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Inhibition of ovicidal activities and fertility in a water beetle, *Cybister tripunctatus asiaticus* Sharp (Coleoptera, Dytiscidae) following topical application of Diflubenzene, a Chitin inhibitor

by **N. S. Sen, A. H. Naqvi & Vineeta Rani**

Abstract: Topical application of a chitin inhibitor, Diflubenzene (Dimilin) to the aquatic beetle, *Cybister tripunctatus asiaticus* affected the reproductive potential of both male and female. Control female mated with control male laid a maximum of 450 eggs and 95 % of them hatched, whereas high concentration treated female mated with similar male oviposited only 102 eggs and the hatching efficiency was reduced to 22 % only. Regarding production and hatching of eggs, males were more significantly affected ($p < 0.01$ & 0.001) by the treatment than the females ($p < 0.05$ & 0.01). However weight-loss in eggs and newly hatched larvae was equally affected ($p < 0.01$) on both the sexes.

Key words: Coleoptera, Dytiscidae – *Cybister* – topical application – Diflubenzene – reduction – ovicidal activities – fertility – weight loss – correlation coefficient.

Introduction

Ovicidal activities and fertility of adult insects have been reported to be adversely affected by topical application of organophosphorous compounds (HANIFFA et al., 1986 and HANIFFA & JOSE, 1987). Secondary plant substances (MATHAVAN et al., 1984) and chitin inhibitors (SEXENA & KUMAR, 1982, CHOCKALINGAM & NOORJEHAN, 1984). Such compounds are extensively used in India on a wide range of insects, especially on majority of the crops, vegetables and stored-grain pests not only because of the low cost involvement but also for their lesser residual effects. The basic toxicity of these compounds is targeted through the central nervous system via inhibition of acetyl cholinesterases (PANT & KATIYAR, 1983). Despite of such existing knowledge, little is known about the inhibitory role played by Diflubenzene [1-(4-Chloropheny1)-3-(2-6-diflurobenzyl) Urea], a chitin syntheses inhibitor (HOLST, 1975 & BUTCHI, 1978) on the physiology of reproduction in insect. The present authors attempt to show the impact of topical application of Diflubenzene, a chitin inhibitor, on the ovicidal activities and fecundity rate in an aquatic beetle, *Cybister tripunctatus asiaticus* Sharp, a fish pest (DUTTAMUNSHI et al., 1979).

Material and Methods

Healthy adults of *C. tri. asiaticus* were collected from a nearby pond and were acclimed to the laboratory conditions of 26.5 ± 2.3 °C and 43 ± 10 % relative humidity. Males and females were allowed to mate 4 times and the eggs laid were separated. Freshly hatched larvae were fed with mosquito larvae (LECLAIR et al., 1986) till they became ready for pupation. After a preliminary bioassay experiment (JOSE, 1986) different sublethal concentrations viz. 0.001 % (low) and 0.003 % (high) of Diflubenzene were prepared in distilled water and were topically applied to 2 of the 3 series of isolated, pre-weighed, healthy larvae (test individuals series II & III), while series I individuals were treated with distilled water alone. After the completion of metamorphosis the adult males and females were separated, weighed and allowed to mate in nine combinations: with control male (CM) and female (CF), low concentration-treated males (LM) with females (LF) and high concentration treated male (HM) with female (HF). The experimental setup was maintained in triplicate for statistical analysis (ZAR, 1974). For each combination, fecundity (egg-output) and egg weight were recorded. The eggs were then allowed to hatch in laboratory conditions (SEN, 1979) and the weights of newly hatched young ones were measured. The abdomen of postoviposited females was dissected out and the eggs in the ovary was counted.

Results and Discussion

Total number of egg output was maximum for CF mated with CM which was significantly reduced in all the test individuals treated with either low or high concentration of Diflubenzene (Table 1). Topical application also resulted in a decrease in hatching rate of eggs (expressed in percentage); the range of decrease was also found to be of a significantly high one (95 %–22 %). Moreover, the eggs retained in the ovary was also reduced from an average value of 22 (CF × CM) to an average value of 7 (HF × HM) indicating an inhibitory effect in ovicidal activity and/or vitellogenesis (Table 1).

