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EFFECT OF TWO CHITIN SYNTHESIS INHIBITORS ON THE REPRODUCTIVE POTENTIAL OF TWO SUCCESSIVE GENERATIONS OF *Tribolium castaneum* (Herbst)

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ABSTRACT

Triflumuron and Diflubenzuron, two chitin synthesis inhibitors (CSIs) showed contact activities against *T. castaneum*. Low concentrations of these compounds significantly decreased the rates of egg laying and egg hatching in two successive generations. Diflubenzuron was found to be more effective and at a concentration of 0.05 ppm it totally stopped egg laying of the treated females. The results suggested that both the CSIs are potent candidates for controlling the population of *Tribolium* beetles in the stored grains and cereals. Quite low concentrations of these compounds can be used as a surface treatment for the control of insect pests of the stored products.

***Key words*:** Chitin synthesis inhibitor, fecundity, fertility, *Tribolium castaneum*

INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst) is a cosmopolitan pest of stored grains, cereals and other food products. The beetle not only damages the commodities quantitatively, but also affects the quality. Heavily infested cereals and flour gives a characteristic pungent odour to the commodities. Infested products when consumed cause gastric disturbances to human (Mondal 1994).

Adult females of *T. castaneum* lay eggs continuously throughout their life (Av. 100 d at 25oC) (Howe 1962). *T. castaneum* has developed resistance to many insecticides (Dyte 1972). Therefore protecting stored products from this beetle is challenging.

In order to offer chemical free products safer methods including physical treatments to the storage environment, biological agents, botanicals, pheromones and insect growth regulators (IGRs) are being tested. The benzoylphenyl ureas are biologically mimetic to the natural chitin synthesis inhibitory (CSI) hormones present in insects. These compounds proved to be potential in managing insect pests of the stored products by interrupting metamorphosis (Mian and Mulla 1982a,b, Mondal and Parween 2000). This group of compounds were also found to inhibit reproductive functions of *T. castaneum* (Mondal and Parween 2000). The CSI compounds are known to have gustatory and contact activities (Fox 1990).

Triflumuron (TFM) and diflubenzuron (DFB) are reported to have contact activities at low concentrations, against the target insects, but are safe for the environment and its biota (Fox 1990). Hence, these two compounds are not necessary to be mixed with the stored cereals or grains, which is a great concern for the consumers. Both these compounds disrupt growth and development, and also decrease the reproductive potentials insects (Mondal and Parween 2000).

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The present work was aimed to examine the effect of contact action of triflumuron and diflubenzuron on the fecundity, fertility and the success rate (% emergence of adults) of the treated population of *T. castanem* for two successive generations.

MATERIALS AND METHODS

CIS compounds used

Starycide 480 mg/ml SC, <Bayer AG, Germany> was the source of triflumuron. Dimilin (a.i. 25% WP) is a product of Crompton Corporation, India, was the source of diflubenzuron. Filter papers were dipped separately in different concentrations of either TFM or DFB, dissolved in distilled water, and then air dried. These treated filter papers were used against the test insect.

Concentrations tested: Three sub-lethal concentrations of each compound were selected. The concentrations of TFM used in this experiment were 0.1, 0.05 and 0.01ppm, and those for DFB were 0.05, 0.01 and 0.005ppm.

Rearing of beetles

T. castaneum used in this experiment were obtained from the stock culture maintained in the Controlled Temperature (CT) room, Integrated Pest Management Laboratory, Institute of Biological Sciences, University of Rajshahi. The beetles were cultured in 500 ml (covered with cloth mesh) containing standard food medium (19:1 whole wheat flour: brewer's yeast; Park and Frank, 1948). A crumpled filter paper was placed inside each beaker for easy movement of the beetles. The beakers were kept in the CT room at a temperature of 30 ± 1^0C.

Experimentation

Large number of eggs of 1-d old, were collected by sieving the culture of *T. castaneum*, kept in glass petri dishes and placed in the CT room. The larvae hatched from theses eggs were collected and released on standard food in glass beakers. Pieces of filter paper were soaked with either of the concentrations, 0.1, 0.05 and 0.01 ppm of TFM, and 0.05, 0.01 and 0.005 ppm of DFB, placed separately according to the concentration within the food, and the larvae were reared until pupation. The foods only were replaced by fresh ones at every 4th day to avoid conditioning.

The pupae were sexed by examining the exogenital process (Halstead, 1963). Male and female pupae from each concentration of each compound were kept in separate beakers.

