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Research Article

Biochemical and Histological Effects of Lambda Cyhalothrin, Emamectin Benzoate and Indoxacarb on German Cockroach, *Blattella germanica* L.

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Abstract

Background and Objective: German cockroach developed its high resistance to insecticide groups very fast, therefore, this study conducted to evaluate spinosad, indoxacarb, lambda cyhalothrin and emamectin benzoate as safe insecticides. **Materials and Methods:** A filter paper contact toxicity bioassay method was used to evaluate the toxicity of the three insecticides on German cockroach. The effect of LC_{30} and LC_{50} of insecticides on some enzymes activity had determined. The histological effects were determined using LC_{30} of tested insecticides. **Results:** The indoxacarb was the most toxic insecticide. The activity of Ach E activity decreased after treated with LC_{30} and LC_{50} of the tested insecticides. Higher increase in α esterase activity was achieved after treating with LC_{30} and LC_{50} of indoxacarb and emamectin benzoate, while β esterase was decreased after treated with LC_{30} of the tested insecticides. The total protein, carbohydrate and lipid contents were decreased after treating with LC_{30} and LC_{50} of the tested insecticides. Three tested insecticides caused noticeable histopathological signs. **Conclusion:** The tested pesticides showed high toxicity to the German cockroach and indoxacarb was the most toxic, also significant biochemical and histological effects were recorded.

Key words: Insecticides, toxicity, biochemical, histological effects, German cockroach

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) is an important insect pest worldwide. It is always associated with indoor environments such as; bathrooms, kitchens and food storage areas[1,2]. German cockroach considered an important insect vector transmitting many pathogens as viruses, fungi, bacteria and protozoa that are harmful to human health[3]. Moreover, the substances produced by German cockroach caused allergic symptoms[4,5]. Control of German cockroach is depending on the chemical insecticides. Unfortunately, chemical insecticides are not safe for human, environment and increasing resistance of German cockroach[6,7]. Newer insecticides such as; fipronil and imidacloprid as well as organophosphate, carbamate and pyrethroid insecticides as gel bait formulations used to control cockroaches [8]. Many cockroaches have developed resistance to insecticide groups, so the insecticide resistance is now a huge practical problem. Therefore, safe and more selective insecticides with new modes of action are urgently needed to reduce resistance development. Thus, new low risks insecticides such as; spinosad and indoxacarb have been developed[9]. Indoxacarb is an oxadiazine pesticide and its main mode of action is blockage of the nerve sodium channels[10]. Moreover, mammals convert indoxacarb into non-toxic metabolites, which contributed to its selective toxicity of insect pests[9]. It is difficult to control German cockroach due to the larger egg ratio per capsule, the shortest developmental period, in addition, the nymphs have relatively enhanced chance to survive because the females always carry the egg capsule protecting eggs from direct exposure to pesticides[11,12]. Emamectin benzoate demonstrated significant larvicidal potential with maximum mortality at 1.5 µg mL^{-1}. The LC_{50} value of 0.269 µg mL^{-1} for 24 h duration depicted the strong toxic efficacy of Emamectin benzoate. Increased efficacy of Emamectin after 48 and 72 h of feeding established the delayed toxic effects[13]. The sub-lethal doses of λ-Cyhalothrin induced metabolic and enzymatic abnormalities to *Trogoderma granarium* in laboratory bioassays and can be effectively controlled by using proper dosage and application of this pesticide[14].

The motivation behind this study to throw a light on the resistance of German cockroach to traditional insecticides and our needs to use new insecticides environmentally safe. So, this study was conducted to evaluate the effect of new low risk insecticides such as; spinosad and indoxacarb beside lambda cyhalothrin on German cockroach adults.

MATERIALS AND METHODS

Tested insecticides: Commercial formulations of:

- Emamectin benzoate (Excellent 1.9% EC) as Avermectins group
- Indoxacarb (avaunt 15% SC) as oxidiazine group and lambda cyhalothrin (lambda 5% EC) as pyrethroids group

Insect rearing: This study carried out at the laboratory of Pesticides Department at Faculty of Agriculture Menoufia University from June-December, 2018. Cockroaches reared in glass boxes (60×30×30 cm) under laboratory conditions at 25±2°C and relative humidity 65±5% under a 12 h dark regime, where the insects fed with pieces of biscuit and cotton soaked with water. Cages covered with cloth sleeve opening at the top. To provide shelters for cockroaches, small plastic tubes were put inside cages. The boxes regulatory cleaned and insect faces removed.

Toxicity experiments: A filter paper contact toxicity bioassay method as described by Jang *et al.*[15] used to evaluate the toxicity of the three tested insecticides. Five concentrations and three replicates of each insecticide prepared and 1 mL of each concentration applied to filter papers (Whatman no. 2), while control filters papers received 1 mL of water. After drying each filter paper placed on 0.5 L glass jars. Groups of 10 adult insects (7-8 days old) of *B. germanica* separately placed on each jar and covered with rubber band and muslin.

After 24 h from treatment the mortality determined and corrected according to earlier published method[16]. Probit analysis done by Proban-PC software to perform and estimate toxicity values and slope of regression line for tested insecticides.

Biochemical assay: The German cockroach, *B. Germanica* adults treated with sub-lethal concentrations of LC_{30} and LC_{50} values of tested insecticides, where 60 insects and 3 replicates used for 2 sub-lethal concentrations in each insecticide. After 24 h from treatment by filter paper contact toxicity method, the insects were kept in deep freezer until biochemical assay.

