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Effects of an inorganic insecticide (boric acid) against *Blattella germanica*: Morphometric measurements and biochemical composition of ovaries

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Pestiferous cockroach species are closely associated with humans and are important for economic and public health points of view. In Algeria, *Blattella germanica* L. (Dictyoptera: Blattellidae) is a serious pest in the urban environment. Conventional insecticides, such as the organophosphates, carbamates, and pyrethroids, have been used widely to control cockroach which have developed resistance to these compounds. Thus, interest has been focused on lesser-used compounds. Boric acid was used as an insecticide for many years, especially against cockroaches. In order to obtain more information on the mode of action of boric acid, which has not been satisfactorily established, a biometric and biochemical study of the ovaries was done following the toxicity assays after having evaluated the toxicity of the boric acid against *B. germanica*. Boric acid was injected at two doses, 77.62 and 194.8 µg/insect, corresponding to LC_{50} and LC_{90}, respectively. The effect of this compound on reproduction was evaluated during the first gonatotrophic cycle (0, 2, 4 and 6 days) of the German cockroach, *B. germanica.* Different morphometric parameters on newly emerged female adults (number of oocytes, volume of basal oocytes, length and width of oocytes) were effectuated. The metabolite amounts, proteins and carbohydrates were determined. The results show that boric acid provokes an inhibition of the oogenesis, with a reduction of the oocyte number at day 2 only for the lower dose, and at days 2 and 4 for the higher dose. A reduction of the volume of basal oocytes was observed for all the tested doses. Vitellogenesis was clearly affected by this treatment. Biochemical analyses revealed a significant reduction of ovarian proteins and carbohydrates with the two tested doses.

Key words: German cockroach, boric acid, reproduction, ovary, biochemical.

INTRODUCTION

Cockroach control was mainly dependent on the use of many types of insecticides. Any of these molecules presents physical and chemical properties, because of their toxicity rate and biotransformation or accumulation which vary from one insecticide to another (Strong et al., 2000). However, the abusive use of conventional insecticides (Appel, 1990; Reid et al., 1990) induced in the long run, not only in cockroaches, and especially in *Blattella germanica*, significant phenomena resistance (Rust and Reierson, 1991; Wen and Scott, 2001; Kristensen et al., 2005), but also the degradation of the ecosystems (McCauley et al., 2000; Long, 2000) and adverse side effects on humans and animals. Indeed, a return to old molecules such as borates found a renewed interest in their cost and ease of application (Hubbard, 1998). Among the borates, boric acid because of its low toxicity, now finds a place in the fight integrated against cockroach and it is true because it is harmless to man

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and its environment and its aversion to cockroaches today (Gore and Schal, 2004; Gore et al., 2004; Call et al., 2004; Kilani-Morakchi et al., 2005; Habes et al., 2006). Boric acid has been the subject for many research and several modes of action have been proposed such as abrasion of the cuticle, the neurotoxic action or disruption of the midgut (Cochran, 1995; and Habes et al., 2006). *B. germanica* is the insect most commonly associated with humans, characterized by a high reproductive potential and this present a major problem in terms of public health (Grandcolas, 1998; Peden and Reed, 2010; Rosenfeld et al., 2010). To better understand the mechanism of action of boric acid and to complete previous works (that previous experiment was made by oral administration) by our team (Habes et al., 2006), its effect was tested by injection, assessing the morphometric parameters (volume of the basal oocyte, the number of oocyte) and biochemical assays (protein and carbohydrates) in *B. germanica.*

MATERIALS AND METHODS

Insects rearing

Cockroaches were reared under the laboratory conditions in plastic boxes (30 x 30 x 30 cm) containing aerated blister packages of eggs that serve as shelters. The insects were fed with watered biscuit and cotton soaked with water. Livestock was kept at a temperature ranging from 25 to 28°C, with relative humidity of 40 to 60% and a photoperiod of 12L: 12D.