Twenty four hour old males and females emerged from each concentration were paired. Such 20 pairs from each concentration of TFM and DFB were placed on filter paper treated with same concentration and same compound in separate vials (each pair in a vial) providing standard food. A similar set of experiment with 20 pairs of untreated adults was maintained on standard food only.

On every third day eggs of each pair were collected, counted and kept separately with appropriate labeling. Number of the hatched larvae was recorded and then released them on food without any further treatment. Male and female pupae from these larvae were collected and allowed to emerge as adults. These adults were paired (N=20) and reared on the same food only, with a regular change after every 4th day. Eggs of each pair were collected and counted at 3-d interval. Number of the hatched larvae was recorded. Rearing of these larvae on food only was continued until the emergence of adults. Numbers of the pupae and adults were recorded.

All the experiments with each concentration of TFM and DFB, and the control were carried out in the CT room at 30oC, and replicated three times.

Statistical analysis

The results were analyzed by analysis of variance. Significant differences at concentration levels were tested according to Tukey test (1953). The percent reduction of oviposition to control (PRC) was calculated according to

Mian and Mulla (1982a) by the following formula:

$$PRC = (1 - T/C) \times 100$$

Where, T = number of eggs in treated medium and C = number of eggs in control/ untreated medium.

RESULTS AND DISCUSSION

Effect on fecundity in two successive generations

Fecundity of the 2nd generation *T. castaneum* was found to be higher than the 1st generation in both treated and untreated batches. The effect was increased with the increased concentration of the compounds. The PRC-values ranged between 23.24 – 27.68 at 0.1 and 0.05 ppm of TFM in two generations, but in case of DFB 0.05 ppm aborted oviposition 100% in 1st generation (Table 1). Analysis of variance showed that concentrations of TFM and DFB affected egg production significantly compared to the control treatment in both generations (Table 2).

Effect on fertility in two successive generations

Fertility rate (%) of the laid eggs was found to be decreased by the compounds in both generations of the beetle; the effect was increased with the increased concentrations of the compounds. Potentiality of TFM to abort embryogenesis was found to be less than that of DFB in 1st generation eggs, but both compounds

Table 1. Effect of TFM and DFB on the fecundity and fertility in two successive generations of *T. castaneum* over 45 day period (n = 20 pairs)

Compound	Concn. (ppm)	1st generation					2nd generation				
		Total no. of eggs laid (mean ±SE)	No. of of eggs / day/ ♀	PRC value	No. of larvae hatched	Fertility (%)	Total no. of eggs laid (mean± SE)	No.of eggs / day/ ♀	PRC value	No. of larvae hatched	Fertility (%)
Control	-	5719a (285.9 ±21.7)	6.3	-	3539	61.9 a	4200a (209.9± 5.4)	4.6	_	2842	67.6 a
TFM	0.1	4390ab (219.5 ±19.6)	4.9	23.2	1710	38.9c	3037b (151.8± 16.4)	3.3	27.6	2092	52.2b
	0.05	4371ab (218.5 ±25.4)	4.9	23.5	2510	57.4b	3105b (155.2± 10.1)	3.4	26.0	3.45	26.0
	0.001	5160a (258±3 2.6)	5.7	9.7	2882	55.8b	4005a (200.2± 8.4)	4.4	4.6	1882	60.6b
DFB	0.05	00	00	-	00	-	00	00	-	00	-
	0.01	5386a (269.3 ±14.8)	5.9	5.8	49	0.9d	1905c (95.2±9. 8)	2.1	54.6	2804	59.7a
	0.005	5535a (276.7 ±27.9)	6.1	3.2	1948	35.2c	4695a (234.7± 12.8)	5.2	11.7	1162	61.0 b

allowed >50% hatching in 2nd generation eggs (Table 1). Against the 1st generation eggs both compounds and all the concentrations acted equally, but the effects significantly differed in 2nd generation (Table 2).

Success rate of 2nd generation T. castaneum

The success rate of adult emergence in 2nd generation was 40.99% in control beetles. Contact action of both compounds affected the developmental stages of 2nd generation significantly, and the effect of DFB was more prominent than that of TFM (Table 3a). All the concentrations of both the compounds kept the emergence rate of the adults below 50%. Toxic effect of TFM was recorded against each developmental stage of *T. castaneum*, whereas, DFB showed toxicity against the larvae but not against the pupae (Table 3a). However, DFB was significantly more toxic to the beetle compared to TFM (Table 3b).

