

EFFECT OF AZADIRACTIN ON LARVAE AND ADULTS OF *CRYPTOLESTES PUSILLUS* (SCHON.) (COLEOPTERA: CUCUJIDAE)

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ABSTRACT

The toxicity of Azadiractin (commercial product Nimbicidin®) to different larval instars and adults of *Cryptolestes pusillus* (Schon.) was recorded after 24 and 48 hours of exposure. The LC₅₀ values were 7.829, 19.402, 404.815, 29.374 and 5.659, 13.962, 12.297, 10.203 µgm cm⁻² for first to fourth instars larvae of 24 and 48 hours respectively. For adult the LC₅₀ were 410.722 and 416.895 µgm cm⁻² of 24 and 48 hours respectively.

Key words: *Cryptolestes pusillus*, Azadiractin, *Azadirachta indica*, LC₅₀.

INTRODUCTION

The flat grain beetle *Cryptolestes pusillus* (Schon.) occurs on stored wheat, wheat flour, rice (husked), bran, corn meal and other stored products. Howe and Lefkovitch (1958) recorded this pest from beans, cassava, cocoa, cowpeas, groundnuts, maize, nere seed, rice, sorghum and wheat. It virtually feeds on all kinds of stored grain and milled cereal products and causes immense damage to stored wheat, flour and other stored commodities in all the tropical and subtropical countries of the world including Bangladesh (Hossain *et al.* 1986). It is cosmopolitan in distribution and is one of the common insect pests of stored grain (Hole *et al.* 1976). Its status as a major pest of grain in the United Kingdom has been described by Freeman (1952). The damage is caused both by the larval and adult stages of *C. pusillus* (Cotton 1963).

The continuous use of chemical pesticides for control of stored grain pests has resulted insecticide resistance (Pacheco *et al.* 1990), sometimes the efficacy of insecticides against storage pests varies greatly after treatment (Pinto *et al.* 1997).

The search for alternative, and environmental friendly insecticides has resulted in the rediscovery of botanicals for plant protection. Azadiractin (AZ), a botanical pesticide derived from the neem tree, *Azadirachta indica* A. Juss., (Meliaceae) (Butterworth and Morgan 1971), is generally considered less harmful to the environment than other more commonly used pesticides. AZ is an example of natural chemical defense by plants, affecting feeding primarily through chemoreception (deterrence) and secondarily through toxic effects (Mordue [Luntz] and Blackwell 1993). It is a tetranortriterpenoid plant limonoid that is highly oxidized with many reactive functional groups in close proximity to each other (Ley *et al.* 1993). The growth regulatory effects of AZ are due to

the disruption of the hormonal control of metamorphosis and moulting (Vardhini *et al.* 1997). These effects are manifested by changes in haemolymph ecdyteroid and juvenile hormone titres due to a blockage and/or delay in the release of those hormones from neurohaemal organs (Barnby and Klocke 1990). AZ, however, was found to be a potent insect growth inhibitor for the larvae of *Tribolium confusum* (Hu and Chiu 1993).

Evaluation of AZ against numerous species of insect pest have demonstrated neem's diverse biological effects: repellence (Chander *et al.* 2000, Sahayaraj and Paulraj 2000, Zahoor *et al.* 2002), feeding deterrence (Isman 1993), reduced growth and abnormal development (Beckage *et al.* 1988), oviposition deterrence (Lohra *et al.* 2001), reduced egg laying due to sterilizing effect (Sharma 1995) and also direct toxicity (Ahmed *et al.* 2001). In the present investigation attempts were made to observe the toxicity of azadiractin as stored grains protectant against larvae and adults of *C. pusillus*.

MATERIALS AND METHODS

The experimental insects (*C. pusillus*) were collected from the Bangladesh Council for Scientific and Industrial Research (BCSIR) laboratories, Rajshahi, Bangladesh and successfully reared in the IPM laboratories, Institute of Biological Sciences, University of Rajshahi, Bangladesh. Mass culture were maintained in petri dish (12cm diam.) and sub culture in small petri dish (6cm diam.) containing food medium and kept in the CT room at 30±1°C. A standard mixture of whole wheat flour with powdered dry yeast in ratio of 19:1 (Khalequzzaman *et al.* 1994) was used as food medium.

Nimbicidin® (0.03% azadiractin, EC, ACI Limited, Bangladesh), was used for the assays. AZ was diluted in acetone and different doses were prepared. Five ml of liquid from each dose was placed in petri dishes (60mm

diam.) separately, uniformly covering the whole area of the petri dish. They were then kept open for sometimes to allow the solvent to evaporate. In case of larval trail 1.5 gm of food was added in the petri dish and 40 larvae were released on them. In case of adult trail, 40 adults were also released inside a petri dish having no food. A control batch was maintained in the similar way having solvent only. Each experiment was replicated five times.