Insecticide and treatment

A commercial formulation of boric acid (H_3BO_4) (Merck) was administered by injection to adult female *B. germanica* that newly emerged. Two lethal doses were used representing LD_{50} and LD_{90} respectively (77.6 and 194.8 µg/insect). 2 µl was injected on the lateral side of abdomen with a micro syringe. The control received 2 µl distilled water.

Sampling and morphometric measurements of ovaries

The ovaries of adult females of *B. germanica* from control and treated series were sampled by dissecting the specimen under a binocular microscope during sexual maturation at different times (0, 2, 4 and 6 days) after the adult emergence. Biometric parameters were considered as the number of oocytes per paired ovaries and volume (V) of the basal oocyte. The various measures were determined using an ocular micrometer previously calibrated. The volume (V) expressed in mm^3 was obtained using the formula of Lambreas et al. (1991): $V = 4\pi / 3 (L / 2) (l / 2)^2$ (l=width, L=length)

Extraction of biochemical components of ovaries

The extraction of principal biochemical components of the ovaries from *B. germanica* adult females was carried out by the method of Shibko et al. (1966). Paired ovaries were sampled at different times in the control and treated series and homogenized in trichloroacid acetic (TCA, 20%). After homogenization with ultrasound (Sonifier B-30) and centrifugation (5000 *g* / min for 10 min), the first supernatant was obtained and was used as the determination of carbohydrates. To the pellet, 1 ml ofan ether / chloroform (v / v) was added and a second centrifugation (5000 rev / min for 10 min) was done to retrieve the pellet which was taken up in 1 ml of distilled water for the estimation of total protein.

Quantification of biochemical components

Ovarian proteins were quantified using the method of Bradford (1976) 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 (100 µl) according to Duchâteau and Florkin (1959). This method uses the anthrone as a reagent and glucose (Sigma) as standard. The absorbance was estimated with a spectrophotometer at a wavelength of 620 nm. Data on ovarian biochemical components were expressed in µg per paired ovaries.

Statistical analysis

The results are represented by the mean and the standard deviation (mean ± SD). Statistical analysis was performed using the software Minitab (Weisberg, 1985). Various tests such as linear regression, test "t" of Student and two ways analysis of variance (ANOVA) was performed ($p \leq 5\%$).

RESULTS

Effect of boric acid on the morphometric measurements of ovaries

Effect on the number of oocytes

The number of oocytes per paired ovaries in the control series increased from emergence to 2 days old ($p < 0.0001$) and decreased at four days ($p = 0.001$). Treatment with boric acid caused a decrease in the number of oocytes at two days ($p = 0.006$) for LD_{50} (=77.62 µg/insect). However, a decrease in the number of oocytes was recorded at two days ($p = 0.009$) and at four days ($p = 0.004$) for the LD_{90} (=194.8 µg/insect) as compared with control (Figure 1A). The statistical analysis revealed a highly significant treatment effect with a dose response relationship (p=0.000)

Effect on the volume

The volume of the basal oocyte in the control series of adult females of *B. germanica* increased significantly up to four days ($p = 0.04$) and was highly significant ($p=0.0001$) at 6 days. In the treated series with boric acid, the volume of the oocyte showed the same values from 0 to 6 days ($p \geq 0.05$) for both doses tested during the gonadotropic cycle. The comparison between control and treatment series revealed a significant decrease in the volume of the basal oocyte up to four days ($p = 0.03$) and high significant at 6 days ($p = 0.0001$) in the

