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SHORT COMMUNICATION

Morphological abnormalities in *Tribolium castaneum* (Herbst) and *Tribolium confusum* Duval due to cyromazine and pirimiphos-methyl treatments alone or in combination

AHM Kamaruzzaman[1], AMS Reza[2], KAMSH Mondal[3], S Parween[2]

[1]*Institute of Biological Sciences, Rajshahi University, Rajshahi 6205, Bangladesh*
[2] *Department of Zoology, Rajshahi University, Rajshahi 6205, Bangladesh*
[3]*Member, Bangladesh Public Service Commission, Agargaon, Dhaka, Bangladesh*

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Abstract
Newly hatched (24 h old) larvae of *Tribolium castaneum* and *T. confusum* were allowed to feed on different doses of cyromazine or pirimiphos-methyl, or on a combined dose of both compounds up to pupation. All the treatments produced deformities at all the life stages. Cyromazine produced a number of abnormalities in the larval stage ($P < 0.001$) of the two species. Both the compounds produced similar type of deformities in the adults, but the effect was slightly more in the female *T. confusum*. The combined action (10 ppm cyromazine + 0.1 ppm pirimiphos-methyl) of the compounds also produced deformities at each stage; and the effects were more pronounced than the effect caused by a single dose of either 10 ppm cyromazine or 0.1 ppm pirimiphos-methyl, and this will produce less stress on the environment and human health.

Key words: abnormalities; *Tribolium*; cyromazine; pirimiphos-methyl

Introduction

Insects surviving insecticidal treatments very often become variously deformed, and the formation of chimeric individuals and elytral deformities in adults are very common. The organophosphorous insecticide pirimiphos-methyl has been reported to produce morphogenetic abnormalities in treated insects including *Tribolium* species (Khan, 1981; Mondal, 1984; Rahman, 1992). The insect growth regulators (IGRs) are also able to produce various morphological abnormalities in treated insects (Stall, 1975). Among the IGRs, chitin synthesis inhibitors (CSIs) interfere with the formation of new cuticle (Hajjar, 1985), and disturb the process of ecdysis. A number of these compounds affects moulting in insects (Hajjar, 1985), among which the triazine compounds are effectively used to control dipteran insects in poultry (Bloomcamp *et al*., 1987), animal house (El-Oshar *et al.,* 1985) and public health (Awad and Mulla, 1984; Nelson *et al*., 1986).

Cyromazine, derived from azido-triazine herbicides (Shen and Flapp, 1990), is effective against dipteran larvae (Fox, 1990); but was found to be active against the larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Bishop *et al.,* 1990; Sirota *et al*., 1993; Sirota and Grafius, 1994). As a CSI compound, cyromazine affects growth and development in different insect species, including *T. castaneum* (Herbst) (Mondal and Port, 1995).

The present study is aimed to assess the abnormalities produced in flour beetles *Tribolium castaneum* (Herbst) and *T. confusum* Duval due to the activities of cyromazine and pirimiphos-methyl alone or in combination, using low doses of both the compounds for protection of the stored products destined for human consumption.

Corresponding author:
Dr. A.M. Saleh Reza
Department of Zoology, Rajshahi University
Rajshahi 6205, Bangladesh
E-mail: salehbgd@yahoo.com

Materials and Methods

Insects used
Laboratory strains of *Tribolium castaneum* and *Tribolium confusum* were used in the present experiment. The insects were collected from the IPM Laboratory, Institute of Biological Sciences, Rajshahi University. The stock cultures of these species were maintained in the laboratory for the last 15 years.

