Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2014; 2(11): 768-772 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2014.v02i11.006

Research Article

Toxicity of Neem Based Pesticides (Nimbecidine and Neemazal) On the Cotton Pest, *Earias vittella*

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Abstract: In the present study, the toxic effect of Nimbecidine and Neemazal on the cotton pest, *Earias vittella* was evaluated. For Neemazal T/S the doses used were 1.0, 1.5, 2.5 and 5.0 µg/insect whereas for Nimbecidine 0.9, 1.1, 1.4 and 2.0 µg/insect was used. A 24, 48, 72 and 96h toxicity test was performed to determine the LD (10, 50 and 90) values. It was found that toxicity was time as well as dose dependent. The result shows that the cotton pest, *Earias vittella* is very sensitive to Nimbecidine as compared to Neemazal. These pesticides are eco-friendly and gives outstanding results for the control of *Earias vittella*. Field experiments are suggested to elucidate the effects of these biopesticides on *Earias vittella*.
Keywords: *Earias vittella*, Toxicity, Biopesticides, LD_{50}.

INTRODUCTION

Natural enemies, parasitoids and predators are the main sources of reduction in the populations of harmful insect pests [1]. Biocontrol agents and neem extracts have been reported as an ecofriendly options for management of insect pests [2, 3]. Neem oil produced non-toxic effects after spray and acted as antifeedant, growth inhibitor and oviposition deterrent against insect pests [4]. Indiscriminate use of insecticides has resulted in killing of natural enemies and environmental pollution problem on the large scale. Adoptions of integrated pest management (IPM) strategies ensure safety of environment. Botanicals pesticide (Neem oil or garlic bulb extracts and papaya leaves extracts), microbial control (*Bacillus thuringiensis*) and biological control (spider, ant, lady bird beetle, *Orius*, myrid bug, *Laius*, *Chrysoperla*, *Trichogramma* etc.) should be integrated for economic management of insect pests [5, 6].

Neem has systemic activity; it is active at low concentrations and degrades rapidly in the environment [7]. Schuster & Stansly [8] tested Azatin EC on two species of green lacewings and found that the Neem product was not toxic to eggs, larvae and adults, topically or residually. On the other hand, Neemazal T/S (formulation dried residues on glass panes) was harmful to larvae of the lacewing, *C. carnea* (Stephen) causing mortality and difficulties with moulting [9, 10]. The cotton pest, *Earias vittella* was found to be very sensitive to the oil of *Eupatorium capillifolium* and *Callistemon lanceolatus* and the effect was time-dependent [11]. It was also observed that Achook which is a natural product and reported to be safe for non-target animals is also toxic to zebrafish at low concentrations [12, 13]. The low yield of Bhindi is due to insect pest such as fruit and shoots borer (*E. vitella*), red cotton bug (*D. koenigii*), leaf roller and red spider mites [14]. Therefore, to prevail over the threat of food contamination, diverse sources of bio-insecticides are being sought to replace the synthetic insecticides.

Hence, plant derivatives being eco-friendly having good potential as insect control agent [15, 16]. The neem based insecticides in the form of neemoil extract provided a good source for the control of different insect [17]. Cotton is the backbone of our textile industry, accounting for 70% of total fibre consumption in textile sector and 38% of the country's export. The production is reduced because of a large number of insect pests in different stages which hamper its growth. About 1326 species have been reported attacking cotton in the world. In India 166 insect pests is reported occurring on cotton causing 50-60% of loss. Among them only 15 species have been reported so far. Genus *Earias* includes a number of species serving as representatives of such injurious insects that cause severe damage to cotton and other economically important Malvaceous plants in different parts of the world. One of the very common and notorious members of this destructive group of individuals is *Earias vittella* fab. (*Earias = fabia*), commonly known as the spotted

bollworm which is now well recognised as a major lepidopterous pest of cotton (Gossypium sps.) and okra (*Abelmoschus esculentus moench*) in India and elsewhere in the tropics. Therefore, the present study was carried out to study the toxic effect of Nimbecidine and Neemazal on the cotton pest, *Earias vittella*, ensuring the reduction of this harmful pest.

MATERIALS AND METHODS

Under laboratory condition the stock of *Earias vittella* was maintained by procuring infested okra fruits from the local fields. As per requirement, different lots of such infested okra fruits were kept in round all glass aquaria (30 x 25 cm) covered by white muslin cloth tightened with elastic rubber band so as to prevent the escape of the full grown larvae. Inside the infested fruits, the larvae steadily grew till pupation by utilizing all the dietary requirements essentially obtained from the developing seeds. The full grown larva (approx. 2.0 cm in size) which has completed its development inside the okra fruit makes its exit and selects a suitable spot *viz.*, the wall of the glass aquaria, the muslin cloth or even the outer surface of the fruit itself to pupate in a tough silken cocoon. Under optimal conditions, the pupal period lasts for 5 to 9 days at the end of which new generation of adult moths emerges after sunset. The adult individual of *E. vittella* has a wing expanse of about 20 to 25 mm. The moths are pea-green with a wedge-shaped white band running medially from base to outer margin. For each experiment freshly emerged moths were used. 15% sucrose solution was served as the adult food for these moths. The laboratory culture of *E. vittella* was maintained on whole okra fruits at $28^0 \pm 2^0$ C temperature and 80 $\pm$ 10% relative humidity.

