Full Length Research Paper

Laboratory evaluation of azadirachtin against the oriental cockroach, *Blatta orientalis* L. (Dictyoptera, Blattellidae): Insecticidal activity and reproductive effects

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In this work, the toxicity of azadirachtin applied topically at various doses (300, 600, 1200, 2400, 4800 and 9000 ng/ insect) was evaluated against newly emerged adults of the oriental cockroach, *Blatta orientalis* L. (Dictyoptera, Blattellidae) under laboratory conditions. Results show that treated insects exhibited toxic symptoms with a dose-dependent mortality. In a second series of experiments, the compound applied topically at its LD_{50} and LD_{90} on newly emerged female adults was investigated on ovarian growth during the first gonadotrophic cycle (0, 2, 4 and 6 days). Azadirachtin was found to reduce the number of oocytes per paired ovaries and the size of basal oocytes. Furthermore, the compound was examined on ovarian biochemical components. Biochemical data revealed a significant reduction of ovarian contents of proteins, lipids and carbohydrates with the two tested doses. The overall results suggest an interference of the compounds with the vitellogenesis.

Key words: Azadirachtin, *Blatta orientalis,* toxicity, reproduction, ovary, biochemical components.

INTRODUCTION

Cockroaches are important pests with public health implications. They are potential vectors of human pathogens, and they are associated with allergies and asthma (Mindykowski et al., 2010; Peden and Reed, 2010). *Blattella germanica*, *Blatta orientalis*, and *Periplaneta americana* are worldwide distributed species (Porcar et al., 2006). Cockroaches are primarily controlled through the use of conventional synthetic insecticides (organophosphates, pyrethroids, and carbamates). The extensive and widespread use of these neurotoxic insecticides has caused some concerns and intensified the search for new environmentally safe and target-specific insecticides. One potential alternative is the use of insect growth regulators (IGRs), and natural pesticide like azadirachtin.

Azadirachtin (AZ) is a triterpenoid isolated from the kernels of the neem tree, *Azadirachta indica* A. Juss. Its isolation, structure determination, synthesis, toxicity, mode of action, spectrum of activity, and practical application have all absorbed the interest of scientists of many disciplines during the past four decades and a resurgence of interest in azadirachtin is observed. As reported by published comprehensive reviews (Mulla and Su, 1999; Mordue et al., 2005), Azadirachtin is able to induce multiple effects in numerous species of economic pests such as antifeedancy, growth regulation, fecundity suppression and sterilization, oviposition repellency or attractancy, and changes in biological fitness. These effects have been shown in several species of cockroaches through oral or topical administration of Azadirachtin or commercial formulations. Most tested species are *P. americana* and *B. germanica* (Qadri and Narsaiah, 1978; Prabhakaran and Kamble, 1996; Shafeek et al., 2004). In Algeria, *B. orientalis* is a serious pest in the urban environment and conventional insecticides were used for many years to control its infestations (Habes et al., 2001). In previous bioassays, several categories of pesticides against *B. germanica*:

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boric acid, an inorganic insecticide (Habes et al., 2006; Kilani-Morakchi et al., 2009a), halofenozide and tebufenozide, edysteroid agonists (Maiza et al., 2004; Kilani-Morakchi et al., 2009b, 2009c), and indoxacarb and benfuracarb (Maiza et al., 2004, 2010) were tested. In order to extend the recent insecticidal bioassays and to obtain more information on the mode of action of AZ, we first evaluated the toxicity of this compound through topical administration on *B. orientalis* adults used as cockroach model. The reproductive capacity is a major factor influencing the infestation of pests. Therefore, in a second series of experiments, we investigated the effects of AZ applied at its LD_{50} and LD_{90} on ovarian parameters and components like proteins, carbohydrates and lipids.

MATERIALS AND METHODS

Insects

Colonies of *B. orientalis* were reared in plastic containers (30 x 30 x 30 cm) and maintained at 27 ± 1°C, 80% relative humidity, and photoperiod of 12:12 h light:dark cycle. The cockroaches were fed with dry dog food and water provided *ad libitum* as previously described (Habes et al., 2006). Adults were classified according to their age in days from adult emergence. The age and the number of insect tested in each bioassay are given with the results.

Insecticide and topical treatment

Azadirachtin (1% AZ, non oil EC, Sipcam Inagra, Valencia, Spain) (courtesy of Pr. G. Smagghe, Laboratory of Agrozoology, University of Ghent, Belgium) was dissolved in acetone and topically administrated (7 µl per insect) at different doses (300, 600, 1200, 2400, 4800 and 9000 ng/ insect) on newly emerged male and female adults. Control insects were treated with acetone (7 µl per insect) alone. All insects were kept under the same conditions.

Toxicity tests

Four replication containing each 20 newly emerged adults (<4 h old, ten females and ten males) per dose were treated topically. Mortality was assessed at 5, 10, 15 and 20 days after the beginning of the experiments and corrected (Abott, 1925). Bioassay data were subjected to probit analysis (Finney, 1971), and estimated lethal doses (LD_{50} and LD_{90} values, that is, the dose causing mortality effects in 50 and 90% of the treated insects, respectively) and their fiducial limits were determined (Swaroop et al., 1966).

