J. Entomol. Res. Soc., 13(3): 53-63, 2011 *ISSN:1302-0250*

Sublethal Effects of Methoxyfenozide, in Comparison to Chlorfluazuron and Beta-Cypermethrin, on the Reproductive Characteristics of Common Cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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ABSTRACT

The sublethal effects of Methoxyfenozide on reproductive bio-characteristics, viz., fecundity, fertility and oviposition were evaluated on *Spodoptera litura* treated as 2nd (L2) and 5th instars (L5) larvae with sublethal doses of methoxyfenozide, corresponding to the LC_{10} and LC_{30}, and compared with the effects induced by chlorfluazuron and beta-cypermethrin. In case of L2 treatment, the results elucidated that LC_{10} of methoxyfenozide and chlorfluazuron significantly ($P<0.05$) reduced the fecundity and fertility as compared to beta-cypermethrin and the control. Moreover, due to LC_{30} of methoxyfenozide, the total egg laying and hatching rate were also significantly ($P<0.05$) reduced by the three pesticides as compared to the control. LC_{10} of methoxyfenozide, chlorfluazuron and beta-cypermethrin could not induce any significant effect on pre-oviposition and oviposition duration. Further, it was noticed that LC_{30} of methoxyfenozide and chlorfluazuron significantly reduced oviposition duration as compared to beta-cypermethrin and control. When LC_{10} was used for the 5th instar larvae, the total number of eggs was markedly reduced by methoxyfenozide and chlorfluazuron as compared to beta-cypermethrin and the control. Likewise, the hatching rate was significantly ($P<0.05$) reduced with respect to all pesticides. Further at LC_{30} concentration, the same trend of insecticidal impact was noticed on fecundity and fertility response of all the tested insecticides. The results revealed that the experimental insecticides at LC_{10} had no significant effect in both pre-oviposition and oviposition duration in comparison with control, while only oviposition duration was significantly reduced at the higher concentration of (LC_{30}) for methoxyfenozide and chlorfluazuron. It is possible to conclude that the effects of methoxyfenozide with its sterilizing properties if used strategically on *S. litura* might induce changes in population dynamics of this pest in vegetable crops and it may be considered potent insecticidal compound for controlling this pest.

Key words: Spodoptera litura, methoxyfenozide, sublethal effect, fertility, fecundity, oviposition.

INTRODUCTION

The cutworm, *Spodoptera litura* (Fabricius), (Lepidoptera: Noctuidae), is a polyphagous insect that has about 150 host species (Rao *et al.*, 1993). At present, this pest is effectively controlled with a specific group of insect growth regulators (IGRs), the benzoylphenyl ureas (chitin synthesis inhibitors). However, due to their intensive use, this pest is likely to develop high level of resistance to these compounds as has been observed with the majority of organosynthetic insecticides (Smagghe *et al.*, 2003).

Over the past three decades, efforts have been made to develop novel insecticides with selective properties that are designed to act on specific biochemical sites or physiological processes of the target pest (Ishaaya *et al*., 2005). IGRs are biorational insecticides with novel modes of action which disrupt the physiology and development of the target pest, such compounds tend to be selective and generally less toxic to non-target organisms than conventional insecticides (Biddinger and Hull, 1995; Gurr *et al*., 1999). Ecdysone agonists are one of the most important groups of IGRs, and widely used against many insect pests in fields. In general, sublethal effects caused by ecdysone agonists include delayed developmental rates (Adel and Sehnal, 2000; Biddinger *et al*., 2006), reduced larval and pupal weight (Pineda *et al*., 2007), adult deformities (Sundaram *et al*., 2002) and reduced fecundity and fertility (Seth *et al*., 2004; Saenz-de-Cabezon *et al*., 2005; Pineda *et al*., 2009). Therefore, the survivors of an ecdysone agonist treatment could negatively impact the population dynamics of lepidopteran pest species (Pineda *et al*., 2009). As an important member of the ecdysone agonist family, methoxyfenozide (RH-2485) is highly specific to lepidopteran pests, but has a low toxicity towards other insect orders (Smagghe *et al*., 2003), and is widely used all over the word against many economically important horticultural, agronomic and forest pests (Chandler *et al*., 1992; Smagghe *et al*.,1996ab; Cadogan *et al*., 1997; Palli and Retnakaran, 2001). Methoxyfenozide was reported to be safer for beneficial organisms than conventional products (Medina *et al*., 2004; Schneider *et al*., 2004, 2008). Its favorable ecotoxicological profile and short period of persistence in the environment made it a good choice for integrated pest management (IPM) programs in ornamental and vegetable crops (Pineda *et al*., 2006).

