TOXICITY OF NICOTINE DERIVATIVES AND THEIR EFFECT ON THE PROTEIN PATTERN OF 4TH IMMATURE STAGE OF RED COTTON STAINER *DYSDERCUS KOENIGII* (FABR) (HEMI PTERA: PYRROCHORIDAE)

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ABSTRACT

Toxicity of Nicotine dust and Nicrotine cyanurate was observed against the 4th immature stage of *Dysdercus koenigii* (Febr.) by injection method. Mortality rams were noted after 24 hours of treatments. LD_{50}'s were calculated as 7.2µg/nymph and 9µg/nymph for nicotine dust and nicotine cyanurate respectively. Changes in the flow of protein metabolites occur with higher and lower doses of both the compounds.

Introduction

Cotton is one of the cash crop of Pakistan. In the year 1979-80 Pakistan had earned 262.5 million and 158.6 million US dollars by exporting raw and yarn cotton respectively (Ahmed 1983). Cotton seeds are also utilized in manufacturing of vegetable ghee and other oil commodities (Rah *et.* at, 1983). Cotton plants suffer heavy damages each year due to attack of various insect pests which destroy its different parts. These pests include borers, bollworms, white flies, aphids and jassids.

The red cotton stainer *Dysdercus koenigii* is one of the pests which attack cotton *when* the bolls are formed. It usually sucks the moisture contents of the cotton seeds and stain the lint by its excreta (Rad *et. al,* 1983).

The use of plants derivatives as pesticides is a very old and traditional *way* of controlling insects. During recent years the Pest management people are diverting their attention towards the natural products for controlling insect pests. The nicotine alkaloids were among the first materials to be used as insecticide and thus can be used as a toxicant to insects. Nicotine penetrates directly into intugement and spiracles. It acts directly on the ganglia of the insect central nervous system, producing excitation at low concentration and paralysis at higher concentration probably due to a direct action at the synapse. McIndoo (1916, Al-Levolt (1917), Morril (1921), Campbell *et. al,* (1933), Westegate and Glover (1934), Savchenko (1936), Teiji *et. al,* (1966), Wiackowski (1968) and Elderirawi *et. al*, (1970) used nicotinoid compounds and solutions for the control of different insect

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pests.

Recently, in Pakistan, Ayuh (1982), Ahmed (1983), Azmi *et al,* (1985), Akhter *et. al,* (1986, 87) and Zaidi *et. al,* (1988) used different nicotine *derivative* for the control of insect pests.

The aim of the present study was to ascertain the suitability of nicotine dust and nicotine cyanurate for the control of this pest.

Material and Methods

a) Collection of insects:

The adults of *Dysdercus koenigii* (Fabr.) were collected from the premises of Karachi University Campus and were kept in the breeding glass chimneys. Approximately six pairs *were* kept in each chimney. Twigs of *Withenia somnifera* (L.) (alternate food plant) were provided as food which were immersed in a beaker of water. Soaked cotton seeds were also provided to the bugs.

b) Rearing technique:

For toxicity determination, the insects were reared following Ahmed and Mohammad (1983) method in the laboratory, to allow the ready made provision of insects of uniform age, size, (disease free) etc. for experiments.

c) Preparation of chemicals:

Samples of dust and cyanurate product were obtained from Dr. M.A.A. Beg, Director of PCSIR Laboratories, Karachi, which were especially prepared using new methodology.

Five percent stock solution of nicotine dust was prepared by using 5mg. of nicotine dust in 95m1 of distilled water. Six different conceal rations vis. 0.25%, 0.5%, 1.0%, 2.0% 3.0%, 4.0% were prepared by applying Charles formula CI Vt = CZVZ. In the same way six concentrations of nicotine cyanurate (extract) were also prepared from the 5% stock solution.

d) Method of treatment:

For the determination of the toxicity of the above compounds-injection method was adopted by using manual micro-applicator so that accurate dose could be injected. The volume of the injection was kept constant i.e. 1 ul/nymph. Newly moulted 4th immature stages *of Dysdercus koenigii* (Fabr.) *were* treated with selected concentrations of nicotine dust and nicotine cyanurate after preliminary experiments.

