Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(3): 362-366

Received: 28-04-2015
Accepted: 30-05-2015

Amel Djemaoun
Laboratory of Applied Animal Biology, Department of Biology, Faculty of Science, University Badji Mokhtar, Annaba, 23000-Annaba, Algeria.

Dahbia Habes
Laboratory of Applied Animal Biology, Department of Biology, Faculty of Science, University Badji Mokhtar, Annaba, 23000-Annaba, Algeria.

Noureddine Soltani
Laboratory of Applied Animal Biology, Department of Biology, Faculty of Science, University Badji Mokhtar, Annaba, 23000-Annaba, Algeria.

Correspondence:
Dahbia Habes
Laboratory of Applied Animal Biology, Department of Biology, Faculty of Science, University Badji Mokhtar, Annaba, 23000-Annaba, Algeria.

Effects of ingested indoxacarb (Oxadiazine) on biochemical composition of ovaries in *Blattella germanica* (Dictyoptera, Blattellidae)

Amel Djemaoun, Dahbia Habes, Noureddine Soltani

Abstract
Cockroaches are generally controlled by conventional insecticides such as organochlorines, organophosphates and carbamates. However, these conventional neurotoxins possess strong secondary effects on the environment. In this context, the efficacy of a commercial formulation of indoxacarb (30% WG) was evaluated on *Blattella germanica* L. (Dictyoptera, Blattellidae), the most common species in Algeria. In a first series of experiment, the compound was applied by ingestion incorporated into the diet at different doses (0.125, 0.25, 0.50, 0.75, 1 and 2%) on adult females during a exposure period of 10 days under laboratory conditions. In a second series of experiment, the insecticide was tested by ingestion at the dose LD_{30}= 0.127 %, on newly emerged adult females. Its effect was investigated on ovarian contents of proteins, carbohydrates and lipids. Results showed that treatment caused disoriented movements followed by tremors and paralysis. Moreover, biochemical analyzes revealed that the insecticide at the sublethal dose decreased the ovarian levels of proteins, carbohydrates and lipids. These biochemical modifications observed in *B. germanica* ovaries suggested an interference of indoxacarb with the reproductive process.

Keywords: *Blattella germanica*, Indoxacarb, Toxicity, Ovaries, Biochemical components.

1. Introduction
Cockroaches are generally associated with grossly unsanitary conditions [1] and are considered as the most important pests in the urban environment [2]. Several species of cockroach are known to carry pathogenic or potentially pathogenic bacteria on or within their body and are important for medical and public health points of view [3, 4]. In fact, infestations are frequently associated with human sensitization to cockroach allergens and the development of allergic respiratory diseases [3, 4] and its ability to build up large infectious populations. Neurotoxic insecticides, such as organophosphates carbamates and pyrethroids, are commonly used to control cockroach infestations [5] but these compounds have led to the development of insect resistance [8]. In Algeria, the German cockroach, *Blattella germanica* L., is the most common domiciliary cockroach species and conventional insecticides were widely used for many years to control their infestations [6, 7]. Because secondary effects of these conventional insecticides in environment, alternative new insecticides were searched for controlling domestic cockroaches [8] such as boric acid [6, 7], ecdysteroid agonists and tebufenozide [9, 10] or indoxacarb [11, 12]. Indoxacarb belongs to a new class of insecticides, the oxadiazole. It is reported to act via blockage of the sodium channels in the insect nervous system resulting in paralysis and dead [13] and to exhibit insecticidal activity against a wide range of insect pests with low ectotoxicological risks [14, 15]. Indoxacarb was found toxic when applied orally [14] and also by topical application [14, 11, 12]. The objective of the present work is to evaluate the efficacy of Indoxacarb as a chemical control by applied orally and its effect on some biological and biochemical aspects and the most toxic mode of application of adult females of *B. germanica.*

2. Material and methods
2.1. Animals
The present study was carried out from September 2013 to December 2014 at laboratory of Applied Animal Biology, Department of Biology, Faculty of Sciences, University Badji-Mokhtar of Annaba, Algeria. Colonies of *B. germanica* were reared in plastic containers (30 x 30 x 30 cm) and maintained at 27 ± 1 °C, under a 12 light: 12h dark regime and 80 ± 1%

relative humidity. The cockroaches were provided ad libitum with water and dog food pellets (France) as described previously [9].