The results revealed that both compounds decreased the reproductive potentials of *T. castaneum* in two successive generations. Both of these CSI compounds showed contact action against all the life stages of the beetle, at very low concentrations. The success rate of the beetle to survive and produce live progeny was measured by the adult emergence (%) in the 2nd generation. DFB at 0.05 ppm totally inhibited egg production of the 1st generation beetles, whereas, TFM allowed 27.54 % of adult emergence in the 2nd generation at 0.1 ppm. There are published reports on the reduced fecundity and embryocidal effects of *T. castaneum* and *T. confusum*, when the beetles were allowed to feed on TFM treated food (Mian and Mulla 1982a, Parween 2003, 2004, Parween *et al*. 2001, Mazid *et al*. 2004).

When the immature or adult stages of *Tribolium* beetles were fed on Dimilin, the effects on the successive generations was exhibited by the inhibition of the population buildup (Faragalla *et al.*, 1985). Similar is the case for triflumuron (Parween 2000). Microdoses of troflumuron applied to larval or adult diet of *T. castaneum*, both male and female reproductive systems of the treated beetles were found to be disrupted (Parween 2001, Parween and Reza 2005). The cumulative effect of each of these two compounds on all the life stages would contribute negatively to the build up of the following successive generations.

CONCLUSION

From the present results it could be suggested that both triflumuron and diflumuron are potent candidates for controlling *Tribolium* bee-

Table 2. Analysis of variance of the fecundity and fertility of *T. castaneum* in two successive generations

Parameter	Factor	F-value (level of significance)	
		1st generation	2nd generation
Fecundity	Compounds	7.30 (NS)	12.67 ($p<0.05$)
	Concentrations	32.88 ($p<0.001$)	18.72 ($p<0.05$)
	Compounds x Concentrations	19.94 ($p<0.05$)	23.16 ($p<0.001$)
Fertility	Compounds	2.01 (NS)	215.60 ($p<0.001$)
	Concentrations	2.81 (NS)	177.18 ($p<0.001$)
	Compounds x Concentrations	-	52.40 ($p<0.05$)

tles in the grain and cereal storage system. Very low concentrations of these compounds can be used as a surface treatment at the stores. Such treatment will not become a hazard to the store environment and the stored produce, as the compounds degrade rapidly (Fox 1990).

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Table 3a. Success rate of 2nd generation of *T. castaneum* fed on TFM and DFB up to adult emergence (N= eggs of 20 pairs collected up to 12 days from start of oviposition)

Treatment	Mean Number± SE (Range)					
	Concentration (ppm)	Egg	Larva	Pupa	Adult	Emergence (%)
Control	_	381.27^{a}±44.58 (96-632)	235-93^{a}±29.45 (47-467)	156.27^{ac}±16.63 (45-316)	156.27^{a} ± 16.63 (45-316)	40.99
TFM	0.1	292.6^{a}±49.12 (1-602)	114 ^{ab}±19.91 (0-240)	91.13^{c}±19.46 (0-220)	80.67^{ab} ±19.25 (0-214)	27.54
	0.05	343.47^{a}±37.72 (0-498)	192.13^{d}±22.92 (0-328)	160.6^{a}±17.26 (0-278)	125.53 ^{a} ±12.32 (0-189)	36.55
	0.01	291.4^{a}±32.11 (4-452)	167.33^{d}±18.06 (0-256)	124.47^{c} ±11.78 (0-168)	106.8 ^{a} ±10.61 (0-151)	36.65
DFB	0.05	0	0	0	0	_
	0.01	359.07^{a}±42.08 (168-664)	3.27^{abc}±1.64 (1-20)	3.20^{b} ±1.61 (1-20)	3.20^{c} ±1.61 (1-20)	0.89
	0.005	369.00^{a}±34.86 (64-578)	129.87^{ab}±9.49 (27-177)	96.27^{c} ±7.42 (26-131)	96.27^{ab} ±7.42 (20-131)	26.09

Note: Means with same letter do not significantly differ from each other.

Table 3b. Analysis of variance of the successive rate of life stages of 2nd generation *T. castaneum*

Factors	F-value (Levels of significance)			
	Egg	Larva	Pupa	Adult
Treatment	10.02 (p< 0.05)	215.60 (p< 0.001)	137.11(p< 0.001)	86.15 (p< 0.001)
Concentration	45.34 (p< 0.001)	177.18 (p< 0.001)	62.59 (p< 0.001)	74.21 (p< 0.001)
Treatment x Concentration	27.52 (p< 0.001)	52.40 (p< 0.001)	35.16 (p< 0.001)	24.11 (p< 0.001)

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