

Full Length Research Paper

Impact of hexaflumuron, a chitin synthesis inhibitor, on growth, development and reproductive performance of the progeny in *Callosobruchus maculatus* after adult treatments

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Accepted 02 November, 2018

Hexaflumuron, a benzoylphenylurea derivative, was tested topically at four doses (0.5, 1, 1.5 and 2 µg/insect) on adults of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and first evaluated on fecundity, hatchability and viability of eggs, longevity and morphometric of oocytes. Our data show that the compound reduced the longevity and the fecundity. In addition, treatment affected growth and development of oocytes and egg-viability as evidenced by measurements the number of oocytes per ovaries and the size of basal follicle, respectively. In a second series of experiments, this IGR don't induced significant reduction of the percentage-hatchability but its affect very significantly the viability rate of eggs laid by F1 females. Duration of the embryonic and post – embryonic development of eggs laid by treated females increase when the concentration varied from 0 to 2 µg/µl.

Key words: Hexaflumuron, *Callosobruchus maculatus*, eggs development, fecundity, longevity, morphometric of oocytes, viability.

INTRODUCTION

In recent years, the toxicity of insecticides to humans and wildlife has caused much public concern and led to the use of more target-specific chemicals (Paoletti and Pimentel, 2000). Because of secondary effects of conventional insecticides, the insect growth regulators (IGRs) are receiving more practical attention to provide for safer foods and a cleaner environment. Among these compounds the benzoylphenylurea derivatives

(BPUs) cause abnormal cuticular deposition and abortive molting in insects by interfering with chitin biosynthesis (Casida and Quistad, 1998; Oberlander et al., 1998). Diflubenzuron, the first insecticide, based on interference with cuticle deposition (Mülder and Gijswijt, 1973), have been the subject of intensive investigation. It was found to be effective on several insect species (Grosscurt, 1978; Soltani, 1984 ; Soltani et al., 1984 ; Soltani and Soltani-Mazouni, 1992 ; Khebbeb et al., 1997). Since the introduction of DFB, a number of other BPU derivatives have been developed such as flucycloxuron, hexaflumuron and triflumuron (Soltani et al., 1996, 1999 ; Bendjedou et al., 1998; Peppuy et al., 1998; Rehim and Soltani, 1999).

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These compounds have been found to interfere with chitin biosynthesis (Soltani et al., 1993, 1996). Other IGRs were reported to inhibit ecdysteroid synthesis (Chebira et al., 2000 ; Soltani-Mazouni et al., 2000) or to mimic the action of 20-hydroxyecdysone (Soltani et al., 2002). Saponin extract from alfalfa roots, azadirachtin from the neem seed oil, synthetic ecdysteroid agonist RH-2485, and the juvenoid hydroprene disturb the development and reproduction of *Tropinota squalida* (Coleoptera: Scarabeidae) (Hussein et al., 2005).

Callosobruchus maculatus (F.) (Coleoptera : Bruchidae) is a major insect pest of stored grain, in Africa and Asia, and whose larval development occurred inside seeds from several Leguminosae species (Ouedraogo et al., 1996). *Vigna unguiculata* (Walp.) was considered as the major host plant of this pest (Janzen et al., 1977 ; Jackai and Daoust, 1986). In developing countries, biopesticides, viewed as a means to reduce the load of synthetic chemical pesticides, have been exploited (Hall and Menn, 1999). Thus, plant vegetable oils were tested against several Bruchidae species (Gbolade and Adebayo, 1993 ; Seck et al., 1993; Don-Pedro, 1996a; Rajapakse, 1996 ; Keita et al., 2001; Raja et al., 2001; Kellouche and Soltani, 2004; Kellouche et al., 2004). In addition, laboratory and field tests have evaluated the toxicity of several IGRs against some stored product pests (Mc Gregor and Kramer, 1975; Oberlander et al., 1975; Kramer and Mc Gregor, 1979 ; Mian and Mulla, 1982 ; Abo El-Ghar, 1992 ; Soltani-Mazouni, 1994 ; Soltani-Mazouni and Soltani, 1995; Soltani et al., 1996 ; Herbert et al., 1997; Peppuy et al., 1998). In the present study, conducted under in vivo conditions, we investigated the effect of a benzo-yphenylurea derivative, hexaflumuron, on growth and development, adult reproductive potential and egg hatchability of *C. maculatus*. This IGR is tested for the first time for control insects that develop inside grains.

