sub>, or only acetone in control. The experimental duration extended for ten days. The residue diet and feces deposited by experimental insects were collected, dried out in an oven at 45°C for 72h (U-UL, Memmert, Germany), and reweighed. After the experiment, the initial dry weight of each cockroach was converted into the fresh/dry weight (FW/DW) ratios. The same process was utilized to measure the initial dry weight of the artificial diet cubes used in various treatments. These things were weighted by using a very accurate scale (A&D GF300P Prescription Scale, Japan).

The Nutritional indices were computed by applying the following equations:

- $AD = 100(F - V) / F$
- $ECD = 100G / (F - V)$
- $ECI = 100G / F$
- $RGR = G / DI$
- $RCR = F / DI$

In these equations, **D** for duration of experiment, **F** for dry weight of food eaten, **G** for dry weight gained by insects, **I** stands for mean dry weight of insect during **D**, and **V** for dry weight of feces produced [28, 29].

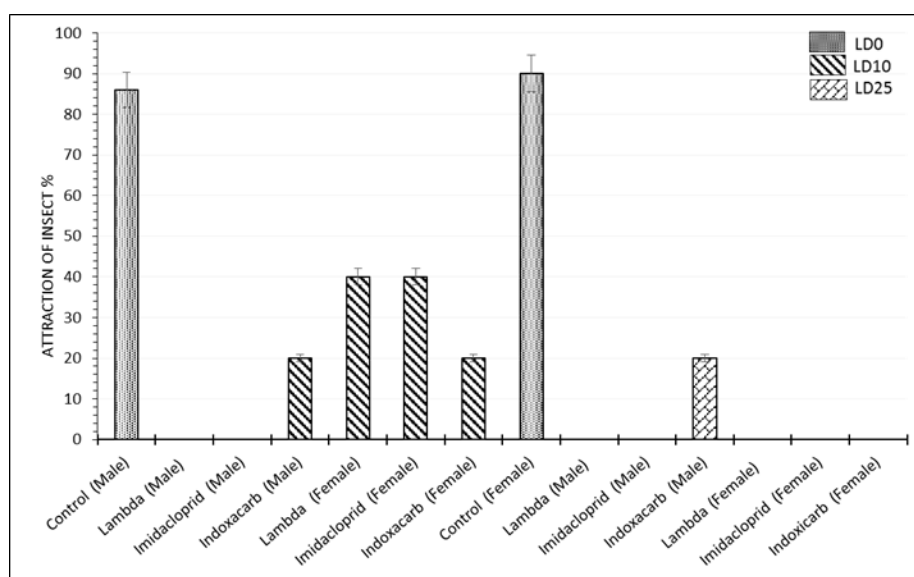
### 2.6. Statistical Analysis

The mortality data were corrected by Abbott's equation in order to compute natural mortality in the control. Paralyzed insects were considered dead. The dose-mortality relation was consistent with observed data. The PoLoPlus 2.0 [30] was employed to determine various LD indices, including LD<sub>10</sub> and LD<sub>25</sub>, for each chemical insecticide [31]. Data from Excess Proportion Index (EPI), and nutritional indices were subjected to factorial ANOVA, and Tukey's HSD test ( $P < 0.05$ ) to isolate the different treatments. On top of that, the binary logistic regression and odds ratio were used to assess the aggregation and attraction of adult *B. germanica*. Tukey multiple comparison test ( $P < 0.05$ ) to isolate the different treatments was used to examine the insecticidal sublethal effects on the weight of food eaten by adult *B. germanica*, body weight and weight gained after feeding. Minitab™17 software was used to conduct all statistical analyses (Minitab Inc., State College, PA, USA).

## 3. Results and Discussion

### 3.1. Sublethal effects of insecticides on aggregation behavior of *Blattella germanica* adults

The results implied that a lower aggregation belonged to the male and female cockroaches exposed to LD<sub>10</sub> or LD<sub>25</sub>, compared to cockroaches treated with acetone only (control). There was a significantly strong relation between doses (LD<sub>10</sub> and LD<sub>25</sub>) and control (LD<sub>0</sub>) (Deviance table:  $X^2 = 63.91$ ,  $P < 0.001$ ) (Figure 2).



**Figure 2.** Sublethal effect of insecticides on aggregation behavior of male and female *B. germanica*, using the “Four-choice” olfactometer

The analysis of data from Excess Proportion Index (EPI) using “Four-choice” olfactometer device indicated that the aggregation response of adult cockroaches decreased after treatment with LD10 or LD25 compared to the control (Dose:  $d.f=2$ ;  $F= 87.50$ ;  $P<0.001$ ) (Table 1).

There were no significant differences among types of insecticides also, no significant difference between insect sexes in their effects on aggregation behavior (Insecticide: Deviance table:  $X^2= 0.52$ ;  $P> 0.05$ ) and (Sex: Deviance table:  $X^2=1.08$ ;  $P> 0.05$ ).

