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COMPARATIVE POTENTIAL OF CHITIN SYNTHESIS INHIBITORS AGAINST *Trogoderma granarium* E. (COLEOPTERA: DERMESTIDAE) FOR STORED WHEAT MANAGEMENET IN PAKISTAN

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Chitin synthesis inhibitors (CSIs) have specific mode of action on insects and lower toxicity against non-target organisms and are potential alternative to control insect populations which are resistant to traditional insecticides. Therefore, to assess the safe alternatives to commonly used organophosphate and pyrethroid, against which insects are resistant already, in stored grain insect management, three commercially available (CSIs): Flufenoxuron (0.0015, 0.003 and 0.006ppm), Triflufenuron (0.02, 0.04 and 0.08ppm) and Lufenuron (0.01, 0.02 and 0.04ppm) were applied to evaluate their potential side effects (mortality and transgenerational effects) on *Trogoderma granarium*, one of the world's most destructive pests of grain products and seeds, in wheat grains at 3, 6, 9, 12, 15, and 18 days interval during 2008-2010 at storage management cell of department of Entomology, University of Agriculture Faisalabad. The tested larval instars of *T. granarium* were released in pesticides mixed flour in vials. As a result, the highest concentration of each CSI at the longest exposure interval showed greater effectiveness than lower concentrations and shorter exposures. Triflumuron at 0.08 ppm gave rise to 42% larval mortality after 18 days of exposure, 44% reduction in adult emergence, and 60% fewer F_1 progeny. The effectiveness of these CSIs was found to be positively correlated with treatment concentration and duration. These findings provide coherence understanding in storage pest management in Pakistan where wheat in mills and goodowns damaged annually due to *T. granarium*.

Keywords: Wheat storage, arthropods, adult emergence, insect pest control, insect growth regulators, larval mortality, *Trogoderma granarium*, weight reduction

INTRODUCTION

Arthropods can cause qualitative and quantitative losses by destroying an estimated 18–26% of annual crop production worldwide (Culliney, 2014). Insect contamination is a fundamental problem in the food industry and export (Rajendran, 2002). The khapra beetle, *Trogoderma granarium* E. (Coleoptera: Dermestidae), is the most widely distributed and the primary destructive insect pest of stored products in tropical regions (Howe, 1965; Bakr *et al*., 2008). This insect has been recognized among the first 100 of the "World's Worst" invaders. It is one of the most serious pests of stored wheat in Indo-Pakistan (Azeem *et al*., 1976; Ram and Singh, 1996; Khattak *et al*., 1996).

Serious infestation of cereal grains by *T. granarium* make them unpleasant, indigestible, and unmarketable. Infestation level at 75% damage grains in wheat and some other cereal reduces total protein and carbohydrate contents of stored wheat and some other cereals (Jood and Kapoor, 1993; Jood *et al*., 1996). During storage, losses can reach from 2.2 to 5.6% in weight and 24% of seed viability with 15% grain infestation in India (Prassad *et al*., 1977). In Pakistan, the loss by this insect ranges from 0.2 to 2.9% over 1 to 10.5 months (Irshad *et al*., 1988).

