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Full Length Research Paper

Tebufenozide effects on the reproductive potentials of the mediterranean flour moth, *Ephestia kuehniella*

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The Mediterranean flour moth, *Ephestia Kuehniella* Zeller (Lepidoptera: Pyralidae), is an important pest in stored products worldwide, and is one of the major pests in flour mills in Algeria. Because of environmental consideration, alternative approaches to neurotoxic insecticides, as well as safe, effective, and sound integrated pest management strategies pest control agents such as the insect growth regulator (IGRs) was developed. Among these IGRs, the bisacylhydrazine derivatives are nonsteroidal ecdysteroid agonists that mimic the action of moulting hormones and induce a precocious and incomplete moult in several insect orders. The reproductive potentials of adult *E. kuehniella* under laboratory conditions were evaluated using topical bioassays of an ecdysteroid agonist, RH-5992 (Tebufenozide), on pupae of *E. kuehniella*. This compound reduced the pupae development period with a dose response relationship. Data showed that tebufenozide, topically applied on newly exuvied pupae, significantly affected reproductive parameters in adults such as fecundity, fertility, egg laying period and embryonic duration of development. In addition, length and width of eggs were also reduced by tebufenozide treatment. Moreover, these parameters were affected more when males treated with pesticide (Tebufenozide) were mated with untreated females or treated males and treated females were mated than when treated females were mated with untreated males and when both males and females were untreated. In a second series of experiment, tebufenozide, topically applied on newly emerged pupae, was evaluated on weight and biochemical composition of gonads of newly emerged adults. Results showed a drastic decrease of gonadic weight and carbohydrate and lipid contents in testicles and ovaries, but, only protein contents of testicles were decreased by tebufenozide treatment. Thus, it seemed that males should be more sensitive than females to tebufenozide treatment.

Keywords: Tebufenozide, reproductive potential, gonads, *Ephestia kuehniella*.

INTRODUCTION

In insects, ecdysteroids regulate many developmental and physiological processes (Gäde et al., 1997) and are considered as potential specific target sites for pest control (Dinan, 1989). In the past decade, a new class of insect growth regulators (IGRs), namely benzoylhydrazines, has been discovered as the first nonsteroidal ecdysteroid agonists that induce a precocious and incomplete molt in several insect orders. One of these IGRs, tebufenozide (RH-5992) has great potential for use as chemical protection in the stored product industry, especially against lepidoptera (Hami et al., 2005). These compounds mimic the biological activity of the natural insect molting hormone 20-hydroxyecdysone (20E) by binding to the ecdysteroid receptor complex in a competitive manner with Ecdysteroids (Wing, 1988).

Although this nonsteroidal ecdysteroid agonist was developed with an aim of disturbing the larval development, substantial effects were observed on lepidoptera reproduction. Indeed, studies carried out on several species belonging to various families (Crambidae, Noctuidae, Pyralidae and Tortricidae) showed that the tebufenozide affected ovarian development (Smagghe and Degheele, 1994a,b; Salem et al., 1997) and reduced fecundity (total of laid eggs) (Smagghe et al., 1996b; Biddinger and Hull,

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Table 1. Effect of tebufenozide on pupae development period (in days) of *Ephestia kuehniella* (mean ± SD, n= 6 to 28).

Treatment	Males	Females
Control	9.71 ± 2.00 (28)a	12.27 ± 1.38 (22)a
LD50	8.16 ± 0.83 (6)b	10.90 ± 1.28 (10)b
LD90	8.00 ± 0.50 (6)b	9.10 ± 2.07 (10)b

Values of each sexes followed by different letter in the same column are significantly different at 5% level.

1999; Knight, 2000; Rodriguez et al., 2001) as well as the fertility of females (Charmillot et al., 1994; Smagghe and Degheele, 1994a,b; Knight, 2000) and males (Carpenter and Chandler, 1994; Sun et al., 2000). However, it is important to mention that this analogue affects the reproductive potential of the two sexes of species where the role of the ecdysone in the reproduction was not necessarily shown. In order to extend these findings, the present study was designed to evaluate the insecticidal activity of tebufenozide (RH-5992) applied topically on newly exuvied pupae of *E. kuehniella* on adult reproduction. Because sexual maturation in this lepidoptera species was carried out during pupae development, tebufenozide was applied on newly exuvied pupae.