MATERIALS AND METHODS

REARING OF INSECTS

Stock cultures of *C. maculatus* were maintained in glass jars (18 cm height, 11 cm diameter) containing seeds of *V. unguiculata*. Insects were kept at $30 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH under almost continuous darkness as previously described (Kellouche and Soltani, 2004).

Insecticide and treatments

Hexaflumuron (technical grade 98.7 %) was kindly supplied by Dow Elanco Speciality (Indianapolis, USA). Serial dilutions were

prepared in acetone and several doses (0, 0.5, 1, 1.5 and 2 $\mu\text{g}/\text{insect}$) were applied topically on newly emerged adults (<24h-old). Acetone (1 μl per individual) was used as control. The insecticidal assay was conducted with five replicates each of 30 adult insects (15 males and 15 females) placed in glass box (14cm diameter, 2cm height) containing food (50 g of *V. unguiculata* seeds).

Morphometric measurements of ovaries

Females were sampled from control and treated series at different moments during the adult life (0, 1, 2 and 3 days) and ovaries dissected according to Ahmed et al. (1976). The number of oocytes per ovaries and the size of basal oocytes were determined, respectively.

Determination of longevity, fecundity, hatching rate

Newly emerged adults of *C. maculatus* were collected from rearing containers. Control and treated series were reared under standard conditions. The longevity of both males and females, the total number of eggs laid per female (fecundity) and the percentage of egg hatching were determined.

Growth and development

Freshly eggs laid were collected from controls and treated series and placed under standard conditions. The time taken for adult emergence was determined. The number and the body weight of descendants of the first generation were scored in all series. The survival rate during the post-embryonic development was calculated (number emerged adults on number eggs laid x 100).

Statistical analysis

Data are presented as the mean \pm SD. The age and the number of insects tested per series are given with the results. To detect differences among treatments, data were subjected to analysis of variance (MINITAB, version 12.21; PA State College, USA).

RESULTS

Effects On Morphometric Parameters Of Ovaries

On number of ovocytes per paired ovaries

In the control, the number of ovocytes by pair of ovaries varied from 45 to 49 according to the age of the females. This number decreased significantly in females, 1 - 3 days, whose parents have been treated with 2 μg of hexaflumuron (Table 1).

On the ovocytes basal size

In the control, the basal ovocytes size didn't vary according to the age of the females. The length of basal ovocytes de-

Table 1. Effect of hexaflumuron on the number of oocytes per paired ovaries in F1 female adults issued from treated adults (2 µg/µ l) (m ± s, n = 40 per age, ns: don't differ significantly, *: difference just significant, ***: highly significant difference; in each colon the means followed by same letter doesn't differ significantly at P < 0.05).

Age of females (days)	0	1	2	3
Control	45.9 ± 7.6 (a)	49.2 ± 8.9 (a)	48.3 ± 8.3 (a)	49.2 ± 8.0 (a)
Treated series	48.6 ± 6.0 (a)	45.6 ± 6.7 (b)	41.4 ± 7.6 (b)	42.3 ± 7.1 (b)
Anova	F = 3.08 P = 0.0797 ns	F = 4.13 P = 0.0431 *	F = 14.79 P = 0.000 ***	F = 16.33 P = 0.000 ***

Table 2. Activity of hexaflumuron on the width and the length (mm) of basal oocytes of F1 adult females issued from treated adults (2µg/µl) (m ± s, n = 40 per age, * : just significant difference, ** : significant difference, ns : don't differ significantly; for each size, means followed by same letter doesn't differ significantly, P < 0.05).

Age of females (days)	Width		Length	
	Control	Hexaflumuron	Control	Hexaflumuron
0	0.28 ± 0.04 (a)	0.29 ± 0.04 (a)	0.47 ± 0.07 (a)	0.42 ± 0.08 (b)
1	0.27 ± 0.05 (a)	0.27 ± 0.05 (a)	0.42 ± 0.09 (a)	0.43 ± 0.07 (a)
2	0.26 ± 0.05 (a)	0.25 ± 0.06 (a)	0.40 ± 0.09 (a)	0.42 ± 0.09 (a)
3	0.29 ± 0.04 (a)	0.26 ± 0.05 (b)	0.43 ± 0.08 (a)	0.40 ± 0.08 (a)
Anova (2 ways) - width		Age Factor : F obs = 4.40 ; P = 0.005 ** Treatment Factor : F = 4.42 ; P = 0.036 * Interaction : F = 2.49 ; P = 0.060 ns		
Anova (2 ways) - length		Age Factor : F obs = 3.38 ; P = 0.019 * Treatment Factor : F = 1.07 ; P = 0.301 ns Interaction : F = 3.27 ; P = 0.022 *		

creased just significantly according to the age of females descended from treated parents (2 µg), the width has not been affected significantly by this treatment (Table 2).