Eighty nymphs were selected for each experiment. Nymphs were not treated with solvent because the concentrations were prepared in distilled water. Seven concentra-

tions, each of nicotine dust and nicotine cyanurate were injected with 10 nymphs in each hatch. A control batch was also kept for observations. Average values were calculated and mortality curve was drawn on log-log graph paper to fold out the lethal dose (LD_{50}) for each compound.

e) Thin layer chromatography:

To observe the effect of nicotine derivatives on the protein pattern of the immature stage, thin layer chromatography was done on Camag TLC apparatus. For this slurry of silica gel was made by mixing 20 gm of silica gel in 50m1 of distilled water. The mixture was poured into hopper, a film of 500 micron thickness was prepared over the standard 20x20 cm glass plates. The plate were first air dried and then kept in an oven at a temperature of 80°C for complete drying.

Fifteen nymphs each for 0.25% (lowest concentration) and 5.0%(highest concentration) were injected alongwith an untreated (control) batch. After 24 hours these nymphs were taken out, ground and homogenized in 4 ml methanol. For TLC experiments fresh homogenates of treated and untreated (control) insects were then centrifuged at 2500 rpm. for about 15 minutes. The supernatants thus obtained were collected in test tubes and marked respectively for each concentration. The samples were concentrated by evaporation at room temperature. Concentrated supernatants were then spotted on the silica gel coated plates by the help of micorpipette (10µl). After spotting, plates were air dried and were kept for 45 minutes in the chambers having methanol as solvent. Ninhydrin (1% solution), a protein indicator was sprayed on the plates which were then kept in the oven for 5-8 minutes at 80°C to develop protein spots, because some reagents need heating for colouration. The "RF" values of different spots were calculated.

Results

Nicotine Dust Extract

Fourth immature stage of the *Dysdercus koenigii* (Fable) were treated with 2.5, 5.0, 10, 20, 40 and 50 pg of nicotine dust extract having concentrations of 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0% respectively. The mortality rates were 24, 28, 58, 70, 80, 86 and 92% respectively (Table-1) and the LD50 was calculated as 7.2 $_{f}$ig/nymph (Fig.1). Statistical analysis revealed that mortality rate at 95% confidence limit increased with increase in dose. The highest range 76.29 - 107.71 was observed with the highest dose (50 µg).

The nymphal duration was increased to a lesser extent in this compound. In lower doses nymphal period was prolonged upto 4 to 5 days. Treated 4th immature stage took about 8 days to moult into the 5th stage instar which was 4-5 days longer than the normal period. In the same way the nymphal duration from 5th to adult was also observed in lower doses and a slight increase was noted (Fig-2). It was also noted that the adult male and female emerging from the nymph were not able to copulate.

Nicotine Cyamrate Extract

For nicotine cyanuratc seven doses i.e., 2.5, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 fag having the concentrations 0.25, 0.50, 1.0, 2.0, 3.0, 4.0 and 5.0% respectively were injected. Average mortality rates were 28, 42, 64, 68, 70, 74 and 78% respectively (Table-2) and the LD_{50} was calculated as 9 tglnymph (Fig-3).

The morphological abnormalities *were* not observed with this compound as the mortality rate was quite high. It was noted that its lower doses were more toxic than nicotine dust (Table-2). Fourth nymphal duration was increased in the lower doses (2.5 and 5.0 µgs), and took 2-4 days more than the normal ones to moult (Fig-4). Usually most of the 5th instar died but those which survived took a long period to become adult. (Fig-4). These adults were not able to copulate either with each other or with the normal adults.

