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Effects of cypermethrin alone and in combination with leaf and seed extracts of neem against adult *Tribolium castaneum* (Herbst)

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Abstract: Cypermethrin was applied either alone or in combination with leaf and seed extracts of neem (*Azadirachta indica* A. Juss) to four strains of the adult red flour beetle *Tribolium castaneum* (Herbst) and the mortality was assessed after 24 and 48 hrs of treatments. The LD_{50} values were calculated as 0.0072, 0.0105, 0.0056 and 0.0124 μgcm^{-2} after 24 hours and 0.0041, 0.0076, 0.0046 and 0.0097 μgcm^{-2} after 48 hours of treatment for the Local, CR-1, FSS-II and CTC-12 strains respectively. The results showed synergism when applied in combined doses of cypermethrin and leaf and seed extracts with petroleum spirit, ethyl acetate and methanol. Leaf extract in acetone had an insignificant effect showing its inertness with cypermethrin excepting 24 hrs after treatment of Local strain and 48 hrs after treatment of CTC-12 strain. Seed extract in methanol in Local strain showed insignificant effect after 24 hrs of treatment but a synergistic effect occurred after 48 hrs of treatment.

Key words: Cypermethrin, *Azadirachta indica*, *Tribolium castaneum*, synergism

Introduction

The intensification of food production has led to several problems in the post harvest phase including the major concern of pest infestation during storage. This is further aggravated by the increased attention paid to maintenance of buffer stocks to provide food security for a country. Pest problems have increased side by side with the increase in the quantity of food stockpiled and the longer duration of storage. Such pest problems are more acute in the tropics than in temperate zones because the environment in the former is more conducive to the growth and development of pests.

The development of resistant strains of stored grain pests to malathion and more recently to fenitrothion has posed serious problems for the grain industries and has prompted extensive studies of alternative grain protectants. Preliminary work in the laboratory has shown that the synthetic pyrethroids cyfluthrin and cypermethrin have the potential to control prevalent resistant strains of the many storage pests (Bengston *et al.*, 1987).

The search for combinations of pesticides with plant derivatives that have a synergistic effect on the pest, whilst having no detrimental effect on stored product, is an important area of study. Especially natural insecticides, like Rotenone (Brannon, 1947), Ryania dust (Reed & Filmer, 1950) and Sabadilla (Blum & Kearns, 1957) could be synergistic. The neem tree (*Azadirachta indica* A. Juss) is one of the most promising botanical insecticide at present (Khalequzzaman & Islam, 1997; Sutherland *et al.*, 2002; El Shafie & Basedow, 2003; Aliero, 2003; Greenberg *et al.*, 2005) and its products are known to have strong pesticidal properties (Isman, 1999; Walter, 1999; Khalequzzaman & Khanom, 2000; Khanom & Khalequzzaman, 2000; Mancebo *et al.*, 2002). Evaluation of extracts and pure compounds against numerous species of insect pests have demonstrated neem's diverse biological effects: repellence (Ma *et al.*, 2000; Hou *et al.*, 2004), feeding deterrence (Weathersbee & Tang, 2002), reduced growth and abnormal development (Isman, 1993), oviposion deterrence (Singh & Singh, 1998, Lale & Mustapha, 2000, Liang *et al.*, 2003), reduced egg laying due to sterilizing effect (Xie *et al.*, 1995; Rahim, 1998; Lale & Abdulrahmanb, 1999) and also direct toxicity (Makanjuola, 1989, Iloba & Ekrakene, 2006). The present investigations were carried out to observe the effects of cypermethrin as stored grains protectant; and combined effect of cypermethrin with leaf and seed extracts of neem on four strains of adult *Tribolium castaneum* (Herbst).