Little is known about the sublethal effects in adults emerged from larvae that have been exposed to sublethal concentrations of methoxyfenozide so far. Therefore, using *S. litura* as the target, present study was aimed to explore the reproductive effects in adults emerged from the 2^{nd} and 5^{th} larvae instar that had been treated with sublethal concentrations of methoxyfenozide, in comparison to chlorfluazuron and beta-cypermethrin, two of common used insecticides against *S. litura*.

MATERIALS AND METHODS

Insects

The insects used were taken from a laboratory culture of *S. litura*, which was established from adults obtained from the Institute of Plant Protection (IPP), Chinese Academy of Agricultural Sciences (CAAS). The culture was routinely maintained on soybean-based artificial diet at standard conditions of 27 ± 2°C, 75 ± 5% r.h. and 16:8 (L:D) photoperiod in growth chambers. Newly hatched larvae were reared communally in groups of about 80–100; later the larvae were reared individually on the fresh diet, which was changed at every two days interval. In the present experiments, the insects were transferred to experimental diets immediately after moulting to the 6^{th} (final) instar. After the pupation, the pupae were collected and placed in four walls square wooden emergence cage with diameter of (40cm×40cm×40cm) lined by thin gauze. Each cage contained about 40~50 pupae. After adult emergence, the wax paper was used around the internal wall cage to let the adults copulate and oviposit freely.

Chemicals

Pesticides used were methoxyfenozide (24% SC, RH-2485 (Runner)), as one of moulting hormone agonists, purchased from Dow Agro Science Co. (China), chlorfluazuron (5% EC, Atabron), as another IGR of chitin synthesis inhibitor brought from Ishihara Sangyo Kaisha, Ltd (Japan), and beta-cypermethrin (4.5% EC, HiFu), as a common traditional chemical against *S. litura*, obtained from Jiangsu Yangnong Chemical Group Co., Ltd.

Dose-Response Bioassays

Dose-response bioassays were conducted to determine the toxicity of methoxyfenozide and other two insecticides against *S. litura* larvae. Leaf-dip method as described by Tabashnik and Cushing (1987) was used in the bioassay. At least five concentrations per replicate of three chemicals were prepared from serial dilutions using, tap water (for 2nd instar larvae, ranging from 0.02 to 100 ppm, 0.1 to 50 ppm, and 1.0 to 400 ppm for methoxyfenozide, chlorfluazuron and beta-cypermethrin,respectively; for 5th instar larvae, ranging from 4 to 200 ppm, 1.0 to 100 ppm, and 1.0 to 400 ppm for methoxyfenozide, chlorfluazuron and beta-cypermethrin,respectively). Cabbage leaves were cut in suitable size (3.5×3.5 cm) and dropped in the solution for 10s then after that they were dried for 4-6 hours. The dried leaves were then put individually into Petri dish with 20 cm diameter, and the ten 2nd instar (L2) or 5th instar larvae (L5) were released on the leaves. Three dishes per concentration were used for each replicate. Distilled water was used as control. Larvae were reared on treated diet for 24h,and then moved on to fresh leaves free from chemicals. Another 24h later, the treated larvae were checked and the number of dead and survival were recorded. Probit analysis was conducted on the dose-response data using POLO PC program (LeOra Software, 1987), and then the two doses (LC_{10} and LC_{30}) were estimated for methoxyfenozide, and chlorfluazuron, beta-cypermethrin respectively.