2.2. Insecticide and treatment

Indoxacarb a commercial 30WG formulation (water dispersible granule, 30% active ingredient) was obtained from DuPont, Wilmington, DE, USA). The compound was incorporated (w/w) into the diet and given as food to the tested insects. Control cockroaches were provided with untreated diet. Different concentrations (0.125, 0.25, 0.50, 0.75, 1 and 2%) were orally administered to newly emerged male and female adults. The bioassay was conducted with three replicates each containing 13 insects per dose. Mortality was observed at 10 days follow-up treatment to sublethal concentration (LD_{30}) and lethal (LD_{90}) were estimated.

2.3. Collection of samples and extraction of ovarian biochemical components

An extra series of newly molted adult females was treated orally with indoxacarb at its LD_{30} (0.127 % = W/W) as determined above The extraction of principal biochemical components of the ovaries from *B. germanica* adult females was carried out by the method of Shibko *et al*. [16]. Paired ovaries were sampled at different times during the first gondaotorophic cycle in control and treated series and homogenized in trichloro acid acetic (TCA, 20%). After homogenization with ultrasound (Sonifier B-30) and centrifugation (5000 g / min for 10 min), the first supernatant is got back and will be of use to the dosage of carbohydrates. In the base 1, we add 1 ml of mixture ether / chloroform (V/V) and the second centrifugation (5000 g / min for 10 min), the base 2 will then be taken back in 1 ml of water distilled for the estimation of total proteins and the second supernatant is got back and will be of use to the quantification of lipids.

2.4. Quantification of biochemical components

Ovarian proteins were quantified using the method of Bradford [17] in an aliquot (100 µl) using the Coomassie Brilliant Blue G 250 (BBC) as a reagent and bovine serum albumin (BSA) as standard (Sigma). The reading of absorbance was performed at a wavelength of 595 nm. The determination of carbohydrates was performed on an aliquot (100µl) according to Duchâteau and Florkin [18]. This method uses anthrone as a reagent and glucose (Sigma) as standard. The absorbance was estimated with a spectrophotometer at a wavelength of 620 nm. Lipid amounts were estimated based on the vanillin method of Goldsworthy *et al*. [19]. Data on ovarian biochemical components were expressed in µg/ mg ovaries.

2.5. Statistical Analysis

Results are represented as the mean ± S.E.M. The toxicity was performed using Graph-Pad prism version 5.00 for Windows Software, La Jolla California, U.S.A., www.Graphpad.com [20]. The percentages of mortality were analyzed using non-linear sigmoid curve fitting. The effect of treatment on biochemical parameters was assessed using Student's *t*-tests at $P < 0.05$. Statistical analysis was performed using MINITAB version 16 software [21].

3. Results and Discussion

3.1. Insecticidal activity

Treated adults showed symptoms of poisoning by inhibition of the insect locomotory activity followed by paralysis and death. Our data also revealed that the mortality varied from 33.33 ± 5.77 % at a dose of 0.125% to 96.66±0.00% at a dose of 2% (Table 1). No mortality was recorded in the control series. The LD_{30} and LD_{90} values (95% fiducial limits, R2=0.96) are given in Table 2. The insecticide effects are correlated to the concentration of indoxacarb and the time of exposure ($p < 0.001$). One-way ANOVA followed by a Tukey test revealed 5 groups of concentrations.

Table 1: Corrected mortality (%) as function of the concentration of indoxacarb (0.125,0.25, 0.5,0.75,1,2.) at the duration of treatment (10 days) after oral application on newly emerged *B. germanica* adults(Means± SD: established on three replicates each containing 13 cockroaches).

Doses (%)	0.125	0.25	0.5	0.75	1	2
Corrected mortality (%)	33.33±5.77	46.67±5.77	66.67±15,28	73.33±5.77	83.33±5.77	96.66±5.77

Table 2: Lethal and sublethal doses of indoxacarb when the compound was orally applied on newly emerged adults of *B. germanica*. The data are expressed as lethal LD50 and sublethal LD30 doses together with the corresponding 95 % fiducial limits (95 % FL) determined at day 10 following treatment.