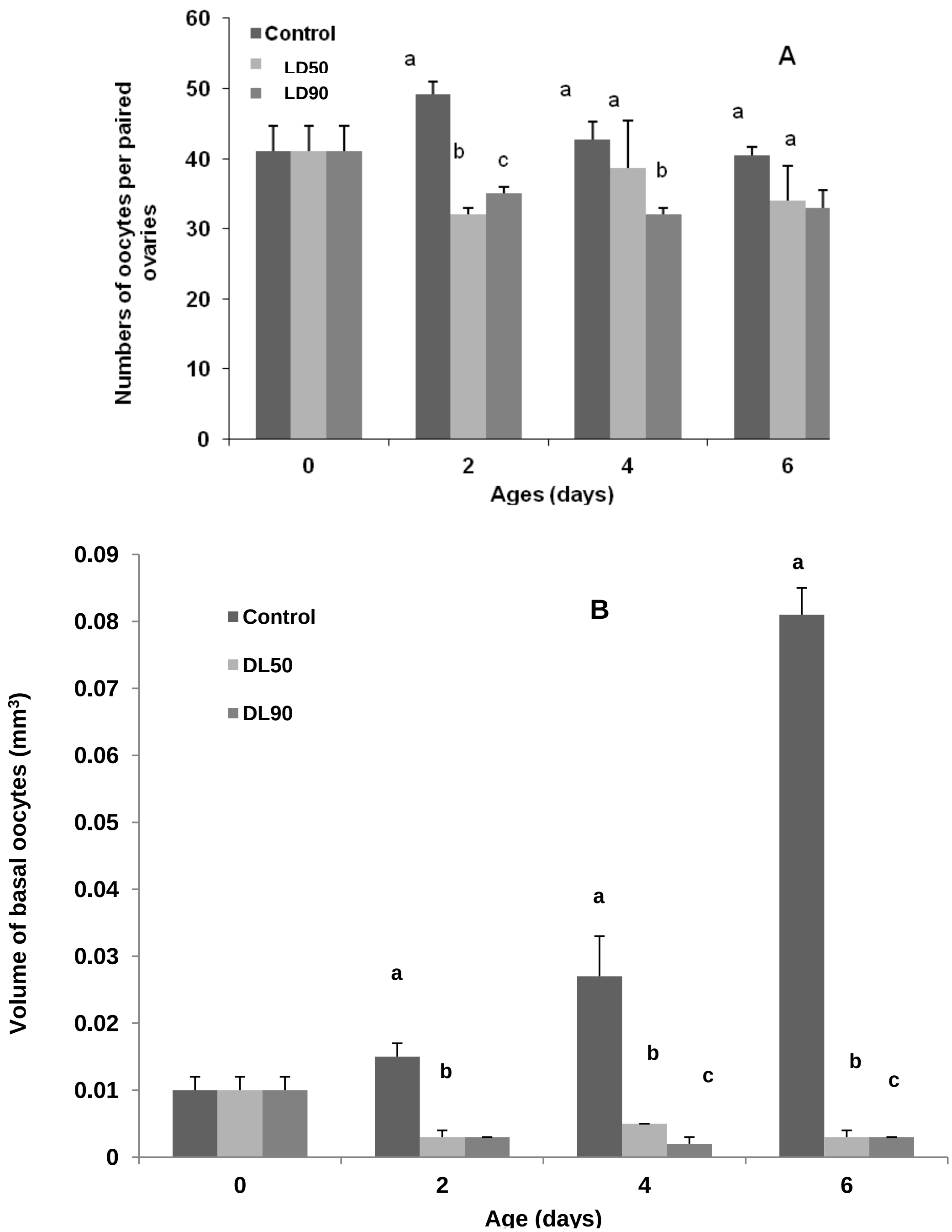

Figure 1. Effect of of boric acid applied by injection (77.62 and 194.8 µg/insect) on newly emerged female adults of *B. germanica* on the volume of basal oocyte (B). Values are presented by the mean ± SD (n=6-8) (mean values of the same age followed by different letters are significantly different p<0.05

treated series with LD_{50}. However, the LD_{90} dose caused a significant decrease at 4 days (p = 0.02) and at 6 days (p= 0.0001) compared to the controls. However, the comparison between the treated series reveals no significant difference (Figure 1B). “The volume of basal oocytes of treated series that increased during the gonadotropic cycle of B. germanica was disrupted after treatment with boric acid.