The biochemical assay carried out to determine the effect of LC_{30} and LC_{50} of tested insecticides on total proteins, total carbohydrates, total lipids, α and β-esterase and acetyl cholinesterase activity.

Preparation of insects for analysis: The insects prepared as described earlier[17], where it was homogenized in distilled water (50 mg 1 mL^{-1}). Homogenates centrifuged at 8000 rpm for 15 min at 2°C in a refrigerated centrifuge. The deposits discarded and the supernatants referred as enzyme extract and stored.

Acetylcholinesterase determination: AchE (acetylcholinesterase) activity measured by using acetylcholine bromide (AchBr) as substrate[18].

Nonspecific esterases: Alpha esterases (α-esterases) and beta esterases (β-esterases) determined using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively[19].

Total proteins: Total proteins determined by using method of Bradford[20].

Determination of total carbohydrates: Total carbohydrates extracted and prepared for assay[21], while it estimated in acid extract of sample by the phenol-sulphuric acid reaction[22].

Determination of total lipids: Total lipids estimated using phosphor-vanillin reagent prepared by dissolving of 0.6 g pure vanillin in 10 mL ethanol and completed to 100 mL with distilled water, then 400 mL of phosphoric acid were added[23].

Histological assay: Alive adults of German cockroach after 24 h of treatment with LC_{30} of each insecticides using filter paper contact method were fixed in 10% formal saline for 24 h, the autopsy samples were taken from insects and gonads washing using tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Samples were cleared in xylene and embedded in paraffin at 56° in hot air oven for 24 h, then sectioning the Paraffin bees wax tissue blocks at 4 microns thickness by sledge microtome. The obtained tissue sections collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for examination research with the aid of stereo microscope[24].

Statistical analysis: All experiments contained 3 replicates (insects homogenates) and the results of biochemical determinations pooled from triplicate determinations. The results analyzed by one-way analysis of variance (ANOVA) of F-test using[25]. When the ANOVA statistics were significant ($p<0.05$) means compared by the Duncan's multiple range test.

RESULTS

Toxicity of tested insecticides: Results in Table 1 cleared that indoxacarb was the most toxic insecticide followed by emamectin benzoate whereas lambda cyhalothrin was the least toxic one.

As presented in Table 2, the respective values of LT_{50} and LT_{90} (h) for lambda cyhalothrin on German cockroach adults are 83.21 and 637.24 h at 0.1 ppm and 2.20 and 85.99 h at 100 ppm for emamectin benzoate are 61.42 and 1201.53 h at 0.1 ppm and 2.70 and 71.40 h at 100 ppm and are 37.82 and 957 h at 0.1 ppm and 1.10 and 22.19 h at 100 ppm. As for indoxacarb, the LT_{50} and LT_{90} were 37.8 and 957 h at 0.1 ppm and it were 1.1 and 22.2 h at 100 ppm.

Effect on enzymes activity

Acetyl cholinesterase activity (AchE): Results in Table 3 indicated that there were significant decrease in AchE activity after treated with LC_{30} of emamectin benzoate and lambda cyhalothrin, while no significant decrease was detected with indoxacarb. Results in Table 3 indicated that adults significantly decreased AchE activity as 244, 285.33 and 213.33 mg g^{-1} b.wt. for indoxacarb, emamectin benzoate and lambda cyhalothrin and LC_{50} were 1.56, 5.75 and 32.79, respectively (Table 1) compared with control.

α-esterase activity: The data in Table 4 showed that there was a significant increase in α-esterase activity in German cockroach adults treated with LC_{30} of Indoxacarb and emamectin. On the contrary, lambda cyhalothrin showed no significant effect on α-esterase activity compared with control.

Table 1: Toxicity of indoxacarb, emamectin benzoate and lambda cyhalothrin on German cockroach, *Blattella germanica* adults

Insecticides	LC_{50} (ppm)	Slope (±SE)	Confidence limits (95%)
After 24 h			
Lambda cyhalothrin	32.79	0.567±0.197	6.835-1229.107
Emamectin benzoate	5.75	0.421±0.171	0.204-141.223
Indoxacarb	1.56	0.526±0.176	0.057-0.843
After 48 h			
Lambda cyhalothrin	0.63	0.347±0.168	3.866E-17-7.389
Emamectin benzoate	0.17	0.433±0.178	3.230E-7-1.515
Indoxacarb	0.19	0.589±0.195	0.0006-1.057
After 72 h			
Lambda cyhalothrin	0.12	0.556±0.198	0.0001-0.810
Emamectin benzoate	0.08	0.535±0.201	4.356E-6-0.613
Indoxacarb	0.03	0.600±0.237	9.144E-8-0.296

Table 2: Lethal times of tested insecticides on *B. germanica*. adults as LT_{50} and LT_{90} (h) together with the corresponding 95% fiducial limits (FL 95%)