Ovarian growth parameters

As above, newly emerged female adults (<4 h after adult emergence) were treated topically with AZ at its LD_{50} and LD_{90} as determined before. Each treated female was immediately paired with one untreated male in a plastic box (9.5 x 6.5 x 2 cm) containing food and water. Adult females from control and treated series were sampled at 0, 2, 4 and 6 days during the adult life and their ovaries dissected out. After removal of circumovarian fat body, the number of oocytes in each paired ovaries was recorded. The volume of the basal oocyte was calculated according to Lambreas et al. (1991). Six to ten replications were done for each series.

Ovarian biochemical components

Paired ovaries were collected at various times from the control and treated adult females and analyzed individually as described previously (Loucif-Ayad et al., 2010). Carbohydrates, lipids and proteins were extracted in 1 ml of trichloroacetic acid (20%) from the same sample according to the procedure of Shibko et al. (1966). Quantification of the amounts of carbohydrates was performed based on the anthrone procedure of Duchâteau and Florkin (1959), total proteins based on Bradford (1976), and lipids based on the vanillin method of Goldsworthy et al. (1972). Six females were used per lethal dose for the morphometric measurements of ovaries and data on ovarian biochemical components were expressed in µg per paired ovaries.

Statistics

Results were expressed as mean ± standard error (SE). Data of toxicity were subjected to a one-way analysis of variance (ANOVA). When the analysis of variance was significant (p<0.05), mean values obtained were separated by LSD test. In the other experiments, the comparison of mean values was made by Student's *t*-test. All statistical analyses were performed using MINITAB Software (Version 13.31, Penn State College, PA, USA).

RESULTS

Insecticidal activity

The percentage of corrected mortality of *B. orientalis* was determined as a function of the doses of AZ (300, 600, 1200, 2400, 4800 and 9000 ng/insect) and the duration of treatment (5, 10, 15, and 20 days). As illustrated in Table 1, our data show that treatment is widely correlated to the concentration of AZ and the mortality is time-dependent (p < 0.001). LD_{50} (ng/insect) calculated by probit analysis at selected times after topical application on newly emerged adults of *B. orientalis* were 1937.51, 1349.32, 577.21 and 335.46 ng/insect, at 5, 10, 15 and 20 days respectively. The mortality recorded in controls was 8.33 ± 1.3%. These data proved that the toxicity of azadirachtin increased with the dose and the duration of treatment (Table 2). Results from one-way ANOVA followed by LSD test revealed three groups of concentrations at five days, four groups at ten days, three groups at 15 days, and three groups at 20 days. The values of LD_{50} and LD_{90} (ng/insect), and their corresponding 95% fiducial limits after treatment of newly emerged adults of *B. orientalis* are presented in Table 2.

Ovarian morphometric measurements

As shown in Figure 1A, the number of oocytes per paired ovaries in the controls increased during the sexual maturation (0 to 2 days old; p< 0.001), and decreased at days 4 and 6 (beginning of ovulation) (p< 0.001). Treatment with Azadirachtin on newly emerged females of *B. orientalis* reduced significantly the number of oocytes, at days 4 (p = 0.008) and 6 (p = 0.023) for the

Table 1. Corrected mortality (%) as function of the dose of azadirachtin (300, 600, 1200, 2400, 4800 and 9000 ng/ insect) and the duration of treatment (days) after topical application on newly emerged adults of *B. orientalis*. Data are expressed as means ± SE based on four replicates each containing 20 cockroaches. For each exposure time, mean values followed by different letters are significantly different by LSD test.

Doses (ng/insect)	5 days	10 days	15 days	20 days
300	5.80 ± 3.87[a]	9.39 ± 0.40[a]	31.51 ± 5.65[a]	53.63 ± 10.90[a]
600	14.39 ± 7.07[a]	37.27 ± 5.45[b]	59.39 ± 3.23[b]	78.18 ± 3.63[b]
1200	38.38 ± 4.71[b]	43.33 ± 8.88[b]	62.42 ± 1.61[b]	84.54 ± 7.87[b]
2400	55.80 ± 6.90[b]	56.36 ± 2.42[b]	87.57 ± 3.83[b]	96.96 ± 4.04[b]
4800	76.51 ± 9.59[c]	81.21 ± 6.46[c]	96.96 ± 4.04[c]	100 ± 0.00[c]
9000	91.16 ± 6.22[c]	96.96 ± 4.04[d]	100 ± 0.00[c]	100 ± 0.00[c]

Table 2. Toxicity of azadirachtin by topical application to newly emerged male and female adults of *B. orientalis:* LD_{50}, LD_{90} (ng/ insect) and their fiducial limits (95% FL) as function of the exposure time (days).

Time (days)	Linear regression	Slope	LD_{50} (95% FL)	LD_{90} (95% FL)
5	y = 1.960x- 1.443	3.21	1937.51 (1862.26 - 2015.80)	8732.18 (8181.69 - 9319.69)
10	y = 1.906x - 0.966	3.32	1349.32 (1295.46 - 1405.41)	6346.27 (5935.79 - 6785.12)
15	y = 3.088x - 0.927	2.09	577.21 (562.87 - 591.90)	1500.93 (1442.10 - 1562.15)
20	y = 2.671x - 1.746	2.35	335.46 (325.85 - 345.35)	1012.67 (966.54 - 1060.98)

LD_{50}, and at all the tested ages (2, 4 and 6 days) during the experimental period for the LD_{90} (p = 0.038 for two days; 0.003 for four days and 0.002 for six days) as compared to controls of the same age (Figure 1). ANOVA revealed a significant effect of the compound on the number of oocytes as function of the dose (p<0.001) and the duration of treatment (p<0.001) during the first days of adult life. Treatment affected the number of oocytes per paired ovaries with a dose-response relationship.