LD_{30} (95%FL) (%)	LD_{50} (95%FL) (%)	LD90 (95%FL) (%)	Slope
0.127 (0.07-0.20)	0.262 (0.19-0.35)	1.720 (0.92-3.21)	1.151 (0.75-1.59)

These symptoms of poisoning can be explained by the neurotoxic activity of indoxacarb following a blockage of neuronal voltage dependent sodium channels in muscle and nerves cells [15]. These symptoms were also observe and described previously in *B. germanica* after treatment with boric acid [6, 7], or after topical application of indoxacarb, spinosad [10, 12], and also after topical application tebufenozide [9]. In *B. germanica*, indoxacarb was more efficacious than boric acid [7], spinosad and Tebufenozide [11, 9]. Indoxacarb was more toxic by topical application (LC 50= 17.2 ppm at 6 days) than oral application (LD50= 0.262% at 10 days) against *B. germanica*. This insecticide was also toxic after topical application against Hemiptera like *Lygus lineolaris* and *Geocoris punctipes* [22] and Lepidoptera *Ostrinia nubilalis* [23]. This compound exhibits also strong activity against lepidopteran pests [23]. Thus, many conventional pesticides have been replaced by newer low risk insecticides with different mode of action like biopesticides [24] or insect growth regulators [11].

3.2. Effect on amounts of carbohydrates, total proteins and lipids in the ovaries

Indoxacarb was applied *in vivo* by ingestion at the dose LD30 =0.127%, on newly emerged adult females of *B. germanica*. The effect of this insecticide was evaluated at different ages (0. 1. 2. 3.4.5 and 6 days) after emergence on ovarian content of proteins, carbohydrates and lipids. Indoxacarb was reported to be more effective following ingestion than after topical treatment [11, 12]. This was correlated with its action as a sodium channel blocker insecticide. It is observed that indoxacarb affects the rate of proteins, carbohydrates and lipids (Figure.1). In the control, the contents of main components of ovaries (proteins, carbohydrates and lipids) increased and showed a peak at day 4 after adult emergence related with the vitellogenesis process. The ovarian protein content was affected in the treated series at 1.2 and 4.5.6 (p=0.000) (Figure 1A). Treatment reduced significantly also the ovarian carbohydrates and lipids content with a dose–response relationship at all ages (one, two, three, four, five and six days) with the LD30 (p=0.004, 0.006, 0.012,0.001,0.000 and 0.0009 respectively) as compared to the controls (Figure 1B and 1C). These effects were dose-dependent. ANOVA showed a significant effect of the compound (P< 0.001) as function of the dose (p=0.001) and the different ages (p=0.001) for all ovarian components.

In *B. germanica*, the vitellogenesis and maturation of oocyte depends on juvenile hormone III synthesized by the corpora allata [24]. The relative activity of corpora allata in adult female is dependent and modulated by intrinsic signals that originate in the brain and ovaries, which can be influenced by the social status of the female [10,11]. Yolk or growth phase is the accumulation of various materials and energy (proteins, carbohydrates and lipids). The yolk proteins are formed from exogenous proteins from the hemolymph or endogenous proteins synthesized by the oocyte itself. The yolk platelets are composed of mucoproteins or glycoproteins, and other reserves that are in the form of glycogen. Vitellogenin is a yolk precursor protein in oocytes of insects [23] and is synthesized in fat and secreted in the hemolymph and then sequestered by endocytosis in developing oocytes [24]. Oocyte maturation depends on metabolites collected from the hemolymph and *in situ*, materials synthesized by the ovary [25], but depend mainly on the protein, a major component of yolk [7]. The quantity of lipids available for the reserves seems to be the result of a balance between the catch of food and the requests for reserves by processes such as reproduction, maintenance and growth, and this balance is disturbed by any toxic product [26, 24]. The lipids represent the independent source of energy in insects and are transported via the haemolymph; from their synthesis site of storage, to be used at the time of vitellogenesis [26]. Carbohydrates are mobilized mainly from glycogen reserves in the fat body, under neuropeptide induction, resulting in increased level of soluble carbohydrates in haemolymph [27] and as energy elements play a crucial role in the physiology of the insects [29]. The biochemical composition of the ovaries of adult female *B. germanica* was assessed at different ages during sexual maturity. It was reported that Indoxacarb at sublethal concentration caused feeding deterrent activity of *S. litura* (F.) larvae [30].

The results show a change in the concentrations of protein, carbohydrates and lipids during the first four days of adult correlated with vitellogenesis [28, 31].

Our results show that indoxacarb test at the LD30, affected the vitellogenesis by a reduction of ovarian contents of proteins, carbohydrates and lipids. Furthermore, inhibition of vitellogenesis in *B. germanica* was also observed during treatment with other types of insecticides such as boric acid [7], the benfuracarb, a carbamate and acetamiprid imidacloprid a neonicotinoid and azadiractin [24, 28, 31].