Effects of boric acid on the biochemical composition of ovaries

Boric acid was applied *in vivo* by injection of two doses: LD_{50} (77.62 µg/insect) and LD_{90} (194.8 µg/insect), on newly emerged adult females of *B. germanica*. The effect of this insecticide was evaluated at different ages (0, 2, 4 and 6 days) after emergence on ovarian content of

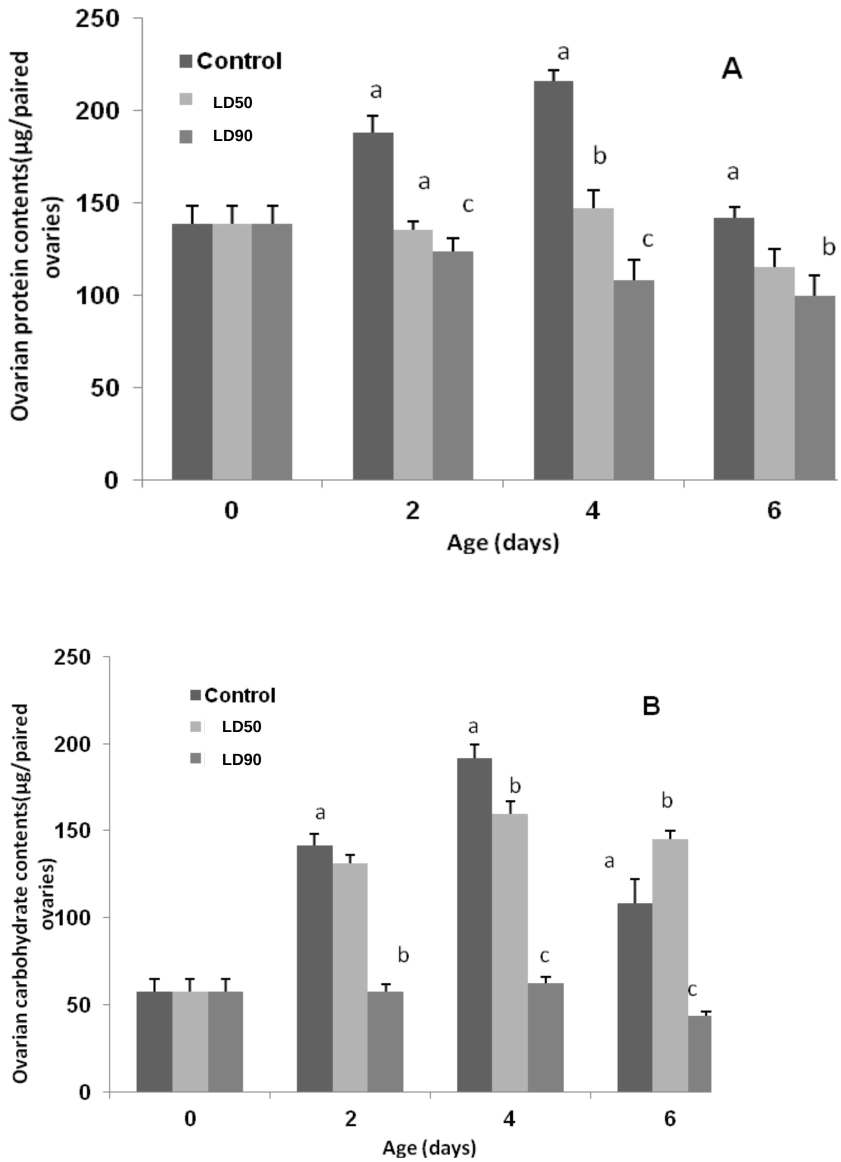

Figure 2. Effect of of boric acid applied by injection (77.62 and 194.8 µg/insect) on newly emerged female adults of *B. germanica,* on the ovarian content (µg/paired ovaries) of proteins (A) and carbohydrates (B). Values are presented by the mean ± SD (n=6-8) (Mean values of the same age followed by different letters are significantly different p<0.05).

proteins and carbohydrates. In the control, the contents of main components of ovaries (proteins 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=0.01, 0.004 and 0.003 respectively) and LD_{90} (p= 0.0004, 0.01 and 0.01 respectively) as compared to the controls (Figure 2A). The ovarian carbohydrate content was also reduced in the treated series at 2 (LD_{90}), 4 (p=0.001 for LD_{50} and 0.0001 for LD_{90}) and six days (p=0.0001 for both tested doses) (Figure 2B). 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 duration treatment (p=0.001) for all ovarian components.