To enhance storability and lengthen shelf-life, many physical treatments (such as heat treatment and sweating) and chemical ones (such as plant extracts, fungicides and plant growth regulators) treatments are widely used in the postharvest industry (Hao *et al*., 2010; Ali *et al*., 2012). Chemical products are the main tool in postharvest to control insects; Among them phosphine and methyl bromide are the simplest-to-use compounds and better cost-effective (Shaaya *et al*., 1997; Hidalgo *et al*., 1998; Islam *et al*., 2010) but different experiments have shown that *T. granarium* has evolved resistance against this fumigant (Bell *et al*., 1984; Rajendran, 2002) as well as methyl bromide is proven to be involved in ozone depletion phenomenon (MBTOC, 2010). Contact insecticides have drawback by selecting insect resistance via natural selection, environmental pollution, residual contamination, lethal effects on non-target organisms and reducing biological diversity (Desneux *et al*., 2007; Biondi *et al*., 2012). Therefore, a new category of insect pest control products, called insect growth regulators (IGRs), was used for stored grain insect pest management. Available IGRs included juvenile hormone, chitin synthesis inhibitors, and ecdysteroid agonists (Graf, 1993; Oberlander *et al*., 1997) were potentially used against different insects. Chitin synthesis inhibitors are benzoylphenylurea compounds, which inhibits chitin synthase; an enzyme which is responsible in formation of chitin (Zhang and Yan Zhu, 2013), resulting in deformation of cuticle (Reynolds, 1987). Acting on chitin makes them unique insecticides, which can overcome the problem of insect resistance against other conventional insecticides. The chitin synthesis inhibitors (CSIs), have been shown to cause the depolarization of the vesicle membrane through inhibition of the K+ channel, which leads to their inhibition of chitin synthesis (Matsumura, 2010). Lefunuron, Triflumuron and Flufenoxuron have been used against insect pests like fleas (Smith, 1995), *Tribolium castaneum* (Coleoptera) (El-Sayed *et al*., 1984, Sagheer *et al*., 2012), cat fleas (Dean *et al*., 1998), *Spodoptera littoralis* (Lepidoptera) (Clarke *et al*., 1990) and *Schistocerca gregaria* (Orthoptera) (Bakr *et al*., 2008). These studies provided data for the control of these species, but the effect of CSIs on other species are necessary, including on *T. granarium.*

The first benzoylphenylurea class of chemistry was discovered by Philips-Duphar Company in the 1972 (Verloop and Ferrell, 1977), which resulted in the development of more PBU derivatives like Flufenoxuron, which was developed at Shell Research Ltd. (now BASF AG) (Sun *et al*., 2015). Triflumuron was discovered by Bayer (now Bayer AG), which is the first commercial product of the second generation BPU (Sun *et al*., 2015). Lufenuron was developed by Ciba-Geigy and tested on many insects from 1990 onwards (Sun *et al*., 2015). The insecticidal activity of the BPU is unusual in that it can be observed only in immature insects and is affective only at moulting stage by inhibiting the chitin biosynthesis. The importance of this mode of action is the absence of chitin biosynthesis in animal and plants makes it affective only against insects.

Keeping this in mind, we aimed to test the comparative efficacy of three Chitin Synthesis Inhibitors (CSIs); Flufenoxuron, Triflumuron, and Lufenuron on the mortality, larval weight, pupation, adult emergence, and F_1 progeny of *T. granarium* on stored wheat grains under laboratory conditions. The potential CSIs with their effective concentration and exposure duration would lead to sustainable integrated insect pest management of stored products in Pakistan.

MATERIALS AND METHODS

Insect culture**:** A mixed population of *T. granarium* was collected from storages of the Faisalabad food department, Government of Punjab, Pakistan, and allowed to develop in earthen pots (2.5 L) containing whole wheat grains. These pots were placed in a growth chamber (30±2°C; 60-65% humidity) in the darkness. After 72 hours, adults were removed by sieving. Wheat with insect eggs was moved to 1.5 L plastic jars fastened with a muslin cloth and rubber bands and 400 g of wheat grains were supplied (Ali *et al*., 2012). These plastic jars were then placed in an incubator at the same environmental conditions mentioned. A homogeneous population of *T. granarium* was obtained after 28-32 days (Nadeem *et al*., 2011). Third instar larvae *T. granarium* were used in the experiments for contact toxicity.

Chitin synthesis inhibitors (CSIs)**:** Flufenoxuron, Triflumuron, and Lufenuron were purchased from FMC (Ltd), Warble (Ltd) and Sygenta (Ltd) in Pakistan, respectively. The three CSIs, Flufenoxuron 10 DC (0.0015, 0.003, and 0.006 ppm), Triflumuron 20 EC (0.02, 0.04, and 0.08 ppm) and Lufenuron 50 EC (0.01, 0.02, and 0.04 ppm) were tested. The concentrations were chosen according to (Ram and Singh 1996) and mentioned that higher concentrations have ovicidal and reproduction inhibition effect on *Tribolium castaneum* (Herbst).