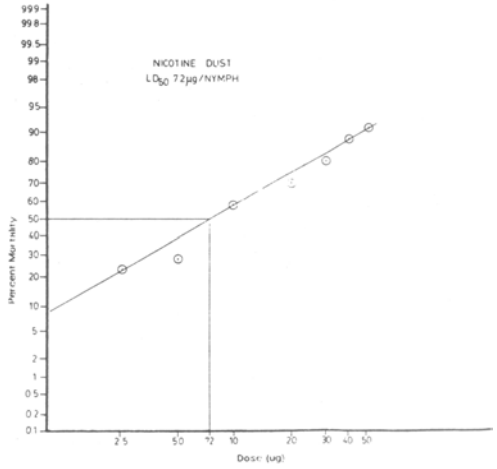


Fig. 1

Period of nymphal prolongation of *Dysdercus koenigii* when treated with nicotine dust

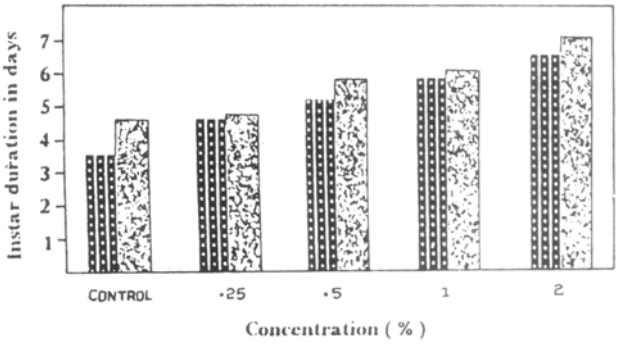


Fig. 2

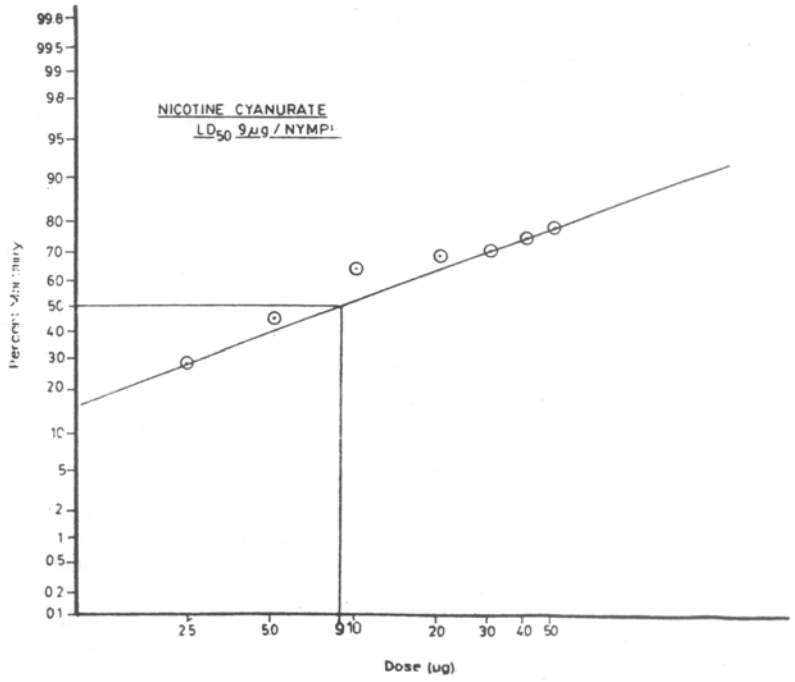


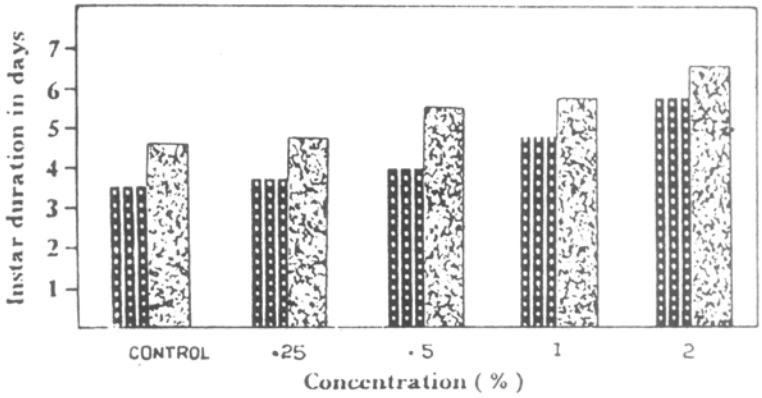
Fig 3

Comparison of Chromatographic Plates

The protein pattern of the 4th immature stage of *Dysdercus koenigii* (Fabr.) was found to be different in treated and untreated nymphs.

In both nidoine dust and nicotine cyanurate the protein metabolities followed the same pattern and it was found that the rate of flow of metabolities in both compounds was quite similar in higher and lower doses. In lower doses the rate of flow was similar to that in the control ones but in the highest dose the metabolities were quantitatively less.

Period of nymphal prolongation of *Dysdercus koenigii* when treated with nicotine cyanurate



The separation of metabolities was not conspicuous in the highest dose and their colour was faint as compared to that in the control ones. The "Rf" values are given in table 3 for nicotine dust and nicotine cyanurate respectively.

Discussion

McIndoo (1916) reported that when nicotine compounds were applied to the larvae of the houseflies, they showed toxicity but no abnormality at any stage. The compounds were applied topically. The present results confirm his findings as no abnormalities and higher toxicity was noted.

Al-Levolt (1917) used nicotine sulphate as a stomach poison and repellent against tent caterpillar (Malacosoma piusialias) and codling moth. The 50% of the codling moth population was killed with 1: 400 nicotine sulphate. In the present experiments the nicotine dust and nicotine cyanurate extract killed 50% of the population of *D. koenigii* at 7.2 µg/nymph and 9.0 µg/nymph respectively.

