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IMPACT OF TRIFLUMURON ON REPRODUCTION AND DEVELOPMENT OF RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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Red flour beetle, *Tribolium castaneum*, is a worldwide serious pest of dried, stored, durable agricultural commodities, and of many value-added food products and non-food derivatives of agricultural products. Sublethal effects of three concentrations (0.01, 0.02 and 0.04ppm) of triflumuron were evaluated on two field collected strains of *T. castaneum* at last larval instar for development and reproduction impacts. Larval mortality increased significantly both with increase in concentration and exposure period. Exposed larvae exhibited reduction in weight and increase in duration of larval developmental duration compared to control. Subsequent development of pupae and emergence of adults was seriously prohibited. However, the increase in fecundity and egg hatchability reduced significantly at all concentrations compared to control. Finally, subsequent development of surviving F_1 larvae, pupae and adults was also severely interdicted. In view of these impressive ovicidal and reproduction inhibition effects of triflumuron against *T. castaneum* and having low toxicity to non-target organisms, it should be tested in flour mills, ware houses and food storages for the promising replacement of synthetic grain protectants e.g. pyrethroids and organophosphates

Keywords: Stored grains, flour, insect pests, triflumuron, chitin synthesis inhibitor, fecundity, growth.

INTRODUCTION

Extensive use of fumigants and conventional insecticides has resulted in increased resistance among insect pests of stored products and negative public reaction to their use (Hamid *et al*., 1988; Bell, 2000; Philips and Throne, 2010). In addition, there is evidence that the treatment of stored grain commodities with fumigants and conventional insecticides may, in some cases, result in the presence of residues in products prepared from treated grain (Mukerjee *et al*., 1973; Bullock, 1974; Norman, 2000). These factors have prompted a search for alternative control measures which are effective against the stored grain pests, safe to the mammals and have minimal impact on the environment (Oberlander *et al.,* 1997; Kostyukovsky and Trostanetsky, 2006; Phillips and Throne, 2010). Insect growth regulators have been observed effective against various stored grain pests, show very low toxicity to mammals and, non-target organisms and are rapidly degradable in the environmental conditions (Oberlander *et al*., 1997; Kostyukovsky *et al*., 2000; Phillips and Throne, 2010).

Insect growth regulators include compounds that may affect moulting and metamorphosis by mimicking juvenile hormone activity (juvenile hormone agonists) or antagonizing juvenile hormone activity (ecdysteroids agonists) or by interfering with cuticle formation (chitin synthesis inhibitors) such as benzoylphenyl ureas (Oberlander *et al*., 1997; Phillips and Throne, 2010). Among them, chitin synthesis inhibitors (CSIs) have been to be effective at killing all immature stages of a wide range of insect species, including the internal grain feeders (Ammar, 1988; Desmarchelier and Allen, 1992; Elek and Longstaff, 1994; Daglish and Wallbank, 2005). Although such compounds do not mimic insect hormones, they prevent normal moulting of larval insects by inhibiting the chitin synthesis (Oberlander *et al*., 1997); however, currently, the precise mechanism of this inhibition is unknown (Cohen, 1993). It can be hypothesized that CSIs disrupt hydrogen bonding between closely packed chitin synthase units essential for the formation of crystalline microfibrils that severely disrupt the moulting, and the weak and soft cuticle triggers desiccation and death of insects (Cohen, 2001).

The application of CSIs have widely been reported to affect insect reproduction and development (Smagghe *et al*., 1996; Parveen *et al*., 2000; Parween *et al*., 2001). Although in some cases the oviposition of the treated females was found to be increased (El-Sayed *et al*., 1985), but the hatching percentage of the eggs oviposited by treated females was found to be significantly reduced (El-Sayed *et al*., 1985). The ovicidal effects of these compounds have been investigated for several insects by treating either adults or the medium in which eggs were deposited (Parween, 2003; Kostyukovsky and Trostanetsky, 2006; Trostanetsky and Kostykovsky, 2008).

The aim of the present study was to investigate the sub-lethal effects of triflumuron on growth, development and reproductive performance of *T. castaneum*.

MATERIALS AND METHODS

Insect collection and standardization : Adults of the red flour beetle (*Tribolium castaneum*) were collected from grain markets of Multan and Faisalabad and were designated as ML and FD strains, respectively. The cultures of both the strains were maintained for three generations before treatment in glass jars containing wheat flour plus yeast medium and placed in cooled incubator (SANYO-MIR-254) at 30 ± 2ºC and 70 ± 5 % relative humidity and a light regime of 12 L: 12 D.