Treatments	Concentration (ppm)	LT_{50} (h)	Slope	Fiducial limits (95%)	LT_{90} (h)	Fiducial limits (95%)
Lambda cyhalothrin	0.1	83.2	1.5±0.4	2450.5-30424.3	637.2	10735.3-3.1E+6
	1	54.8	1.2±0.3	1548.9-13072.6	644.9	10769.5-8.9E+5
	10	16.6	0.8±0.2	441.2-3678.1	634.7	8042.4-9.8E+5
	50	12.1	0.8±0.2	323.3-2578.1	558.2	6910.6-9.2E+5
	100	2.2	0.8±0.2	65.9-303.6	85.9	1547.0-54724.8
Emamectin benzoate	0.1	61.4	0.9±0.2	1527.8-20340.2	1201.5	14752.0-3.1E+6
	1	20.8	0.9±0.2	608.5-3730.3	400.5	6773.5-2.9E+5
	10	11.6	0.9±0.2	336.3-1960.7	311.2	5051.2-3.4E+5
	50	5.3	1.0±0.2	175.2-676.7	89.7	1998.1-30991.9
	100	2.7	0. 9±0.1	83.3-342.9	71.4	1442.4-32463.5
Indoxacarb	0.1	37.8	0.9±0.19	969.3-10527.1	957	11860.7-2.1E+6
	1	25.6	1.0±0.18	728.8-5035.8	506.9	8061.5-4.5E+5
	10	6.7	1.2±0.2	230.0-806.4	78.6	1956.4-21276.6
	50	3.1	1.1±0.2	108.1-351.0	38.9	1005.1-9816.3
	100	1.1	0.9±0.2	35.2-126.0	22.2	535.0-7079.0

Table 3: AchE activity in German cockroach, *Blattella germanica* adults treated with LC_{30} and LC_{50} of tested insecticides

Insecticides	Indoxacarb	Emamectin benzoate	Lambda cyhalothrin	Control
24 h after treated with LC_{30}				
Mean activity (mg g^{-1} b.wt.)	349.67 ± 11.55^{a}	232.67 ± 5.77^{b}	191.33 ± 8.16^{c}	362.76 ± 5.74^{a}
Change (%)	-3.68	-35.8	-47.24	-
Activity ratio	0.96	0.64	0.53	-
LSD (0.05)	29.14			
24 h after treated with LC_{50}				
Mean (mg g^{-1} b.wt.)	244 ± 12.70^{c}	285.33 ± 11.54^{b}	213.33 ± 10.61^{d}	362.76 ± 5.74^{a}
Change (%)	-32.72	-21.33	-41.78	-
Activity ratio	0.67	0.79	0.59	-
LSD (0.05)	27.77			

Same letters means no significant difference at 5% level, each value is the mean of three samples±SE, LC_{30}: Lethal concentration and LSD: Least significant difference

Table 4: Activity of α-esterase and β-esterase in, *B. germanica* treated with LC_{30} and LC_{50} of tested insecticides

Insecticides	Indoxacarb	Emamectin benzoate	Lambda cyhalothrin	Control
α-esterase				
24 h after treated with LC_{30}				
Mean activity (mg g^{-1} b.wt.)	522.67 ± 5^{a}	492.67 ± 8.4^{b}	385.67 ± 5.3^{b}	408.67 ± 1.7^{b}
Change (%)	+27.87	+20.55	-5.63	-
Activity ratio	1.28	1.21	0.94	-
LSD (0.05)	32.36			
24 h after treated with LC_{50}				
Mean (mg g^{-1} b.wt.)	526.67 ± 6.1^{b}	576.33 ± 8.2^{a}	450.00 ± 2.9^{c}	408.67 ± 1.7^{b}
Change (%)	+28.88	+41.03	+10.11	-
Activity ratio	1.29	1.41	1.10	-
LSD (0.05)		41.45		
β-esterase				
24 h after treated with LC_{30}				
Mean activity (mg g^{-1} b.wt.)	544.67 ± 1.4^{a}	555.00 ± 8.2^{a}	491.33 ± 5.8^{b}	571.33 ± 5.8^{a}
Change (%)	-4.67	-2.86	-14	-
Activity ratio	0.95	0.97	0.86	-
LSD (0.05)	27.90			
24 h after treated with LC_{50}				
Mean (mg g^{-1} b.wt.)	688.00 ± 3.4^{a}	631.67 ± 8.2^{a}	495.00 ± 2.9^{c}	571.33 ± 5.8^{b}
Change (%)	+16.92	+6.94	-13.36	-
Activity ratio	1.17	1.11	0.87	-
LSD (0.05)	46.16			

Same letters means no significant difference at 5% level, each value is the mean of three samples±SE

β esterase activity: Treatment of German cockroach adults with LC30 and LC50 after results in Table 4 show that the lambda cyhalothrin significantly decreased β esterase activity.

Table 5: Total protein, total carbohydrate and total lipid contents in German cockroach, *Blattella germanica* treated with LC_{30} and LC_{50} of tested insecticides