In the control series, the volume of basal oocytes also increased during this period (0, 2, 4 and 6 days) varying from 0.0096 ± 0.0006 mm^3 at 0 day to 0.0453 ± 0.0008 mm^3 at six days. Treated females (LD_{50} and LD_{90} respectively) had significantly smaller volumes at days 2 (p = 0.026 and 0.001), 4 (p = 0.002 and 0.001) and 6 (p = 0.027 and 0.011) (Figure 1B). The ANOVA indicated a significant effect (p≤ 0.001) of treatment, age and interaction treatment age.

Ovarian biochemical components

In the control, the contents of main components of ovaries (proteins, lipids and carbohydrates) increased and showed a peak at day 4 after adult emergence related with the vitellogenesis. Treatment reduced significantly the ovarian protein content with a dose-response relationship at all ages (two, four and six days) with the LD_{50} (p =0.003, 0.004 and 0.014 respectively) and LD_{90} (0.002, 0.001 and 0.034 respectively) as compared to the controls (Figure 1C). The ovarian lipid content was also reduced in treated series at 2 (p = 0.039 for LD_{50} and 0.012 for LD_{90}), 4 (p = 0.022 for LD_{50} and

0.004 for LD_{90}) and 6 days (p = 0.015 for LD_{50} and 0.001 for LD_{90}) (Figure 2C). Finally, the compound at the two tested doses also reduced significantly the carbohydrate contents (p=0.010, <0.001 and 0.007 for LD_{50} and p = 0.007, <0.001 and 0.001 for LD_{90}) in ovaries at all tested ages (two, four and six days respectively) (Figure 2C). These effects were dose-dependent. ANOVA showed a significant effect of the compound (p<0.001) as a function of the dose (p<0.001) and the duration of treatment (p<0.001) for all ovarian components.