MATERIALS AND METHODS

Experimental animals

Last instars larvae of *E. kuehniella* were collected from stock colony and reared on wheat flour at 27°C and 80% relative humidity. Pupae were staged according to their age in days from pupal ecdysis.

Insecticides and treatment

RH-5992 (tebufenozide) of technical grade was kindly provided by Dr. G. R. Carlson (Rohm and Haas, Spring House. PA). All other chemicals were of analytical grade. The compound was dissolved in acetone (2 µl per insect) and topically administered at two doses: (0.002 and 0.025 µg/insect, corresponding respectively to LD50 and LD90).

Pupae development

Tebufenozide was topically applied at two doses (0.002 and 0.025 µg/insect) on male and female newly ecdysed pupae. Male and female pupae development period was examined at adult emergence and compared with control.

Reproductive potential

Tebufenozide was applied at 0.002 µg/insect on male and female newly ecdysed pupae. After adult emergence, four categories of couples (numbered I, II, III and IV) were formed and mated according to whether the male, the female or both the two were treated (I: Untreated male and untreated female as control couple; II: Treated male and untreated female; III: Untreated male and treated female; IV: Treated male and treated female). The following parameters were measured: preoviposition duration (days between mating and egg laying beginning), egg laying duration, fecundity (total of laid eggs), fertility (number eggs hatched compared to the totality of laid eggs), embryonic duration of development and length and width eggs. Experiment was carried out with ten replications for each couple (20 insects per group).

Weight and biochemical composition of gonads

Tebufenozide was topically applied at two doses (0.002 and 0.025 µg/insect) on male and female newly ecdysed pupae. Testicles and ovaries of newly emerged adults were removed and weighted. Gonads were then homogenised in trichloroacetic acid (20%, w/v) and carbohydrate, lipid and protein of testicles and ovaries were extracted according to Shibko et al. (1966) and evaluated according to Duchateau and Florkin (1959), Goldsworthy et al. (1972) and Bradford (1976), respectively.

Statistics

Mean values (±SD) were compared between control and tested series by Student's t-test at 5% level.

RESULTS

Pupae development period

The effect of LD50 and LD90 of tebufenozide (0.002 and 0.025 µg/insect respectively), has been examined on pupae development period of *E. kuehniella*. Data showed that tebufenozide treatment significantly reduced this period in males and females ($P<0.01$) (Table 1) compared with control of the same sexes.

Reproductive potential

Data (Table 2) showed that tebufenozide treatment increased ($P<0.05$) preoviposition duration and embryonic duration of development, and decreased ($P<0.01$) egg laying duration, fecundity and fertility. Moreover, this compound affected more couples with treated-male alone or treated-male and female than couples with treated-female alone.

Table 2. Effect of Tebufenozide on reproductive potential parameters of ***Ephestia*** *kuehniella* (mean ± SD, n = 10).

Parameter	Couples			
	I	II	III	IV
Preoviposition duration (days)	1.01 ± 0.107a	1.66 ± 0.309b	1.75 ± 0.134c	2 ± 0.10d
Egg laying duration (days)	7.33 ± 0.31a	5.67 ± 0.88b	4.50 ± 0.26c	4,25 ± 0.32d
Fecundity (eggs/female)	186.67 ± 2.52a	73.32 ± 6.21b	165 ± 8.01c	126.33 ± 8.04d
Fertility (%)	96.13 ± 0.208a	19.13 ± 1.83b	85.53 ± 4.01c	10.55 ± 0.69
Embryonic duration of development (days)	5.35 ± 0.47a	7.66 ± 0.57b	5.50 ± 0.50a	7.50 ± 0.50b

I: Untreated male and untreated female as control couple; **II**: Treated male and untreated female; **III**: Untreated male and treated female; **IV**: Treated male and treated female.
Values followed by different letter in the same line are significantly different at 5% level.

Table 3. Effect of tebufenozide (LD50) on egg size of adult ***Ephestia*** *kuehniella* (mean ± SD, n = 10).