Bioassays**:** Stock solutions of the three CSIs were prepared in acetone and kept at 4°C in the dark until use in small beakers (250 mL) covered with parafilm. Solutions of the CSIs were further prepared from these stock solutions to obtain the different final concentrations in ppm. Wheat grains sterilized in the micro-oven were exposed to those concentrations by thoroughly mixing. Grains were allowed (10 min at room temperature) to ventilate before larvae were released. Wheat grains treated with acetone were used for larval feeding as control. Twenty larvae were placed in 50 g wheat grains per experimental glass jar covered with muslin cloth and tied with rubber bands to prevent insects to escape. The larva mortality was recorded at 3, 6, 9, 12, 15, and 18 days after releasing in glass jars. The larvae were considered dead when they stayed immobile when touched with a fine brush. Larval weight was recorded on the eighteenth day with an electronic weighing balance. After pupation, individuals of all the treatments were moved to untreated wheat grains under the same environmental conditions, and the pupae and adults recorded. The adults emerging from each treatment were counted visually to record the F1 progeny. All experiments were repeated three times in a completely randomized design at the same environmental conditions as rearing one except that the L14:D10 photoperiod was used.

***Statistical analysis*:** Data of larva mortality, larva weight, pupation, adult emergence, and F_1 progeny for each CSI were subjected to statistical analysis with the software STATISTIX (2005). The least significance test (LSD) was used to compare the different exposure periods (3, 6, 9, 12, 15 and 18 days) and different concentrations for three CSIs, Flufenoxuron (0.0015, 0.003, 0.006 ppm), Triflumuron (0.02, 0.04, 0.08 ppm) and Lufenuron (0.01, 0.02, 0.04 ppm).

RESULTS

Triflumuron was more effective in inducing significant larval mortality than Flufenoxuron or Leufenuron (Table 1). Mortality of *T. granarium* was higher with the increased concentration of CSIs and exposure duration. Highest mean mortality was recorded at 18 days after exposure treatments among all tested chitin synthesis inhibitors (hereafter known as CSIs) that was accounted that 42.4% for Triflumuron (0.08 ppm), 40.7% for Leufenuron (0.04 ppm), and 34.5% for Flufenoxuron (0.006 ppm). Lowest mean mortality was recorded at 3 days after exposure treatments among all tested CSIs that was accounted 0% for Triflumuron (0.01 ppm), 1.7% for each Leufenuron (0.02 ppm) and Flufenoxuron (0.0015,0.003 and 0.02 ppm).

Larval weight loss with the different CSI (Table 2) indicates that feeding inhibition was highest with Lufenuron (56.4%) at 0.04 ppm followed by Flufenoxuron (56.1%) at 0.006 ppm and Triflumuron (30.7%) at 0.08 ppm. Pupation was correlated with the concentration of CSI and exposure duration. Again, Triflumuron (42.4%) at 0.08 ppm was more effective than Lufenuron (39.7%) at 0.04 ppm and Flufenoxuron (34.4%) at 0.006 ppm.

However, Triflumuron gave maximum reduction (60.1%) at 0.08 ppm in the F_1 progeny of *T. granarium* followed by Lufenuron (55.4%) at 0.04 ppm and Flufenoxuron (53.2%) at 0.006 ppm. Among all the tested CSIs, lowest percent

Table 1. Larval mortality (Mean±SD) of *Trogoderma granarium* exposed to different concentrations (ppm) of each Flufenoxuron, Triflumuron and Lufenuron for 3, 6, 9, 12, 15 and 18 days after treatment under laboratory conditions.