DISCUSSION

Insecticidal activity

Recent insecticides such as IGRs and natural pesticide are presumed to have little impact on many beneficial organisms (Dhadialla et al., 2005; Mordue et al., 2005), but effects are highly variable depending on the species and studied developmental stage. In this study, we evaluated the topical toxicity of AZ on newly emerged adults of *B. orientalis.* Our results revealed that AZ caused lethal effects on *B. orientalis,* which may affect population dynamics via reduction in survival. Our results showed that this compound resulted in a dose-and-time-dependent mortality since the LD_{50} value (ng/insect) decreased as a function of the time. The harmlessness of neem derivatives products towards many predators and parasitoids has been reported extensively in the literature even though several negative delayed effects of these compounds were recorded mostly under laboratory conditions (Schmutterer, 1997). Nevertheless, AZ effects
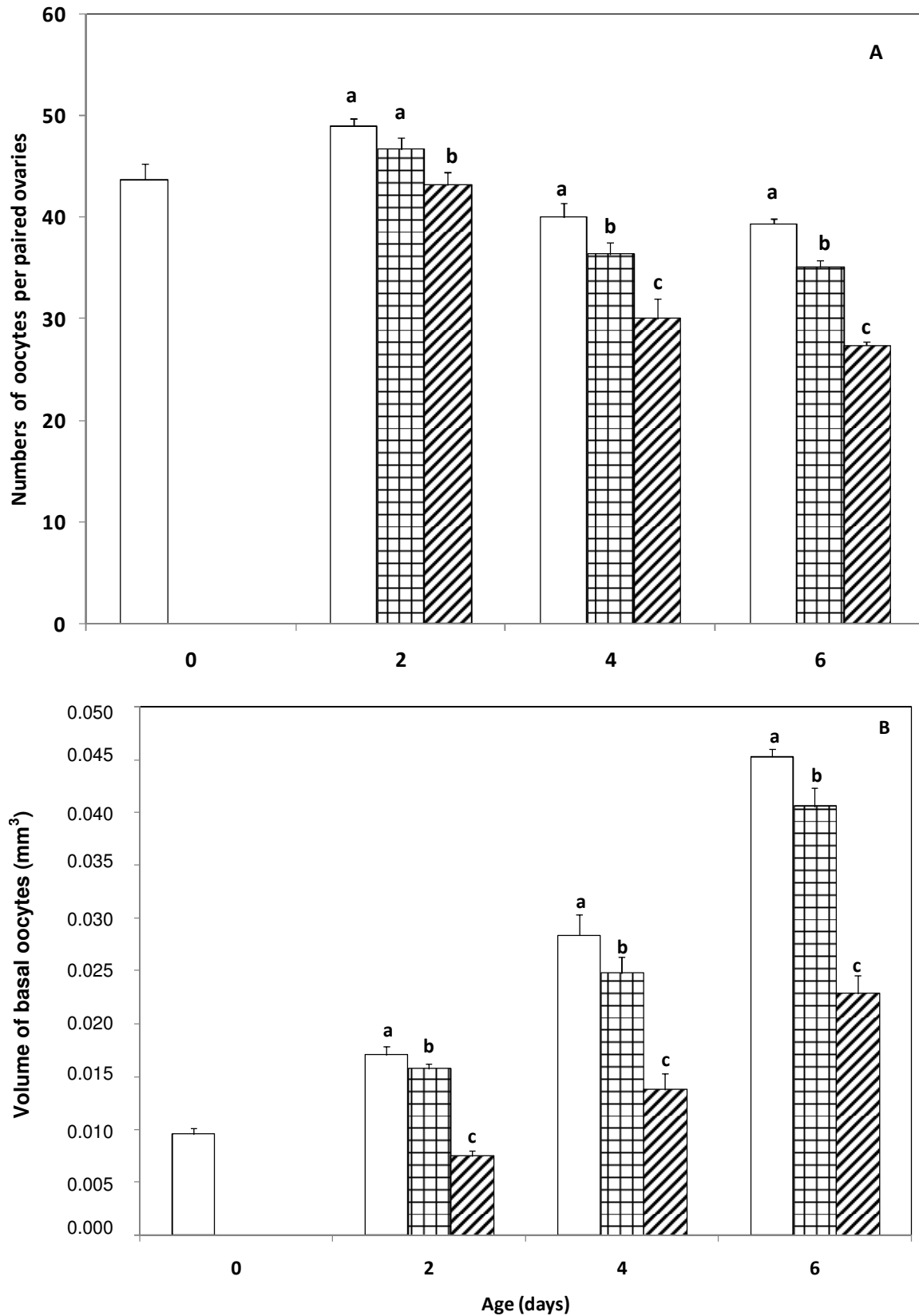
Figure 1. Effect of topical application of azadirachtin (1937,51 and 8732,18 ng/insect) on newly emerged female adults of *B. orientalis* on the numbers of oocytes per paired ovaries (A), the volume of basal oocytes (B). Values is the mean ± SE (n=6) (Mean values of the same age followed by different letters are significantly different (p<0.05).
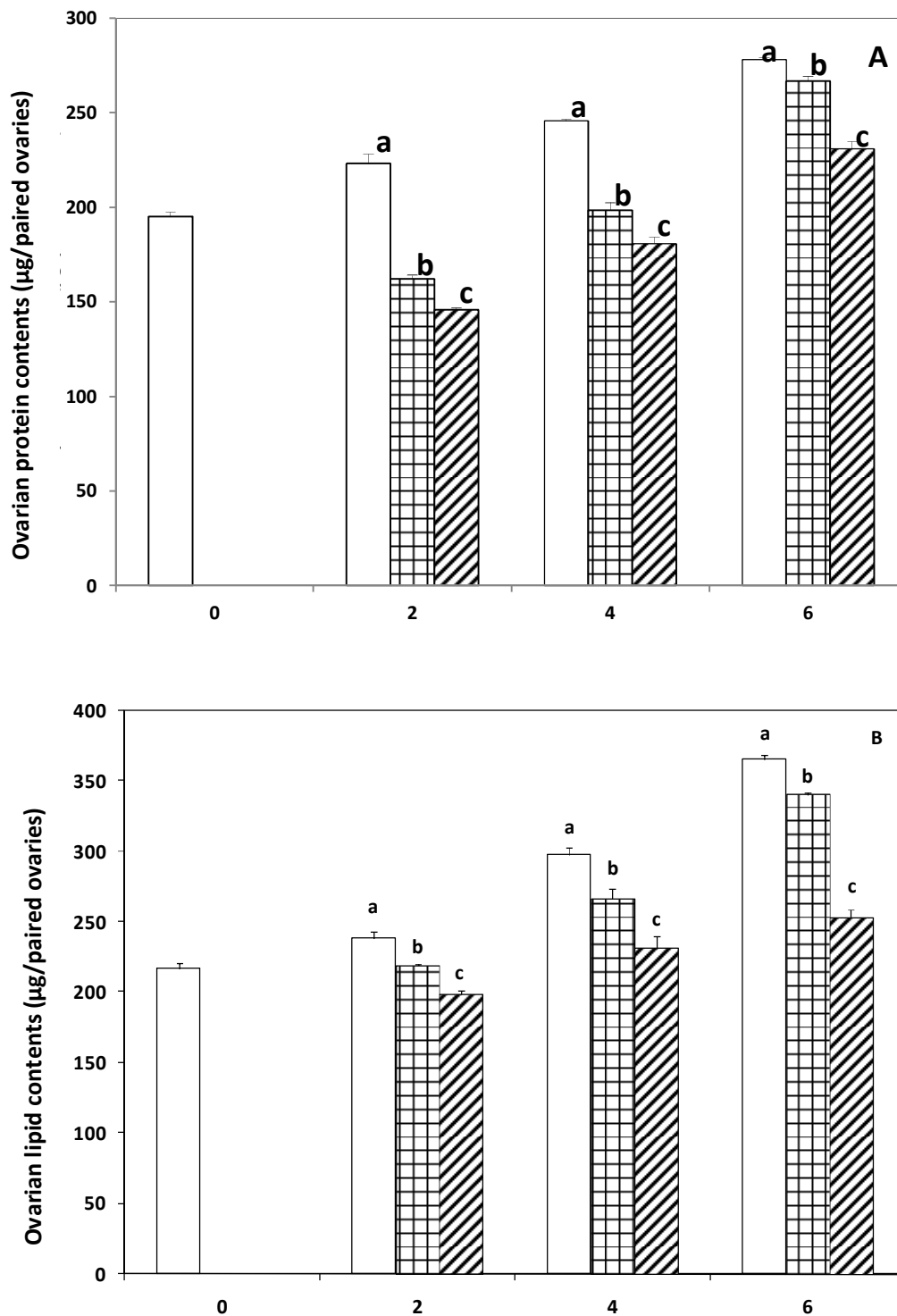
Figure 2. Effect of topical application of azadirachtin (1937,51 and 8732,18 ng/insect) on the ovarian content (µg/paired ovaries) of proteins (A), lipids (B), and carbohydrates (C) in newly emerged female adults of *B. orientalis* Values is the mean ± SE (n=6) (mean values of the same age followed by different letters are significantly different (p<0.05).