Mortality was recorded 24 and 48 hours after treatment. Mortality was corrected for using Abbott's formula (Abbott 1925). The observed data was subjected to probit analysis according to Finney (1947) and Busvine (1971) using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, UK. Heterogeneity is tested by a chi-square test, if the probability is greater than 5% an automatic correction of heterogeneity was introduced and the regression lines were drawn by using Microsoft-XLs.

RESULTS AND DISCUSSION

The LC_{50} , 95% fiducial limits, regression equation and χ^2 values calculated are presented in Table 1. In the larvae, the LC_{50} was estimated to be 7.829, 19.402, 404.815, 29.374 $\mu\text{gm cm}^{-2}$ and 5.659, 13.962, 12.297, 10.203 $\mu\text{gm cm}^{-2}$ for first through fourth instars, treatment after 24 and 48 hours respectively. The LC_{50} of the adults were estimated to be 410.722 and 416.895 $\mu\text{gm cm}^{-2}$, treatment after 24 and 48 hours respectively. The LC_{50} values gradually increased from the lowest in first instar attaining a maximum in the 4th instar indicating that earlier stages are more susceptible to azadirachtin. Adults were more tolerant to AZ than the larvae. All chi-square values were significant indicating good fit of the regression lines (Fig.1). Following the criteria of overlapping fiducial limits, the data shows that the only statistically significant differences in susceptibility to AZ are that 3rd instar were less susceptible than 1st instar, the 4th instars are less susceptible than 1st instar, 3rd and 4th instars and the adult stage is less susceptible than all instars.

Khan *et al.* (1994) reported that the LD_{50} values was 12.0 $\mu\text{g /cm}^2$ for *T. castaneum* adults using a neem formulation. Vardhini *et al.* (1997) observed higher concentrations of AZ resulted in death of adults after a few hours. Xie *et al.* (1996) studied the flour-disk bioassay of AZ on three stored product pests and found that mortality of *T. castaneum* was caused entirely by its toxic action. Ahmed *et al.* (1999) observed the LC_{50} of crude neem extract as 1300 $\mu\text{gm cm}^{-2}$ against *T. castaneum* adults. In another experiment Ahmed *et al.* (2001) observed the mortality of *T. castanum* was 18,

30, 52, 66 and 86% at neem extract concentration of 649.35, 974.0, 1298.7, 1623.37 and 1948.05 $\mu\text{g /cm}^2$ respectively after 24 h. of treatment. Ulrichs and Mewis (2000) observed that a single treatment with the neem product, in concentrations of 0.01, 0.1, 0.2 and 1.0 g azadirachtin/kg rice, increased the mortality rate. Neemazal-W (10% AZ) was evaluated for mortality and mortality values of *T. castaneum* adults were 22.2, 97.8 and 100% at 50, 500 and 1000 ppm after 14 days post-treatment, respectively (El-Lakwah and El-Kashlan, 1990). As far as known, no work was done on larval and adult mortality of *C. pusillus* was recorded previously and hence comparison of the data could not be done.

CONCLUSION

The neem insecticide Nimbicidin was found to be toxic to larval and adult stages of *C. pusillus*. However, larval stages were more susceptible than the adult stage. These results indicate that insecticides from the neem tree might be effective controls for this pest species.

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Table 1: LC₅₀, 95% fiducial limits, regression equation and χ^2 -values for *C. pusillus* larval and adult mortality, after 24 and 48 h. exposure to azadirachtin treated surfaces.

Stage	Dura. of treatment	Dose	Mortality (%)	LC ₅₀	95% fiducial limits		Regression equations	χ^2 (df)
					Lower	Upper		
1 st	24h	21.200	66.05	7.829	5.018	12.21	Y=4.081565+1.027598X	0.1409407 (2)
		10.610	58.18					
		5.300	43.60					
	48h	2.650	31.55	5.659	3.700	8.656	Y=4.139548+1.143003X	0.1863537 (2)
		21.200	76.05					
		10.610	60.60					
2 nd	24h	5.300	50.65	19.402	9.327	40.36	Y=3.741628+0.9770814X	1.253723 (2)
		2.650	37.03					
		21.200	56.05					
	48h	10.610	35.28	13.962	7.403	26.33	Y=3.959764+0.9085267X	1.389185 (2)
		5.300	26.05					
		2.650	23.08					
3 rd	24h	21.200	40.28	404.814	3.010	5.444	Y=4.557836+0.1695883X	1.693036 (2)
		10.610	37.83					
		5.300	46.05					
	48h	2.650	30.58	12.297	3.513	43.03	Y=4.537216+0.4246389X	0.5755421 (2)
		21.200	53.10					
		10.610	49.03					
4 th	24h	5.300	48.08	29.373	7.571	113.9	Y=4.004294+0.6782833X	0.6551848 (2)
		2.650	35.53					
		21.200	50.28					
	48h	10.610	34.08	10.203	6.621	15.72	Y=3.858164+1.131932X	2.381111 (2)
		5.300	29.63					
		2.650	25.60					
Adult	24h	21.200	71.58	410.722	6.285	268.3	Y=2.78219+0.8485762X	0.0867347 (2)
		10.610	43.13					
		5.300	34.08					
	48h	2.650	30.38	416.895	5.614	309.5	Y=2.960819+0.778301X	0.1180036 (2)
		21.200	14.03					
		10.610	8.05					
		5.300	5.53					
		2.650	2.55					
		21.200	14.63					
		10.610	12.08					
		5.300	6.63					
		2.650	4.08					