Egg size	Couples			
	I	II	III	IV
Length (µm)	530.7 ± 1.11a	488.1 ± 0.187b	523.9 ± 1.36c	412.5 ± 2.33d
Width (µm)	326.4 ± 4a	294.4 ± 10.8b	297 ± 9b	292.5 ± 13.8b
Volume (μm^3)	29.62 ± 0.9a	22.1 ± 2.3b	24.3 ± 3.4b	19.01 ± 2.3b

I: Untreated male and untreated female as control couple; **II**: Treated male and untreated female; **III**: Untreated male and treated female; **IV**: Treated male and treated female.
Values followed by different letter in the same line are significantly different at 5% level.

Egg size

Obtained results (Table 3) showed that tebufenozide treatment significantly decreased both egg length and width ($P<0.05$) and egg volume ($P<0.01$).

Weight of gonads

Results (Table 4) showed that tebufenozide treatment involved a significant decrease ($P<0.01$) of testicle and ovarian weight with a dose-response relationship.

Testicular lipid, carbohydrate and protein concentrations

Obtained results showed that tebufenozide treatment caused a significant decrease on carbohydrate ($P<0.05$) and lipid ($P<0.01$) concentrations in both testicles (Figure 1) and ovaries (Figure 2) with a dose-response relationship. On the other hand, only protein content in testicles was decreased ($P<0.01$) compared to control. In adult ovaries, protein concentrations were unaffected by tebufenozide treatment ($P>0.1$).

DISCUSSION

Many developmental and physiological processes in insects are orchestrated by 20E, JH and other neurohormones such as eclosion hormone. Molting and metamorphosis have been extensively studied in several representative insects (Nijhout, 1994; Riddiford, 1994,

Table 4. Effect of Tebufenozide (LD50 and LD90) on newly exuvied pupae, on weight of gonads (mg) of newly emerged adults of *Ephestia kuehniella* (mean ± SD; n = 10).

Treatment	Testicles	Ovaries
Control	1.58 ± 0.69a	5.42 ± 3.04a
LD50	0.93 ± 0.53b	3.63 ± 1.24b
LD90	0.86 ± 0.24c	3.50 ± 1.53 c

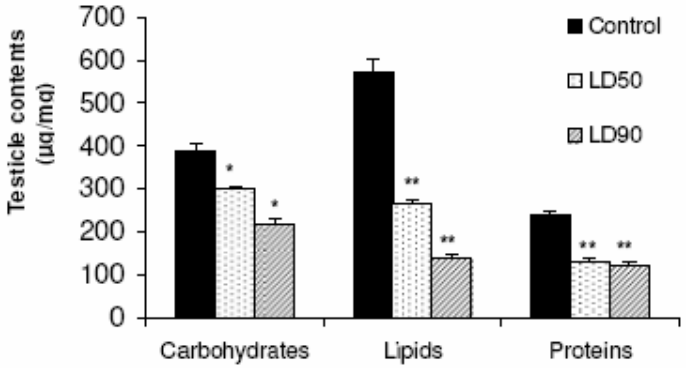

Figure 1. Effect of Tebufenozide on testicular concentrations of carbohydrates, lipids and proteins (µg/mg of testicle) of males of *Ephestia kuehniella* (mean ± SD; n = 3; Different from control *P<0.05; **P<0.01).

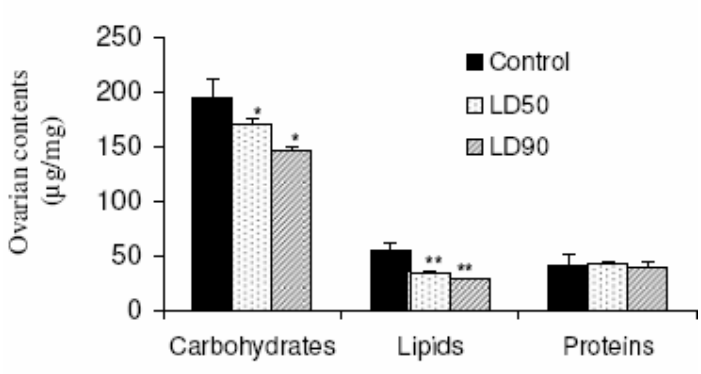

Figure 2. Effect of tebufenozide, (LD50 and LD90) on ovarian concentrations of carbohydrates, lipids and proteins (µg/mg of ovary) of females of *Ephestia kuehniella* (mean ± SD; n = 3; Different from control *P<0.05; **P<0.01).