**3.2. Sublethal effects of insecticides on sex communicative behavior of adult male to untreated virgin females of *Blattella germanica*.**

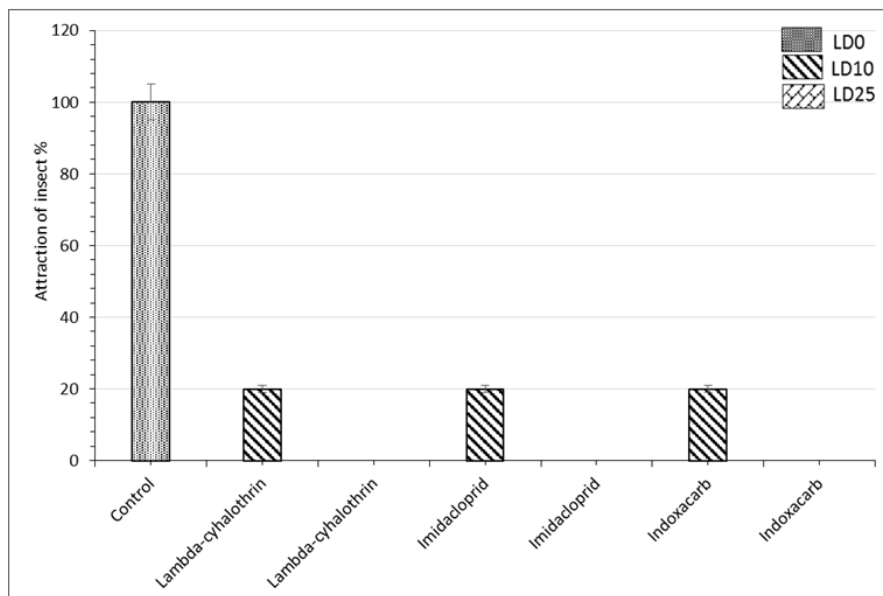
The results showed that the sexual communication of male exposed to LD10 or LD25 to untreated virgin females was decreased compared to male treated with acetone only in control (LD0). Accordingly, a significant difference was observed in effects between sublethal doses and control (Deviance table:  $X^2= 35.07$ ;  $P<0.001$ ) (Figure 3). On the other hand, there was no significant difference in effects among types of insecticides (Deviance table:  $X^2= 6.62$ ;  $P>0.05$ ). Excess Proportion Index (EPI) was also used to examine sexual attraction of the treated males with insecticidal sublethal doses to untreated virgin females of *B. germanica* using the “four-choice” olfactometer device. The EPI data was analyzed, and a significant difference observed in the effects between insecticidal sublethal doses and control (Dose:  $d.f= 2$ ;  $F=87.50$ ;  $P<0.001$ ) (Table. 2).

**Table 1.** Excess Proportion Index (EPI) for measuring aggregation behavior of male and female *B. germanica* in various insecticides using the “Four-choice” olfactometer device.

Treatment			Mean	EPI
Insecticide	Sex	Dose		
Lambda-cyhalothrin	Male	LD <sub>0</sub>	0.733 a	0.7
		LD <sub>10</sub>	-0.600 bc	-0.6
		LD <sub>25</sub>	-1.00 c	-1.0
	Female	LD <sub>0</sub>	0.733 a	0.7
		LD <sub>10</sub>	-0.600 b	-0.6
		LD <sub>25</sub>	-1.00 c	-1.0

Imidacloprid	Male	LD <sub>0</sub>	0.800 a	0.8
		LD <sub>10</sub>	-0.600 bc	-0.6
		LD <sub>25</sub>	-1.00 c	-1.00
	Female	LD <sub>0</sub>	0.800 a	0.800
		LD <sub>10</sub>	-0.600 bc	-6.00
		LD <sub>25</sub>	-1.00 c	-6.00
Indoxacarb	Male	LD <sub>0</sub>	0.600 a	0.6
		LD <sub>10</sub>	-0.600 bc	-0.6
		LD <sub>25</sub>	-0.800 c	-0.8
	Female	LD <sub>0</sub>	0.866 a	0.8
		LD <sub>10</sub>	-0.600 bc	-0.6
		LD <sub>25</sub>	-1.000 c	-1.0

Means followed by similar letters within a column are not significantly different at  $P < 0.05$



**Figure 3.** Sublethal effect of insecticides on sexual communication of adult male to untreated virgin females *B. germanica*, using the “Four-choice” olfactometer device.

**Table 2.** Excess Proportion Index (EPI) for sexual communication of adult male to untreated virgin females *B. germanica*, in different insecticides using the “Four-choice” olfactometer device.