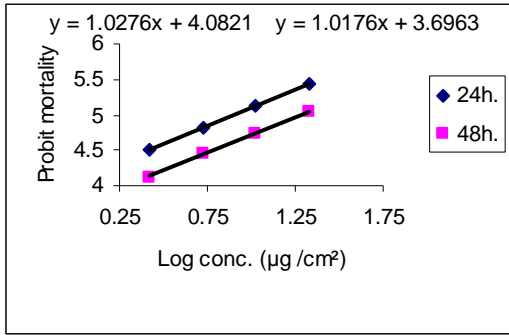


Fig. 1 Regression line of azadirachtin on probit mortality at 24 and 48h. exposure of 1st instar larvae of *C. pusillus*

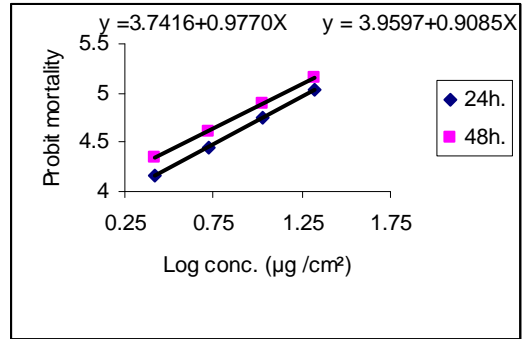


Fig. 2 Regression line of azadirachtin on probit mortality at 24 and 48h. exposure of 2nd instar larvae of *C. pusillus*

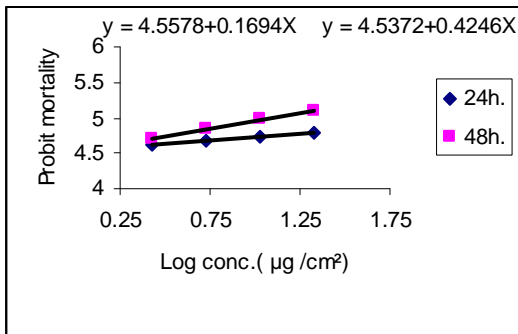


Fig. 3 Regression line of azadirachtin on probit mortality at 24 and 48h. exposure of 3rd instar larvae of *C. pusillus*

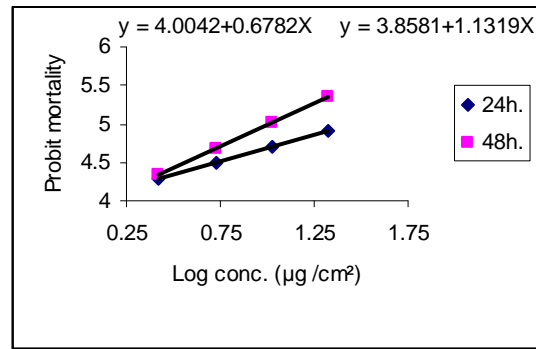


Fig. 4 Regression line of azadirachtin on probit mortality at 24 and 48h. exposure of 4th instar larvae of *C. pusillus*

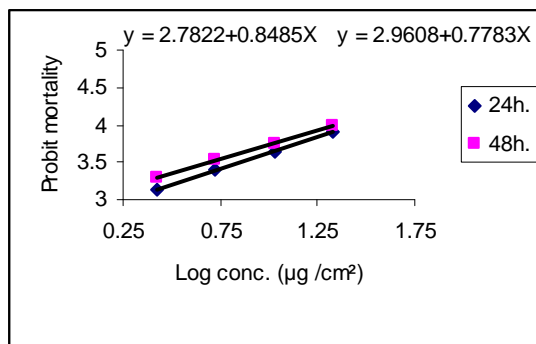


Fig. 5 Regression line of azadirachtin on probit mortality at 24 and 48h. exposure of adult of *C. pusillus*

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