Materials and Methods

Test insects: Four strains of the flour beetle, *T. castaneum viz*., Local, CR-1, FSS-II and CTC-12 were used for the study. The Local strain was collected from flourmills and other three strains were collected from Crop Protection Lab., Department of Agriculture and Environmental Science, University of Newcastle upon Tyne, U.K. The exotic strains were successfully reared for more than ten years in the Crop Protection and Toxicology Lab., Institute of Biological Sciences, Rajshahi University. The cultures were maintained in jars (1000 ml) and sub-cultured in beakers (500 ml) with food medium and kept in an incubator at 30°±0.5°C. A standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 19:1

(Khalequzzaman *et al*., 1994) was used as food medium throughout the experimental period.

Neem leaf and seed extractions: Neem leaves and seeds were collected and dried in shade. Extractions were done in a Soxhlet`s apparatus with solvent in the process described by Feuerhake & Schmutterer (1982). The solvents used were petroleum spirit, ethyl acetate, acetone and methanol serially on the same stock of leaves and seeds. After completing extraction, the mixed solvent was removed from the extract with a vacuum rotary evaporator.

Mortality tests: Residual film method (Busvine, 1971) was used to test the mortality of the adults of *T. castaneum*. The test insecticide was cypermethrin [(RS)–alpha-cyano-3-phenoxybenzyl (IRS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] commercially available as Fenom 10 EC of Novartis (Bangladesh) Limited. The insecticide was diluted in acetone and different doses were made. The doses were 0.01572, 0.00786, 0.00393, and 0.00196 μgcm^{-2} including a control dose (only acetone). One ml of liquid from each dose was dropped on petridishes (90 mm) separately, covering uniformly the whole area of the petridish. They were then kept open for sometimes to dry-up. Four plastic rings (30 mm) were placed inside a petridish and 10 adult beetles were released within each ring. The rings within the petridish were served as replications. The doses were calculated by measuring the actual amount of active ingredient (μg) present in one ml of the solvent divided by the surface area of the petridish. The mortality was recorded after 24 and 48 hrs of treatment. The mortality percentage was corrected using Abbott's formula (Abbott, 1925), and the observed data was subjected to probit analysis according to Finney (1947) and Busvine (1971).

Before actual experiments ad-hoc trials for each leaf and seed extracts were done to have a clear idea of the dose-mortality response. The dose at which mortality took place between 10-20 percent was selected as low dose. After completion of the above experiments the lowest dose of insecticide (0.00196 μgcm^{-2}) and the lowest doses of neem leaf extracts (33.96, 123.82, 67.76 and 86.27μgcm^{-2} for extracts in petroleum spirit, ethyl acetate, acetone and methanol, respectively) were mixed and thus four combined doses were made. Again another four combined doses were made by mixing the same dose of insecticide and the lowest doses of neem seed extracts (44.41, 155.78, 116.53 and 110.13 μgcm^{-2} for extracts in petroleum spirit, ethyl acetate, acetone and methanol respectively). The combined doses were then applied on four strains of adult *T. castaneum* in the same procedure detailed above.

The mortality was recorded after 24 and 48 hrs of treatment and the mortality was tested using chi-square based on an expected mortality for the sum of the two individual effect as given by Mather (1940) as follows.

$$\chi^2 = \frac{(N_C X_1 X_E \quad X_C N_1 N_E)^2 N_1 N_E}{X_1 X_E N_C \{N_1 N_E (N_1 N_E \quad X_1 X_E) + N_C N_E X_E Y_1 + N_C N_1 X_1 Y_E\}}$$

Where, N_1, N_E and N_C are the total number of insects used in the treatments with cypermethrin, extracts (leave and seed) and their combined doses respectively; X_1, X_E and X_C represent the total number of insects surviving in the treatments of cypermethrin, extracts (leave and seed) and their combined doses respectively; and Y_1 and Y_E denote the number of insects killed in individual treatment of cypermethrin and neem extracts, respectively. Significant chi-square values are expected to indicate that observed mortality of the combined chemicals is greater and synergism may occur. The combined effects on adult mortality were classified on the basis of criteria for synergism (Hewlett, 1960) as described by Benz (1971).