Insecticides	Indoxacarb	Emamectin benzoate	Lambda cyhalothrin	Control
Total protein content				
24 h after treated with LC_{30}				
Mean (mg g^{-1} b.wt.)	8.96 ± 0.58^{b}	7.83 ± 0.48^{c}	8.70 ± 0.90^{b}	9.88 ± 0.39^{a}
Change (%)	-8.85	-20.35	-11.50	-
LSD (0.05)	0.30			
24 h after treated with LC_{50}				
Mean (mg g^{-1} b.wt.)	9.35 ± 0.41^{b}	9.31 ± 0.18^{b}	9.21 ± 0.17^{b}	9.88 ± 0.39^{a}
Change (%)	-5.36	-5.77	-6.78	-
LSD (0.05)	0.19			
Total carbohydrates content				
24 h after treated with LC_{30}				
Mean (mg g^{-1} b.wt.)	11.07 ± 0.66^{b}	11.03 ± 0.40^{b}	5.57 ± 0.38^{b}	15.4 ± 0.81^{a}
Change (%)	-28.11	-28.38	-50.84	-
LSD (0.05)	1.15			
24 h after treated with LC_{50}				
Mean (mg g^{-1} b.wt.)	13.47 ± 0.58^{b}	10.57 ± 0.37^{c}	10.47 ± 0.57^{c}	15.4 ± 0.81^{a}
Change (%)	-12.53	-31.36	-32.02	-
LSD (0.05)	1.07			
Total lipids content				
24 h after treated with LC_{30}				
Mean (mg g^{-1} b.wt.)	7.20 ± 0.57^{b}	7.00 ± 0.29^{b}	6.52 ± 0.26^{c}	10.57 ± 0.33^{a}
Change (%)	-31.88	-33.77	-38.32	-
LSD (0.05)	0.52			
24 h after treated with LC_{50}				
Mean (mg g^{-1} b.wt.)	9.37 ± 0.57^{b}	9.38 ± 0.42^{b}	9.93 ± 0.59^{b}	10.57 ± 0.33^{a}
Change (%)	-11.35	-11.26	-6.05	-
LSD (0.05)	0.54			

Same letters means no significant difference at 5% level, Each value is the mean of three samples ±SE

Effects on main contents

Total protein: The obtained results in Table 5 showed that significant decrease in total protein content after treated with LC_{30} and LC_{50} indoxacarb, emamectin benzoate and lambda cyhalothrin.

Total carbohydrate: The obtained data presented in Table 5 revealed that there were significant differences between the three tested insecticides and the control treatment as a result of treating German cockroach adults with LC_{30} and LC_{50} of indoxacarb, emamectin benzoate and lambda cyhalothrin.

Total lipids: High decreases in total lipids content (Table 5) detected after treated with LC_{30} of indoxacarb, emamectin benzoate and lambda cyhalothrin,

Histological effects

Histological alterations induced by three different insecticides on the *B. germanica* adult females: The normal histological structure of the mid gut of *Blattella germanica*, illustrated in Fig. 1a. The midgut composed of simple epithelial cells which rest on a basement membrane. This membrane covered with a muscle layer. The food particles separated from the epithelium by a thin sheath, the peritrophic membrane (Fig. 1).

Treated *B. germanica* adults for 24 h with LC_{30} of each insecticide (Lambda cyhalothrin, Emamectin benzoate and Indoxacarb) caused noticeable histopathological signs in the midgut of the treated individuals comparing to control ones (Fig. 1b-d). The treatment elicited degeneration of the midgut epithelium in some parts (Fig. 1b-d). The treatment also caused destruction of the epithelium of the midgut leaving empty spaces (Fig. 1c).

The normal histological structure of malpighian tubules (MT) of the *B. germanica* adults is composed of simple ciliated epithelial cells which rest on a basement membrane (Fig. 2a). *The B. germanica* treated with LC_{30} of the three insecticides caused extensive architecture destruction of the MT epithelium (Fig. 2b-d). Severe MT damage was observed after treatment with Emamectin benzoate and Indoxacarb with cells lacking nuclei (Fig. 2c-d).

The ovary of *B. germanica* is of panostic type. Different sizes of oocytes were found in the healthy control ovary (Fig. 3a). Each oocyte contained nucleus and yolk granules subsequently fill the oocytes. This oocyte is surrounded by a

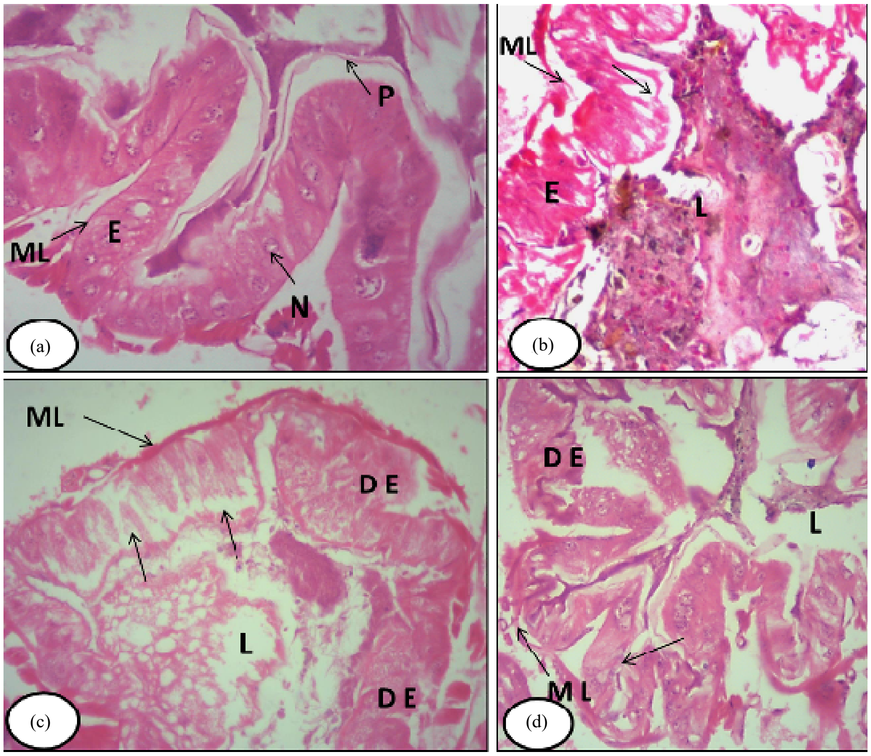

Fig. 1(a-d): Transverse sections in midgut of *Blattella germanica* female stained with H/E. (a) Control midget and (b-d) Treatment with the three insecticides (b = Lambda cyhalothrin, c = Emamectin benzoate and d = Indoxacarb) induced destruction of epithelium leaving empty spaces (arrows) and degeneration of epithelial cells in some parts
DE: Degenerated epithelium, E: Epithelium, L: Gut lumen, ML: Muscle layer, N: Nucleus, P: Peritrophic membrane