IGR: Triflumuron 20 SC, a CSI, obtained from Warble Ltd. (Pakistan), was used in this study at three concentrations including 0.01, 0.02 and 0.04ppm.

Bioassay :A stock solution of triflumuron at a concentration of 0.04ppm was prepared in acetone and remaining concentrations were prepared from this stock solution. These prepared concentrations were then mixed thoroughly in 50g of wheat flour. Flour treated with acetone alone was used as control. The treated culture was ventilated for 24 hours to evaporate all of the acetone from the samples. A group of 20, last instar larvae of ML and FD strains of *T. castaneum* were placed separately in 100-ml glass jars containing treated flour. Each treatment was replicated thrice. *T. castaneum* larvae were exposed to treated flour up to pupation and remaining stages were shifted to untreated flour. The jars were kept in darkness at 28 ± 2 ºC and 70 ± 5% relative humidity. Data regarding larval mortality was taken after 1, 3 and 5 days of treatment. Larval weight was taken at 5th day of treatment with the help of electronic weighing balance and larval duration before pupae was recorded for each individual in all the treatments. After pupation, individuals of all the treatment were shifted to untreated wheat flour under same conditions and development of pupae and adult population was recorded. The adults emerging from each treatment were allowed to feed on untreated flour for twenty days. Afterwards, adults of each treatment were placed in untreated wheat flour for 2 days for oviposition and number of egg laid, hatching percentage, F_1 larvae, pupae and adults were recorded.

Statistical analysis: Data of larval mortality was corrected by Abbott's formula (Abbott, 1925) and data of % corrected larval mortality and all the other treatments were analyzed statistically by using software Statistix. The significant results were compared using Tukey's HSD test. The analyzed data is presented as mean ± standard error.

RESULTS

Flour treated with 0.01, 0.02 and 0.04ppm concentrations of triflumuron caused 1.7, 5.1 and 11.9% larval mortality in the ML strain and 3.4, 8.5 and 13.6% larval mortality in the FD strain of *T. castaneum* after 24 hours of treatment (Table 1). Larval mortality was increased with increase in concentration ($F= 50.15$; $p < 0.001$), exposure period ($F= 69.57$; $p < 0.001$) and due to effect of strains ($F= 6.90$; $p < 0.05$, Table 1). At 0.04ppm concentration of triflumuron, 29.8 and 36.2% larval mortality was observed in the ML and FD strains, respectively compared to control after 120 hours of treatment (Table 1).

Table 1. Mean percent corrected mortality ± SE of *T. castaneum* larvae exposed to flour treated with different concentrations of triflumuron at various exposure periods

Strain	Conc. (ppm)	Exposure Period (hours)		
		24	72	120
ML	0.04	11.9±1.69 b	22.4±2.98 b	29.8±1.75 a
	0.02	5.1±1.69c	15.5±1.72 Bb	26.3±3.03 a
	0.01	1.7±1.69b	5.2±1.72a b	12.3±1.75 a
FD	0.04	13.6±2.93 b	24.1±3.44 ab	36.2±4.56 a
	0.02	8.5±2.93b	19.0±4.56 ab	31.0±1.72 a
	0.01	3.4±0.00b	6.9±0.00b	17.2±2.98 a

Means followed by same letter(s) within each row (denoted by lower-case letters) are not significantly different by Tukey's HSD test at P = 0.05.

Larval weight was reduced by 9.22 and 10.49% at 0.04ppm in the ML and FD strains, respectively, compared to control (Figure 1). This inhibition effect increased with increase in concentration ($F= 13.21$; $p < 0.01$, Figure 1), but there was no significant effect of strains on % reduction in larval weight ($F= 0.30$; $p > 0.05$, Figure 1). Larval duration was increased significantly at all the concentrations ($F= 8.89$; $p < 0.01$, Figure 2), causing 37.15 and 40.05% (at 0.04ppm) prolongation in larval duration in ML and FD strains, respectively compared to control treatment (Figure 2). Similarly pupation from surviving larvae was seriously affected at all the concentrations ($F= 25.36$; $p < 0.001$, Table 2). At 0.04ppm, pupae were inhibited by 51.9 and 56.6% in the ML and FD strains, respectively compared to control. However, no significant differences in pupae inhibition were found due to the effect of strains ($F= 1.90$; $p > 0.05$, Table 2). Emergence of adults was inhibited by 66.7 and 75.5% at 0.04ppm in the ML and FD strains,

respectively compared to control treatment (F= 51.01; p < 0.001, Table 2).