CSIs	ppm	Percent mean mortality days after treatments					
		3	6	9	12	15	18
	0.0015	1.7±1.7g	5.0±0.0efg	5.0±0.0efg	6.8±1.7efg	11.8±1.7def	13.8±1.7cde
	0.003	1.7±1.7g	5.0±2.9efg	5.0±2.9efg	10.1±1.7def	18.6±1.7bcd	22.4±3.0bc
Flufenoxuron	0.006	3.3±1.7fg	8.3±1.7def	11.7±1.7d-g	16.9±3.4bcd	25.4±1.7ab	34.5±1.7a
	0.02	1.7±1.7f	6.6±1.7ef	3.3±1.7f	8.6±1.8def	12.1±0.0de	20.3±1.7c
	0.04	3.3±1.7f	6.6±1.7ef	11.7±1.7de	15.6±19.0cd	22.4±0.0c	32.2±1.7b
Triflumuron	0.08	3.3±1.7f	8.3±1.7def	15.0±1.7cd	17.2±0.0c	31.0±1.7b	42.4±1.7a
	0.01	0.0±0.0h	3.3±1.7h	5.0±0.0gh	8.4±0.0e-h	10.3±1.7e-h	16.9±1.7c-f
	0.02	1.7±1.7h	6.7±1.7fgh	10.0±0.0e-h	16.1±2.1c-f	19.0±4.6b-e	27.1±4.5bc
Lufenuron	0.04	3.3±1.7h	10.0±2.9e-h	16.7±1.7c-f	23.7±0cdef	29.3±1.7b	40.7±1.7a

Means followed by similar letters in the column are not significantly different by LSD test α =0.05.

Table 2. Larval weight (mg), pupation (%), adult emergence (%) and F_1 progeny of *Trogoderma granarium* after exposure to different concentrations of CSIs, each of Flufenoxuron, Triflumuron and Lufenuron for 3, 6, 9, 12, 15 and 18 days after treatment under laboratory conditions.

CSIs	Conc. (ppm)	Larval weight (mg)	Weight decrease (%)	Pupation (%)	Pupation decrease (%)	Adult emergence	Adult decrease (%)	F1 progeny	F1 decrease (%)
Flufenoxuron	Control	5.5± 0.48a	-	19.3 a	-	19.3 a	-	195.3 a	-
	0.0015	4.3± 0.34ab	21.9	16.7 b	13.9	16.7 c	13.8	153.7 b	21.3
	0.003	3.4±0.43bc	37.9	15.0 b	22.4	14.7 c	24.1	125.7 c	35.7
	0.006	2.4±0.52 c	56.1	12.7 c	34.4	12.0 d	37.9	91.3 d	53.2
Triflumuron	Control	3.5±0.99 a	-	19.7 a	-	19.7 a	-	205.3 a	-
	0.02	2.5± 0.27a	28.4	15.7 b	20.3	15.7 b	20.3	138.0 b	32.8
	0.04	2.8± 0.43a	19.9	13.3 c	32.2	13.3 c	32.2	105.0 c	48.9
	0.08	2.5±0.40 a	30.7	11.3 d	42.4	11.0 d	44.1	82.0 d	60.1
Lufenuron	Control	2.1± 0.21a	-	19.3 a	-	19.3 a	-	199.3 d	-
	0.01	1.8±0.30ab	31.3	16.3 b	15.5	16.3 b	15.5	141.0 c	29.3
	0.02	1.2±0.23bc	40.8	14.3 b	25.9	14.3 c	25.9	104.0 c	47.8
	0.04	0.6±0.06c	56.4	11.7 c	39.7	11.7 d	39.7	89.0 d	55.4

Means followed by similar letters in the columns are not significantly different, by LSD test at α = 0.05.

(21.3%) F_1 progeny was recorded at 0.0015 ppm of Flufenoxuron. The transgenerational effect of tested CSIs was recoded substantially significant in Triflumuron.

