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Reproductive effects of a neonicotinoid insecticide (Imidacloprid) in the German Cockroaches *Blattella germanica* L. (Dictyoptera, Blattellidae)

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Abstract

Imidacloprid a neonicotinoid insecticide was administrated by injection to newly emerged adults of the German cockroach *Blattella germanica* (Insecta, Dictyoptera) at 0.0002% and 0.0005% doses corresponding to the LD₅₀ and LD₉₀ respectively. The insecticidal effect of compound was investigated on ovarian growth during the first gonadotrophic cycle (0, 2, 4 and 6 days) under laboratory conditions. Results showed that imidacloprid reduced the number of oocytes per paired ovaries and the size of basal oocytes with a dose-response relationship. Furthermore, the compound was examined on ovarian biochemical composition. Biochemical analysis revealed that the insecticide at the two tested doses reduced significantly the contents of proteins, lipids and carbohydrates as compared with control series. The overall results suggest an interference of the compounds with the vitellogenesis.

Keywords: *Blattella germanica*, Imidacloprid, Reproduction, Ovaries, Biochemical components.

1. Introduction

In addition to its high reproduction performance, *Blattella germanica* [1] pestiferous insect is the carrier of many pathogen and allergen factors in habitable environments [2, 3]. Neurotoxic insecticides such as organophosphates, carbamates and pyrethroids have been widely used to control cockroach. Unfortunately, many of these chemicals are harmful to man and beneficial organisms and cause ecological disturbances [4]. Furthermore a rapid development of resistance was noted in the most prevalent cockroach, the German cockroach, *B. germanica* [5]. Thus many organo-synthetic pesticides have been replaced by biorational or more target-specific chemicals with novel mode of action like insect growth regulators or low risk compounds such as boric acid [6, 7]. Previously we have reported that ingested acid boric exhibited a neurotoxic action [8] and affected reproduction of *B. germanica* [9]. Moreover among the most recent advances in cockroach control consist in use of biopesticides [10, 11, 12]. Imidacloprid is a biopesticide belonging to neonicotinoid insecticide class which acts agonistically on insect nicotinic acetylcholine receptors [13]. Imidacloprid is effective against a wide range of nuisance and public hygiene insect species like *Aphids* [14], *Aedes aegypti* [15], *Bemisia tabaci* [16], *Tribolium castaneum* and *Cryptolestes ferrugineus*, *Sitophilus oryzae*, *Rhyzopertha dominica* and *Oryzaephilus surinamensis* [17], *Gromphadorhina portentosa* [12]. Moreover it was reported that imidacloprid against sucking insects is safe for natural enemies of other pests such as spiders and some predatory beetles and bugs [18, 19, 20]. Recently it was reported that imidacloprid can be used with reasonable environmental safety and low toxicity toward non target aquatic organisms [21]. The neurotoxicity of imidacloprid against American cockroach and housefly has been well documented [22, 23]. Additionally the toxic effects of imidacloprid on oviposition and reproduction have been reported in any species like *Aedes aegypti* [15], *Tetranychus cinnabarinus* [24]. However, to our knowledge, no study has tested the potential of using imidacloprid in oviposition to control the German cockroach *B. germanica*. Thus in the current paper we investigated the insecticidal activity of imidacloprid for controlling this pest.

2. Materials and Methods

The present study was carried out from December 2013 to June 2014 at Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, University Badji Mokhtar of Annaba

2.1. Insects

Colonies of *B. germanica* were reared in plastic boxes (30x30x30 cm) at $26 \pm 1^\circ$, 70% relative humidity under a 12:12 dark-light photoperiod cycle. The cockroaches were fed with watered biscuit and cotton soaked with water.

2.2. Insecticide and treatments

The commercial formulation of imidacloprid, (Confidor 200 SL {1-[(6-chloro-3-pyridinyl) methyl]-*N*-nitro-2-imidazolidinimine}) (courtesy by Pr. G. Smaghe, Laboratory of Agrozoology, Gent University, Belgium) was dissolved in acetone and administrated by injection (3 μ l/insect) to newly emerged adult of *B. germanica*. The effect of compound was evaluated at two doses 0.0002% and 0.0005% corresponding to LD50 and LD90 respectively. The controls received 3 μ l of acetone.

2.3. Morphometric measurements of ovaries

Adult females from control and treated series were sampled at 0, 2, 4 and 6 days of adult life, during the first gonadotrophic cycle [25], and their ovaries dissected out. After removal of circum ovarian fat body, the number of oocytes in each paired ovaries was recorded. The volume of basal oocytes was calculated according to Lambreas *et al.* [26].