Similar reports on reduction in fecundity and percentage of hatching were reported in insects due to applications of moulting inhibitors (SAXENA & KUMAR, 1982; CHOCKALINGAM & NOORJE-

HAN, 1984), Pesticides (Kuribayashi, 1981) and antifeedants (ANSARI & KHAN, 1973, 1978). SINGH et al., 1986 were of the opinion that the insecticides interfer with endocrine process in insects and inhibit their development and fertility (LEWALLEN, 1964). The adverse effects in egg laying and hatching in *C. tri. asiaticus* could be attributed to the interference of toxicants on the synthesis and incorporation of macromolecules in the ovary (HALL et al., 1976; SRINIVASAN & KESHAVAN, 1979) and/or due to inhibition in the synthesis of required neurosecretory hormones (ADAMS, 1974; MC DANIEL & BERRY, 1974).

Increase in concentration of topical application to males and females also resulted in an inevitable weight-loss for the eggs. Table 1 also reveals that either one way – (either male or female) or both way – (both male and female) treatment affects the mating potential of males more significantly than of females, when fecundity and hatching of eggs are concerned. DAVIES, 1965; GILLOTT & FRIEDAL, 1977 and SUBRAMANIUM, 1984 stated that in addition to supplying sperms, mating in insects initiates oogenesis, accelerates egg maturation, stimulates ovulation, promotes oviposition and enhances the total egg output. The present findings conform their statement in the sense that even the low concentration treated individuals (LM or LF) are very conspicuous inhibitors of all those stages of ovicidal activities which culminate into an overall reduced hatching rate for the treated insects.

Correlation coefficient analysis confirmed that the inhibition in egg output and hatching in treated females was highly significant when mating was allowed with treated male ($p < 0.01$ and 0.001). It also showed that the treatment affected the reproductive potential of the females significantly (egg output: $p < 0.05$; hatching: $p < 0.01$). The inhibition of fecundity and fertility of the eggs by the toxicant showed a clear dosedependency and is very similar to the results obtained in *Musca domestica* (SRINIVASAN & KESHAVAN, 1979) and *Bombyx mori* (HANIFFA et al., 1986). The weight-loss of eggs as well as freshly emerged larvae is again dose-dependent and the efficacy of the toxicant (Diflubenzene) is equally exhibited in both male and female ($p < 0.01$; Table 1).

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Table 1. Showing the effect of topical application of Diflubenzene on the fecundity and ovidical activities in C. tri. asiaticus

Mating Male	Pairing Female	Number of eggs laid	Percentage reduction in Hating (%)	Number of eggs retained	Egg weight (mg)	First instar weight (mg)
C	C	458 ± 9.1	4.8 ± 0.66	22.7 ± 1.73	0.66 ± 0.03	0.74 ± 0.021
C	L	352 ± 9.3	10.7 ± 2.64	18.9 ± 3.51	0.60 ± 0.01	0.70 ± 0.016
C	H	265 ± 8.0	21.3 ± 1.99	14.6 ± 3.19	0.58 ± 0.02	0.71 ± 0.026
L	C	380 ± 21.6	28.2 ± 2.81	19.0 ± 2.06	0.62 ± 0.02	0.73 ± 0.028
L	L	290 ± 32.7	43.4 ± 3.21	13.7 ± 1.63	0.60 ± 0.01	0.73 ± 0.018
L	H	242 ± 28.7	52.1 ± 5.11	8.7 ± 1.36	0.57 ± 0.03	0.71 ± 0.116
H	C	321 ± 16.6	44.3 ± 1.51	14.7 ± 1.15	0.60 ± 0.03	0.74 ± 0.012
H	L	166 ± 5.6	57.9 ± 2.65	10.0 ± 1.73	0.53 ± 0.02	0.72 ± 0.015
H	H	102 ± 6.0	87.9 ± 4.57	7.0 ± 2.0	0.51 ± 0.05	0.70 ± 0.0126
CORRELATION COEFFICIENT						
Male	P	< 0.01	< 0.001	–	< 0.01	< 0.01
Female	P	< 0.05	< 0.01	–	< 0.01	< 0.05

C = control, L = low concentration treated, H = high concentration treated,
P < 0.05 = significant, < 0.01 = highly significant.