Two commercial neem based pesticides *viz.*, Neemazal T/S (Azadirachtin 1.0%, other limonoids 3.0%, oil fatty acids, glycerol esters 46.3%, polyethylene monosorbitol oleate 49.7%) and Nimbecidine (0.03% Azadirachtin, 90.57% neem oil, 5.0% hydroxyel, 0.50% epichlorohydrate and 3.0% aromax) used during the present study were provided by M/S EID Parry (India) Ltd. Chennai and M/S T. Stanes & Co. Ltd. Coimbatore respectively.

For toxicity test, freshly emerged *E. vittella* was procured from the stock culture. The insects were anaesthetized by ether. A 24, 48, 72 and 96 h toxicity test was performed to determine the LD (10, 50 and 90) values. The pesticides were diluted in acetone to make different concentrations as per requirement. The doses were selected by the trial and error methods and were expressed in the terms of μg/insect. The insects were treated topically on the thorax and abdomen by micro pipette not exceeding 0.01 ml/insect.

The toxicity tests were conducted in 250 ml Borosil glass beakers covered by white muslin cloth tightened by elastic rubber bands. Ten insects were placed in each beaker and six such replicates were made for each dose of the pesticide. For Neemazal T/S the doses used were 1.0, 1.5, 2.5 and 5.0 μg/insect whereas for Nimbecidine 0.9, 1.1, 1.4 and 2.0 μg/insect was used. Each experiment was accompanied with a control having same number of insect with same volume of the acetone without the pesticide.

Mortalities of *E. vittella* were recorded for different treated periods *viz.*, 24, 48, 72 and 96 h at different doses. The lethal dose (LD 10, 50 and 90), upper and lower confidence limits (UCL and LCL), slope values, *t* ratio and heterogeneity were calculated by the POLO computer programme [18]. The regression coefficient (r) was determined between treated time and different values of LD_{50} [19].

RESULTS AND DISCUSSION

Toxicity test experiments are the most essential part of the toxicological studies. With these experiments various toxic doses of the toxicant to a particular test animal can be established. The results are shown in tables 1 and 2. It is evident from both the tables that the LD_{50} values decreases with the increase in treatment period. It means that the toxicity of these pesticides increases with the advancement of time. In other words, the mortality of insects increases with increase of time. Table 1 shows that the LD_{50} value of Neemazal T/S after 24 hours was 4.95 μg/insect which decreased to 0.99 μg/insect after 96 h of treatment. Similarly, there was a decrease in LD_{50} value from 1.93 μg/insect (24 h of treatment) to 0.88 μg/insect after 96 h of treatment of Nimbecidine (Table 2). From Tables 1 and 2 it is evident that the Nimbecidine is more toxic than Neemazal T/S. The doses of Nimbecidine required for killing the insect is lower than the doses of Neemazal T/S.

The action of both the pesticides is dose as well as time-dependent. The slope values shown in the Tables 1 and 2 are steep and heterogeneity factors (Chi-square) is less than 1.0 which indicates the result found to be within 95% confidence limits of LD_{50} values. The regression test (*t* ratio) is greater than 1.96 and the potency estimation test, *g* value, is less than 0.5 at all probability levels (90%, 95% and 99%).

Table-1: Toxic effect of Neemazal T/S against *Earias vittella**

Treated period (h)	Effective dose (µg/insect)	Confidence limits LCL (µg/insect)	Confidence limits UCL (µg/insect)	Slope value	t ratio	g value	Heterogeneity
24	LD_{10} 0.55	0.138	0.924	1.343±0.33	4.035	0.236	0.19
	LD_{50} 4.95	3.458	11.581				
	LD_{90} 44.55	16.282	773.322				
48	LD_{10} 0.40	0.176	0.694	1.520±0.32	4.666	0.176	0.28
	LD_{50} 2.83	2.206	4.089				
	LD_{90} 19.71	9.898	101.591				
72	LD_{10} 0.25	0.052	0.485	1.509±0.32	4.616	0.180	0.20
	LD_{50} 1.77	1.299	2.291				
	LD_{90} 12.56	7.018	50.125				
96	LD_{10} 0.25	0.084	0.431	2.159±0.39	5.516	0.126	0.48
	LD_{50} 0.99	0.668	1.244				
	LD_{90} 3.88	2.974	6.389				

*****Batches of ten insects were taken for topical treatment of four doses of Nemazal T/S (diluted in acetone). Mortality was recorded every 24 h. Each set of experiment was replicated six times. The control groups were treated with acetone simultaneously. Regression coefficient showed a significant ($P < 0.05$) negative regression between treated time and different values of LD_{50}.