Sublethal effects of Methoxyfenozide on fecundity and fertility of *S. litura* larvae 2nd or 5th instar

The second instar (L2) or fifth instar (L5) larvae were moved into treated cabbage leaves in Petri dishes for 48 h., and then moved out to fresh cabbage leaves free from chemicals. The larvae were reared to pupate and emerge. The adults were collected and paired to mate in glass bottle (diameter 18.0 cm; height 18.0 cm). Wax paper was circled around the wall of the glass bottle for oviposition. The ovipositional substarte (wax papers) were checked everyday to collect egg masses and eggs count (total number of eggs laid by female and the fertility (percentage of eggs hatched**).** The pre-ovipostion and oviposition duration were also observed. Three female *S. litura* adults were randomly chosen from each treatment for egg counting and pre-ovipostion, oviposition duration recording.

Data analysis

Differences in total Eggs/femal, egg hatching rate, pre-oviposition duration and oviposition duration were statistically analyzed by one- way ANOVA followed by Tukey's post hoc tests among the all treatments. An alpha level of 0.05 was used for all tests. All statistical analysis was performed with the statistical program SPSS 17.0.

RESULTS

The effect of Toxicity of tested pesticide on different instar larva of *S. litura*

Based on the preliminary results of the bioassay for the present experiments, presents probit-log concentration regression equations were obtained for the 2nd and 5th instar larvae of *S. litura*, and LC_{10} and LC_{30} values with respect to 2nd and 5th instar larvae, respectively, were estimated (Table 1).

Table 1. The toxicity of tested pesticides to larva of *Spodoptera litura*

Pesticides	Instar	Regression Equation Y=aX+b	χ^2	LC_{10} (ppm)	LC_{30} (ppm)
Methoxyfenozide	2nd	Y=0.6259X+4.8212	6.52	0.0173	0.2803
Chlorfluazuron		Y=0.8467X+4.6487	3.24	0.0797	0.6246
Beta-cypermethrin		Y=0.2310X+3.9400	4.66	0.6613	2.7256
Methoxyfenozide	5th	Y=1.4646X+2.8363	6.69	4.0021	13.1594
Chlorfluazuron		Y=1.7724X+4.0066	2.57	0.6878	1.8393
Beta-cypermethrin		Y=1.9619X+3.726	3.26	0.9904	2.4083

Sublethal effects of Methoxyfenozide on the reproductive parameters of *S. litura* adult treated as 2nd instar larvae (L2)

Data pertaining to sublethal effects of methoxyfenozide on the reproductive parameters of *S. litura* treated larvae 2nd instar is shown in Table 2. The results showed that the total number of eggs laid by female derived from treated L2 (LC_{10} of methoxyfenozide and chlorfluazuron) was significantly ($P<0.05$) reduced compared to the control. On the other hand, no significant ($P>0.05$) difference was noticed on total number of eggs laid by the female when LC_{10} beta-cypermethrin was applied in the same stage as compared to the control. Further it was noticed that that methoxyfenozide (LC_{10} to L2) significantly ($P<0.05$) lowered hatching rate to 61.5 % as compared to (72.3% and 78.8 % reduction due to chlorfluazuron and beta-cypermethrin treatment, respectively. When LC_{30} concentration of methoxyfenozide, chlorfluazuron and beta-cypermethrin was applied to L2; the total number of eggs laid was significantly ($P<0.05$) reduced to (149.0±92.1, 236.7±52.3 and 1250.0±114) respectively, as compared to the control (1751.3±47.5). It was also found that methoxyfenozide, chlorfluazuron and beta-cypermethrin significantly ($P<0.05$) decreased hatching rates to (31.2%, 40.8% and 66.9 %) respectively compared to the control (82.7%).

Table 2. Sublethal effects of methoxyfenozide on the reproductive characteristics (fecundity and fertility) of *S. litura* adult treated as 2nd instar larvae (L2) as compared to chlorfluazuron and beta-cypermethrin.

Treatments and Index		Methoxyfenozide	Chlorfluazuron	Beta-cypermethrin	Control
LC_{10}	Total Eggs	188.7±40.5b	555.3±75.2b	1930.0±130.5a	2136.3±697.2a
	Hatching Rate (%)	61.5±6.9c	72.3±5.5bc	78.8±3.5b	89.2±2.4a
LC_{30}	Total Eggs	149.0±53.2c	236.7±30.2c	1250.0±65.8b	1751.3±27.4a
	Hatching Rate (%)	31.2±0.2d	40.8±2.0c	66.9±6.8b	82.7±1.3a

* The data indicate Means±SE followed by the same small letters within a row are not significantly different (p≤0.05).