DISCUSSION

Larval mortality of *T. granarium* was substantially induced by Triflumuron at highest concentration as compared to other CSIs considered in this study. These results are like the larval mortality of *Tribolium castaneum*, which increased as the dose rate of triflumuron and duration of exposure (Sagheer *et al*., 2011). The application of 1 ppm of spinosad on wheat caused about 50% mortality of adults of *Liposcelis bostrychophila* B., *L. decolor* P. and *L. paeta* P., although progeny was like that of untreated control (Nayak *et al*., 2005). Larvicidal activity of triflumuron and flufenoxuron towards red flour beetle has also been reported a positive effect on larval weight, percent pupation and percent adult emergence, as well as time taken for adult emergence (Parween, 2003; Salokheab, 2003). Vegetable oils (chamomile, sweet almond and coconut) tested against *Rhyzopertha dominica* gave over 95% control (Amin, 2007).
Exposure of *T. granarium* larvae against different CSIs indicates relatively higher feeding inhibition percentage with Lufenuron. There could be two possibilities for this response, (1) due to the benzoyl acyl urea of CSIs that might have reduced chitin synthesis in khapra beetle resulting in larval growth inhibition as weight reduction and/or (2) food intake was lower than expected as the decrease in weight of *T. castaneum* was recorded when exposed to monoterpenoids (Stamopoulos *et al*., 2007).
Percent pupation of *T. granarium* was recorded highest for Triflumuron at longest exposure period and highest concentration applied. The larval stage of *T. granarium* did not increase with the CSI what differ for that reported for *T. castaneum* with dietary ZR-512 and ZR-619 at the concentrations of 10-1000 ppm (Ishaaya and Yablonski, 1976). A maximum of reduction in adult emergence was observed with the Triflumuron (44.1%) followed by Lufenuron (39.7%) and Flufenoxuron (37.9%). This result was similar to those of Methoprene, Pyriproxifen, RH-5849, and Tebufenazide which affected the development of susceptible and actellic-resistant strains of *T. castaneum* and susceptible ones of *R. dominica* and *Sitophilus oryzae* but without effect on the mortality of parental adults (Kostyukovsky *et al*., 2000).
Reduction in the trans-generational progeny was recorded highest with Triflumuron. These results agree with the reduced development of the *T. castaneum* progeny when its adults were treated with novaluron, a chitin synthesis inhibitor (Trostanetsky and Kostyukovsky, 2008). Another experiment agreed with our experiment in which novaluron (1 ppm) treated whole wheat grains were exposed to the adults of *T. castaneum* and showed same effects as to those of treated flour at the same concentration: egg hatching was significantly reduced and only 1% of the eggs hatched after 18 days. Similarly, Kostyukovsky and Trostanetsky (2006) reported the 100% reduction in hatching rate when adults were removed from treated surfaces of Petri dishes to untreated flour. The CSIs, Hydroprene and Pyriproxyfen, gave effective residual control by inhibiting adult emergence from larvae of primary stored grain insect pests (Arthur *et al*., 2009). Comparable results were obtained from the extracts of leaves and stems of *Psychotria* spp. and showed significant effects on the hatching rate, parameters of caterpillar body (weight and length and width of head capsule), repellency, and mortality of *S. zeamais* and *Spodoptera frugiperda* (Tavares *et al*., 2013).
In summary, reduction in emergence of adults of *T. granarium* by the CSIs Triflumuron, Lufenuron and Flufenoxuron indicating that these chemical compounds greatly reduced its development. Triflumuron was most effective from all three chitin synthesis inhibitors, causing more mortality, reducing adult emergence and minimizing F_1 progeny. In general, effects increased significantly both with increase in concentration and exposure period. Our results are similar to results obtained from treating these CSIs against *T. castaneum* (Sagheer *et al*., 2012). As these chitin synthesis inhibitors are also effective against several other species including *T. castaneum* (Sagheer *et al*., 2012), *R. dominica* and *S. oryzae* (Daglish and Wallbank, 2005), they could be expected to control a range of pest species in future. Further biochemical and ultrastructural studies of treated *T. granarium* with these chitin synthesis inhibitors could reveal the mechanism involved in inhibition of chitin synthesis and reduction in F_1 progeny.

***Conclusion*:** Overall, the present study leads to a useful strategy in contribution to the development of environmentally safe control measures against stored grain insects. Especially when it is urgent need to find alternative methods to the currently used fumigants, methyl bromide, which has been banned due to its ozone depletion potential. In addition, it has high level of activity against pests with low toxicity to mammals and its specific mode of action. Therefore, chitin synthesis inhibitors may be a better replacement for commonly used organophosphates and pyrethroid insecticides.

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