1996). The morphological and ultrastructural changes that occur in the epidermis during insect growth and development are dependent upon the regulation of gene expression with different concentrations of 20E in the absence or presence of JH. Any interference by IGRs with a hormone action in the homeostasis of one or more of these hormones with exogenous sources of the hormone or with synthetic analogs (agonists or antagonists) would result in the disruption or abnormal development of the target insect. In the present study, tebufenozide (RH-5992), a nonsteroidal ecdysteroid agonist exhibited insecticidal effects on *E. kuehniella*, when administered by topical application on newly ecdysed pupae.

This compound has a similar hormonal activity with the natural hormones in the insects and particularly in lepidoptera of which the principal effect is to cause incomplete and lethal moults (Dhadialla et al., 2005). In addition, the ingestion of this insecticide on the larval stage induced on *Spodoptera exigua* and *Choristoneura fumiferana* an early moult and anomalies such as a double cephalic capsule and an incomplete cuticle (Retnakaran et al., 1997 a,b; Smagghe et al., 1996a). Other agonists induced early and incomplete moults, on *Ostrinia faxiatus* treated with RH-5849, (Darvas et al., 1992), on *Culex pipiens* (Boudjelida et al., 2005) and on *E. kuehniella*, (Hami et al., 2005), treated with RH-0345. Our data on reproduction parameters clearly indicated that fecundity and fertility were reduced when couples were formed and mated with treated males and females. Moreover, these results showed that tebufenozide effect was more pronounced on couples with treated males than on couples with treated females. Although egg size was reduced by tebufenozide, the weight of testicles was more affected by this insecticide than that of ovaries. Moreover, the biochemical composition of the testicles was also more reduced than that of the ovaries, especially carbohydrate and lipid concentrations.

The current observed effects of ecdysone agonists on egg formation together with previous studies on oviposition (Wing and Ramsay, 1989; Lawrence, 1992; Smagghe and Degheele, 1992, 1994a, b; Dhadialla et al., 1998; Farinos et al., 1999) indicated that a single topical application or oral feeding may halt egg laying in a dose dependent manner after 2 - 4 days in various lepidopteran, coleopteran and dipteran insects. Several studies showed that the tebufenozide reduced the fertility of the females and/or the males of many lepidopteran species like *Helicoverpa zea* (Carpenter and Chandler, 1994), *Argyrotaenia velutiana* and *Choristoneura rosace-ana* (Sun et al., 2000). Carpenter and Chandler (1994) also reported that the tebufenozide prevented the transfer of the spermatozoids during the copulation of *Helicoverpa zea*.

Ovaries of treated females were reduced in mass, which may indicate a status of resorption of the oocytes. Similarly, continuous exposure to RH-5992 and RH-2485-treated surfaces significantly reduced the mean number of eggs laid and the percent of eggs that hatched in *Argyrotaenia vellutinana* and *Choristoneura rasaceana* (Sun et al., 2000). The chorion of insect eggs has a protective role which is essential to normal development of the embryo. Ultrastructural observation of basal oocytes of *E. kuehniella* showed that RH-5992 and RH-0345 caused a significant reduction of the chorion thickness (Hami et al., 2005).

However, tebufenozide impact on males could be explained by the important reduction of the lipid content of testicles. This reduction could be reflected on prostaglandin synthesis via polyunsaturated fatty acids (Stanley-Samuelson et al., 1988; Uscian and Stanley-Samuelson, 1993; Stanley-Samuelson and Pedibothla; 1996). Indeed, several work showed that these prostaglandins act on the reproduction by activating egg-laying behaviour on several species of which *Acheta domesticus* (Destephano and Brady, 1977), *Teleogryllus commodus* (Loher et al., 1981; Stanley-Samuelson and Loher, 1986) and *Bombyx mori* (Setti and Ramaiah, 1980). Although this mechanism does not exist on all species (Stanley-Samuelson, 1994), the prostaglandins were detected in ovaries of *Locusta migratoria* (Lange, 1984), *Musca domestica* (Wakayama et al., 1986) and Tenebrio molitor (Howard et al., 1992).

Thus, it appears that tebufenozide, topically applied on newly exuvied pupae of *E. kuehniella*, induces not only early moults (as evidenced by the decrease of pupae development period), but also significantly reduces reproductive potential of adults, in particular by falling fertility, fecundity and biochemical composition of gonads.

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