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Efficacy of commercial neem-based insecticide, Nimbicidine® against eggs of the red flour beetle *Tribolium castaneum* (Herbst)

Dipali Rani Das, Selina Parween and Saiful Islam Faruki*
Department of Zoology, Rajshahi University, Rajshahi-6205, Bangladesh
*Corresponding author; email: faruki64@yahoo.com

Abstract: The efficacy of different concentrations of the commercial neem-based insecticide, Nimbicidine® was evaluated against the eggs of the red flour beetle *Tribolium castaneum* (Herbst). The insecticide significantly inhibited the hatching, pupation and adult emergence of the beetle. The latent effects of Nimbicidine® on the next generation progenies were expressed by significant reductions in the growth of larvae, pupation and adult emergence coupled with lengthened developmental period, but the sex ratio was unaffected.

Key words: *Tribolium castaneum*, Azadirachtin, hatching, pupation, adult emergence, growth and development, sex ratio

Introduction

The post-harvest losses and quality deterioration caused by storage pests are major problem throughout the world (Hill, 1990). Synthetic insecticides have been successfully used to protect stored grains from insect infestations but their indiscriminate and massive use have created serious problems such as hazards to the environment including human health and non-target organisms (Sighamony *et al.*, 1986), residues in food grains (Fishwick, 1988), environmental pollution (Wright *et al.,* 1993; WMO, 1995), and development of resistant strains (Champ & Dyte, 1976; Zettler, 1982; Zettler & Cuperus, 1990; Yusof & Ho, 1992; White, 1995). Finding safe alternatives to synthetic insecticides to protect stored grains and grain products from insect infestations are highly desirable.

Recently, attention has been given to the possible use of plant products or plant derived compounds as promising alternatives to synthetic insecticides in controlling insect pests of stored products (Jahan *et al.*, 1989; Mondal *et al.,* 1989; Khalequzzaman & Islam, 1992; Senguttuvan *et al.*, 1995; Liu & Ho, 1999; Ohazurike *et al.,* 2003; Umoetok & Gerard, 2003). The effectiveness of many plant derivatives for use against stored grains pests has been reviewed by Jacobson (1983, 1989) and Golob & Webley (1980). Joseph *et al.* (1994) and Haque *et al.* (2000) studied the growth inhibitory effects of some commercially available plant extracts on the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae).

Many biologically active compounds have been isolated from neem, *Azadirachta indica* A. Juss, including triterpenoids, azadirachtin (Butterworth & Morgan, 1971) and melantriol (Lavie *et al.*, 1967), which reduced the feeding of insects. Azadirachtin is a mixture of seven isomeric compounds as Azadirachtin-A to Azadirachtin-G of which Azadirachtin-E is the most effective insect growth regulator (Verkerk & Wright, 1993). Azadirachtin possess insecticidal, ovicidal, antifeedant, and growth inhibiting effects against many insect pests (Akou-Edi, 1984; Schmutterer, 1990; Vietmeyer, 1992; Nawrot & Harmatha, 1994), including the storage pests (Jilani & Su, 1983; Ivbijaro, 1983a, b; Makanjuola, 1989). The present investigation, therefore, was undertaken to evaluate the pesticidal activity of Nimbicidine® on eggs, and the growth and development of next generation progenies of *T. castaneum* developed from treated eggs.

Materials and Methods

The test insects: T. castaneum was collected from the stock culture maintained at the Insect Research Laboratory, Department of Zoology, Rajshahi University on standard food medium (wheat flour: Brewer's yeast, 19:1) in plastic containers.

Source of the test compound: The experimental compound, Nimbicidine®, a commercial formulation of Azadirachtin EC (0.03% a. i.) was produced by The Stanes & Co. Ltd., India and marketed by the ACI Crop Care, Bangladesh. The compound is an extract of neem seed, which acts as repellent, antifeedant and growth regulator. The concentrations used in the present experiment were 0.5-, 1-, 2-, 4-, 8- and 16 $ml.kg^{-1}$ and prepared by mixing the requisite amounts of Nimbicidine® with the food medium.