Results and Discussion

The LD_{50} values, 95% confidence limits, regression equations and chi-square values for cypemethrin at 24 and 48 hrs after treatment on adult *T. castaneum* are presented in Table 1. From the regression equations LD_{50} values were calculated and all values of χ^2 showed insignificant indicating the good fit of the regression lines. It was observed that cypermethrin was very toxic to the adult flour beetles and mortality increased with the increasing exposure time. Local and FSS-II strains were more susceptible towards cypermethrin whereas CR-I and CTC-12 were moderately susceptible. CTC-12 is a recognized malathion resistant strain (Lloyd & Ruczkowski 1980) but it was found susceptible to cypermethrin. The LD_{50} values have been calculated as 0.0072, 0.0105, 0.0056 and 0.0124 μgcm^{-2} for Local, CR-1, FSS-II and CTC-12 strains respectively after 24 hours of treatment. The values were 0.0041, 0.0076, 0.0046 and 0.0097 μgcm^{-2} in the above mentioned strains respectively after 48 hrs of treatment.

Table 1. $LD_{50,}$ 95% confidence limits and regression equations for cypermethrin against different strains of adult *T. castaneum*.

Hrs of treatment	Strains	LD_{50} μgcm^{-2}	95% CL (Lower–Upper)	Regression equations (χ^2 at 2 df)
24	Local	0.0072	(0.0051–1.0200)	Y=3.7627+1.4382X (2.26)
	CR-1	0.0105	(0.0071–0.0157)	Y=3.4684+1.4942X (0.24)
	FSS-II	0.0056	(0.0041–0.0076)	Y=3.8879+1.4896X (0.28)
	CTC-12	0.0124	(0.0080–0.0192)	Y=3.3688+1.4925X (0.25)
48	Local	0.0041	(0.0031–0.0053)	Y=3.8061+1.9599X (0.89)
	CR-1	0.0076	(0.0054–0.0107)	Y=3.7317+1.4406X (1.22)
	FSS-II	0.0046	(0.0033–0.0064)	Y=4.0063+1.4959X (0.08)
	CTC-12	0.0097	(0.0066–0.0143)	Y=3.5682+1.4497X (0.68)

Table 2. χ^2-values based on expected mortality of the adult *T. castaneum* for the sum of the individual effects of cypermethrin and neem extracts

Plant parts	Hrs of Treatment	Extraction Solvents	Strains	(χ^2-values)			
				Local	CR-1	FSS-II	CTC-12
Leaf	24	Petroleum spirit	Local	9.76**	7.56**	7.81**	17.57***
		Ethyl acetate	CR-1	7.88**	12.13***	5.35*	7.12**
		Acetone	FSS-II	6.38*	0.901	0.27	1.02
		Methanol	CTC-12	7.72**	13.48***	4.12*	8.40**
	48	Petroleum spirit	Local	15.01***	10.81***	20.16***	24.80***
		Ethyl acetate	CR-1	13.00***	14.97***	13.95***	14.77***
		Acetone	FSS-II	0.456	0.994	2.06	8.81**
		Methanol	CTC-12	11.20***	9.77**	6.53**	12.47***
Seed	24	Petroleum spirit	Local	19.20***	14.97***	12.12***	18.71***
		Ethyl acetate	CR-1	9.77**	17.44***	11.53***	5.03*
		Acetone	FSS-II	0.99	3.02	0.27	3.56
		Methanol	CTC-12	3.62	10.04***	4.30*	4.74*
	48	Petroleum spirit	Local	17.55***	15.26***	10.61***	23.97***
		Ethyl acetate	CR-1	5.71*	6.72**	11.99***	4.33*
		Acetone	FSS-II	1.24	4.83*	2.28	0.99
		Methanol	CTC-12	8.72**	14.48***	3.94*	5.98*