DISCUSSION

In the present study, the number of oocytes per pair of ovaries in *B. germanica* increased to 2 days, then decreased from the fourth day in the control series. The decrease in the number of oocytes observed at 4 days is explained by spawning, which occurs at the time of the cycle gonadotropin (Tine et al., 2011; Kilani-Morackchi et al., 2009). Boric acid caused a decrease in the number of oocytes for the two doses. The treatment of newly emerged *B. germanica* females by injection show that boric acid disrupts oogenesis by causing a reduction in morphometruc measurements of ovaries. The effects of boric acid on reproduction could possibly be explained by their neurotoxic action; they could act secondarily in the process of endocrine regulation. Ovarian activity also requires a dual hormonal control by juvenile hormone and the ecdysteroids (Wang et al., 2004). In B. germanica, the vittelogenesis and maturation of oocyte depends on juvenile hormone III synthesized by the corpora allata (Kilani-Morakchi et al., 2009).

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 (Schal et al*.,* 1997). Disruption of development and reproduction was well observed after *in vivo* application of azadiractin on *Blatta orientalis* (Tine et al., 2011). The treatment *in vivo* with RH-0345, an ecdysteroid agonist, and flucycloxuron (FCX) a chitin synthesis inhibitor, affect the morphometric parameters on *Tenebrio molitor* (Taibi, 2003; Mazouni-Soltani et al., 2001). 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 (Harnish et al., 1998) and is synthesized in fat and secreted in the hemolymph and then sequestered by endocytosis in developing oocytes (Raikhel and Dhadialla, 1992). Oocyte maturation depends on metabolites collected from the hemolymph (Telfer et al., 1981) and *in situ*, materials synthesized by the ovary (Indrasith et al., 1998), but depend mainly on the protein, a major component of yolk (Cassier et al., 1997).

The accumulation of these biochemicals and energy in the ovaries of adult female *B. germanica* wasassessed at different ages during sexual maturity as a result of measurement of ovarian protein and carbohydrates. The results show a change in the concentrations of protein and carbohydrates during the first four days of adulthood correlated with vitellogenesis (Maiza et al., 2004a). Our results show that boric acid test at the LD_{50} and LD_{90}, affected the vitellogenesis inhibiting ovarian content of protein and carbohydrate, and reduction in the concentration of these two metabolites was observed with an age effect, a treatment effect and treatment-age interaction. Then, inhibition of vitellogenesis in *B. germanica* was also observed during treatment with other types of insecticides such as benfuracarb, a carbamate and acetamiprid a neonicotinoid (Maiza et al., 2004). Maiza et al., 2010 also reported that the application of indoxacarbe in *B. germanica* reduced the that the application of several agonists of molting of the hormone, such as HR-2485, HR-5992 and HR-0345 on female *Eupolybothrus nudicornis* (Myriapoda) harvested spring or fall, reduced the protein concentrations in the ovarian tissue (Daas et al., 2003) Similarly, KK42, an inhibitor of ecdysteroids synthesis, disrupts the vitellogenesis inhibiting ovarian concentrations of proteins in *T. molitor* (Soltani-Mazouni et al., 2001). The application of an analogue of the molting hormone, HR-0345, also affects the ovarian content of protein and carbohydrates in B. germanica (Rouiba, 2002), but also in *T. molitor* (Taibi et al., 2003). The reduction rates of these various metabolites in the ovaries of *B. germanica* after treatment with boric acid can be explained by its neurotoxic action and thus 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 (Belles, 2005).

Conclusion

Morphometric and biochemical studies demonstrated that boric acid affects 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 suggested an interference of boric acid with the vitellogenesis process.

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