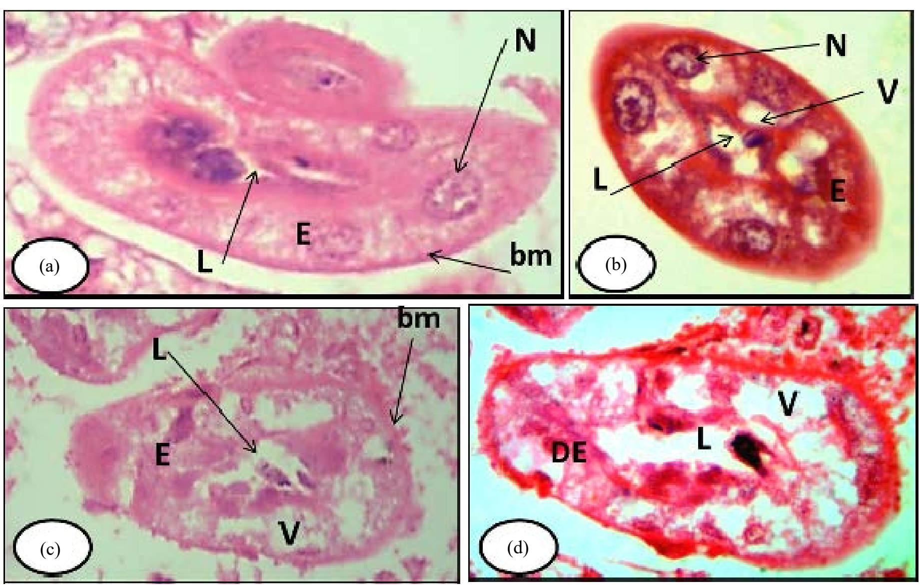

Fig. 2(a-d): Transverse sections in Malpighian tubules (MT) of *Blattella germanica* female stained with H/E (a) Control MT and (b-d): Treatment with the three insecticides (b = Lambda cyhalothrin, c = Emamectin benzoate and d = Indoxacarb) induced cytoplasmic vacuoles in the epithelium
bm: Basement membrane, DE: Degenerated epithelium, E: Epithelium, L: Lumen, N: Nucleus, V: Vacuole

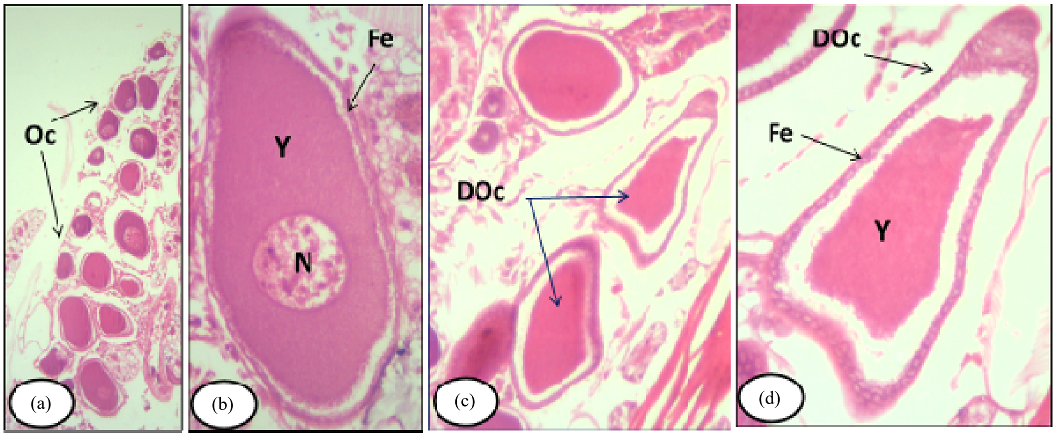

Fig. 3(a-d): Transverse sections in ovariole of *Blattella germanica* female stained with H/E. (a,b): Control (ovariole and oocyte) (c,d): Treatment with Indoxacarb induced malformed oocyte with small amount of yolk granules
Doc: Deformed oocyte, Oc: Oocyte, N: Nucleus, Fe: Iron, Y: Yolk granules