Maiza *et al.* [11] also reported that the application of indoxacarb in *B. germanica* reduced the ovarian contents and that the tebufenozide, imidacloprid affected the reproduction of the German cockroach [9, 31]. Ecdysteroids are synthesized by the follicle cells in the ovaries and are available to develop embryos and pre-hatching larvae [26]. The application of an analogue of the molting hormone, halofenozide, affects the ovarian content of protein and carbohydrates in *B. germanica* [10].

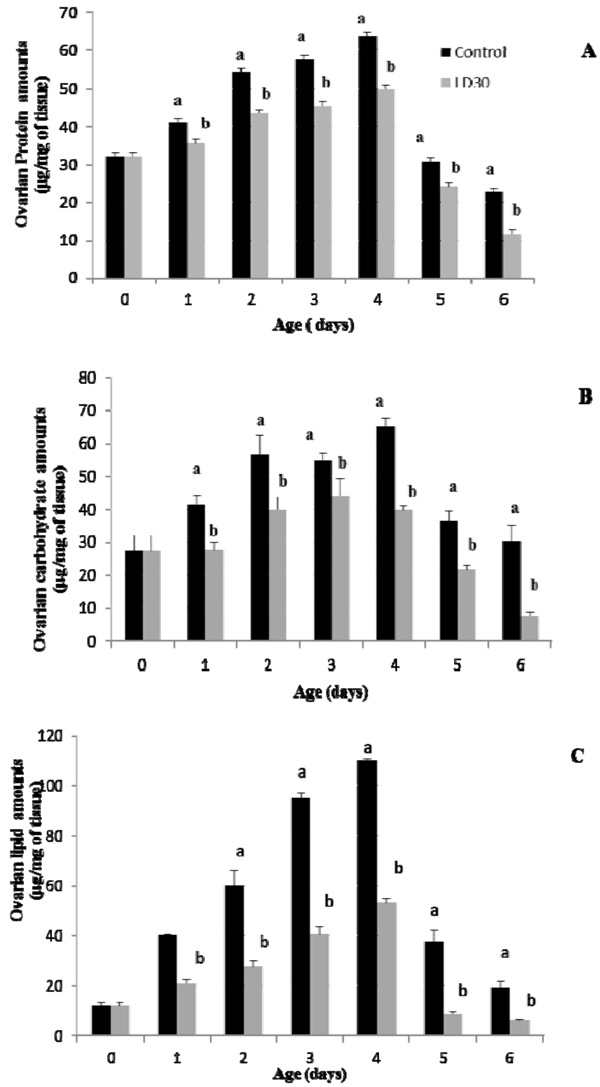

Fig 1: Effect of indoxacarb applied by ingestion (LD_{30}=0.127%) on newly emerged female adults of *B. germanica,* on the ovarian content (µg/mg of tissue) of proteins (**A**) and carbohydrates (**B**). Values are presented by the (Mean ± SD; n=6-8; values of the same age followed by different letters are significantly different p<0.05).

Captopril applied topically on newly emerged adult females of *T. molitor* was found to reduce significantly both carbohydrate, lipid, protein and RNA amounts of ovaries [32]. Similarly, these biochemical modifications also observed in *Ephestia kuehniella* suggested an interference with the reproductive events probably *via* the vitellogenesis process and/or its endocrine regulation [33]. This is supported by the reduction of both vitellogenin titers in *N. bullata* and oviposition in *S. littoralis* [32].

The reduction rates of these various metabolites in the ovaries of *B. germanica* after treatment with indoxacarb can be probably explained by its neurotoxic action and an interference with the HJ and ecdysteroids that can lead to a disruption of the endocrine regulation controlling vitellogenesis. The decline in juvenile hormone interferes with synthesis and release of vitellogenin by the fat body in the hemolymph and their incorporation into the oocytes [34]. Although the activation metabolism of indoxacarb was proven in lepidopteran insects, different resistance levels to indoxacarb might be observed because of the presence of various resistance mechanisms in *Plutella xylostella* [35].

4. Conclusion
Indoxacarb exhibited insecticidal activity against *B. germanica.* It was found more toxic by topical application than oral application. The current biochemical study revealed that this compound at the tested dose (LD30= 0.127%) also affected reproduction as evidenced by a reduction in ovarian constituents with. The overall data suggested an interference of indoxacarb with the vitellogenesis process.