on the mortality changes as a function of the insect order; *Schistocerca gregaria* and many species of Lepidoptera are among the most sensitive, being deterred by as little as 0.007 ppm, whereas Hemiptera and Coleoptera species are much less sensitive with EC_{50} values of around 100 ppm or more (Mordue et al., 2005).
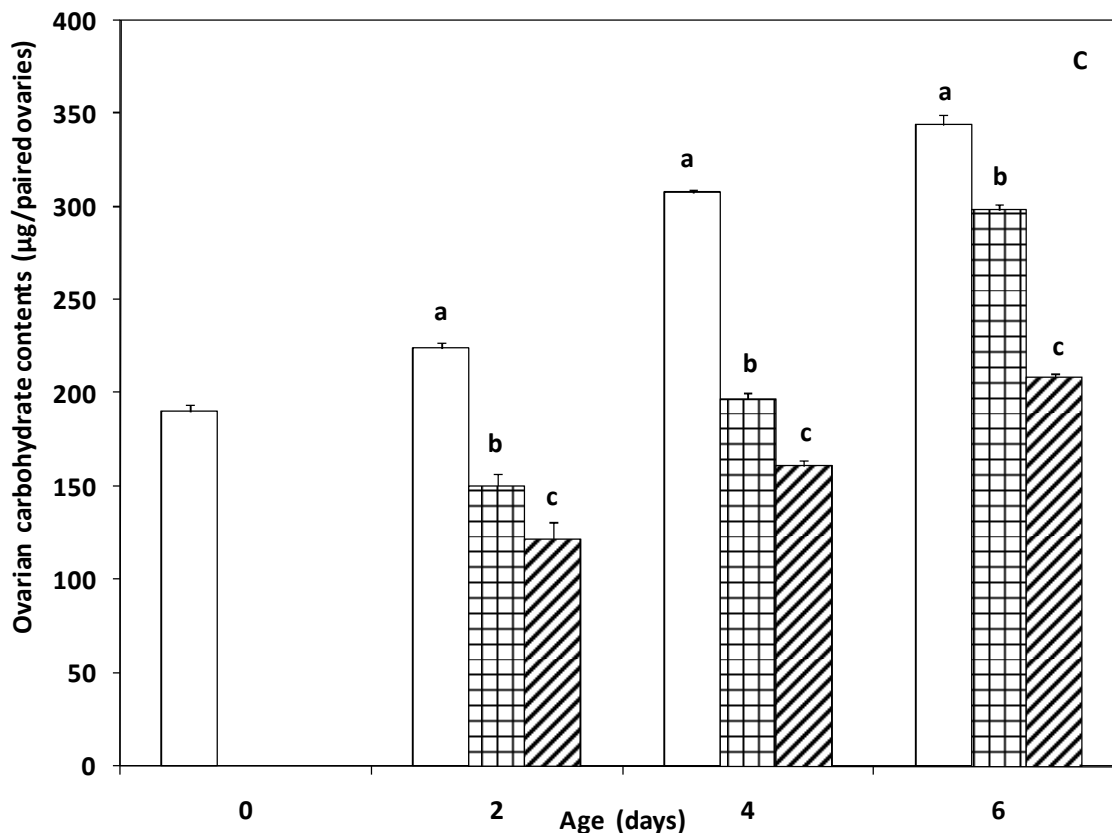
Figure 2. continue.

Numerous studies have been done on the anti-feedant and insect growth-regulatory effects of neem derivatives (Andreu et al., 2000). The study of Shafeek et al. (2004) was carried out to understand the mechanism of neurotoxicity of AZ in the cockroach, *P. americana.* They suggested that AZ exerts excitatory action on the electrical activity in the nervous system of cockroach by interfering with the ion channels in the nerve membrane, the probable target of several insecticides. Tang et al. (2002) found little impact in the survival of adults of the Aphidiinae *Lysiphelbus testaceipes* when exposed to citrus leaves previously dipped in AZ suspensions.