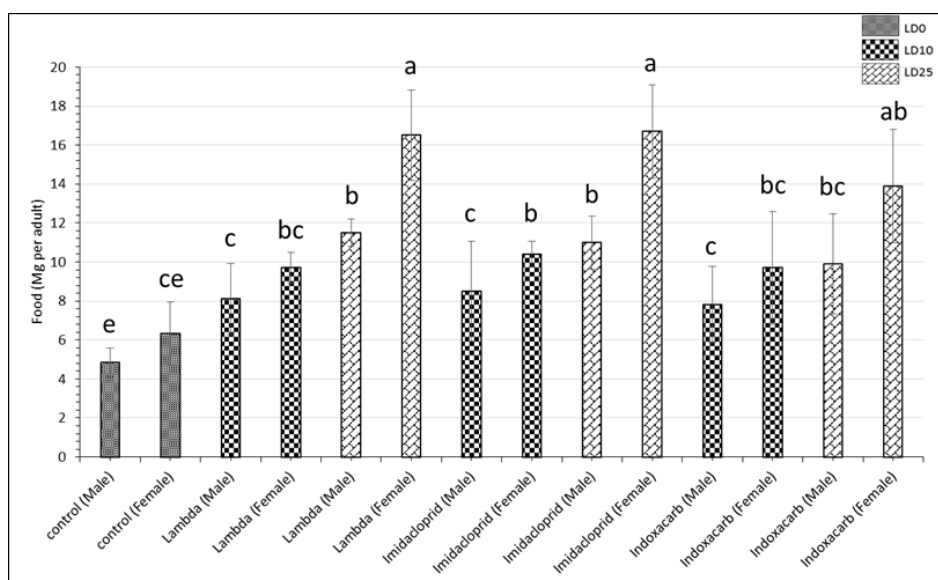
Treatment		Mean	EPI
Insecticide	Dose		
Lambda-cyhalothrin	LD <sub>0</sub>	0.800 a	1.0
	LD <sub>10</sub>	-0.600 b	-0.6
	LD <sub>25</sub>	-1.000 c	-1.0

Imidacloprid	LD <sub>0</sub>	0.800 a	1.0
	LD <sub>10</sub>	-0.600 bc	-0.6
	LD <sub>25</sub>	-1.000 c	-1.0
Indoxacarb	LD <sub>0</sub>	0.600 ab	0.6
	LD <sub>10</sub>	-0.600 bc	-0.6
	LD <sub>25</sub>	-8.000 c	-8.0

Means followed by similar letters within a column are not significantly different at  $P < 0.05$ .

### 3.3. Effect of sublethal doses of insecticides on amount of food intake by adult *Blattella germanica*

The results indicated that the treated cockroaches with LD<sub>10</sub> or LD<sub>25</sub> of lambda-cyhalothrin, imidacloprid, and indoxacarb were not fed as successfully as untreated cockroaches from 0 to 48 hours of the test time, but after 48 hours to the end of the experimental duration, treated cockroaches were being fed far more effectively than untreated cockroaches (Figure 4).



**Figure 4.** Feeding efficiency of both male and female of *B. germanica* in different insecticidal sublethal effects columns signaled with different letters are significantly differing ( $P < 0.05$ )

In addition, we observed that treated females were being fed as much as the treated males. So, the insecticidal sublethal doses had a significant correlation between feeding efficacy and cockroach's sex (dose:  $P < 0.001$ ,  $d.f.=2$ ,  $F=107.85$ ,  $n=90$ ; sex:  $P < 0.001$ ,  $d.f.=1$ ,  $F=48.82$ ,  $n=90$ ). On the other hand, there was a significant difference in the interaction between the dose of insecticide and the sex of cockroach ( $P \leq 0.05$ ,  $d.f.=2$ ,  $F=5.60$ ,  $n=90$ ).

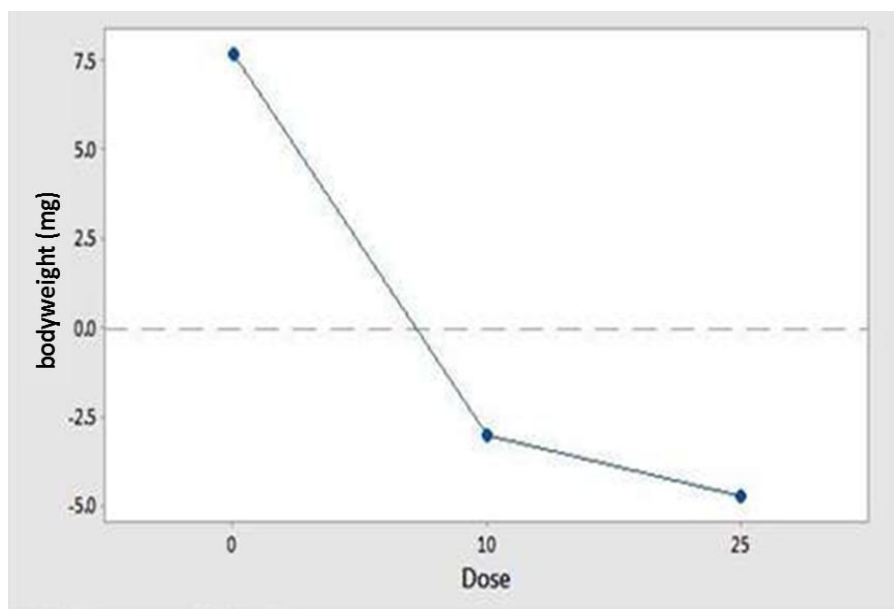
Furthermore, there was no significant difference between the types of applied insecticides. Also, no significant difference was observed between the type and the dose of insecticides on feeding efficacy of the German cockroach ( $P > 0.05$ ,  $d.f.=2$ ,  $F=0.09$ ,  $n=90$ ;  $P > 0.05$ ,  $d.f.=4$ ,  $F=2.70$ ,  $n=90$ ;  $P > 0.05$ ,  $d.f.=2$ ,  $F=0.01$ ,  $n=90$ ) respectively.