5. Acknowledgements
This work was supported by the National Fund for Scientific Research to Pr. N. Soltani and the Ministry of High Education and Scientific Research of Algeria (CNEPRU and PNR projects).

6. References
1. Ebelling W. Ecological and behavioural aspects of Cockroach management, MR. Gorham ed, Ecology and management of food- Industry pests. FA Technical Bulletin 4.Association of official Analytical chemist, Arlington.VA, 1991, 85-113.
2. Cloarec A, Rivault C, Fontaine F, Le Guyader A. Cockroaches as carrier of bacteria in multi-family dwelling. Epidemiol. Infect 1992; 109; 483-490.
3. Mindykowski B, Jaenicke E, Tenzer S, Cirak S, Schweidardt T, Schild H *et al.* Cockroach allergens Per a 3 are oligomers. Dev Comp Immunol 2010; 34(7):722-33.
4. Nejati J, Keyhani A, Moosa-kazemi SH, Mohammadi M, Mahjoob M, Boostan Bakhsh A. Cockroaches' bacterial infections in wards of hospitals, Hamedancity. Asian Pacific Journal of Tropical Disease 2012; 2(5):381-384.
5. Rust MK, Owens JM, Reierson DA. Understanding and controlling the German cockroach. New York-Oxford. Oxford University Press, 1995, 265.
6. Habes D, Morakchi S, Aribi N, Farine JP, Soltani N. Boric acid toxicity to the German cockroach, *Blattella germanica*: alterations in midgut structure, and acethylcholinesterase and glutathione Stransferase activity. Pestic. Biochem. Physiol 2006; 84:17-24.
7. Habes D, Messiad R, Gouasmia S, Grib L. Effects of an inorganic insecticide (boric acid) against *Blattella germanica*: Morphometric measurements and biochemical composition of ovaries. Afr. J. Biotechnol 2013; 12(18):2492-2497.
8. Valles SM, Koehler PG, Brenner RJ. Comparative insecticide susceptibility and detoxification enzyme activities among pestiferous Blattodea Comp. Biochem. Physiol. Part C124, 227, 1999.
9. Kilani-Morakchi S, Badi A, Aribi N, Farine JP, Soltani N. Toxicity of tebufenozide, an ecdysteroid agonist, to Blattella germanica (Blattodea: Blattellidae). African Entomology 2014; 22(2):337-342
10. Kilani-Morakchi S, Aribi N, Farine JP, Smagghe G, Soltani N. Effects of tebufenozide on ovarian growth and sexual behaviour in the German cockroach (*Blattella* germanica). Comm. Appl. Biol. Sci. Ghent Univ 2009; 74(2): 429-436.
11. Maiza A, Aribi N, Soltani N. Effect of an oxadiazine indoxacarb, on the biochemical composition of ovaries in the german cockroach. Commun. Appl. Biol. Sci. Ghent Univ 2010; 75(3):359- 366.
12. Maiza A, Aribi N, Smagghe G, Kilani morakchi S, Bendjedid M, Soltani N. Sublethal effects on reproduction and biomarkers by spinosad and indoxacarb in cockroaches *Blattella germanica.* Bulletin of Insectology 2013; 66 (1):11-20.
13. Nehare S, Moharil MP, Godki BS, Lande GK, Bisane KD, Thkare AS *et al.* Biochemical analysis and synergist suppression of indoxacarb resistance in *Plutella xylostella* L. J. Asia-Pacifique Entomol 2010; 13:91-95.
14. Mahmoudvand M, Abbasipour H, Garjan AS, Bandani AR. Sublethal effects of indoxarb on the diamondback moth, *Plutella xylostella* (L) (Lepidoptera: Yponomeutidae). Appl Entomol Zool 2011; 46:75-80.
15. Wing KD, Sacher M, Kagaya Y, Tsurubuchi Y, Mulderig L, Connair M *et al.* Bioactivation and mode of action of the oxadiazine indoxacarb in insects. Crop Protection 2000; 19:537-545.
16. Shibko S, Koivistoinen P, Tratnyek CA, Newhall AR, Friedman L. A method for sequential quantitative separation and determination of protein, RNA, DNA, lipid, and glycogen from a single rat liver homogenate or from a subcellular fraction. Analytical Biochemistry 1966; 19:514-528.
17. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities on protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976 ; 72:248-254.
18. Duchâteau G, Florkin M. Sur la trehalosemie des insectes et sa signification. Archives Internationales de Physiologie et Biochimie 1959 ; 67(2):306-314.
19. Goldsworthy J, Mordue W, Guthkelch J. Studies on insect adipokinetic hormones. General and Comparative Endocrinology 1972; 18:545-551.
20. GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.
21. MINITAB statistical software standard version 13, 2003 (Penn State College, PA, USA) S. Weisberg. Minitab reference manual PA. States College. Minitab, 1985, 232.
22. Tillman PG, Hammes GG, Sacher M, Connair M, Brady EA, wing KD. Toxicity of a formulation of the insecticide indoxacarb to the tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae), and the big-eyed bug, *Geocoris punctipes* (Hemiptera: Lygaeidae). Pest Management Science. 2001; 58:92-100.
23. Alves AP, Allgeier WJ, Siegfried BD. Effects of the synergist S,S,S-triphorotrithioate on indoxacarb toxicity and metabolism in the European *Ostrinia nubilalis* (Hübner). Pesticide Biochemistry and Physiology 2008; 90:26-30.
24. Tine S, Aribi N, Soltani N. Laboratory evaluation of azadiractin against the oriental cockroach, *Blatta orientalis* L. (Dictyoptera, Blattellidae): Insecticidal activity and reproductive effects. Afr. J. Biotechnol 2011; 10(85):19816-19824.
25. Raikhel AS, Dhadialla TS. Accumulation of yolk proteins in insect oocytes. Ann. Rev. Entomol 1992; 37:217-251.
26. Lafont R, Dauphin- Villemant C, Warren JT, Rees H. Ecdysteroid chemistry and biochemistry. In: Gilbert LI, Iatrou K, Gill SK, editors. Comprehensive molecular insect science. Oxford: Elsevier 2005; 3:125-195.
27. Gäde G. Regulation of intermediary metabolism and water balance of insects by neuropeptides. Annual Rev. Entomol 2004; 49:93-113.
28. Maiza A, Kilani-Morakchi S, Farine JP, Smagghe G, Aribi N, Soltani N. Reproductive effects in german cochroaches by ecdysteroid agonist RH-0345, juvenile hormone analogue methoprene and carbamate benfuracarb. Commun. Appl. Biol. Sci. Ghent Uni 2004; 69:257-266.
29. Kaufmann C, Brown MR. Regulation of carbohydrate