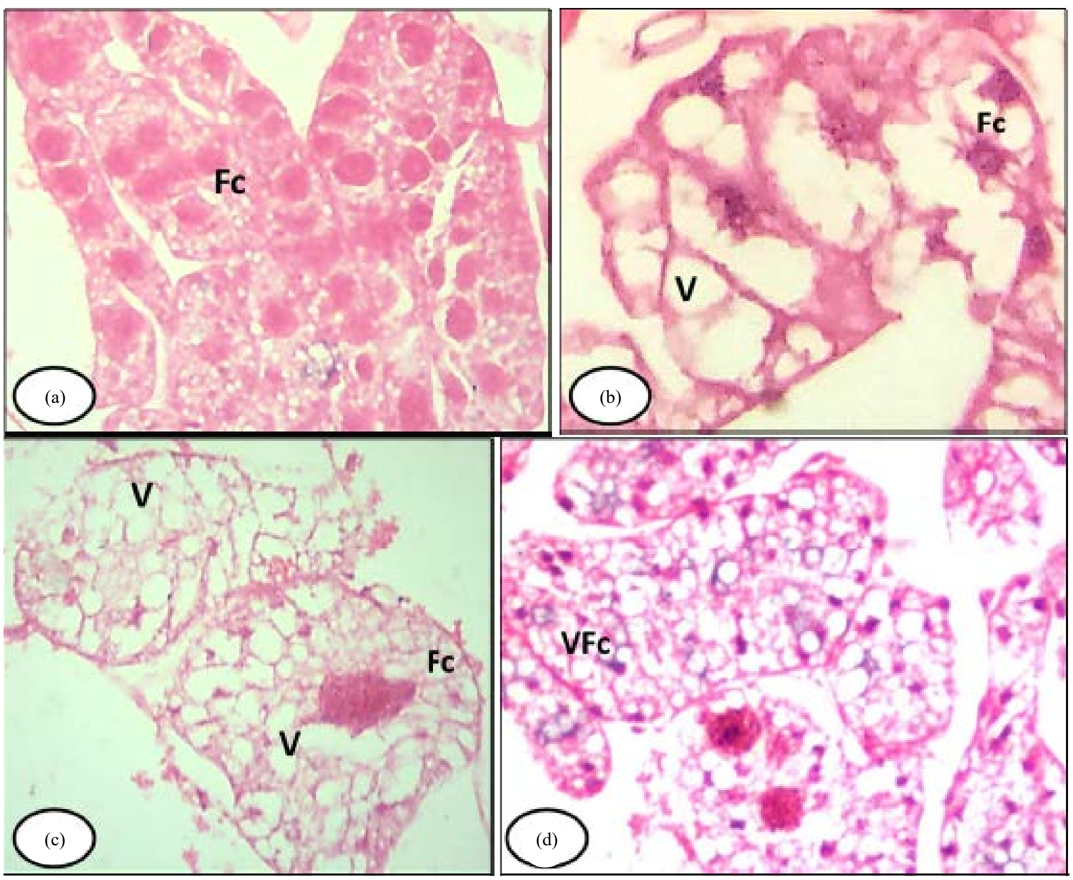

Fig. 4(a-d): Transverse sections in fat-body tissue (Fc) of *Blattella germanica* female stained with H/E, (a): Control showing normal cells and (b-d): Treatment with three insecticides (b = Lambda cyhalothrin, c = Emamectin benzoate and d = Indoxacarb) elicited cytoplasmic vacuoles in the fat tissue cells
Fc: Fat cells, V: Vacuole

normal follicular epithelium (Fig. 3b). In comparison with control, the ovary of the treated individuals with Indoxacarb contains some malformed oocytes (Fig. 3c-d). These oocytes appeared elongated instead of being round and contain small and abnormal distributed yolk granules (Fig. 3d). The malformed oocytes have vacuolated follicular epithelium (Fig. 3d). Comparing to control ovary, Lambda cyhalothrin and Emamectin benzoate were not significantly altered the structure of the ovaries of the treated individuals.

The fat body of *B. germanica* adults consists of a large number of lobes. Each lobe contains different types of cells: trophocytes, mycetocytes and urate cells. The abundant trophocytes (adipocytes) cells arranged in the periphery of the fat body lobe. The mycetocytes are located in the center of the lobe and surrounded by urate cells. All fate body cells of the control *B. germanica* were visible in normal shape as shown in Fig. 4a. The treatment of *B. germanica* with the three insecticides caused many alterations in the fat body cells (Fig. 4b-d).

DISCUSSION

The tested pesticides showed high toxicity to the German cockroach and Indoxacarb was the most toxic, also showed significant biochemical and histological effects, this were in agreement with authors who found that indoxacarb was toxic against German cockroach male and female adults. Upon topical treatment with indoxacarb results in a dose and time-dependent mortality since the LC_{50} value (ppm) decrease with the increase of duration of treatment[26].