### 3.4. Effect of sublethal doses of insecticides on body weight of *Blattella germanica*

Loss of bodyweight was observed in adults after exposure to LD<sub>10</sub> or LD<sub>25</sub> of lambda-cyhalothrin, imidacloprid, and indoxacarb. Also, losing some bodyweight was proportional to increasing doses LD<sub>10</sub> to LD<sub>25</sub> in comparison with the control (LD<sub>0</sub>) as follows: LD<sub>0</sub> = 7.66  $\mu\text{g}$ , LD<sub>10</sub> = -3.03  $\mu\text{g}$ , and LD<sub>25</sub> = -4.70  $\mu\text{g}$ .



The results clearly represented a significant difference between doses of insecticide ( $P < 0.001$ ,  $d.f. = 2$ ,  $F = 57.65$ ). On the other hand, there were no significant differences among the types of insecticides on a cockroach's body weight ( $P > 0.05$ ,  $d.f. = 3$ ,  $F = 0.35$ ) (Figure 5).



**Figure 5.** Sublethal effect of lambda-cyhalothrin, imidacloprid, and indoxacarb on insect bodyweight, 0 = LD<sub>0</sub>, 10 = LD<sub>10</sub>, 25 = LD<sub>25</sub>. ( $P < 0.05$ )

### 3.5. Effect of sublethal doses of insecticides on nutritional indices of adult *Blattella germanica*

The analyzed data of the nutritional indices showed that there were significant differences between (LD<sub>10</sub> and LD<sub>25</sub>) of lambda-cyhalothrin, imidacloprid, and indoxacarb and the control group (Dose:  $P < 0.05$ ,  $d.f. = 2$ ,  $F = 119.02$ ), also, a significant difference was observed between genders of cockroach regarding RCR (Sex:  $P < 0.05$ ,  $d.f. = 1$ ,  $F = 61.51$ ). Concerning RGR, there was significant difference between insecticidal sublethal doses. ( $P < 0.05$ ,  $d.f. = 2$ ,  $F = 334.14$ ). In addition, there was a significant difference between doses of insecticides in ECI ( $P < 0.05$ ,  $d.f. = 2$ ,  $F = 132.34$ ). Not only this, there was also a significant difference in terms of AD between doses of insecticide and insect gender (Dose:  $P < 0.05$ ,  $d.f. = 2$ ,  $F = 251.25$ , sex:  $P < 0.05$ ,  $d.f. = 1$ ,  $F = 113.21$ , and dose\*sex:  $P < 0.05$ ,  $d.f. = 2$ ,  $F = 12.99$ ). Moreover, a significant difference was observed in terms of ECD between the type and the dose of insecticide (insecticide:  $P < 0.05$ ,  $d.f. = 2$ ,  $F = 10.55$ ; dose:  $P < 0.05$ ,  $d.f. = 2$ ,  $F = 213.64$ ; insecticide\* dose:  $P < 0.05$ ,  $d.f. = 4$ ,  $F = 9.65$ ); whereas, no significant differences were observed in the other studied parameters (Table 3).

The olfactometers are devices designed to study the changes in insects' behaviors. However, the study was conducted using a "four-choice" olfactometer device to measure the effects of sublethal doses of lambda-cyhalothrin, imidacloprid, and indoxacarb on aggregation behavior and sexual communication of adult German cockroaches. The current study has described some impacts of sublethal doses of these commonly used insecticides on communicative abilities of *B. germanica*.