Morril (1921) used nicotine dust against 3 species of the insects viz grape leaf-hoppers, melon aphid and woolly apple aphid. It was proved to be effective and quick acting against woolly apple aphid. In the present study, the two nicotinoid compounds were used against cotton stainer which is also a heterometabolous insect and thus same results were achieved.

Campbell *et al.* (1933) compared the toxicity of nicotine with anabasine, methylanabasine and luprine against two spp. of culicine mosquito larvae viz *Culex pipiens territans, C. quinquefaciatus*. In the present experiment nicotine dust and nicotine cyanurate extract were used against a bug and similar results were noted.

Table-I

Data of statistical analysis indicating range of mortality at 95% confidence limit after 24 hours of Nicotine dust extract treatments

Dose in μg/nymph	Mortality (%)	S.D.	S.E.	Range at 95% confidence limit
Control	6	±5.47	2.45	1.20 - 10.80
2.50	24	±16.73	7.50	9.29 - 38.70
5.00	28	±21.09	9.45	9.46 - 46.52
10.00	58	±35.63	15.97	26.69 - 89.31
20.00	70	±29,15	13.07	44.35 - 95.12
30.00	80	±14.14	6.34	67.58 - 92.42
40.00	86	±8.98	4.02 7	8.11 - 93.89
50.00	92	±17.80	8.01	76.29 - 107.71

Table-2

Data of statistical analysis indicating range of mortality at 95% confidence limit after 24 hours of Nicotine cyanurate extract treatments

Dose in μg/nymph	Mortality (%)	S.D.	S.E.	Range at 95% confidence limit
Control	8	± 4.47	2.00	4.07 - 11.90
2.50	28	±8.36	3.74	20.30 - 35.34
5.00	42	±13.03	5.84	30.50 - 53.34
10,00	64	±32.86	14.73	32.20 - 92.80
20.00	68	±13.03	5.84	56.00 - 79.45
30.00	70	±35.35	15.84	38.00 - 101.06
40.00	74	±32.86	14.75	45.00 - 103.04
50.00	70	±32.56	14.75	47.12 - 104.88