metabolism and flight performance by a hypertrehalosaemic hormone in the mosquito *Anopheles gambiae*. Journal Insect Physiol 2008; 54:367-377.

30. Vishal M, Rahman SMA, Kuldeep S, Nayak SK. Antifeedant effects of sub-lethal concentrations of some novel insecticides against tobacco caterpillar, Spodoptera litura (Fab*.)*. J.Plant Protect. Environ 2005; 2:118-122.
31. Messiad R, Habes D, Soltani N. Reproductive effects of a neconicotinoid insecticide (Imidacloprid) in the German Cockroaches *Blattella germanica* L. (Dictyoptera, Blattellidae). Journal of Entomology and Zoology Studies 2015; 3(2):01-06
32. Soltani-Mazouni N, Hami M, Gramdi H. Sublethal effects of methoxyfenozide on reproduction of the Mediterranean flour moth, *Ephestia Kuehniella* Zeller. Invertebrate Reproduction & Development 2012; 56(2):157-163.
33. Soltani- Mazouni N, Bouteldja D, Lemeire E, Smagghe G. *In vivo* effect of ACE inhibiting in mealworms on ovarian composition and ecdysteroid amounts. Commun. Agric. Appl. Biol Sci 2007; 72(3):623-627.
34. Belles X. Vitellogenesis directed by juvenile hormone, *in:* Reproductive Biology of Invertebrates, Part B: Progress in Vitellogenesis, A.S. Raikhel (Ed), Science Publishers, Enfield, USA/Plymouth, UK 2005; 12:157-197.
35. Fu-Gen LI, Guo-Min AI, Dong-Yun ZOU, Ying JI, GU Bao-Gen, Xi-Wu GAO. Characterization of Activation Metabolism Activity of Indoxacarb in Insect s by Liquid chromatography - Triple Quadrupole Mass Spectrometry. Chinese Journal of Analytical Chemistry 2014; 42(4):463-468.