*=P<0.05, **=P<0.01; ***=P<0.001

The results of the combined action of cypermethrin and neem extracts are presented in Table 2. Leaf extracts of neem shows synergistic effect on cypermethrin to adult *T. castaneum*. It was observed that neem leaf extracts in petroleum spirit, ethyl acetate and methanol offered synergism with cypermethrin having all significant χ^2-values after 24 and 48 hrs of treatments. But it was interesting to note that acetone extract having insignificant χ^2-value shows their inertness with cypermethrin excepting 24 hrs after treatment of Local strain (P<0.05) and 48 hrs after treatment of strain CTC-12 (P<0.01). The combined action of neem seed extracts and cypermethrin also showed similar results as shown in leaf extracts. Here extracts in petroleum spirit, ethyl acetate and methanol with cypermethrin showed significant χ^2-values suggesting synergistic effect on *T. castaneum*. Acetone extract again showed insignificant results in all strains except CR-1 at 48 hrs after treatment (P<0.05). It was also interesting that methanol extract in Local strain showed insignificant χ^2-value after 24 hours of treatment but the synergism occurred after 48 hrs of treatment (P<0.01).

The toxic effect of cypermethrin on four strains of the red flour beetle indicated that the CTC-12 was more resistant to cypermethrin than the remaining strains. Champ & Campbell-Brown (1970) reported that CTC-12 was 18.3 times more resistant to malathion than CTC-4. Lloyd & Ruczkowski (1980) reported that the CTC-12 is resistant to malathion. According to Wool & Manheim (1980) CTC-12 is not only tolerant to malathion but also to many other insecticides.

Yadav (1987) studied the toxicity of three pyrethroids viz., deltamethrin, cypermethrin and permethrin against 13 stored grain pests. Karnatak & Khari (1991) reported that deltamethrin and cypermethrin were significantly superior among the synthetic pyrethroids and mortality was directly correlated with the dose concentration. Gul-e-Rukhsana *et al.* (1993) studied the toxic effects of neem oil, deltamethrin and perfecthion against *Callosobruchus analis* under laboratory conditions and the LD_{50} of those compounds were found to be 46.25%, 0.00625% and 0.00084082% for neem oil, deltamethrin and perfecthion respectively. Naqvi *et al.* (1995) determined the toxic and teratogenic effects of Coopex (25 E.C), pyrethroid and neem extract (N-7) against 3rd instar larvae of *Musca domestica* L. where LC_{50} of Coopex was 0.0029% and neem extract was 3.8%.

It is evident from the present results that almost all the combined doses offered synergism having significant χ^2-values. According to Hewlett (1960) and Metcalf (1967) the synergists inhibit the enzymes responsible for toxicant degradation. Othaki *et al.* (1968) and Othaki & Williams (1970) showed that the insect body contains enzymes for the degradation of hormones like the moulting hormone. Azadirachtin, a tetranorterpenoid first isolated from the neem tree, *A. indica* (Butterworth & Morgan, 1971), is known for its potent insecticidal effects. It acts as a phagodeterrent, antifeedant and growth-disrupting substance and is most effective against Lepidoptera (Mordue & Blackwell, 1993).

Leaf and seed extracts used in the present experiment showed to some extent similar synergistic results to that of Dyte & Rowlands (1970) who reported higher mortality of *T. castaneum* adults in combined doses of insecticides (e.g., Fenitrothion, Bromoxon and Malaoxon) and synergists (Sesamex, SKF, 525 and PAOB-1) in comparison with the mortality due to individual action of the chemicals. This result is also similar to that of Ishaaya *et al.* (1983) who reported higher mortality of *T. castaneum* in combined doses of insecticide (e.g., *trans*- and *cis*-cypermethrin) and synergist (piperonylbutoxide). Mondal (1990) also observed a similar effect of an insecticide (pirimiphosmethyl) and a synergist (methylquinone) on *T. castaneum.*

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