All the tested concentrations of triflumuron caused significant reduction in oviposition in the both strains of *T. castaneum*. At the concentration of 0.04ppm, 80.1 and 86.0% reproductive control (PRC) was obtained in the ML and FD strains, respectively compared to control and this effect was significantly different at all concentrations in both strains (F= 158.54; p < 0.001, Table 3). However, PRC was not significantly different between the strains (F= 4.07; p > 0.05, Table 3). Hatching % of eggs was significantly reduced at all the concentrations. Egg sterility of 92.0 and 94.5% taken place at 0.04ppm in the ML and FD strains, respectively compared to control (F= 306.71; p < 0.001, Table 3). The egg sterility (%) was not statistically different between the strains (F= 4.90; p > 0.05, Table 3).

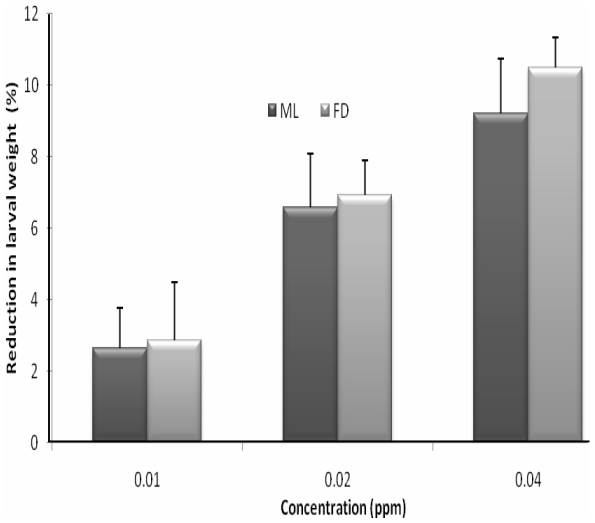

Figure 1. Mean percent reduction in larval weight of *T. castaneum* due to exposure of larvae to various concentrations of triflumuron

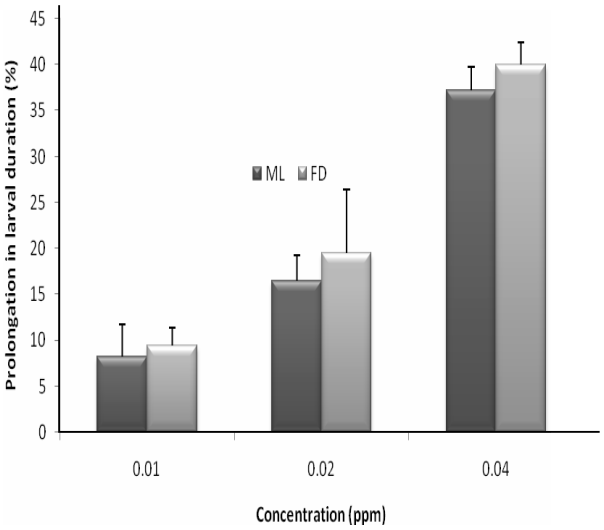

Figure 2. Mean percent prolongation in larval duration of *T. castaneum* due to exposure of larvae to various concentrations of triflumuron.

Table 2. Effect of different concentrations of triflumuron on development of pupae and adults in *T. castaneum*

Strain	Conc. (ppm)	Mean ± SE Pupae (% inhibition)[a]	Mean± SE Adults (% inhibition)[b]
ML	0.04	8.3± 0.33 (51.9± 1.92)	5.3± 0.33 (66.7± 2.08)
	0.02	10.0± 0.57 (42.3± 3.33)	7.7± 0.33 (52.1± 2.08)
	0.01	13.7± 0.88 (26.4± 5.08)	11.3± 0.66 (29.2± 4.16)
	0.00	17.3± 0.33 -	16.0± 0.57 -
FD	0.04	7.7± 0.66 (56.6± 3.77)	4.0± 0.57 (75.5± 3.53)
	0.02	9.3± 0.88 (47.2± 4.99)	7.3± 0.88 (55.1± 5.39)
	0.01	13.0± 1.00 (21.2± 5.66)	10.7± 0.66 (34.7± 4.08)
	0.00	17.7± 0.33 -	16.3± 0.33 -

a=100*(1-t/c), where t is the number of pupae in treated flour, and c is the number of pupae in control.

b=100*(1-t/c), where t is the number of adults in treated flour, and c is the number of adults in control.