Compounds used
The tested compounds were a chitin synthesis

Table 1 Larval deformities produced by cyromazine or pirimiphos-methyl alone and by their combination in *T. castaneum* (N = 250)

Treatment (ppm)	Pupal recovery (%)	No. of abnormal larvae	No. of larval-pupal intermediates	Total no. of abnormal larvae (%)
Control (0)	241 (96.40)	-	-	-
Cyromazine				
10	228 (91.20)	09±0.23[a]	04±0.64[a]	13±0.42 (5.20)[a]
20	215 (86.00)	14±0.04[b]	07±0.5[a]	21 ± 0.67 (8.40)[b]
30	197 (78.80)	17±0.31[b]	09±0.33[ab]	26±0.34 (10.40)[bc]
Pirimiphos-methyl				
0.1	230 (92.00)	07±0.41[a]	00	07± 0.48 (2.80)[a]
0.2	196 (78.40)	15±0.36[b]	04±0.06[a]	19 ± 0.39 (7.60)[b]
0.4	180 (72.00)	19±0.1[b]	05±0.72[a]	24±0.05 (9.60)[b]
Cyromazine + Pirimiphos-methyl 10 + 0.1	190 (76.00)	16±0.7	08±0.48	24±0.55 (9.60)[b]

Note: data with the same letters do not differ significantly from each other (P > 0.05 DMRT)

inhibitor, cyromazine and an organophosphorous insecticide pirimiphos-methyl. Cyromazine was kindly supplied by the Ciba Geigy as Larvedex ca 98.4% wp formulation. Pirimiphos-methyl was purchased from the local agrochemical shop.

Doses used

Doses were prepared by mixing required amount of each of the compound and mixed with standard food medium (19:1, whole wheat flour: Brewers' yeast) to obtain the doses in ppm unit.

The doses prepared for cyromazine were 10, 20 and 30 ppm and those for pirimiphos-methyl were 0.1, 0.2 and 0.4 ppm. The single combined dose used was prepared as 10 ppm cyromazine + 0.1 ppm pirimiphos-methyl (lowest doses of both the compounds). The doses of cyromazine and pirimiphos-methyl chosen were based on the mortality tests of the compounds on the larvae of *T. castaneum* and *T. confusum* (Kamaruzzaman *et al.,* 1999; Kamaruzzaman, 2000).

Experimentation

Newly hatched larvae (12 h old) of both species were collected separately from sub-cultures of the beetles. The larvae were released in food medium treated with different doses of either cyromazine or pirimiphos-methyl or cyromazine + pirimiphos-methyl. The larvae were reared up to pupation. After every three days the food material was replaced by a fresh one treated with same dose and compound.

The pupal recovery (%) was recorded. The pupae were sexed according to Halstead (1963) and kept separately for emergence of adults. Adult recovery (%) was recorded. The morphologically abnormal individuals were separated. The deformed characters were studied, and the number of abnormal individuals was counted for each life stage. A set of control larvae was reared similarly on untreated food.

Fifty larvae of each species were used for each treatment, each dose and control. The experiments were carried at 30±1 ^{0}C in an incubator, without controlling light and humidity, and replicated five times.

Statistical analyses

The abnormalities produced by different treatments alone or in combination and in different species of *Tribolium* were tested for significance using analysis of variance (ANOVA). The effect of the different doses of each treatment with respect to control was tested with Duncan's Multiple Range Test (DMRT).

Results

Abnormal characters produced

Cyromazine treatment produced various types of abnormalities in the larvae and adults of both species. Abnormalities produced by cyromazine were recorded as follows:

a) Larval abnormalities
- i) reduced body size,
- ii) swelling in the integument/cuticular lesions,
- iii) stiffness of the cuticle,
- iv) incomplete metamorphosis: larviform pupae, pupal head with larval body, and pupa with larval skin.

Table 2 Larval deformities produced by cyromazine pirimiphos-methyl alone and by their combination in *T. confusum* (N = 250)

Treatment (ppm)	Pupal recovery (%)	No. of abnormal larvae	No. of larval-pupal intermediates	Total no. of abnormal larvae (%)
Control (0)	240 (96.00)	-	-	-
Cyromazine				
10	230 (92.00)	10±0.88[a]	05±0.72[a]	15±0.49 (6.00)[a]
20	221 (84.40)	15±0.53[ab]	06±0.6[a]	21±0.55 (8.40)[b]
30	209 (83.60)	21±0.55[b]	08±0.46[a]	29±0.32 (11.60)[c]
Pirimiphos-methyl				
0.1	227 (90.80)	08±0.52[a]	00	08±0.51 (3.20)[a]
0.2	198 (79.20)	12±0.62[ab]	05±0.46[a]	17±0.33 (6.80)[b]
0.4	195 (78.00)	14±0.55[b]	06±0.31[a]	20±0.05 (8.00)[bc]
Cyromazine + Pirimiphos-methyl				
10 + 0.1	200 (80.00)	15±0.43[b]	07±0.06[a]	22±0.71 (8.80)[bc]

Note: data with the same letters do not differ significantly from each other (P > 0.05 DMRT)

b) Adult abnormalities
- i) bent abdomen,
- ii) incomplete elytra.