**Table 3.** Nutritional indices of adult male and female *B. germanica* treated by different insecticides.

Treatments		Sex	RCR	RGR	AD	ECI	ECD
Insecticide	Dose						
Control	LD <sub>0</sub>	Male	0.116 cd	0.063 a	55.518 e	95.920 a	43.373 a

		Female	0.086 d	0.035 b	42.919 d	130.978 a	31.369 b
Lambda-cyhalothrin	LD <sub>10</sub>	Male	0.213 b	-0.024 cd	-11.038 c	161.437 b	-9.195 c
		Female	0.139 c	-0.030 c	1.386 b	193.485 b	-6.154 c
Imidacloprid	LD <sub>10</sub>	Male	0.210 b	-0.014 cd	-6.834 c	169.496 b	-4.845 c
		Female	0.140 c	-0.012 c	-8.517 b	207.512 b	-6.134 c
Indoxacarb	LD <sub>10</sub>	Male	0.208 b	-0.028 cd	-13.522 c	155.431 b	-5.979 c
		Female	0.148 c	-0.005 c	-3.227 b	213.559 b	-7.563 c
Lambda-cyhalothrin	LD <sub>25</sub>	Male	0.307 a	-0.025 d	-8.058 b	229.565 b	-6.244 c
		Female	0.192 b	-0.018 cd	-9.496 a	329.665 b	-5.820 c
Imidacloprid	LD <sub>25</sub>	Male	0.297 a	-0.035 d	-11.802 b	219.575 b	-10.173 c
		Female	0.253 b	-0.034 cd	-13.304 a	333.672 b	-8.871 c
Indoxacarb	LD <sub>25</sub>	Male	0.275 a	-0.036 c	-12.917 b	197.495 b	-10.636 c
		Female	0.175 b	-0.019 cd	-10.796 a	277.603 b	-6.709 c

Where (RCR) Relative Consumption Rate, (RGR) Relative Growth Rate, (AD) Approximate Digestibility, (ECI) Conversion of Ingested food, (ECD) Efficiency of Conversion of Digested food. Means followed by the same letters within the same column are not significantly different at  $P < 0.05$  (post-hoc Tukey test).

The obtained results implied that the aggregation ability of adult males and females decreased after being treated with LD<sub>10</sub> or LD<sub>25</sub> four times. On the other hand, the sexual attraction of cockroach males to untreated virgin females also reduced due to sublethal dose exposure.

Both adult male and female cockroaches had a lower response to aggregation and sex pheromones which might be in account of interference with antenna-receptor neuron sites used to detect pheromones and other odors. Therefore, the odors became desensitized, decreased, or delayed by the findings of this might explain why the aggregation behavior of male and female cockroaches exposed to sublethal doses was decreased [32-35]. Disruption of sexual communication after the exposure to insecticidal sublethal doses was observed. Consequently, the male's ability to locate sex pheromone released by untreated virgin adult females was impaired. A delayed receptivity signaled during the test also indicates that treated male cockroaches were less responsive to sex pheromone. Our results were also compatible with those in [32,35-37].

In addition, the results showed that the sublethal doses of lambda-cyhalothrin, imidacloprid, and indoxacarb are likely to impact the cockroach's olfactory system. Because all these insecticides belong to the neuronal insecticides, they could have stronger effects on the odorant receptors (OR) and olfactory sensory neurons (OSN) [38].

Exposure to insecticidal sublethal doses several times might lead to accumulating them in an insect's body. Therefore, they would ultimately impact neuronal function and result in inadequate response to aggregation, attraction, and other olfactory functions. The results of this study indicated that long-term insecticide exposure to low doses could have negative effects on the aggregation behavior and sexual communication of *B. germanica*. In fact, the sublethal doses of insecticides have various influences on several insect species in agricultural and urban ecosystems; therefore, the application of sublethal insecticides is likely induction on the side effects on their behavior [39], and physiology [40].

The evidence collected in this study suggested that the sublethal dose of insecticide reduces the response of the insect's olfactory system to odor stimuli. As a result, either olfactory receptors (ORs) in insect's antenna are damaged or sites of Odorant Binding Proteins (OBPs) in sensillum lymph are occupied or changed by other odors.

Results of experiment observed that the feeding efficiency of adult male and female *B. germanica* after being exposed to LD<sub>10</sub> or LD<sub>25</sub> of lambda-cyhalothrin, imidacloprid, and indoxacarb were primitively decreased, concordant with [14] but after 48 h. of experimental time, the feeding efficiency and nutritional indices were significantly increased. These increases were curvilinearly achieved with an increased insecticide dose. Therefore, treated cockroaches with sublethal doses were

continuously being fed, which might lead to getting sufficient nutrients for detoxification processes and normal insect growth [41].

To explain the augmentation in food efficiency, the differences between nutritional indices in the treatment groups (LD<sub>10</sub> and LD<sub>25</sub>) and control (LD<sub>0</sub>) were assessed. There was a reduction in RGR (relative growth rate), ECD (efficiency of conversion of digested food), and the bodyweight of treated-cockroach adults with sublethal doses of lambda-cyhalothrin, imidacloprid, and indoxacarb. Also, AD (approximate digestibility) of adult *B. germanica* decreased after its exposure to insecticidal sublethal doses. Most probably, the treated samples tried to overcome and recompense for this problem by food-increasing consumption. The results were consistent with Nathan's findings [37]. AD (Reduced digestibility) of the samples was due to the fast passing of food through the digestive tract when the target insect overate [41]. These results are consistent with findings by Nathan suggesting that the ECD and RGR of fourth-instar larvae of *Cnaphalocrocis medinalis* were significantly reduced after being exposed to botanical insecticide extracted from Chinaberry tree [42, 43]. Jansen and Groot also mentioned that fraxinellone might reduce the RGR of Asian corn borer [44]. The RGR reduction might have been caused by the irrecoverable damage to the mid-gut lumen cells.