Experimental procedures: One hundred 24 h-old eggs were released on Nimbicidine®-treated foods separately. An equal number of eggs of the same age were released on untreated food as controls. The eggs were exposed to treated food for 24h, and were transferred to clean Petri dishes separately for each concentration to record their hatching percentage. The newly hatched larvae were transferred to untreated food separately for each concentration. The food medium was changed with a fresh one after every 7 days to avoid conditioning. The larvae were kept in the food medium up to pupation, and the following parameters were recorded: (i) Larval survival (%) on 12th day after hatching, and (ii) Pupal and adult recoveries (%). To assess the effects of Nimbicidine® on the next generation progeny, adults emerging from the treated eggs were kept separately in Petri dishes provided with the fresh food. Eighteen-day old adults were allowed to oviposit for three consecutive days. Eggs were incubated and neonates were transferred to fresh food up to pupation with regular changing of the food medium. The following parameters for the progeny were recorded: (i) Weight of 12- and 16-day old larvae; (ii) Developmental period; (iii) Pupal and adult recoveries (%); (iv) Weight of male and female pupae; and (v) Weight of male and female adults. All the experiments were replicated three times and conducted at mean room temperatures of 30±2ºC without any humidity and light control.

Results and Discussion

Effect on egg-hatching and survival of life stages: Hatching of *T. castaneum* eggs was inhibited significantly due to Nimbicidine® treatment at all concentrations ($F_{6,12}$ = 52.06, P<0.001; Table 1). The insecticide reduced the egg hatching up to 18.60% at higher concentrations. The percentages of larval, pupal and adult survival reduced up to 20.00, 20.25 and 21.79% respectively at 16 ml.kg^{-1} of Nimbicidine® as compared to control and the reduction was statistically significant (P<0.001) in all the life stages (Table 1).

Table 1. Effects of egg treatment with Nimbicidine® on hatching and survival of various life stages in *T. castaneum* (N = 300)

Concentrations (ml.kg^{-1})	Hatching (%) Mean±SE	% reduction@	Survival (%)					
			Larvae		Pupae		Adults	
			Mean ± SE	% reduction@	Mean ± SE	% reduction@	Mean ± SE	% reduction@
0.0	86.0±0.58^a	—	80.0±0.58^a	—	79.0±0.58^a	—	78.0±0.58^a	—
0.5	82.0±0.58^a	4.65	78.0±0.58^a	2.50	77.0±0.58^a	2.53	76.0±0.58^a	2.56
1.0	82.0±0.58^a	4.65	78.0±1.16^a	2.50	78.0±1.16^a	1.27	76.0±0.58^a	2.56
2.0	71.0±0.58^{bc}	17.44	67.0±1.16^c	16.25	66.0±0.58^{bc}	16.46	65.0±0.58^b	16.67
4.0	75.0±2.89^b	12.79	71.0±0.58^b	11.25	68.0±1.16^b	13.92	66.0±1.16^b	15.38
8.0	70.0±0.05^c	18.6	67.0±1.16^c	16.25	67.0±1.16^b	15.19	65.0±0.58^{bc}	16.67
16.0	70.0±1.16^c	18.6	64.0±0.58^d	20.00	63.0±0.58^c	20.25	61.0±0.58^c	21.79
F-values	52.06***		188.14***		69.00***		109.56***	

Means in each column followed by the same letter are not significantly different by Tukey's test (P < 0.05); @% reduction over control; *** P< 0.001

Effect on growth of various developmental stages of next generation progenies: The latent effects of Nimbicidine®-treated eggs on the weight of larvae, pupae and adults of next generation *T. castaneum* are presented in Table 2. The insecticide significantly reduced the weight of larvae ($F_{6,12}$= 8.56 and 12.28, P<0.001 for 12- and 16-day old larvae, respectively), which gradually reduced at increasing concentrations. However, the weights of pupae and adults were not affected.

Effect on developmental period and next generation progeny formation: Nimbicidine®-induced developmental periods, pupal and adult recoveries and sex ratio in *T. castaneum* are shown in Table 3. The larval period was not affected compared to that of the control but the insecticide significantly lengthened the pupal period in the next generation progenies ($F_{6,12}$ = 8.11, P<0.01). In addition, it significantly reduced pupation as well as adult emergence. The reduction in pupation and adult emergence was increased respectively up to 13.45% and 14.73% as the concentrations were increased. However, Nimbicidine® failed to produce any appreciable change in adult sex ratios in *T. castaneum.*

Table 2. Effects of Nimbicidine® treatment on the weight (mg) of larvae, pupae and adults in next generation *T. castaneum* progenies