The German cockroach resistant to organophosphates, carbamates, pyrethroids and fipronil were susceptible to indoxacarb[11]. Also, indoxacarb showed larvicide and adulticide activity against *Anopheles gambiae* resistant to pyrethroid insecticides[27]. Emamectin benzoate and cypermethrin induce significant effects on population of *H. armigera*. The LC_{50} values on larval stage were 1.75 and 127.74 µg a.i./mL for emamectin benzoate and cypermethrin, respectively[28]. The maximum AChE activity was recorded in emamectin benzoate treated *H. armigera* larval samples followed by chlorpyrifos, lufenuron, lambda cyhalothrin and bifenthrin, respectively [29]. Emamectin benzoate demonstrated significant larvicidal potential after 48 and 72 h of feeding[13]. Emamectin benzoate and abamectin demonstrated high efficiency against 4th instar larvae of *Culex pipiens* (42.60 and 43.61 h) and imidacloprid were the least effective (232.08 h) based on the LT_{50} values[30]. The sub-lethal doses of λ-Cyhalothrin induced metabolic and enzymatic abnormalities to *Trogoderma granarium* in laboratory bioassays and can be effectively controlled by using proper dosage and application of this pesticide[14]. The acetyl cholinesterase activities in the head of treated 4th instar larvae of *S. littoralis* with emamectin benzoate, abamectin and spinosad after 24, 48 and 72 h clearly reduced compared with control[31]. Significant decrease detected in acetyl cholinesterase and increase α and β esterase activities after treated 2nd and 4th instar larvae of cotton leave worm, *Spodoptera littoralis* with LC_{50} of indoxacarb[32]. The increasing activity of several detoxification enzymes such as; esterases protected the insect from insecticides poisoning as a part of defense mechanism or added stress on enzyme expression system to synthesize new and higher amount of detoxification enzymes where could be the possible reasons for arrested growth and mortality[33]. Indoxacarb at LD_{30} decreased ovarian level contents of protein, carbohydrates and lipids in *B. germanica*[34]. Total protein content and total lipids significantly decreased in haemolymph of treated 4th instar larvae of *S. littoralis* with emamectin benzoate, abamectin and spinosad at all tested periods 24, 48 and 72. Treated 2nd instar larvae of *S. littoralis* with indoxacarb caused 32.6 and 82.7% decrease in the content of carbohydrates and lipids in larvae than their value in the control hour[31]. Total main metabolites protein, carbohydrates and lipids in the late third instar larvae of *Culex pipiens* decreased under the stress of lambda cyhalothrin and leufenuron[35]. Indoxacarb exhibited high level of toxicity with LC_{50} (0.3983 ppm) on the 4th instar larvae of *Spodoptera littoralis*. Furthermore, a significant reduction in the digestive enzymes (amylase, trehalase and invertase) recorded[36]. Several histological changes in the body wall (integument), midgut, fat bodies and malpighian tubules as well as muscles of the treated *Spodoptera littoralis* larvae induced by LC_{50} of emamectin benzoate[37]. The LC_{50} and LC_{90} of indoxacarb significantly reduced the amount of proteins, carbohydrates and lipids in German cockroach ovaries. Which mean that indoxacarb induced changes in ovaries contents and this lead to histological alternations[26]. The ovaries of sterile insects indicated a degeneration of ovarian follicle cells, defective vitellogenesis[38].

CONCLUSION

The tested pesticides showed high toxicity to the German cockroach and Indoxacarb was the most toxic, also showed significant biochemical and histological harmful effects, therefore, it could be considered as the mode of action of these insecticides on German cockroach.

SIGNIFICANCE STATEMENT

This study discovered that indoxacarb was very toxic on German cockroach and induced significantly biochemical and histological harmful effects that can be beneficial for integrated management programs for German cockroach and this study will help the researchers to uncover the critical areas of the mode of action of the tested insecticides on German cockroach that many researchers were not able to explore.

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REFERENCES

1. Phillips, A.K. and A.G. Appel, 2010. Fumigant toxicity of essential oils to the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol., 103: 781-790.
2. Nasirian, H., H. Ladonni, M. Aboulhassani and M. Limoee, 2011. Susceptibility of field populations of *Blattella germanica* (Blattaria: Blattellidae) to spinosad. Pak. J. Biol. Sci., 14: 862-868.
3. Tawatsin, A., 2006. Novel repellents derived from phytochemicals against mosquito vectors and common cockroaches in Thailand. Ph.D. Thesis, School of Graduate Studies, Mahidol University, Thailand.
4. Arruda, L.K., L.D. Vailes, V.P.L. Ferriani, A.B.R. Santos, A. Pomes and M.D. Chapman, 2001. Cockroach allergens and asthma. J. Allergy Clin. Immunol., 107: 419-428.
5. Nasirian, H., 2010. An overview of German cockroach, *Blattella germanica*, studies conducted in Iran. Pak. J. Biol. Sci., 13: 1077-1084.
6. Chang, K.S., E.H. Shin, C. Park and Y.J. Ahn, 2012. Contact and fumigant toxicity of *Cyperus rotundus* steam distillate constituents and related compounds to insecticide-susceptible and resistant *Blattella germanica*. J. Med. Entomol., 49: 631-639.
7. Jung, W.C., Y.S. Jang, T.T. Hieu, C.K. Lee and Y.J. Ahn, 2007. Toxicity of *Myristica fragrans* seed compounds against *Blattella germanica* (Dictyoptera: Blattellidae). J. Med. Entomol., 44: 524-529.
8. Wang, C., M.E. Scharf and G.W. Bennett, 2004. Behavioral and physiological resistance of the German cockroach to gel baits (Blattodea: Blattellidae). J. Econ. Entomol., 97: 2067-2072.
9. Wing, K.D., M. Sacher, Y. Kagaya, Y. Tsurubuchi, L. Mulderig, M. Connair and M. Schnee, 2000. Bioactivation and mode of action of the oxadiazine indoxacarb in insects. Crop Prot., 19: 537-545.
10. Lahm, G.P., S.F. Mccann, C.R. Harrison, T.M. Stevenson and R. Shapiro, 2001. Evolution of the Sodium Channel Blocking Insecticides: The Discovery of Indoxacarb. In: Agrochemical Discovery Insect, Weed and Fungal Control: Symposium Series 774, Baker, D.R and N.K. Umetsu (Eds.)., American Chemical Society, Washington, DC., USA., pp: 20-34.
11. Chai, R.Y and C.Y. Lee, 2010. Insecticide resistance profiles and synergism in field populations of the German cockroach (Dictyoptera: Blattellidae) from Singapore. J. Econ. Entomol., 103: 460-471.
12. Fasulo, T.R., 2002. Stored product pests and German cockroach. Bug tutorials. University of Florida/IFAS. CD-ROM. SW-165.
13. Dagar, V.S and S. Kumar, 2018. Emamectin benzoate: Potential larvicide and antifeedant agent against cotton Boll worm *Helicoverpa armigera* (Lepidoptera: Noctuidae). J. Applied Natural Sci., 10: 564-571.
14. Shakoori, F.R., A. Feroz, A. Gondal, S. Akram and T. Riaz, 2018. Impact of λ-cyhalothrin on carbohydrate metabolizing enzymes and macromolecules of a stored grain pest, *Trogoderma granarium*. Pak. J. Zool., 50: 1467-1474.
15. Jang, Y.S., Y.C. Yang, D.S. Choi and Y.J. Ahn, 2005. Vapor phase toxicity of marjoram oil compounds and their related monoterpenoids to *Blattella germanica* (Orthoptera: Blattellidae). J. Agric. Food Chem., 53: 7892-7898.
16. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
17. Amin, T.R., 1998. Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd.). Ph.D. Thesis, Faculty of Science, Cairo University, Egypt.
18. Simpson, D.R., D.L. Bulland and D.A. Linquist, 1964. A semi microtechnique for estimation of cholinesterase activity in boll weevils. Ann. Entomol. Soc. Am., 57: 367-371.
19. Van Asperen, K., 1962. A study of housefly esterases by means of a sensitive colorimetric method. J. Insect Physiol., 8: 401-416.
20. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
21. Crompton, M and L.M. Birt, 1967. Changes in the amounts of carbohydrates, phosphagen and related compounds during the metamorphosis of the blowfly, *Lucilia cuprina*. J. Insect Physiol., 13: 1575-1592.
22. DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.
23. Knight, J.A., S anderson and J.M. Rawle, 1972. Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. Clin. Chem., 18: 199-202.
24. Bancroft, J.D., A. Stevens and D.R. Turner, 1996. Theory and Practice of Histological Techniques. 4th Edn., Churchill, Livingston, New York, London, San Francisco, Tokyo.
25. CoHort, 2008. CoStat 6.400: User's Manual. CoHort Software Inc., Monterey, CA., USA.
26. Maiza, A., N. Aribi, G. Smagghe and N. Soltani, 2010. Effect of an oxadiazine, indoxacarb, on the biochemical composition of ovaries in the German cockroach. Commun. Applied Biol. Sci. Ghent Univ., 75: 359-366.