Pirimiphos-methyl alone or in combination with cyromazine produced a similar type of deformities in both species, as follows:
- i) reduced larval body size,
- ii) larval-pupal intermediates,
- iii) adultoids and incomplete elytra in adults.

The abnormal individuals with incomplete metamorphosis at larval-pupal transformation were categorized as larval abnormality and the adultoids as pupal abnormality.

Percentages of abnormalities produced

Cyromazine produced a higher percentage of abnormal larvae in *T. confusum* than *T. castaneum* (Tables 1, 2). Effect of cyromazine on the normal growth of larvae was found to be dose related ($P < 0.001$, $F = 87.219$), and was similar in both species ($P > 0.05$, $F = 0.981$). The number of abnormal larvae was higher than the number of larval-pupal intermediates, in both species of *Tribolium.*

Pirimiphos-methyl produced comparatively less number of abnormal larvae in both species ($P > 0.05$, $F = 1.31$), though the effect varied with the doses ($P < 0.001$, $F = 84.283$) (Tables 1, 2).

The combined effect of the IGR and the insecticide produced a greater effect on the morphogenesis of the larvae of both species ($P > 0.05$, $F = 0.4$). The effects of the combined treatment was more than the effects recorded with the lower doses of either cyromazine or pirimiphos-methyl ($P < 0.001$, $F = 211.6$) (Tables 1, 2).

Cyromazine produced similar abnormal adults at same extent in both sexes of *T. castaneum* (Table 3), whereas the effect was slightly greater in the females of *T. confusum* (Table 4). The percentages of abnormal adults produced were not significant between the species ($P > 0.05$, $F = 0.067$); but significant differences of the effect was observed between the doses in *T. confusum* ($P < 0.05$, $F = 9.25$).

Pirimiphos-methyl treatments also produced similar percentage of adult abnormalities in both the species ($P > 0.05$, $F = 0.72$), which did not vary between the sexes of *T. castaneum* (Table 3), but the females were more affected than the males of *T. confusum* (Table 4). The adult morphogenesis of the beetles slightly varied among the doses ($P < 0.01$, $F = 10.47$).

The combined treatment of cyromazine (10 ppm) and pirimiphos-methyl (0.1 ppm) produced a greater effect on the adult morphogenesis than the single treatment with either cyromazine or pirimiphos

Table 3 Adult abnormalities produced by cyromazine or pirimiphos-methyl alone and by their combination in *T. castaneum* (N = 250)

Treatment (ppm)	Total Adult recovery (%)	Male (no.)	Female (no.)	No. of abnormal individuals (%)	
				Male	Female
Control (0)	237 (94.80)	115	122	-	-
Cyromazine					
10	218 (87.20)	117	101	09±0.06 (7.69)[a]	06±0.21 (5.94)[a]
20	204 (81.60)	109	95	09±0.2 (8.26)[a]	07±0.13 (7.37)[a]
30	188 (75.20)	100	88	09±0.11 (9.00)[a]	12±0.31 (13.64)[a]
Pirimiphos-methyl					
0.1	209 (83.60)	115	94	08±0.21 (6.96)[a]	07±0.07 (7.45)[a]
0.2	189 (75.60)	95	94	09±0.05 (9.47)[a]	09±0.23 (9.57)[a]
0.4	168 (67.20)	96	72	09±0.21 (9.37)[a]	13±0.15 (18.05)[b]
Cyromazine + Pirimiphos-methyl					
10 + 0.1	185 (74.00)	107	78	19±0.25 (17.76)[b]	15±0.22 (19.23)[b]

Note: data with the same letters do not differ significantly from each other (P > 0.05 DMRT)

methyl at the same dosage (Tables 3, 4). The combined treatment produced similar effects in both species of *Tribolium* (P > 0.05, F = 0.034).