Table 3. Oviposition and egg hatching in *T. castaneum* due to exposure of larvae to triflumuron- treated diet

Strain	Conc. (ppm)	Mean ± SE Eggs Laid	Mean ± SE Eggs Hatched

		(PRC)[a]	(% Sterility)[b]
ML	0.04	24.7 ± 1.20	8.7 ± 0.66
		(80.1± 0.96)	(92.0± 0.61)
	0.02	36.3± 1.20	18.0± 0.57
		(70.7± 0.96)	(83.4± 0.53)
	0.01	66.0± 2.51	36.3± 1.20
		(49.9± 2.02)	(66.5± 1.10)
	0.00	124.0± 6.80	108.3± 4.09
		-	-
FD	0.04	18.3± 2.60	6.3± 0.88
		(86.0± 1.98)	(94.5± 0.76)
	0.02	37.3± 3.38	18.0± 1.52
		(71.5± 2.58)	(84.4± 1.32)
	0.01	65.7 ± 2.72	36.33 ± 1.45
		(46.8± 2.08)	(68.6± 1.25)
	0.00	131.0± 5.13	115.7 ± 5.69
		-	-

a=100*(1-t/c), where t is the number of eggs laid by treated adults, and c is the number of eggs laid by untreated adults.
b=100*(1-t/c), where t is the number of eggs hatched in treated flour, and c is the number of eggs hatched in control.
F_1 larvae resulting from hatched eggs were reduced significantly at all the concentrations of triflumuron. At concentration of 0.04ppm, 94.8 and 96.5% F_1 larvae were inhibited compared to control treatment in the ML and FD strains, respectively ($F= 424.10$; $p < 0.001$, Table 4). F_1 pupae were inhibited by 96.7 and 98.0% at 0.04ppm compared to control in the ML and FD strains, respectively and this effect was significantly different at all the concentrations ($F= 841.35$; $p < 0.001$, Table 4) and between the strains ($F= 11.70$; $p < 0.01$, Table 4). Finally, at the concentration of 0.04ppm, emergence of F_1 adults was suppressed by 98.0 and 99.3% compared to control in the ML and FD strains, respectively. Inhibition of F_1 adult emergence was significantly different at each concentration ($F= 511.97$; $p < 0.001$) and in both strains ($F= 15.18$; $p < 0.01$, Table 4).

Table 4. Effect of different concentrations of triflumuron on development of F_1 larvae, pupae and adults in *T. castaneum*

Strain	Conc. (ppm)	Mean ± SE F_1 Larvae (% inhibition)[a]	Mean ± SE F_1Pupae (% inhibition)[b]	Mean ± SE F_1 Adults (% inhibition)[c]
ML	0.04	5.0±0.57	3.0±0.57	1.7±0.33
		(94.8±0.59)	(96.7±0.62)	(98.0±0.39)
	0.02	12.0±0.57	8.3±0.33	4.7±0.33
		(87.6±0.59)	(90.9±0.36)	(94.5±0.39)
	0.01	27.7±0.88	22.0±0.57	12.7±0.33
		(71.5±0.90)	(76.0±0.62)	(85.2±0.39)
	0.00	97.0±5.50	91.7±5.20	85.3±4.66
		-	-	-
FD	0.04	3.7±0.66	2.0±0.57	0.7±0.33
		(96.5±0.64)	(98.0±0.58)	(99.3±0.36)
	0.02	12.0±1.00	8.0±0.57	4.3±0.33
		(88.4±0.96)	(91.9±0.58)	(95.3±0.36)
	0.01	27.3±0.88	21.7±0.33	12.0±0.57
		(73.6±0.85)	(78.0±0.33)	(87.0±0.62)
	0.00	103.7±3.28	98.7±3.28	92.3±0.33
		-	-	-

a=100*(1-t/c), where t is the number of larvae in treated flour, and c is the number of larvae in control.
b=100*(1-t/c), where t is the number of pupae in treated flour, and c is the number of pupae in control.
c=100*(1-t/c), where t is the number of adults in treated flour, and c is the number of adults in control.