27. N'Guessan, R., V. Corbel, J. Bonnet, A. Yates and A. Asidi *et al*., 2007. Evaluation of indoxacarb, an oxadiazine insecticide for the control of pyrethroid-resistant Anopheles gambiae (Diptera: Culicidae). J. Med. Entomol., 44: 270-276.
28. Parsaeyan, E., M. Saber and M. Bagheri, 2013. Toxicity of emamectin benzoate and cypermethrin on biological parameters of cotton bollworm, *Helicoverpa armigera* (Hubner) in laboratory conditions. J. Crop Protect., 2: 477-485.
29. Khan, M.B., M.Z. Ashraf, S. Freed and M. Bilal, 2018. Activity of acetylcholinesterase and acid and alkaline phosphatases in different insecticide-treated *Helicoverpa armigera* (Hubner). Environ. Sci. Pollut. Res., 25: 2903-2910.
30. Elghareeb, T.A., M.A.I. Ahmed, I.A. Mohamed, S.M.M. Saleh and H.A. Ezz El-Din, 2018. Synergistic action of glyphosate on novel pesticides against *Culex pipiens* L. (Diptera: Culicidae) mosquitoes under laboratory conditions. Aust. J. Basic Applied Sci., 12: 45-52.
31. Megahed, M.M.M., M.F. El-Tawil, M.M.M. El-Bamby and W.L. Abouamer, 2013. Biochemical effects of certain bioinsecticides on cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Res. J. Agric. Biol. Sci., 9: 308-317.
32. Gamil, W.E., F.M. Mariy, L.A. Youssef and S.A. Halim, 2011. Effect of Indoxacarb on some biological and biochemical aspects of *Spodoptera littoralis* (Boisd.) larvae. Ann. Agric. Sci., 56: 121-126.
33. Wheeler, D.A and M.B. Isman, 2000. Effect of *Trichilia americana* extract on feeding behavior of Asian armyworm, *Spodoptera litura*. J. Chem. Ecol., 12: 2791-2800.
34. Djemaoun, A., D. Habesand and N. Soltani, 2015. Effects of ingested indoxacarb (Oxadiazine) on biochemical composition of ovaries in *Blattella germanica* (Dictyoptera, Blattellidae). J. Entomol. Zool. Stud., 3: 122-126.
35. Shaurub, E.S.H and N.M.A. El-Aziz, 2015. Biochemical effects of lambda-cyhalothrin and lufenuron on *Culex pipiens* L. (Diptera: Culicidae). Int. J. Mosquito Res., 2: 122-126.
36. Khaled, A.S and S.M. Farag, 2015. Toxicological, biological and biochemical impacts of indoxacarb and methoxyfenozoid on the larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.). (Lepidoptera: Noctuidae). Egypt. Acad. J. Biolog. Sci., 7: 25-36.
37. Abo-El-Mahasen, M.M., 2016. Histological effects of emamectin benzoate on larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae). Egypt. J. Biol. Pest Control, 26: 147-152.
38. Fathpour, H., A. Noori and B. Zeinali, 2007. Effects of a *Juvenoid pyriproxyfen* on reproductive organ development and reproduction in German Cockroach (Dictyoptera: Blattellidae). Iran. J. Sci. Technol., 31: 89-98.