Sublethal effects of methoxyfenozide on the reproductive characteristics (fecundity and fertility) of *S. litura* adult treated as 5th instar larvae(L5).

For the 5th instar larvae treated with LC_{10} of methoxyfenozide, the results revealed that total number of eggs was significantly ($P<0.05$) reduced to 162.7±32.1 and 415.3±110.5, by both methoxyfenozide and chlorfluazuron, respectively as compared to the control (1751.3±47.5) (Table 4.). However, no significant ($P>0.05$) effect was induced by Beta-cypermethrin on total number of eggs compare to the control . Furthermore, methoxyfenozide, chlorfluazuron and beta-cypermethrin significantly ($P<0.05$) lowered hatching rate to the following percentages, 30.8 %, 32.9 % and 53.9 %, respectively. When LC_{30} of methoxyfenozide, chlorfluazuron or beta-cypermethrin was applied to the larvae 5th instar; the total number of eggs laid was significantly ($P<0.05$) reduced as compared to the control. Similarly, the hatching rate was significantly ($P<0.05$) decreased to 26.0 %, 31.7 %and 42.8 % respectively, as compared to the control (Table 3).

Table 3. Sublethal effects of methoxyfenozide on the reproductive characteristics (fecundity and fertility) of *S. litura* adult treated as 5th instar larvae (L5).

Treatments and Index		Methoxyfenozide	Chlorfluazuron	Beta-cypermethrin	Control
LC_{10}	Total Eggs	162.7±18.5b	415.3±63.8b	1096.0±123.5a	1279.7±79.2a
	Hatching Rate (%)	30.8±0.1b	32.9±0.2b	53.9±12.1b	75.7±2.4a
LC_{30}	Total Eggs	50.3±16.2b	281.7±73.3b	354.7±114.3b	1082.3±126.9a
	Hatching Rate (%)	26.0±0.0b	31.7±1.4b	42.8±3.1b	74.1±3.9a

* The data indicate Means±SE followed by the same small letters within a row are not significantly different (p≤0.05).

Sublethal effects of methoxyfenozide on the oviposition of *S. litura* adult when larva 2nd instar was treated.

Our results showed that LC_{10} of methoxyfenozide, chlorfluazuron and beta-cypermethrin applied to the larvae 2nd instar had no effect ($P>0.05$) on pre-oviposition duration compared to the control, which ranged from 2.0 to 2.7

days (Table 4). Likewise, the above three pesticides at LC_{10} did not affect (P>0.05) oviposition duration (4.3, 4.0 and 5.3 days due to methoxyfenozide, chlorfluazuron and beta-cypermethrin, respectively as compared to the control (5.0 days). When LC_{30} of methoxyfenozide, chlorfluazuron or beta-cypermethrin was applied, there was no significant difference found in pre-oviposition duration, however the oviposition duration was significantly (P<0.05) reduced by methoxyfenozide and chlorfluazuron as compared to beta-cypermethrin and control

Table 4. Sublethal effects of methoxyfenozide on the oviposition of *S. litura* adult treated as 2nd instar larvae (L2) as compared to chlorfluazuron and beta-cypermethrin.

Reproductive index		Methoxyfenozide	Chlorfluazuron	Beta-cypermethrin	Control
LC_{10}	Pre-Oviposition Duration (d)	2.3±0.0a	2.7±0.7a	2.0±0.0a	2.3±0.3a
	Oviposition Duration (d)	4.3±1.2a	4.0±0.5a	5.3±0.7a	5.0±0.5a
LC_{30}	Pre-Oviposition Duration (d)	2.7±0.3a	2.0±0.5a	2.3±0.3a	1.7±0.3a
	Oviposition Duration (d)	2.3±0.3bc	1.7±0.3c	3.0±0.0ab	4.0±0.5a

* The data indicate Means±SE followed by the same small letters within a row are not significantly different (p≤0.05).