The RGR was reduced with increasing insecticide dose, showing that the diet was not appropriate for the insect and might have acted as an inhibitor. Consequently, the treated adult German cockroach did not have adequate specific ingredients for metabolic processes and normal growth [45].

RCR and ECI were useful in determining nutritional indices [46]. RCR is used to measure food consumption and observes the proportion of feeding relation with the weight of cockroaches at a certain time [47].

ECI is the overall measure of an insect's ability to convert food for development and growth and AD measures the approximate digestibility [48]. On the other hand, Djemaoun's results from biochemical analyses revealed that the sublethal dose of indoxacarb decreased the ovarian protein level, carbohydrates, and lipids in adult *B. germanica*. These biochemical modifications might affect reproductive processes [49]. These results explain why insects lost bodyweight after being exposed to an insecticidal sublethal dose in this investigation. Reduction of AD, RGR, and ECD due to insecticidal sublethal doses exposure have either a direct or indirect relation with an insect's fertility and longevity, making them too weak against diseases and natural enemies [50,51]. Thus, we observed that the nutritional indices (i.e. AD, RGR, and ECD) of adult cockroaches treated to sublethal doses of lambda-cyhalothrin, imidacloprid, and indoxacarb were negatively altered, and other nutritional indices (i.e. RCR, ECI, and FDI) were positively changed. On the other hand, the negatively affected bodyweight and normal growth of the insect were powerfully related to the amount of food consumed by the German cockroach. These results are concordant with [46].

The effects of insecticides and chemical products on an insect's metabolism and the correlation between insects and their food intake could be explained using nutritional indices [46, 52]. Consumption of more food could be utilized for detoxification, leaving insufficient essential components for normal growth and be directly associated with the developmental delay of treated populations of the German cockroach.

#### 4. Conclusion

This study focused on the impacts of sublethal doses of lambda-cyhalothrin, imidacloprid, and indoxacarb which are widely used to control insect pests either in agricultural fields or urban areas, on the aggregation behavior, sexual communication, feeding efficiency, body weight, and nutritional indices of German cockroaches. Certain negative effects were observed on aggregation behavior, sexual communication, body weight, and some of the nutritional indices. On the other hand, the positive effects of insecticidal sublethal doses on feeding efficiency, and on other nutritional indices of German cockroach was also observed. In case of reducing pesticide use or in an ecotoxicological context with the presence of insecticide residues, our investigation confirms the need for evaluating the effects of sublethal doses and for explaining their impacts on behavioral and physiological patterns of German cockroaches. In addition to other control agents, we should take sublethal doses of these chemicals into account when developing an integrated pest management program against this household pests.

