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Toxic, Residual, and Teratomorphic Effect of a Neem Extract (N-9) in Comparison to Coopex 25 WP (Permethrin + Bioallethrin) against *Musca domestica* L. (Holland Strain)

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Abstract: The toxicity and residual effect of a topically applied crude extract of neem (N-9) and a pyrethroid (Coopex) against second-instar larvae of *Musca domestica* L. (Holland strain) was studied and compared. Toxic and residual studies revealed that Coopex (permethrin + bioallethrin) was more toxic and persistent than N-9. LD_{50} of Coopex 25 W.P. and N-9 was found to be 0.8 and 5.6 µg/larva, respectively; however, deformed pupae and partial emergence of adult flies were observed when only N-9 was applied.

Key Words: *Musca domestica*, neem extract, pyrethroids, residual effect, second instar larva

Introduction

Musca domestica is a serious health threat to human beings and livestock by transmitting many infectious diseases (Khan and Ahmed, 2000a). To control them indiscriminately, pesticides are used, which results in the development of resistance in the insect pests, environmental pollution, and health hazards. Among the scores of pesticides, pyrethroids are widely used for their rapid, paralytic knockdown effect and very low mammalian toxicity. Pyrethroids are safer than other conventional insecticides (Ahmed et al., 2001). In the search for safer pesticides, research on the potential of neem products is being conducted internationally (Khan and Ahmed, 2000a, 2000b) and there is a general trend these days to reduce the risk to human life (Azmi et al., 2006).

The present investigation was an attempt to determine the toxicity and residual effect produced by a pyrethroid, Coopex 25 W.P. (permethrin + bioallethrin) in comparison to a crude neem extract (N-9). Abnormalities produced by N-9 were also observed. Neem products are less toxic, biodegradable, and safe.

Materials and Methods

Toxicity Determination

Rearing of *Musca domestica* L. (Holland strain) was carried out according to Ashrafi et al. (1966) and second-instar larvae were used to test the topically applied compounds. Coopex 25 W.P., a commercial product of M/S. Wellcome Pakistan Limited, which contains permethrin + bioallethrin, and N-9, an extract of sun-exposed dried ripe berries of neem, were obtained from Dr. B.S. Siddiqui of the H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan. Five doses of each compound were used, which were selected after preliminary experiments (Tables 1 and 2). LD_{50} of both compounds was determined by plotting average mortality values of post 24 h treatment on log-probit graph paper.

Residual Effect

The residual effect of both compounds was determined by introducing 20 fresh, untreated second-instar larvae into the feeding medium, which was mixed with a known quantity of pesticides for toxicity

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Table 1. Estimation of the toxicity of a crude neem extract (N-9) against larvae of *Musca domestica* L. (Holland Strain) 24 h after treatment, showing the mortality range at the 95% confidence limit.

Dose (µg/larva)	% Mortality Mean	± S.E.	Range at 95% confidence limit
Control	0	-	–
0.78125	8	3.8	0.552 – 15.448
1.5625	28	6.7	14.868 – 41.132
3.125	40	4.5	31.180 – 48.820
6.25	52	3.8	44.552 – 59.448
12.5	62	6.7	48.868 – 75.132

Table 2. Estimation of the toxicity of Coopex (25 W.P.) against larvae of *Musca domestica* L. (Holland strain) 24 h after treatment, showing the mortality range at the 95% confidence limit.

Dose (µg/larva)	% Mortality Mean	± S.E.	Range at 95% confidence limit
Control	0	-	–
0.15	26	3.4	19.339 – 32.54
0.3	43	3.2	36.728 – 43.272
1.5	51	4.1	42.964 – 59.036
3.0	70	-	70.000 – 70.000
4.5	90	3.7	82.748 – 97.252

determination. Mortality readings were taken after 24 h. A control was also run simultaneously.

Abnormalities

To check for abnormalities after 24 h in the treated feeding medium, live larvae treated with each concentration were transferred separately into untreated feeding medium and observed for abnormalities until adulthood.

Results

Toxicological studies of Coopex 25 W.P. and N-9 revealed that Coopex was a better larvicidal than N-9. Mortality curves were drawn to determine the LD_{50} value of each compound. The data were statistically analyzed as shown in Tables 1 and 2. The LD_{50} of N-9 was 5.6 µg/larva and that of Coopex was 0.8 µg/larvae. Residual studies revealed that Coopex was better than the N-9 because Coopex was effective for 9 days, whereas N-9 was effective for only 3 days (Tables 3 and 4).

However, the N-9 produced certain abnormalities in the development of second-instar larvae up to the adult stage, including deformation of pupae and the partial emergence of adults. These types of abnormalities were observed with all the tested concentrations of N-9. As far as Coopex is concerned, there were no such abnormalities as those caused by N-9.

Discussion

Toxic Effect

Redfern et al. (1981) reported 83% mortality in second- and fourth-instar larvae of *Spodoptera frugiperda* in response to treatment with 10 µg of Azadirachtin (insecticide). In the present case, 4.5

Table 3. Residual effect of a crude neem extract (N-9) on mean mortality percentage (at highest and lowest doses).