Sublethal effects of methoxyfenozide on the oviposition of *S. litura* adult derived from treated 5th instar larvae

Our results indicated that when 5th instar larvae (L5) were treated with LC_{10} of methoxyfenozide, chlorfluazuron or beta-cypermethrin, no significant (P>0.05) difference was observed in the pre-oviposition duration with respect to control (Table 5). The results also showed that when at LC_{10} of methoxyfenozide, chlorfluazuron and beta-cypermethrin, the oviposition duration was not affected. When LC_{30} concenetration of these different insecticides was used, there was no significant (P>0.05) influence on the pre-oviposition duration with respect to methoxyfenozide, chlorfluazuron and beta-cypermethrin in comparison to the control, however the oviposition duration was significantly (P<0.05) reduced by methoxyfenozide and chlorfluazuron as compared to beta-cypermethrin and control (Table 5).

Table 5. Sublethal effects of methoxyfenozide on the oviposition of *S. litura* treated as 5th instar larvae (L5) as compared to chlorfluazuron and beta-cypermethrin.

Reproductive index		Methoxyfenozide	Chlorfluazuron	Beta-cypermethrin	Control
LC_{10}	Pre-Oviposition Duration (d)	2.7±0.3a	2.3±0.9a	2.3±0.3a	2.0±0.0a
	Oviposition Duration (d)	2.3±0.3a	3.0±1.2a	3.0±0.6a	4.3±0.3a
LC_{30}	Pre-Oviposition Duration (d)	2.7±0.3a	2.3±0.3a	2.0±0.0a	2.0±0.0a
	Oviposition Duration (d)	2.0±0.6b	2.3±0.3b	3.3±0.3a	4.3±0.3a

* The data indicate Means±SE followed by the same small letters within a row are not significantly different (p≤0.05).

* The data indicate Means±SE followed by the same small letters within a row are not significantly different (p≤0.05).