Effects On Reproductive

Capacity On the fecundity

The results showed a highly significant reduction (P<0.0001) of the number of eggs laid by each female treated with the higher dose (2 µg/ µl), compared with the control (Table 3).

The number of eggs laid by the first generation females was higher than the one of the females treated, especially with the higher doses (Table 3). The treatment undergone by the parents didn't affect the egg laying of the F1 females.

On the eggs hatching rate

The hatching rate of the eggs in the control didn't differ significantly the one of the eggs laid by the females treated with the dose of 0.5 µg/µl (F=20.19, DF=99, P<0.0001). The hatching of eggs laid by the females treated with the doses higher than 0.5 µg/µl has been reduced more than the one of the eggs laid by the F1 females (Figure 1). The hatching rate of eggs laid by F1 females was not affected by the treatment (F=2.86, DF=99, P=0.5).

On the eggs viability rate

The viability or post-embryonic survival rate of the eggs laid by the treated females was significantly reduced (F=27.04, DF=99, P=0.000), compared with the control. In F1 females, this rate was significantly (F=7.5, DF=99, P<0.0001) different between the control and the treatments

Table 3. Effect of hexaflumuron on the fecundity of *C. maculatus* female ($m \pm s$; $n=5$ replicates each of 15 pairs, ns: don't differ significantly, *: just significant difference, **: significant difference ***: highly significant difference at $P < 0.05$, in each colon the means followed by same letter doesn't differ significantly).

Doses ($\mu\text{g}/\text{insect}$)	0	0.5	1	1.5	2
Treated females	69.0 ± 13.7 (a)	65.0 ± 13.2 (b)	69.4 ± 11.9 (a)	51.8 ± 2.7 (b)	33.0 ± 12.6 (b)
F1 females	74.4 ± 15.2 (a)	81.6 ± 1.8 (a)	81.6 ± 5.4 (a)	80.4 ± 3.3 (a)	84.0 ± 7.4 (a)
Anova	F = 0.35 P = 0.573 ns	F = 7.75 P = 0.024*	F = 4.34 P = 0.071 ns	F = 19.46 P = 0.002**	F = 60.07 P = 0.000***

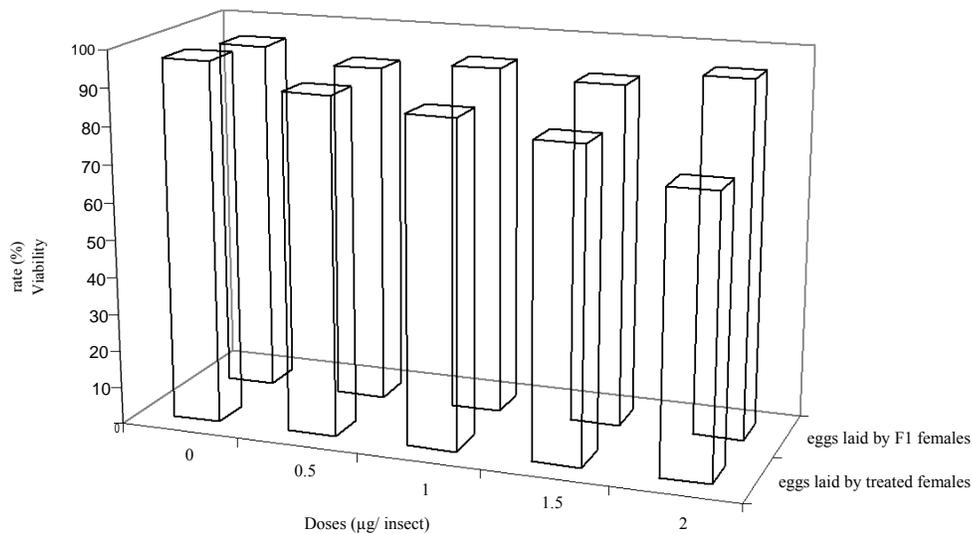


Figure 1: Hatching rate of eggs laid by treated and F1 females in *C. Maculatus*.