Table-3

Rf values of Nicotine dust and Nicotine cyanurate treated and untreated five protein metabolities (pm 1-pm 5) in 4th immature stage of *D. koenigii*

	pm-1		pm-2		pm-3		pm-4		pm-5	
	N.D.	N.C.	N.D.	N.C.	N.D.	N.C.	N.D.	N.C.	N.D.	N.C.
CONTROL	0.0625	0.0625	0.1250	0.1250	0.4062	0.2031	0.4062	0.4062	0.6250	0.6250
0.25%	0.0468	0.03125	0.0937	0.0937	0.03750	0.2031	0.3750	0.4062	0.6031	0.5937
0.50%	0.03125	0.0231	0.0937	0.0937	0.1562	0.1718	0.3437	0.3281	0.5781	0.5781

N.D. = Nicotine dust
N.C. = Nicotine cyanurate
p.m. = Protein metabolite

Westegate and Glover (1934) studied the toxicity of nicotine against larvae *of Cuter plpiens* and they proved that it acts as a promising absorptive chemical as they used it topically. In our experiments nicotinoid compounds were tested by injection method and they proved to be effective and toxic.

Savchenko (1936) used nicotine sulphate and anabasinc sulphate against beflies. He proved that nicotine was more potent and only 0.06 was sufficient to kill 50% of the beadly population. In the present studies 1.0% nicotine dust killed the 58% of the experimental population of 4th immature stage of *Dysdernts koenigii.* The slight difference in the results may be due to difference in insect species.

Brooks and Allen (1940) concluded that for the control of cabbage aphid the nicotine dust is generally considered to be the most satisfactory insecticide. In the present studies nicotine dust showed higher toxicity value i.e. by applying only 7.2 µg/nymph 50% of *D. koenigii* (Fourth instar) was killed.

Smith and Beenel (1947) worked on different nicotinoid compounds against cotton aphid and boll weevils of cotton and reported that nicotine and calcium arsenate showed poor result. In the present experiments nicotine dust and nicotine cyanurate proved to be better controlling agents against red cotton stainer. This difference is probably due to the difference of species.

Teiji *et al* (1966) used nicotine alongwith other insecticides against 4th larval stage of *Culex molests.* They proved that nicotine was more effective than other insecticides and its LD_{so} value was also lower. The present experiment proved that the nicotinoid compounds arc toxic to 4th immature stage of cotton stainer and their $_{LD50}$ values were also very low.

Wiackowski (1968) tested various nicotinoid compounds against larvae of *Ghposopa cornea* and reported that nicotine acts as an inhibitor as well as fast acting agent. In the present experiments nicotine dust and nicotine cyanurate also inhibited the metamorphosis of the red cotton stainer.

Elderfrawi *et al.* (1970) observed the mode of action of nicotine in housefly. They found that the nicotine and its derivatives combine with brain receptors and greatly affect the nervous system of the housely. In the present studies nicotine dust and nicotine cyanurate both proved to be toxic and quick acting as they effect the nervous system.

Azmi *et. al* (1985) used nicotine cyanurate solution and nicotine emulsion against houseflly and LD_{95} for nicotine cvanurate was calculated as 14 µg/fly and for nicotine emulsion was calculated as 15.2 µg/fly. Akhtar *et. al* (1986) used nicotine dust against 3rd instar of *M. domestica* and observed the egg laying capacity which was found to be 30.4 egg/fly at high doses.

Zaidi *et. al.* (1988) used nicotine cyanurate against larvae of rice stemborer. They found these larvae susceptibile to nicotine cyanurate and LD_{50} was calculated as 119 µg/larva. The results of the present studies coincide with those of the above authors as the mortality rate was also higher with higher doses of nicotine cyanurate.

Conclusion

On the basis of above data and previous findings, it may be concluded that the nicotine dust and nicotine cyanurate (indegenous products) may prove a reasonably good control agent. However, more work on the physiology and affect on mammals should be undertaken, before its registration as pesticide.

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