2.4. Biochemical composition of ovaries

The extraction of the different constituents was made following the procedure of [27]. Paired ovaries were collected at various times 0, 2, 4 and 6 days from control and treated adult females and homogenized individually in 1 ml of aqueous trichloro-acetic acid (20%). The ovarian content of proteins,

carbohydrate and lipids was determined respectively according Bradford [28], Duchateau and Florkin [29] and Goldsworthy *et al.* [30]. The ovarian constituents were expressed as μ g per paired ovaries.

2.5. Statistics

Results are represented as means \pm standard deviation (S.D). The significance between control and treated series was estimated using Student's t- test at 5% level. Data were subjected to two-way analysis of variance (ANOVA). All data were statistically analyzed by the MINITAB software (version 15, PA state College, USA).

3. Results

3.1. Effects on ovarian parameters

In controls, the number of oocytes increased between the imaginal moult at day 2 of the first gonadotrophic cycle and decreased at day 4 corresponding to the egg-layer. Imidacloprid injected on newly emerged females of *B. germanica* significantly reduced the number of oocytes recorded at day 2 ($P = 0.001$) and 4 ($P = 0.0001$) as compared to controls (Fig. 1). ANOVA revealed a significant effect of the compound on number of oocytes ($P \leq 0.001$) as function of the duration of treatment during the first day of the adult life. Treatment affected the number of oocytes per paired ovaries.

The volume of basal oocytes also increased during this period from 0.011 ± 0.002 at 0 day to 0.075 ± 0.02 mm³ at 6 days in control series (Fig. 2). Imidacloprid treatment affected the volume of basal oocytes and reveals a significant decrease at days 4 ($P = 0.005$) and 6 (0.002) with no effect dose as compared to control series.

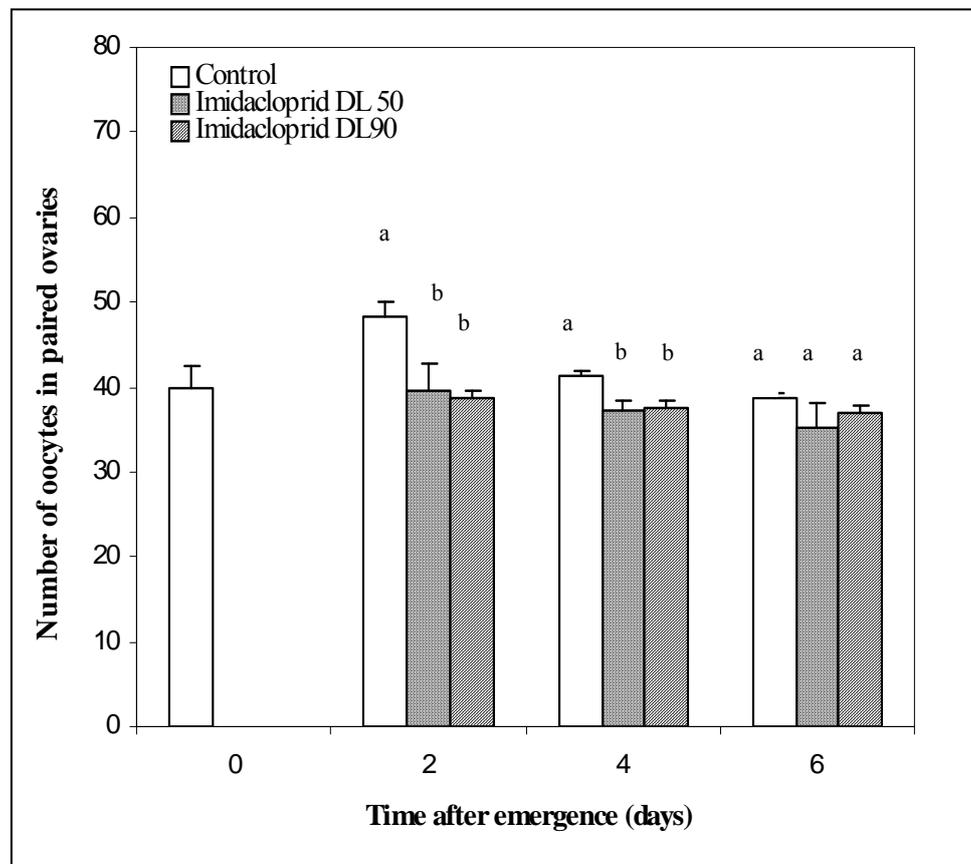


Fig 1: Effects of injected imidacloprid (0.00025 and 0.00050%) on the number of oocytes per paired ovaries during the first gonadotrophic cycle on newly ecdysed females of *B. germanica*. Data are expressed as means \pm SEM. (n = 6-8). Asterisks indicate a significant difference between control and treated series of the same age; $p^* < 0.05$; $p^{**} < 0.01$; $p^{***} < 0.001$.