(Figure 2)

EFFECTS ON GROWTH AND

DEVELOPMENT On the life cycle

The duration of life cycle, egg to adult, didn't differ significantly between the control and the treatment with dose of $0.5 \mu\text{g}$. However, the hexaflumuron lengthened very significantly the total duration of the embryonic and post-embryonic development, when the dose was higher than $0.5 \mu\text{g}/\mu\text{l}$ (Table 4).

On the weight of adult descendants

The male adult descendant weight didn't differ significantly between the control and the treatments with the doses of 0.5 and $1 \mu\text{g}$. This weight decreased just significantly ($F=3.33$, $DF=99$, $P=0.0218$) if the dose of

the parents treatment was higher than $1 \mu\text{g}$ (Figure 3). The hexaflumuron didn't affect significantly the weight of the F1 adult females ($F=1.23$, $DF=99$, $P=0.3011$) (Figure 3). On the adult longevity

The adult individuals (males and females) lived between 6 and 7 days in the control. The hexaflumuron reduced significantly ($F=19.63$, $DF=29$, $P=0.002$) the adult longevity when the bruchidae was treated with the highest dose. On the other hand, this growth regulator didn't affect the longevity of the first generation adults (Figure 4)

DISCUSSION

The results of our study confirm the efficiency of the growth regulators, derived of the benzoylphenylurea, on the development of insect pest of stored products.

The hexaflumuron was the object of some works, it has been tested for example on the weevil of the beet sugar, *Aubeonymus mariaefranciscas* (Coleopter Curculionidae),

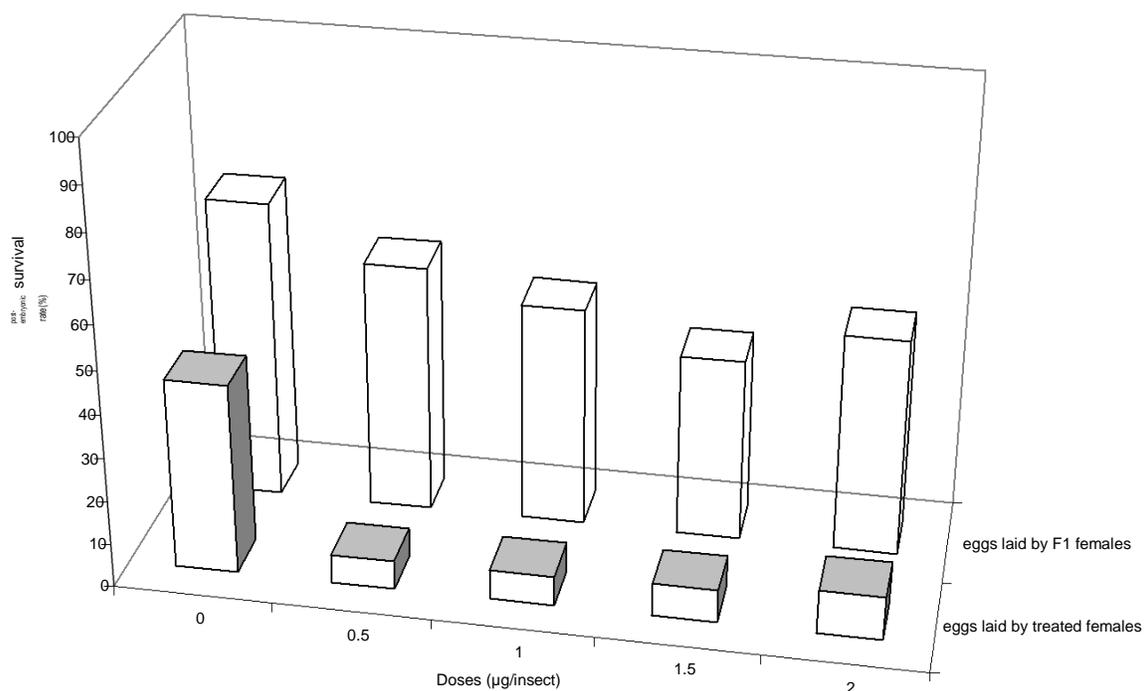


Figure 2. Post-embryonic survival rate in *C. maculatus*.