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## References

- [1] Roth L M. 1970. Evolution and taxonomic significance of reproduction in Blattaria. *Annual Review of Entomology*, **15**: 75-96.
- [2] Schal C J, Gautier Y and Bell W J. 1984. Behavioural ecology of cockroaches. *Biological Reviews*, **59**: 209-254.
- [3] Cochran D G 1999. *Cockroaches: their biology, distribution and control*. Geneva: World Health Organization.
- [4] Jalil N, Keyhani A, Hasan M K S, Mahdi M, Monireh M and Atefeh B. 2012. Cockroaches' bacterial infections in wards of hospitals, Hamedan city, west of Iran. *Asian Pacific Journal of Tropical Disease*, **2**: 381-384.
- [5] Haghi S M, Aghili S, Gholami S, Salmanian B, Nikokar S, Khangolzadeh M and Geravi H. 2014. Isolation of medically important fungi from cockroaches trapped at hospitals of Sari, Iran. *Bulletin of Environment, Pharmacology and Life Sciences*, **3**: 29-36.
- [6] Mueller G A, Pedersen L C, Glesner J, Edwards L L, Zakzuk J, London R E, Arruda L K, Chapman M D, Caraballo L and Pomés A. 2015. Analysis of glutathione S-transferase allergen cross-reactivity in a North American population: Relevance for molecular diagnosis. *Journal of Allergy and Clinical Immunology*, **136**: 1369-1377.
- [7] Thatheyus A and Selvam A D G. 2013. Synthetic pyrethroids: toxicity and biodegradation. *Applied Ecology and Environmental Sciences*, **1**: 33-36.
- [8] O'Reilly A O, Khambay B P, Williamson M S, Field L M, Wallace B and Davies T E. 2006. Modelling insecticide-binding sites in the voltage-gated sodium channel. *Biochemical Journal*, **396**: 255-263.
- [9] Tomizawa M, and Casida J E. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annual Review of Pharmacology and Toxicology*, **45**: 247-268.
- [10] Lapiéd B, Grolleau F and Sattelle D B. 2001. Indoxacarb, an oxadiazine insecticide, blocks insect neuronal sodium channels. *British Journal of Pharmacology*, **132**: 587-595.
- [11] Guedes R N C, Walse S S and Throne J E. 2017. Sublethal exposure, insecticide resistance, and community stress. *Current Opinion in Insect Science*, **21**:47-53.
- [12] Desneux N, Decourtye A, and Delpuech J-M. 2007. The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, **52**: 81-106.
- [13] Williamson S M and Wright G A. 2013. Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees. *Journal of Experimental Biology*, **216**:1799-1807.
- [14] Crawley S E, Kowles K A, Gordon J R, Potter M F and Haynes K F. 2017. Behavioral effects of sublethal exposure to a combination of  $\beta$ -cyfluthrin and imidacloprid in the bed bug, *Cimex lectularius* L. *Pest Management Science*, **73**: 598-603.
- [15] Ishii S Y and Kuwahara. 1967. An Aggregation Pheromone of the German Cockroach *Blattella germanica* L. (Orthoptera: Blattellidae): I. Site of the Pheromone Production. *Applied Entomology and Zoology*, **2**: 203-217.
- [16] Roth L M and Cohen S. 1973. Aggregation in Blattaria. *Annals of the Entomological Society of America*, **66**: 1315-1323.
- [17] Schal C J, Burns E L, Jurenka R A and Blomquist G J. 1990. A new component of the female sex pheromone of *Blattella germanica* (L.) (Dictyoptera: Blattellidae) and interaction with other pheromone components. *Journal of Chemical Ecology*, **16**: 1997-2008.
- [18] Liang D and Schal C. 1993. Volatile sex pheromone in the female German cockroach. *Experientia*, **49**: 324-328.

- [19] Guedes R N C and Cutler C. 2014. Insecticide-induced hormesis and arthropod pest management. *Pest Management Science*, **70**: 690-697.
- [20] Wright, C. 1966. Modification of a vacuum cleaner for capturing German and brown-banded cockroaches. *Journal of Economic Entomology*, **59**: 759-760.
- [21] Piquett P G and Fales J. 1952. Rearing cockroaches for experimental purposes: US Department of Agriculture, Bureau of Entomology and Plant Quarantine.
- [22] Sherman M and Hayakawa M. 1961. Carbon Dioxide as an Anesthetizing Agent for the Flesh Fly, *Sarcophaga peregrina* Robineau-Desvoidy, and the Adzuki-bean Weevil, *Callosobruchus chinensis* L. *Japanese Journal of Applied Entomology and Zoology*, **5**: 151-153.
- [23] Ko A E, Bieman D N, Schal C and Silverman J. 2016. Insecticide resistance and diminished secondary kill performance of bait formulations against German cockroaches (Dictyoptera: Blattellidae). *Pest Management Science*, **72**: 1778-1784.
- [24] Abbott W. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, **18**: 265-267.
- [25] Robertson J L, Jones M M, Olguin E and Alberts B. 2017. *Pesticide bioassays with arthropods. Third Edition*. Taylorfrancis.
- [26] Adams T, Holt G and Sundet W. 1979. Physical and physiological effects on the response of female screwworms, *Cochliomyia hominivorax* (Diptera: Calliphoridae), to carrion odors in an olfactometer. *Journal of Medical Entomology*, **15**: 124-131.
- [27] Sakuma M and Fukumi H. 1985. The linear track olfactometer: an assay device for taxes of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) toward their aggregation pheromone. *Applied Entomology and Zoology*, **20**: 387-402.
- [28] Waldbauer G. 1964. The consumption, digestion and utilization of solanaceous and non-solanaceous plants by larvae of the tobacco hornworm, *Protoparce sexta* (Johan.) (Lepidoptera: Sphingidae). *Entomologia Experimentalis et Applicata*, **7**: 253-269.
- [29] Waldbauer G. 1968. *The consumption and utilization of food by insects*, *Advances in insect physiology*. Elsevier, pp. 229-288.
- [30] Le Ora S, and Berkeley, C A. 1987. *POLO-PC: a user's guide to probit or logit analysis*. City: *LeOra Software* Berkeley, CA.
- [31] Choo L E W, Tang C S, Pang F Y, and Ho S H. 2000. Comparison of two bioassay methods for determining deltamethrin resistance in German cockroaches (Blattodea: Blattellidae). *Journal of Economic Entomology*, **93**: 905-910.
- [32] Wei H-y and Du J-w. 2004. Sublethal effects of larval treatment with deltamethrin on moth sex pheromone communication system of the Asian corn borer. *Pesticide Biochemistry and Physiology*, **80**: 12-20.
- [33] Feltham H, Park K, and Goulson D. 2014. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology*. **23**: 317-323.
- [34] González J W, Yeguerman C, Marcovecchio D, Delrieux C, Ferrero A. and Band B F. 2016. Evaluation of sublethal effects of polymer-based essential oils nanoformulation on the german cockroach. *Ecotoxicology and Environmental Safety*, **130**: 11-18.
- [35] Lalouette L, Pottier M A, Wycke M A, Boitard C, Bozzolan F, Maria A, Demondion E, Chertemps T, Lucas P. and Renault D. 2016. Unexpected effects of sublethal doses of insecticide on the peripheral olfactory response and sexual behavior in a pest insect. *Environmental Science and Pollution Research*, **23**: 3073-3085
- [36] Delpuech J-M, Legallet B, Terrier O, and Fouillet P. 1999. Modifications of the sex pheromonal communication of *Trichogramma brassicae* by a sublethal dose of deltamethrin. *Chemosphere*. **38**: 729-739.
- [37] Pan H, Liu Y, Liu B, Lu Y, Xu X, Qian X, Wu K, and Desneux N. 2014. Lethal and sublethal effects of cycloxaprid, a novel cis-nitromethylene neonicotinoid insecticide, on the mirid bug *Apolygus lucorum*. *Journal of Pest Science*, **87**: 731-738.
- [38] Tricoire-Leignel H, Thany S H, Gadenne C, and Anton S. 2012. Pest insect olfaction in an insecticide-contaminated environment: info-disruption or hormesis effect. *Frontiers in Physiology*, **3**: 58.