Discussion

The mode of action of cyromazine is different from that of pirimiphos-methyl. Being a chitin synthesis inhibitor, cyromazine affects the mechanical properties of the insect cuticle and produces abnormalities in the skin, and resists moulting (Fox, 1990). Inhibition of moulting results in increase of the internal body pressure in the larvae (Fox, 1990), producing swellings on the cuticle (cuticular lesions) (Kotze and Reynolds, 1993; Sirota *et al*., 1993; Sirota and Grafius, 1994). So, larval deformities are common in CSI treated insects. The abnormal characteristics as noted in the present experiment, have been also reported in cyromazine treated Colorado Potato beele (Sirota and Grafius, 1994) and triflumuron (a CSI compound) treated *T. castaneum* (Parween, 1998).

Due to CSI activity insect cuticle often becomes stiff (Fox, 1990). Consequently feeding is often hampered, as reported by Neuman and Guyer (1988), Soltani (1984) and Parween (1996) in different insects. Even is some could survive, the starved larvae were reduced in body size. Pirimiphos-methyl had been reported to avoid feeding on the treated medium *Tribolium* species, and as a result adults loose weight and length (Mondal, 1984b; Rahman, 1992).

Pirimiphos-methyl affected the larval-pupal transformation and produced intermediate forms along with adult abnormalities. Both cyromazine and pirimiphos-methyl affected growth of the emerged adults, which were superficially normal but with deformed elytrae.

The combined action of cyromazine and pirimiphos-methyl to some extent was greater on the morphogenesis of *Tribolium* species, than the action of single treatment of either compound. All the abnormal larvae and larval-pupal intermediates failed to survive long, and the abnormal adults were found to be uncapable to mate or oviposite.

Cyromazine is easily miscible with most of the standard insecticides and fungicides (Fox, 1990), and in the present study was found to reduce the doses of insecticide used, producing the same effect. So, it can be used combined along with traditional insecticides at minimal doses for the protection of the stored products against beetle infestation, which may produce less stress on the environment and human health.

Both *T. castaneum* and *T. confusum* have become resistant against most of the insecticides used. For insect management in the grain and cereal stores, high doses of these insecticides are needed, which affect the human health, the environment and its biota. To overcome this problem, cyromazine could be used with pirimiphos-methyl against *Tribolium* species, since this association would be able to produce abnormal individuals. The abnormal larvae or adults either would die or fail to

Table 4. Adult abnormalities produced by cyromazine or pirimiphos-methyl alone and by their combination in *T. confusum* (N = 250)

Treatment (ppm)	Total Adult recovery (%)	Male (no.)	Female (no.)	No. of abnormal individuals (%)	
				Male	Female
Control (0)	237 (94.80)	116	121	-	-
Cyromazine					
10	219 (87.60)	114	105	08±0.21 (7.02)[a]	06±0.04 (5.71)[a]
20	207 (82.80)	110	97	08±0.03 (7.27)[a]	10±0.22 (10.31)[b]
30	197 (78.80)	105	92	11±0.21 (10.48)[b]	14±0.5 (15.22)[bc]
Pirimiphos-methyl					
0.1	221 (88.40)	116	105	06±0.06 (5.17)[a]	10±0.15 (9.52)[b]
0.2	194 (77.60)	101	93	10±0.22 (9.90)[b]	11±0.21 (11.83)[b]
0.4	188 (75.20)	102	86	14±0.22 (13.72)[bc]	10±0.03 (11.63)[b]
Cyromazine + Pirimiphos-methyl 10 + 0.1	195 (72.78)	103	92	21±0.15 (20.39)[c]	11±0.3 (11.96)[b]

Note: data with the same letters do not differ significantly from each other (P > 0.05 DMRT)

develop further or to reproduce, and ultimately the population would be controlled. Moreover, as very low doses of these compounds could be used, the residues may not create hazard to the stored commodities as well as to the environment.

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