Table 4. Effect of hexaflumuron on the duration of the development cycle (egg to adult) in *C. maculatus* ($m \pm s$, $n = 100$ eggs for each dose, ***: highly significant difference; values followed by the same letter are not significantly different at $P < 0.05$)

Doses (µg/Insect)	0	0.5	1	1.5	2
Duration (days)	23.6 ± 1.8 (a)	23.4 ± 2.5 (a)	25.0 ± 2.9 (b)	26.9 ± 2.3 (b)	26.0 ± 2.2 (b)
Anova	F = 39.11 et P = 0.000 ***				

to know his mode of penetration through the adult cuticle and its excretion; the lower concentration recovered in the eggs slows down the embryonic development and inhibit their hatching (Farinos et al., 1998). Otherwise, this IGR proved to be also very toxic against the larva's of the of *Schystocerca gregaria* stage 2 (Coppen and Jepson, 1996) and the Subterranean termite (Isoptera: Rhinotermitidae) workers who contaminate themselves by trophalaxis and of which it also disrupts the chitin synthesis (Sheets et al., 2000).

Some works reported the effect of other growth regulators, of the same family that the hexaflumuron, against insect pests of stored products. The analogue of the JH (1-(4-methylphenyl)-3,7-dichloro-3,7-dimethyloctane) caused a strong mortality in the larva's of *C. maculatus*, with the doses of 1.5 and 2%, as well as of

important lesions in the ovarian cloths; most mature ovocytes is damaged, the size and the number of ovocytes by ovariole are decreased (Sareen et al., 1992).

Five growth regulators analogues of the JH (MV - 678, RO - 20458, CGA - 29170, CGA 45128 and the Fenoxycarb), in treatment of *V. unguiculata* seeds with different doses (of 10 to 100 mg/kg), induce a significant reduction of the oviposition and the hatching rate of the eggs, in a short time (15 days), and the number of the F1 descendants, in a long-term (6 months), in *C. maculatus* (Abo El - Ghar, 1992).

The terpen extract of *Saussura lappa* Clarke roots, analogous of the juvenile hormone (JH), reduced significantly the fertility, the hatching rate of the eggs and the number of descendants in *C. maculatus*, with the doses of 0.75 and 1.0 µg / adult female (Singh, 1998).