Ovarian growth

In a second series of experiments, AZ was evaluated on ovarian morphometry. In the controls, the number of oocytes per paired ovaries decreased at day 4; this reduction coincides with the beginning of ovulation in *B. germanica* as previously determined (Kilani-Morakchi et al., 2009a). AZ was found to reduce the number of oocytes per paired ovaries and the volume of the basal oocytes as compared to controls during the adult life with effect-dose dependant. As stated by Shafeek et al. (2004), AZ might act negatively on neuropeptides with

myotropic properties regulating ovulation. Galvan et al. (2005) showed that other natural pesticides decreased weight gain and reduced female fertility of *Harmonia axyridis* (Coleoptera). Previous studies reported similar effects on ovarian parameters in *B. germanica* treated with boric acid (Kilani-Morakchi et al., 2009b), benfuracarb, a neurotoxic compound, and halofenozide, an ecdysteroid agonist (Maiza et al., 2004). Methoprene, a juvenile hormone analogue, reduced the number of oocytes per paired ovaries, but increased the volume of basal oocytes (Maiza et al., 2004). In adult females of *Labidura riparia*, AZ has been reported to have marked cytological effects on ovaries resulting in severely reduced ovarian development (Sayah et al., 1996). Sterility effects in females due to interference with vitellogenin synthesis and its uptake into oocytes has been demonstrated in many insects including *Locusta migratoria, Oncopeltus fasciatus, Spodoptera exempta,* and *R. prolixus* (Feder et al., 1988; Tanzubi and McCaffery, 1990). Reduced fecundity has been recorded in *Ceratitis capitata, Liriomyza trifolii, Myzus persicae, S. exempta, Spodoptera littoralis,* and the *Nezara viridula* (Nisbet et al., 1994; Adel and Sehnal, 2000; Riba et al., 2003). Such fertility effects is related to re-absorption of yolk proteins in developing eggs (Rembold and Sieber, 1981) and lower viability of emerging larvae after

treatment of the parental generation (Tanzubil and McCaffery, 1990). Moreover, in *R. prolixus*, reduction in oocyte growth and egg production are caused by reduced ecdysteroid levels in hemolymph and ovaries (Garcia et al., 1991). In *L. migratoria,* effects of AZ on egg development were shown to be related to changes in both juvenile hormone (JH) and ecdysone titers (Rembold et al., 1986).

Biochemical composition of ovaries

In the controls, the low amount of protein content at six days is probably due to the end of vitellogenesis and the preparation of the ovary to new gonadotrophic cycle; whereas, the reduction in lipids and carbohydrates correspond to the energy consumption required to vitellogenesis. In the treated series, AZ induced a significant decrease in ovarian protein, lipid and carbohydrate contents suggesting an interference with the vitellogenesis. The physiological process in less specialized groups like cockroaches is governed mainly by juvenile hormone and a number of peptides are also involved (Bellés and Maestro, 2005; Gade and Hoffmann, 2005). AZ is an anti-feedant and it is well known that in this species feeding affects vitellogenesis (Schal et al., 1997). AZ treatment blocks vitellogenesis and corpus allatum activity as seen by ultrastructural studies and concomitantly increases allatostatin build-up in the pars lateralis. This phenomenon together with the fact that JH treatment rescues the effect of AZ on vitellogenesis suggest that this compound affects the peptides controlling corpus allatum activity in a similar manner to the azadirachtin effects on prothoracicotropic hormone (PTTH) and hence ecdysone production (Mordue et al., 2005). AZ induced several effects in the treated insects. According to Mordue et al. (2005), AZ alters or prevents the formation of new assemblages of organelles or cytoskeleton resulting in the disruption of cell division, blocked transport and release of neurosecretory peptides, and inhibition of spermatozoa formation. It also inhibits protein synthesis in cells that are metabolically active.

The decrease in protein synthesis in AZ treated *Choristoneura rosaceana* is due to an inhibition of midgut esterases (Mordue et al., 2005). Azadirachtin directly inhibits protein synthesis in a variety of tissues via enzymes such as trypsin in the midgut or 20-monooxygenases for ecdysone catabolism by fat body cells. Also, the primary behavioral antifeedant response of insects to AZ is mediated via neural input from the contact chemoreceptors. Inhibition of feeding behavior results from stimulation of deterrent receptors by AZ often coupled with an inhibition of sugar receptors (Mordue et al., 2005).

Our results show a significant decrease in ovarian protein, lipid and carbohydrate contents suggesting an

interference with the vitellogenesis. This reduction of ovarian component contents was also observed in *B. germanica* treated with benfuracarb (carbamate) and insect growth regulators like the ecdysone agonist, halofenozide (Kilani-Morakchi et al., 2009a; Maiza et al., 2010) and the juvenile hormone mimetic, methoprene (Maiza et al., 2004), boric acid (Kilani-Morakchi et al., 2009b), or with indoxacarb (Maiza et al., 2010). In contrast, methoprene increased the ovarian protein content at the lower dose in *B. germanica* (Maiza et al., 2004). In insect predators, AZ also had no significant effects, such as *Chrysoperla carnea* (Medina et al., 2003) and *Orius insidiosus* (Elzen, 2001). The biochemical composition of ovaries is also disturbed after treatments with conventional pesticides in other taxa (Glusczak et al., 2005; Rahimi and Abdollahi, 2005).