Concs. (ml.kg^{-1})	Larvae		Pupae		Adults	
	12 day-old	16 day-old	♂♂	♀♀	♂♂	♀♀
	Mean± SE	Mean± SE	Mean± SE	Mean± SE	Mean± SE	Mean± SE
0.0	1.62± 0.01^a	2.98± 0.02^a	2.67± 0.08	3.06± 0.06	2.10± 0.07	2.24± 0.03
0.5	1.59± 0.01^{ab}	2.89± 0.03^{ab}	2.61± 0.02	2.90± 0.02	2.05± 0.03	2.21± 0.05
1.0	1.55± 0.03^{abc}	2.81± 0.06^{ab}	2.63± 0.05	2.89± 0.10	2.02± 0.06	2.20± 0.03
2.0	1.52± 0.03^{abc}	2.79± 0.05^{ab}	2.63± 0.07	2.86± 0.05	2.01± 0.01	2.21± 0.08
4.0	1.47± 0.08^{bc}	2.77± 0.07^{ab}	2.63± 0.05	2.88± 0.05	2.01± 0.01	2.11± 0.07
8.0	1.45± 0.02^{bc}	2.74± 0.06^b	2.63± 0.03	2.84± 0.10	2.02± 0.02	2.15± 0.03
16.0	1.44± 0.03^c	2.68± 0.02^b	2.63± 0.03	2.85± 0.10	2.02± 0.04	2.19± 0.06
F-values	8.56***	12.28***	0.15^{ns}	2.70^{ns}	0.77^{ns}	2.22^{ns}

Means in each column followed by the same letter are not significantly different by Tukey's test (P < 0.05); *** P<0.001; ns = not significant

The production of eggs, hatching and adult emergence in *Callosobruchus maculatus* and adult emergence in *Sitophilus oryzae* were significantly reduced when raised on cowpeas and maize respectively treated with extracts of neem leaf and seed (Makanjuola, 1989). Similarly, Lale & Abdulrahman (1999) and Lale & Mustapha (2000) reported a significant reduction in egg laying and adult emergence in *C. maculatus* treated with neem seed oil. Ivbijaro (1983a) also reported a significant reduction in egg laying of *C. maculatus* on cowpeas mixed with neem seeds. Neem oil treatment also reduced oviposition, inhibited adult emergence and development of *C. maculatus, C. chinensis* and *C. analis* (Yadav, 1985; Sujatha & Punnaiah, 1985; Babu *et al.*, 1989). That Azadirachtin inhibits the release of prothoracicotropic hormones and allatotropins (Banken & Stark, 1997), thereby affecting metamorphosis in insects (Schmutterer & Rembold, 1995) is well documented. It also alters insect behaviour because of its antifeedant and repellent action, and it modifies insect development by inhibiting the release of prothoracicotropic hormones and allatotropins (Mordue (Luntz) & Blackwell, 1993; Williams & Mansingh, 1996). Previous report by Chander *et al.* (1999) showed Nimbicidine® was effective at 20 mg.cm^{-2} and above against *T. castaneum*. In this study, we have demonstrated that Nimbicidine® has a latent effect on *T. castaneum* progenies as manifested by the reduced growth and development of immature and mature stages and the lesser production of adults. The present results imply that the use of natural products like neem extracts as pesticide would help controlling the storage pests in an environment-friendly way.

Table 3. Effects of Nimbicidine® treatment on the developmental periods, pupal and adult recoveries and sex-ratios in next generation *T. castaneum* progenies

Concentrations (ml.kg^{-1})	Developmental periods (days) (Mean ± SE)		Recovery (%) (Mean ± SE)				Sex-ratios
	Larval	Pupal	Pupal	% reduction$^{@}$	Adult	% reduction$^{@}$	♂:♀
0 .0	21.00 ± 0.58	6.00 ± 0.17^{a}	90.48 ± 0.69^{a}	—	90.48 ± 0.69^{a}	—	1:1.08
0.5	21.67 ± 0.17	6.00 ± 0.33^{a}	85.81 ± 0.89^{ab}	5.16	85.81 ± 0.63^{ab}	5.16	1:1.09
1.0	22.33 ± 0.33	6.33 ± 0.33^{ab}	85.03 ± 1.61^{ab}	6.02	83.26 ± 1.34^{ab}	7.98	1:1.09
2.0	22.33 ± 0.60	6.67 ± 0.58^{abc}	83.38 ± 1.87^{ab}	7.85	82.25 ± 2.21^{ab}	9.1	1:1.10
4.0	22.33 ± 0.44	6.67 ± 0.58^{abc}	82.90 ± 2.10^{ab}	8.38	82.90 ± 2.21^{ab}	8.38	1:1.05
8.0	22.50 ± 0.29	7.33 ± 0.33^{bc}	80.86 ± 1.45^{ab}	10.63	80.37 ± 1.53^{b}	11.17	1:1.29
16.0	22.84 ± 0.73	7.67 ± 0.33^{c}	78.31 ± 3.90^{b}	13.45	77.15 ± 3.58^{b}	14.73	1:1.07
F-values	2.34^{ns}	8.11**	5.86**		6.46**		

Means in each column followed by the same letter are not significantly different by Tukey's test ($P < 0.05$); $^{@}$ % reduction over control, $^{**}P<0.01$, ns = not significant

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