Dose (µg/larva)	I Day	II Day	III Day	IV Day	V Day	VI Day	VII Day	VIII Day	IX Day
12.5	62	24	4	-	-	-	-	-	-
0.78125	8	2	-	-	-	-	-	-	-

Table 4. Residual effect of Coopex (25 W.P.) on mean mortality percentage (at highest and lowest doses).

Dose (µg/larva)	I Day	II Day	III Day	IV Day	V Day	VI Day	VII Day	VIII Day	IX Day
4.5	90	85	85	75	56	55.0	33.0	21	11
0.15	26	25	20	11	5	1.6	3.3	-	-

µg/larva (Coopex) and 12.5 µg/larva (N-9) caused 90% and 62% mortality, respectively, but the higher toxicity of Coopex in this case may have been due to the mode of action. N-9 controls the population by physiological mechanisms, like the IGR effect, while permethrin kills by toxic effect. Higher control by Coopex (90%) was due to its toxic action. Ladd (1984) reported that the LD_{50} of Azadirachtin against *Popillio japonica* was 0.1 µg/larva. The LD_{50} reported by Ladd (1984) is very low in comparison to the present report (5.6 µg/larva), which may be due to the different compounds used and tolerance levels of the insects. Ladd (1984) also reported the direct proportionality of the compound to insect mortality. This observation also supports the present report.

Residual Effect

Meisner et al. (1981) reported the residual effect of some neem products on larvae of *Spodoptera littoralis*. In field trials the neem suspensions on sugar beet leaves had the highest residual activity. On cotton, almost no protection of the leaves was obtained with any of the products tested. On lucern, all the products showed good residual activity after 24 h when applied at 0.6% concentration, whereas at 0.2% only the seed suspension was active. In the present investigation, wheat bran was treated with N-9 in the laboratory and second-instar larvae of *M. domestica* L. were introduced into it. The highest concentration (12.5%) showed the highest residual activity (for 3 days), whereas its lowest concentration (0.78%) showed residual activity until the second day after treatment, which means our findings support the previous work by Meisner et al. (1981). Chavan (1984) reported the residual activity of the neem fraction NP-2. According to his report, 100 ppm of NP-2 was effective up to 9 days (68% mortality), whereas a lower dose of 10 ppm was effective for 8 days (76% mortality). In the present work, residual activity was not as promising. This difference between Charvan's results and ours may be due to the fact he used a different insect and the different nature of the neem fraction Chavan (1984) used, a purified fraction of neem (NP-2), whereas we used the crude extract, N-9. Bostanian et al. (1985) reported the residual effect of cypermethrin, fenvalerate, deltamethrin, permethrin, and azinphos-methyl. They reported that these pesticides were effective until the sixth week after treatment. In the present work the higher tested dose, 4.5 mg/larva, was effective for 6 days and caused 55% mortality to tested larvae. It then gradually decreased to 11% mortality in 216 h (9 days). The difference between Bostanian et al.'s (1985) results and ours may be due to different methods of residual analysis employed.

Teratomorphic Effect

In recent years it has been found that neem products disturb the metamorphosis of insects. Outstanding contributions in this field have been made by Rembold et al. (1981). Saxena et al. (1981) reported developmental abnormalities in larvae of *Cnaphalocrocis medinalis* after treatment with 50% neem oil. They observed that moths were unable to emerge from their pupal cases, as in the

present study in which adult *Musca domestica* did not completely emerge from puparium. Therefore, the above report by Saxena et al. (1981) is comparable with our results. Koul (1984) reported a prolonged developmental period, wing lobes, the development of wingless adults, and larval mortality due to application of Azadirachtin on various stages of *Dysdercus koenigii*. In the present study similar results were obtained using N-9 against *Musca domestica*. Haasler (1984) reported that larvae of *Manduca sexta* reared on a treated diet (1 ppm methanolic extract of seed of neem) turned black. He observed certain morphological changes in the larvae of *M. sexta*, which died during their attempt to molt into second-instars. In our study of *Musca domestica* instars, most of the larvae died after 24 h of treatment at the highest concentration and did not molt normally; therefore, this report confirms Haasler's results. Jotwani and Srivastava (1984) reported that treatment of a neem kernel suspension given to larvae of *Haliothis armigera* produced abnormalities in the larvae and pupae, which did not live long enough to produce another generation. In the present study, abnormal pupae were also noted in response to N-9 treatment. Jahan et al. (1990) reported that preimaginal stages of houseflies failed to emerge or succeeded in emerging only partially after treatment with margosan-O (0.03% Azadirachtin). Our report confirms the previous report by Jahan et al. (1990), as a number of adults failed to come out from the puparium.

Instant and residual effects of the pyrethroid Coopex 25 W.P. in comparison to N-9 revealed that both compounds have acute lethal properties. Additionally, N-9 produced certain abnormalities during the development of flies.

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