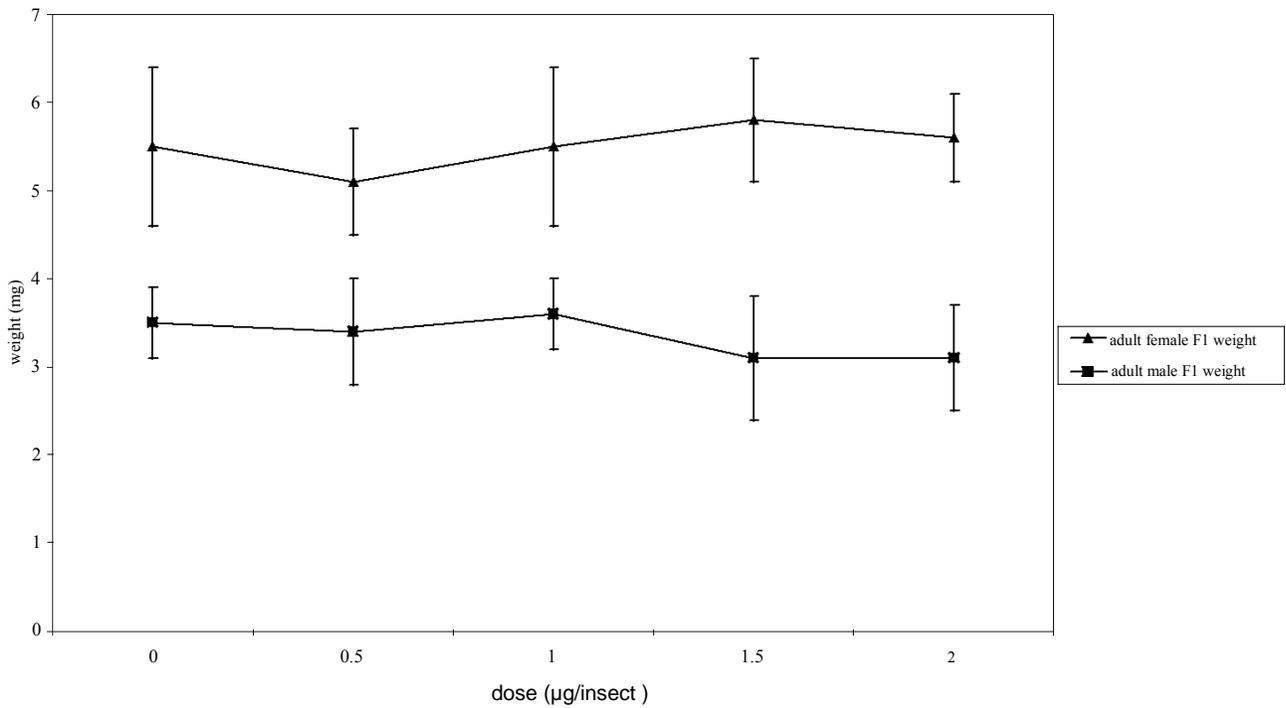


Figure 3. weight of adult descendants in *C. maculatus* regarding the dose of the parents treatment.

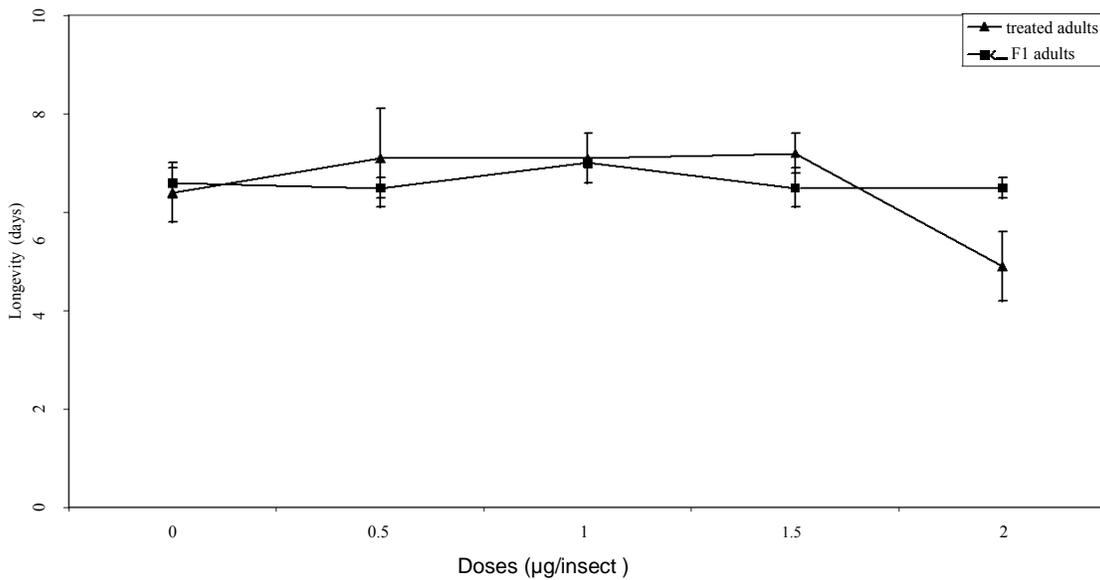


Figure 4. Treated and F1 adult longevity in *C. Maculatus*.

Otherwise, the diflubenzuron reduces longevity and weight of the adults (Soltani, 1984), it also decreases significantly the numbers of oocytes and the weight of

the ovaries (Soltani, 1987; Soltani et al., 1996) as well as the oocytes basal size in *T. molitor* (Soltani – Mazouni, 1994) and *Cydia pomonella* (Lepidoptera: Tortricidae)

(Soltani and Soltani – Mazouni, 1992). These authors suggest that interference of the diflubenzuron with the vitellogenesis can explain the reduction origin of the fertility.

The triflumuron, derivative product of the benzoylphenylurea, increase the length of the larval development and reduced the adults emergence in *Culex pipiens* (Diptera: Culicidae) (Soltani et al., 1999). Wen neonates of *Tribolium castaneum* (Coleoptera: Tenebrionidae) were subjected to sublethal concentrations of flufenoxuron, there were dose-dependant effects on larval weight, percentage pupation and percent adult emergence, as well as time taken for adult emergence (Salokhe et al., 2003).

According to Dalairé et al. (2004), tebufenozide interferes with various aspects of the reproductive biology of males and females of *Choristoneura fumiferana* and *C. rosaceana* (Lepidoptera: Tortricidae). The high accumulation of three insect growth regulators, diflubenzuron, flucycloxuron and halofenozide, in the reproductive system of females and males of *T. molitor*, may explain their strong reproductive effects (Chebira et al., 2006).

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