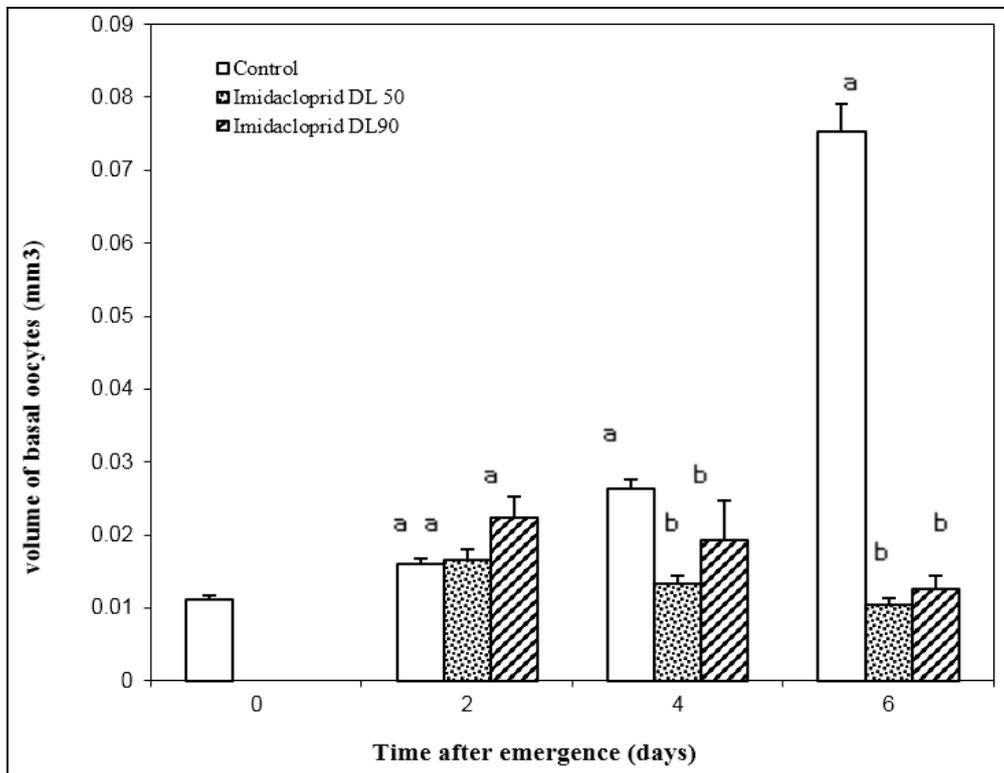


Fig 2: Effects of injected imidacloprid (0.00025 and 0.00050%) on the volume of basal oocyte (mm³) during the first gonadotropic cycle on newly ecdysed females of *B. germanica*. Data are expressed as means ± SEM. (n = 6-8). Asterisks indicate a significant difference between control and treated series of the same age; p* < 0.05; p** < 0.01; p*** < 0.001.