DISCUSSION

The results of this study reveal the strong growth, development and reproductive inhibitory effects of triflumuron against two field strains of *T. castaneum*. Similarly results of other scientists show that CSIs of the benzoyl phenyl urea group, in contrast to juvenoids and ecdysteroids, are very active against beetles that develop inside the grain (Ammar, 1988; Elek and Longstaff, 1994; Oberlander *et al*., 1997; Parween *et al*., 2001; Kostyukovsky and Trostanetsky, 2006). This phenomenon may be attributed to the activity of these compounds on the viability of eggs laid by females that were exposed to treated food. Due to the internal development of these insects it is difficult to check this hypothesis by experiments on *Sitophilus oryzae*. Thus, our experiments with *T. castaneum* might serve as a good model for the evaluating the effect of CSIs on internal feeders.
Triflumuron caused 29.8 and 36.2% mortality (at 0.04ppm) in ML and FD strains of *T. castaneum* larvae respectively at exposure period of 120 hours. Similarly, it has been reported that novaluron, a CSI, caused 83% larval mortality in *T. castaneum* after 168 hours of treatment (Kostyukovsky and Trostanetsky, 2006). Other studies also exhibit the larvicidal activity of CSIs; triflumuron and flufenoxuron against *T. castaneum* (Parween, 2003; Salokhe *et al*., 2003). Due to feeding on triflumuron treated flour, *T. castaneum* larval weight was found to be reduced significantly compared to control. Effects on certain physiological activities, some of the insect growth regulators reduce the body weight of insects (El-Din *et al*., 1990; Smagghe *et al*., 1996; Parveen, 2000). It can be hypothesized that triflumuron might have caused reduction in chitin content of *T. castaneum* that might have resulted in development inhibition and reduction in larval weight. Triflumuron also slightly prolonged the larval duration compared to control. Similar results were reported by Kostyukovsky *et al*. (2000) by exposing *T. castaneum* larvae to food medium treated with methoprene and pyriproxifen. It seems that extension in larval

development will lead to the consumption of more food by larvae but it has been reported that CSIs usually cause stop feeding in *T. castaneum* (Parween, 1996).

When adults emerged from treated larvae were allowed to lay eggs, both egg laying and hatching percentage was reduced drastically. This effect might be due to the transovarial activity of triflumuron that has caused reduction in egg laying and egg hatchability of the treated adults. Adults emerged from CSI treated larvae or reared on treated food mostly produce fewer eggs compared to untreated adults (Nickle, 1979; Saxena and Mathur, 1981; Parween *et al*., 2001). CSIs have been reported to disrupt embryogenesis partially or fully and also disrupt the growth and development of the gonads (Soltani-Mazouni, 1994), resulting in the production of non-viable eggs. Abo-Elghar *et al*. (2004) also reported the ovicidal and development inhibition activity of CSIs against cowpea weevil. Similarly when *T. castaneum* adults were allowed to feed on dimilin-treated sorghum, more than 79 % reproductive inhibition was obtained (Faragalla *et al*., 1985). These effects have also been reported in other insect species. It was found that lufenuron in cattle blood reduced the hatching of eggs of cat fleas (Dean *et al*., 1998) and application of diflubenzuron (a CSI) on the adults of *Chrysoperla carnea* resulted in total inhibition of egg hatching (Medina *et al*., 2002).

In the present experiment, emergence of F_1 larvae, pupae and adults was inhibited seriously compared to control. These results and those of other scientists (Trostanetsky and Kostyukovsky, 2008) conclude that when adults of *T. castaneum* are treated with CSIs, these chemicals are taken up by adults that affect the subsequent development of progeny. This penetration of CSI might be due to contact or ingestion (Cutler *et al*., 2005; Kostyukovsky and Trostanetsky, 2006; Mommaerts *et al*., 2006). Arthur *et al*. (2009) also reported inhibition in adult emergence of stored-product insects due to larval exposure with insect growth regulators.

In view of the impressive larvicidal, growth, development and reproduction impairment effects of triflumuron against *T. castaneum*, further studies are necessary to evaluate the residual efficacy and persistence of triflumuron over extended period of time in mills, warehouses and food storage facilities for the promising replacement of commonly used organophosphate and pyrethroid grain protectants.

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