DISCUSSION

The high effectiveness of methoxyfenozide against Lepidoptera pest has been widely recognized (Moulton *et al*., 2002; Smagghe *et al*., 2003; Pineda *et al*., 2006) but its sublethal effects on the adults derived from treated larvae have not been studied deeply. In the current study, the fecundity and fertility of adult *S. litura* treated as 2nd and 5th instar larvae with methoxyfenozide were significantly and adversely affected. Methoxyfenozide is a potent ecdysone agonist against Lepidoptera by ingestion, this compound is highly active, and to a lesser degree, by contact or topical application (Moulton *et al*., 2002). This diacylhydrazine is five- to ten fold more potent than its analogue (tebufenozide) on *S. littoralis* (Ishaaya *et al*., 1995) and exhibits more activity against budworm/bollworm and diamond back moth with no harm to natural enemies (Le *et al*., 1996; Dhadialla *et al*., 1998; Smagghe and Degheele 1998), and fit well into insect pest management programs. The sublethal effects such as suppression of reproductive capacity and reduction observed in the survivors could have a negative impact on insect population dynamics (Trisyono and Chippendale, 1998; Lopez and Latheef, 1999; Knight, 2000, Pineda *et al*., 2007). Our results indicated that methoxyfenozide significantly reduced both of the total number of eggs laid and their hatching rate in comparison with the control, chlorfluazuron and beta-cypermethrin, which could be due to the persistence of methoxyfenozide for a longer period, together with the high metabolic rate of this compound in larval tissue (Smagghe *et al*., 1999). When the 2nd and 5th instars larvae of *S. litura* were treated with LC_{10} and LC_{30} of methoxyfenozide, the results revealed that the life parameters (fecundity and fertility) were significantly reduced as compared to chlorfluazuron and beta-cypermethrin and the control (untreated). The literature suggests that reductions in fecundity are most likely caused by the ecdysone agonists affecting the female reproductive system. Reports by Smagghe and Degheele (1994) and Smagghe *et al*. (1996a) suggest that ecdysone agonists has a chemosterilizing effect on female Lepidoptera by interfering with ovulation and oviposition in female Lepidoptera which may cause reabsorbtion of ovarioles (Smagghe and Degheele, 1994; Salem *et al*., 1997). In addition, it is well known that the maturation of insect eggs is dependent basically on the materials taken up from the surrounding hemolymph and by materials synthesized by the ovary in situ (Indrasith *et al*., 1988). These materials include proteins, lipids and carbohydrates, all of which are required for embryogenesis (Kanost *et al*., 1990). We suppose that methoxyfenozide could interfere with the accumulation of proteins in the eggs, which also might explain the reduction in fecundity and fertility in *S. litura.* Also, the significant decline in egg fertility may in small part be explained by the fact that methoxyfenozide has been reported to be ovicidal (Trisyono and Chippendale, 1997, 1998). Christian-Luis *et al*., (2010) reported that when the 3rd instars of beet armyworm *Spodoptera exigua* from the methoxyfenozide treatment did not show reduced in fecundity but fertility was significantly reduced compared with untreated control. Also a reduction in fecundity and fertility occurred in adult tufted apple bud moth, *Platynota idaeusalis* (Walker); corn earworm, *Helicoverpa zea* (Boddie); and codling moth, *Cydia pomonella* (L.), when the larvae were fed on diet treated with sublethal doses of its analogue tebufenozide (Carpenter and Chandler, 1994; Brown, 1996; Biddinger and Hull, 1999). Moreover Carpenter and Chandler (1994) reported a significant decline in the fertility of corn earworm *Helicoverpa zea* (treated as larvae with sublethal doses of tebufenozide in diet. In contrast, Ishaaya *et al*., (1995) reported a significant increase in the fecundity of adult *S. littoralis* that when the larvae were fed on leaves treated with a sublethal dose of methoxyfenozide, the reason remains unknown and such a phenomenon warrants further investigation. However, topical application of sublethal doses of an IGRs chlorfuazuron (LD_{10}: 1.00 ng per larva or LD_{30}: 3.75 ng per larva) on newly molted 5th instars had an effect on the reproduction of *S. litura* by reducing fecundity, fertility, and hatchability was observed in laboratory conditions (Perveen and Miyata, 2000). To validate the sublethal effects observed in the reproduction of *S. litura,* further research under semi-field conditions is in progress and will help to increase the practical relevance of the laboratory-based results to know what may happen in the fields. Our results disclosed that when the larvae 2nd and 5th instar were treated with LC_{10} dose of methoxyfenozide the both pre-ovipostion and oviposition duration were not affected, while at higher concentration (I.e., LC_{30}) of methoxyfenozide, the ovipostion duration was significantly reduced. However, the mechanisms of how methoxyfenozide affects the oviposition duration are still unknown, but we suppose that methoxyfenozide might be transported to eggs from females paired with males derived from treated larvae and it might affect oviposition duration and copulation process. This effect could be explained, in this study, by the accumulation/reabsorption of methoxyfenozide in eggs and displaying a degenerative appearance or because males might be less vigorous (transferred less viable sperm). In addition, the presence of this compound in the eggs also might help to understand the loss of egg fertility that registered in another study on S*. littoralis* by Pineda *et al*.(2009), because sperm is affected and eggs are not fertile (Carpenter and Chandler, 1994). Since *Spodoptera species* has developed resistance to the majority of conventional insecticides, the use of insecticide chemistries with different modes of action, such as methoxyfenozide, might be considered a part of an insecticide resistance management program. The present results verified sublethal effects of methoxyfenozide on larval stages of *S. litura.* In addition, we demonstrated that methoxyfenozide negatively affected the reproduction of this pest. These effects are very important from a practical point of view, because offspring can be reduced and as a consequence, the insect population can be maintained below a level of economic loss. Further the use of sublethal doses having anti-reproductive effects must be strategically used before the extensive use of lethal doses of such boirational compounds in order to manage the development of insecticide resistance.

ACKNOWLEDGEMENTS

This research was supported by a grant from Department of Science and Technology, Jiangsu Province, China (BZ2006038), which is greatly appreciated. We also thank Dr. Lu in Institute of Plant Protection (IPP), Chinese Academy of Agricultural Sciences (CAAS), for his kindly providing *S. litura* colony for the reaearch.

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Received: April 11, 2011   *Accepted: October 25, 2011*