3.2. Effects on biochemical composition of ovaries

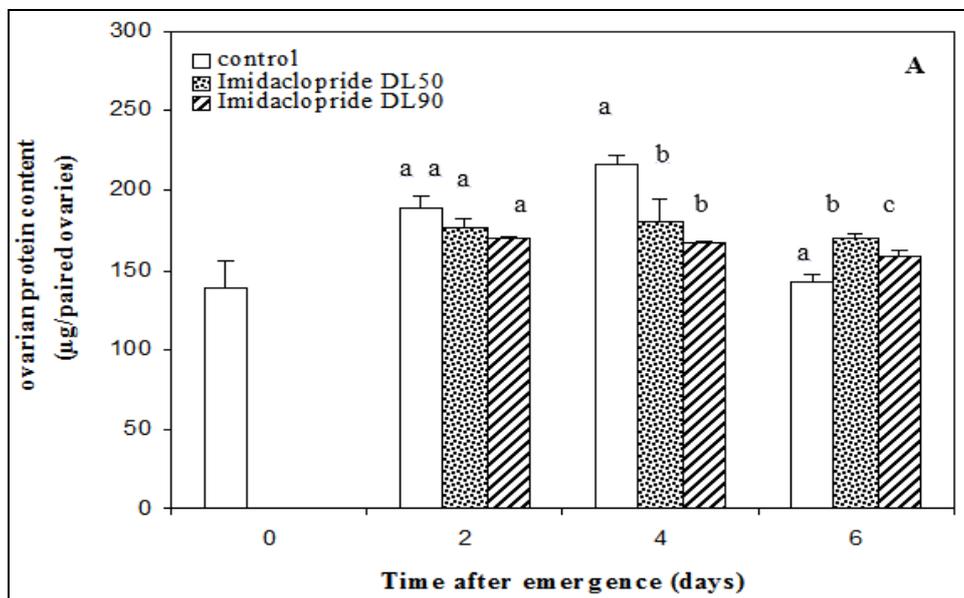
In control, the principal constituents (proteins, lipids and carbohydrates) of ovaries showed a peak at day 4 after adult emergence corresponding to the beginning of egg-laying (Fig. 3). Imidacloprid affected at all tested ages the ovarian carbohydrates content at 2 ($P = 0.0001$), 4 ($P = 0.0001$) and day 6 (0.001). The dose-response relationship was observed at days 2 and 6 after treatment with 0.0005% of imidacloprid but only at days 4 with 0.0002% of insecticide tested as compared to control series (Fig. 3B). The ovarian proteins content was found to decrease only at day 4 ($P = 0.01$) and to increase thereafter at days 6 ($P = 0.0001$) with a dose-response relationship after treatment as compared to control (Fig. 3A). The compound also reduced significantly ($P \leq 0.01$) the lipid ovarian contents at day 2 ($p = 0.004$), 4 ($P = 0.01$) and 6 ($P = 0.001$) with a dose-response relationship at all ages tested.

ANOVA showed a significant effect of the compound ($P < 0.001$) as function of the dose and the duration of treatment for all ovarian constituents.

4. Discussion

4.1. Effects on 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, juvenile hormone [31], and also neurohormones with gonadotropic and antigonadotropic effects [32]. In *B. germanica*, as in all cockroaches studied to date, vitellogenesis and cyclic maturation of oocytes depends upon juvenile hormone synthesis by the corpora allata (CA) [33]