- [39] Tappert L, Pokorny T, Hofferberth J, and Ruther J. 2017. Sublethal doses of imidacloprid disrupt sexual communication and host finding in a parasitoid wasp. *Scientific Reports*, **7**(1):42756
- [40] Xiao D, Zhao J, Guo X, Li S, Zhang F, and Wang S. 2016. Sublethal effect of beta-cypermethrin on development and fertility of the Asian multicoloured ladybird beetle *Harmonia axyridis*. *Journal of Applied Entomology*, **140**: 598-608.
- [41] Stoyenoff, J, Witter J, and Montgomery M. 1994. Nutritional indices in the gypsy moth (*Lymantria dispar* (L.)) under field conditions and host switching situations. *Oecologia*, **97**: 158-170.
- [42] Nathan S S. 2006. Effects of *Melia azedarach* on nutritional physiology and enzyme activities of the rice leaf folder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae). *Pesticide Biochemistry and Physiology*, **84**: 98-108.
- [43] Liu Z L, Ho S H, and Goh S H. 2008. Effect of fraxinellone on growth and digestive physiology of Asian corn borer, *Ostrinia furnacalis* Guenee. *Pesticide Biochemistry and Physiology*, **91**: 122-127.
- [44] Jansen B, and De Groot A. 2004. Inhibition of pyrethroid insecticides on nerve Na-K-ATPase in house flies (*Musca Domestica*). *Natural Product Reports*, **21**: 449-477.
- [45] Schoonhoven L M, Van Loon B, van Loon J J, and Dicke M. 2005. *Insect-plant biology: Oxford University Press on Demand*.
- [46] Parra J R, Panizzi A R, and Haddad M L. 2012. *Nutritional indices for measuring insect food intake and utilization. Insect Bioecology and Nutrition for Integrated Pest Management*. 13.
- [47] Srinivasan R, and Uthamasamy S. 2005. Studies to elucidate antibiosis resistance in selected tomato accessions against fruitworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae). *Resistant Pest Management Newsletter*, **14**: 24-26.
- [48] Koul O, Singh G, Singh R, Singh J, Daniewski W, and Berlozecki S. 2004. Bioefficacy and mode-of-action of some limonoids of salannin group from *Azadirachta indica* A. Juss and their role in a multicomponent system against lepidopteran larvae. *Journal of Biosciences*, **29**: 409.
- [49] Djemaoun A, Habes D, and Soltani N. 2015. Effects of ingested indoxacarb (Oxadiazine) on biochemical composition of ovaries in *Blattella germanica* (Dictyoptera, Blattellidae). *Journal of Entomology and Zoology Studies*, **3**: 122-126.
- [50] González J O W, Gutiérrez M M, Ferrero A A, and Band B F. 2014. Essential oils nanoformulations for stored-product pest control—Characterization and biological properties. *Chemosphere*. **100**: 130-138.
- [51] Farooq M, and Freed S. 2016. Lethal and sublethal effects of mixtures of entomopathogenic fungi and synthetic insecticides on biological aspects of *Musca domestica* L. *Turkish Journal of Entomology*, **40**: 211-225.
- [52] Teimouri N, Sendi J J, Zibaee A, and Khosravi R. 2015. Feeding indices and enzymatic activities of carob moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: pyralidae) on two commercial pistachio cultivars and an artificial diet. *Journal of the Saudi Society of Agricultural Sciences*, **14**: 76-82.