The data obtained in *B. orientalis* showed that AZ affected reproduction. In insects, reproduction comprises a succession of interdependent steps, from sex determination to oviposition, all of which are regulated by certain hormonal factors, including ecdysteroids and juvenile hormone, but also neurohormones with gonadotropic and antigonadotropic effects (Bellés, 1995; Gade and Hoffmann, 2005). The physiological process in less specialized groups like cockroaches is governed mainly by juvenile hormone and a number of peptides (Bellés and Maestro, 2005; Gade and Hoffmann, 2005). In all cockroaches studied to date, vitellogenesis and cyclic maturation of oocytes depends upon juvenile hormone III synthesis by the corpora allata (CA). The relative activity of the CA in adult female is dependent upon and modulated by intrinsic signals which may originate from the brain and ovary and which may be influenced by nutrient status of the female (Schal et al., 1997). To explain these results, different hypotheses can be postulated. Treatment may have caused possible disturbance in the vitellogenesis process via the nervous, neuroendocrine and/or endocrine system.

Conclusion

Azadirachtin exhibited insecticidal activity against *B. orientalis*. Morphometric and biochemical studies demonstrated that the compound also affected reproduction. Data shows that the compound reduced the number of oocytes per paired ovaries, the size of basal oocytes and ovarian constituents with the two tested doses (LD_{50} and LD_{90}). The overall data suggest an interference of azadirachtin with the vitellogenesis.

REFERENCES

Abott WS (1925). A method for computing the effectiveness of insecticide. J. Econ. Entomol. 18: 265-267.

Adel MM, Sehnal F (2000). Azadirachtin potentiates the action of ecdysteroid agonist RH-2485 in *Spodoptera littoralis*. J. Insect Physiol. 46: 267-274.

Andreu J, Albert S, Magi R (2000). Antifeedant activity of *Melia azadirach* and *Azadirachta indica* on larvae of *Sesamia nonagrioides*. Phytoparasitica, 28: 311-319.

Bellés X (1995). Interaction between corpora allata, fat body and ovary in insect reproduction: which controls which? Netherlands. J. Zool. 45: 152- 156.

Bellés X, Maestro JL (2005). Endocrine peptides and insect reproduction. Invertebrate Reprod. Dev. 47(1): 23-37.

Bradford MM (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.

Dhadialla TS, Retnakaran A, Smagghe G (2005). Insect growth- and developmental-disturbing insecticides, in: Gilbert LI, Iatrou K, Gill SK (eds.), Comprehensive Molecular Insect Science, Elsevier, Oxford, 6: 55-116.

Duchateau G, Florkin M (1959). Sur la tréhalosémie des insectes et sa signification. Arch. Int. Physiol. Biochem. 67: 306-314.

Elzen GW (2001). Lethal and sublethal effects of insecticide residues on *Orius insidiosus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Lygaeidae). J. Econ. Entomol. 94: 55-59.

Feder D, Valle D, Rembold H, Garcia ES (1988). Azadirachtin-induced sterilization in mature females of *Rhodnius prolixus*. Z. Naturforsch. C 43: 908-913.

Finney DJ (1971). Probit A nalysis, Third edition. Cambridge University Press, London. p. 333.

Gade G, Hoffmann KH (2005). Neuropeptides regulating development and reproduction in insects. Physiol. Entomol. 30: 103-121.

Galvan TL, Koch RL, Hutchison WD (2005). Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae). Biol. Control. 34: 108-114.

Garcia ES, Gonzales MS, Azambuja P (1991). Effects of azadirachtin in *Rhodnius prolixus*: data and hypotheses. Mem. Inst. Oswaldo Cruz, Rio de Janiero. 86: 107-111.

Glusczak L, Miron DDS, Crestani M, Fonseca MBD, Pedron FDA, Duarte MF, Vieira VL (2005). Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obstusidens*). Ecotoxicol. Environ. Saf. 65(2): 237-241.

Goldsworthy GJ, Mordue W, Guthkelch J (1972). Studies on insect adipokinetic hormones. Genr. Compar. Endocrinol. 18: 545-551.

Habes D, Morakchi S, Aribi N, Farine JP, Soltani N (2001). Toxicity of Boric acid to *Blattella germanica* (Dictyoptera: Blattellidae) and analysis of residue in several organs. Med. Fac. Landbouww. Univ. Gent. 66(2): 525-534.

Habes D, Kilani-Morakchi S, Aribi N, Farine JP, Soltani N (2006). Boric acid toxicity to the German cockroach, *Blattella germanica:* alteration in midgut structure, and acetylcholinesterase and glutathione S-transferase activity. Pestic. Biochem. Physiol. 84: 17-24.

Kilani-Morakchi S, Aribi N, Soltani N (2009a). Activity of boric acid on German cockroaches: Analysis of residues and effects on reproduction. Afr. J. Biotechnol. 8: 703-708.

Kilani-Morakchi S, Aribi N, Farine JP, Smagghe G, Soltani N (2009b). Halofenozide affects sexual behavior, cuticular hydrocarbons and reproduction in the female German cockroach *Blattella germanica* (Dictyoptera, Blattellidae). Belg. J. Zool. 139: 147-155.

Kilani-Morakchi S, Aribi N, Farine JP, Smagghe G, Soltani N (2009c). Effects of tebufenozide on ovarian growth and sexual behaviour in

the German cockroach (*Blattella germanica*). Comm. Appl. Biol. Sci. Ghent Univ. 74(2): 429-436.

Lambreas CI, Galante F, Mena I (1991). Ovarian condition as an indicator of the phonology of *Bubas bubas* (Coleoptera: Scarabeidae). Ann. Entomol. Soc. Am. 84: 190-194.