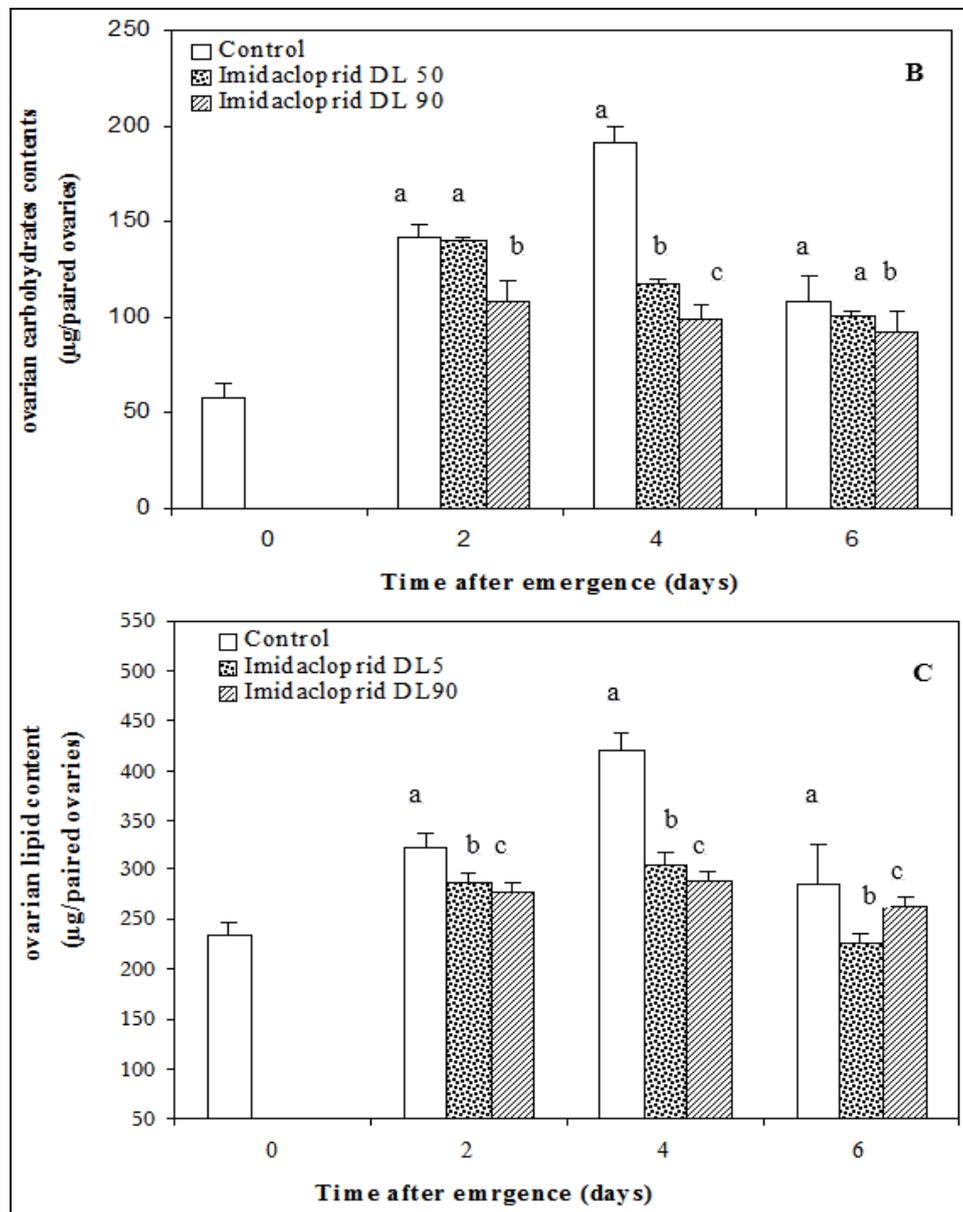


Fig 3: Effects of injected imidacloprid (0.00025 and 0.00050%) on the ovarian metabolites contents ($\mu\text{g}/\text{paired ovaries}$) of protein (A), carbohydrates (B), and lipids (C) in adult females of *B. germanica* during the first 6 days following adult emergence (means \pm SEM; n = 6-8;

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 [34].

The treatment by imidacloprid affected ovogenesis and vitellogenesis process in *B. germanica*. Reduction of oocytes per paired ovaries coincides, in controls, with the beginning of ovulation [33]; imidacloprid was found to reduce the number of oocytes per paired ovaries and the volume of basal oocytes as compared to controls. This disruption of the ovogenesis by imidacloprid may be explained by its neurotoxic action interfering negatively on neuropeptides with myotropic properties regulating ovulation. Our previous studies reported similar effects in ovarian morphometry of *B. germanica* treated with other neurotoxic compound like boric acid [9,35], a carbamate benfuracarb and indoxacarb [36,37] but also other insecticides belonging to various groups of pesticides like ecdysteroid agonist halofenozide, juvenile hormone analogue methoprene [36]. The identical effects were observed after treatment of the same specie with spinosad and azadirachtin a naturally derived from the fermentation of the actinomycete *Saccharopolyspora spinosa* and the neem tree *Azadirachta*

indica respectively [38,37].

Vitellogenesis and vitellogenin synthesis are regulated by hormone and neurohormone [31]. Imidacloprid evaluated on ovarian constituents significantly decreased protein, lipid and carbohydrate contents. The low amount of protein content recorded at 6 days is probably due to the end of vitellogenesis and the preparation of the ovary to new gonadotrophic cycle [9]; whereas, the reduction in lipids and carbohydrates correspond to the energy consumption required to vitellogenesis process. Thus, the neurotoxicity of the compound might interfere with hormones and neurohormones that regulate vitellogenesis and caused disturbance of the endocrine mechanisms and thus the perturbation of vitellogenesis process. Maiza *et al* [36] and Maiza *et al* [37] has shown the reduction of ovarian proteins content in *B. germanica* treated with other neurotoxic compound the benfuracarb and indoxacarb. Acid boric affected similarly the metabolites contents in the same species [9, 35]. Additionally halofenozide decrease significantly the proteins content but the juvenile hormone analogue methoprene increase this contents in the same specie [36]. Treatment of *B. germanica* by naturally compound Spinosad and azadirachtin affected similarly the

vitellogenesis [38, 37].

5. Conclusion

In conclusion, our results reveal that injected imidacloprid affected reproduction in *B. germanica*. Further studies with other modes of application, such as ingestion in order will be conducted to obtain more information on the effect of imidacloprid action in this species.

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