Table-2: Toxic effect of Nimbecidine against *Earias vittella**

Treated period (h)	Effective dose (µg/insect)	Confidence limits LCL (µg/insect)	Confidence limits UCL (µg/insect)	Slope value	t ratio	g value	Heterogeneity
24	LD_{10} 1.09	0.929	1.209	5.162±0.81	6.41	0.093	9.51
	LD_{50} 1.93	1.744	2.273				
	LD_{90} 3.42	2.762	5.065				
48	LD_{10} 0.57	0.308	0.748	3.063±0.66	4.65	0.177	0.28
	LD_{50} 1.50	1.328	1.805				
	LD_{90} 3.94	2.795	8.915				
72	LD_{10} 0.41	0.154	0.603	2.761±0.65	4.22	0.215	0.24
	LD_{50} 1.21	1.016	1.391				
	LD_{90} 3.52	2.504	8.609				
96	LD_{10} 0.44	0.245	0.581	4.251±0.79	5.36	0.134	0.47
	LD_{50} 0.88	0.714	0.991				
	LD_{90} 1.76	1.539	2.282				

*****Batches of ten insects were taken for topical treatment of four doses of Nimbecidine (diluted in acetone). Mortality was recorded every 24 h. Each set of experiment was replicated six times. The control groups were treated with acetone simultaneously. Regression coefficient showed a significant ($P < 0.05$) negative regression between treated time and different values of LD_{50}.

The insecticidal performance of neem products against most insects is dramatic and its toxicity in varying degrees has been reported. It is evident from the results that the two commercial neem based formulations used during the present study are highly toxic to the cotton pests, *Earias vittella.* The main component present in these formulations is Azadirachtin which is reported to have high insecticidal action [20].

The steep slope values indicate that a small increase in the dose of different treatments given in Tables 1 and 2 causes large mortality in the *Earias vittella* with relatively small increase in the dose of the pesticide. Values of 't' ratio greater than 1.96 indicates that the regression is significant. The ratio of any observed difference to its standard deviation which is less than 1.96 will be non-significant [21]. The Chi-square test for goodness of fit (Heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous. Values of the heterogeneity factor less than 1.0 denote that, in the replicate test of random samples, the dose-response lines would fall within 95 percent confidence limits and thus that the model fits the data adequately. The index of significance of potency estimation, 'g' indicates that the value of the mean is within the limits at all probability levels (90, 95 and 99%) as it is less than 0.5.

A large number of literatures are available on the toxic effect of crude neem extracts and neem based formulations in the recent years. But still report on the toxicity of these formulations in adult is scanty. Qadri

& Narsaiah [22] reported an LD_{50} of 1.5 µg/g azadirachtin against *Periplanata americana.* Similarly, Chavan [23] observed 100% mortality of *Culex pipiens fatigans* in 24 h by a 1% petroleum ether extract with 0.2% neem extract, while such a result was obtained after 0.2% pyrethrum extract. Schmutterer & Zebitz [24] reported LC_{50} of various neem extracts ranging from 55 to 90 mg/kg (=mg/l = ppm) against *Aedis aegypti*. Jaipal *et al.* [25] reported that alcohol, ethyl acetate, benzene and petroleum ether extracts of fresh neem leaves showed pesticidal effect on *Rhyzopertha dominica*.

During the toxicity tests Saeed *et al.* [26] calculated the LD_{50} of NfA (petroleum ether, soluble neutral fraction from ripe neem fruits; mixture of triterpenoids) to be 1.4 µg/housefly. The LD_{50} of NfB (Neutral fraction of winter neem leaves; mixture of triterpenoids) was found to be 5.0 µg/cockroach (German Cockroach, *Blattella germanica*) [27, 28]. Similarly, Chakraborti & Chatterjee [29] investigated the toxicity of 4 neem products (azadirachtin, azadirachtin-iodine, neem seed kernel extract *i.e.*, NSKE and neem oil) on the various developmental stages and the adults of *C. cephalomica.* The neem leaf extract was effective to kill 82% of *H. armigera* larvae treated with 6% of the extract after 96 h of treatment [30].

This proves that the *Earias vittella* is more sensitive to Nimbicidine. Sharma & Lal [31] and Singh & Kumar [32] also reported neem products to effective against leafhopper, okra fruit borer. However, contrary results were reported by Bindu *et al.* [2] and Singh & Sharma [33] where they found that Achook was more toxic than Nimbicidine against *E. vittella* on okra. In present investigation, Neemazal was least effective. The difference in toxicity of Nimbicidine and Neemazal may be due to difference in active ingredient (*Azadirachtin* in ppm).

It is concluded from the present results that the two commercial neem based formulations (Nimbicidine and Neemazal) used during the present study are highly toxic to the cotton pests, *Earias vittella*, and Nimbicidine is more potential than Neemazal. Further, there is a need for a wider comparative survey of the toxicity of biopesticides on insect in biochemical terms also.

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