Loucif-Ayad W, Aribi N, Smagghe G, Soltani N (2010). A scientific note on the impact of acaracides on the nutritional biochemistry of *Apis mellifera intermissa* (Hymenoptera: Apidae). Apidologie, 41(2): 135-137.

Maiza A, Kilani-Morakchi S, Farine JP, Smagghe G, Aribi N, Soltani N (2004). Reproductive effects in german cochroaches by ecdysteroid agonist RH-0345, juvenile hormone analogue methoprene and carbamate benfuracarb. Commun. Appl. Biol. Sci. Ghent Uni. 69: 257-266.

Maiza A, Aribi N, Smagghe G, Soltani N (2010). Effect of an oxadiazine indoxacarb, on the biochemical composition of ovaries in the german cockroach. Commun. Appl. Biol. Sci. Ghent Univ. 75(3): 359- 366.

Medina P, Budia F, Del Estal P, Viñuela E (2003). Effects of three modern insecticides, pyriproxyfen, spinosad and tebufenozide, on survival and reproduction of *Chrysoperla carnea* adults. Ann. Appl. Biol. 142: 55-61.

Mindykowski B, Jaenick E, Tenzer S, Cirak S, Schweikardt T, Schild H, Decker H (2010). Cockroach allergens Per a 3 are oligomers. Dev. Comp. Immunol. 34: 99-722.

Mordue Luntz AJ, Morgan ED, Nisbet AJ (2005). In Comprehensive Molecular Insect Science, Gilbert LI, Iatrou K, and Gill SS (eds.). Elsevier, Oxford, UK. 6: 117-135.

Mulla MS, Su T (1999). Activity and biological effects of neem products against arthropods of medical and veterinary importance. J. Am. Mosq. Contr. Ass. 15(2): 133-152.

Nisbet AJ, Woodford JAT, Strang RHC (1994). The effects of azadirachtin-treated diets on the feeding behavior and fecundity of the peach potato aphid, *Myzus persicae*. Entomol. Exp. Appl. 71: 65-72.

Peden D, Reed CE (2010). Enviromental and occupational allergies. J. Aller. Clin. Immunol. 125: 60-150.

Porcar M, Navarro L, Jiménez-Peydro R (2006). Patogenicity of intrathoracically administrated *Bacillus thuringiensis* spore in *Blatta orientalis*. J. Invert. Pathol. 93: 63-66.

Prabhakaran SK, Kamble ST (1996). Effects of azadirachtin different strains of German cockroach (Dictyoptcra: Blattellidae). Environ. Entomol. 25: 130-134.

Qadri SSH, Narsaiah J (1978). Effect of azadirachtin the moiling processes of last instar nymphs of *Periplaneta americana* (Linn.). Indian J. Exp. Biol. 16: 1141-1143.

Rahimi R, Abdollahi M (2005). A review on the mechanisms involved in hyperglycemia induced by organophosphorus pesticides. Pest. Biochem. Physiol. 88: 115-121.

Rembold H, Sieber KP (1981). Inhibition of oogenesis and ovarian ecdysteroid synthesis by azadirachtin in *Locusta migratoria* migratorioides (R and F). Z. Naturforsch. C 36: 466-469.

Rembold H, Schlagintweit B, Ulrich GM (1986). Activation of juvenile hormone synthesis in vitro by a corpus cardiacum factor from *Locusta migratoria*. J. Insect Physiol. 32: 91-94.

Riba M, Martı J, Sans A (2003). Influence of azadirachtin on development and reproduction of *Nezara viridula* L. (Het., Pentatomidae). J. Appl. Entomol. 127: 37-41.

Sayah F, Fayet C, Idaomar M, Karlinsky A (1996). Effect of azadirachtin on vitellogenesis of *Labidura riparia* (Insecta, Dermaptera). Tissue Cell. 28: 741-749.

Shafeek A, Jaya Prasanthi RP, Hariprasad Reddy G, Chetty CS, Rajarami Reddy G (2004). Alterations in acetylcholinesterase and electrical activity in the nervous system of cockroach exposed to the neem derivative, azadirachtin. Ecotoxicol. Environ. Saf. 59: 205-208.

Schal C, Holbrook GL, Bachmann JAS, Sevala VL (1997). Reproductive biology of the German cockroach, *Blattella germanica*: juvenile hormone as a pleiotropic master regulator. Arch. Insect. Biochem. Physiol. 35: 405-426.

Schmutterer H (1997). Side-effects of neem (*Azadirachtina indica*) products on insect pathogens and natural enemies of spider mites and insects. J. Appl. Entomol.121: 121-128.

Shibko S, Koivistoinen P, Tratnyek CA, Newhall AR, Friedman L (1966).

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. Anal. Biochem. 19: 514-528.

Swaroop S, Gilroy AB, Uemura K (1966). Statistical methods in malaria eradication. Geneva: World Health Organisation. Monograph Series. 51: p. 164.

Tang YQ, Weathersbee III AA, Mayer RT (2002). Effects of neem seed extract on the brown citrus aphid (Homoptera: Aphiididae). Environ. Entomol. 31: 172-176.

Tanzubil PB, McCaffery AR (1990). Effects of azadirachtin and aqueous neem seed extracts on survival, growth and development of the African armyworm, *Spodoptera exempta*. Crop Prot. 9: 383-386.