

TOXICITY OF CRUDE NEEM EXTRACTS (N-4) AND (N-9) AGAINST LATE 2ND INSTAR LARVAE OF *MUSCA DOMESTICA* L (PCSIR STRAIN)

WASIF M. KHAN, S.N.H. NAQVI IMTIAZ AHMAD, RAHIL & TABBASUM AND F.A. MOHAMMAD
Department of Zoology, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

The LD₅₀ of crude neem extract (N-4) and (N-9) was found to be 4.80% and 0.47%, respectively. The range of mortality was found to be increasing (at 95% confidence limit) with increase in concentration of solution.

Introduction

During the recent years the 4th generation pesticides i.e. the plant products (Abmad 1988) specially neem *Azadirachta indica* (A. Juss) are being tried as safer pesticide to human beings and their environment (Proceedings of three international neem conferences 1981-1986). Naqvi (1987) not only reported the effects of various neem extracts against different insects including *Musca domestica* L but also thoroughly discussed his results in the light of recent findings. More recently Akhtar *et al.* (1989) and Naqvi *et al.* (1989) reported the toxicity of extracts of spring and winter neem leaves (NPO and NfD), respectively on various insects, finding them effective. Presently the toxicity of crude neem extracts (N-4) and (N-9) prepared by HEY Research Institute of Chemistry, University of Karachi is determined against late 2nd instar larvae of *Musca domestica* L (PCSIR strain) and the results are compared with those of conventional pesticides in order to vouch the efficacy of the products.

Materials and Methods

Rearing of *Musca domestica* was carried out according to Ashrafi *et al.* (1966) with some modifications. The initial strain was obtained through the courtesy of Dr. (Mrs.) Kahkashan Arshad of Zoology Department, University of Karachi. During the rearing period the temperature was maintained at 28-31 °C and relative humidity 60-70%. When pupation completed, pupae were collected and dried on filter papers. The pupae were kept in the cages for emergence, to start new generation.

Compounds:

The extracts used in the experiments to determine the toxicity were N-4 and N-9, crude neem extracts.

Neem Extract (N-4).

It is an ethanolic extract of ripe berries of neem seed and was provided by Dr. Beena Siddiqui of Dr. Saleem-uz-Zainan Siddiqui's Laboratory of Hussain Ebrabim Jamal Institute of Chemistry (HEJ).

Neem Extract (N-9).

N-9 was also obtained through the courtesy of Dr. Beena Siddiqui of HEJ Institute of Chemistry, University of Karachi. It is methanolic extract of UV exposed dried ripe berries of neem (*Azadirachta indica* A. hiss).

Preparation of experimental concentration

For the treatment of house fly larvae, five different concentrations of each compound were selected after a series of preliminary experiments.

To make five different dilution of N-4 a stock solution of 5% concentration was prepared. For this purpose 5 g of crude N- 4 was dissolved in 100 ml of ethanol. After preliminary experiments 5 different concentrations were selected viz 1%, 2%, 3%, 4% and 5% where as the volume was kept constant at 4ml/5 grams of medium.

For N-9, 5% stock solution was prepared as above. Five different concentrations of neem extract were selected after preliminary experiment. These were 0.15625%, 0.3125%, 0.62%, 1.25% and 2.5% and the volume was kept constant at 4 ml/5 gram of bran for each concentration.

All experiments were done on the late 2nd instar larvae of *Musca domestica* L (PCSIR strain). The compounds were applied with diet of larvae. To check the toxicity of each compound wheat bran was used as feeding media of larvae. Five different selected concentrations were used for each compound. Six small plastic bowls were used for each experiment. Five g wheat bran was taken in each bowl then mixed with six ml of distilled water. Then 4 ml each of different concentrations of pesticide was added in different bowls. Each bowl was marked with respective concentration. After mixing the pesticide in bowl, twenty larvae of housefly of approximately same age were transferred in each bowl. Then each bowl was covered with cloth sheet and tied with the help of rubber bands. After the treatment they were placed on shelf in laboratory. A control batch of untreated larvae was also kept for determination of the environmental effects. If mortality in control batch increased by more than 10%, the experiment was discarded. N-4 was prepared in ethanol and N-9 in methanol. So a check batch of ethanol or methanol was taken to check the solvents effect. Readings were taken after 24 hours. All the experiments were repeated for seven times. Toxicity of both the compounds was tested to determine the LD₅₀. The LD₅₀ of both compounds was determined by plotting average mortality values on log-log graph paper.

Results

Crude Neem Extract (N'-4)

N-4 is found to be a mildly toxic compound. The rate of mortality increased with increase in concentration of solution as shown in Table-i. For same doses of different concentrations the significant values were 11.26-20.74, 25.3-30.7, 20.88-55.11, 36.15-51.85, 43.9-64.01 at the concentrations of 1%, 2%, 3%, 4%, and 5% respectively. The range in check was 7.24-12.76. By plotting average mortality values on log-log graph paper the 24 hours LD₅₀ was found to be 4.80% (Figure 1).

Standard deviation, standard error and range of mortality at 95% confidence limit in *Musca domestica* caused by N-4 is given in the Table-1.

Crude Neem Extract (N-9)

N-9 also proved a mildly toxic extract but more than N-4. Mortality rate increased with the increase in concentration as shown in the Table-2. For same doses of different concentration the significant values were 6.65-29.44, 9.95-61.59, 48.42-61.58, 69.26-90.74 and 73.58-102.42, respectively. By plotting average mortality values on log-log graph paper the 24 hours LD₅₀ was found to be 0.470%.

Standard deviation, standard error and range of mortality at 95% confidence limit in *Musca domestica* caused by N-9 is given in the Table-2.

Table-1

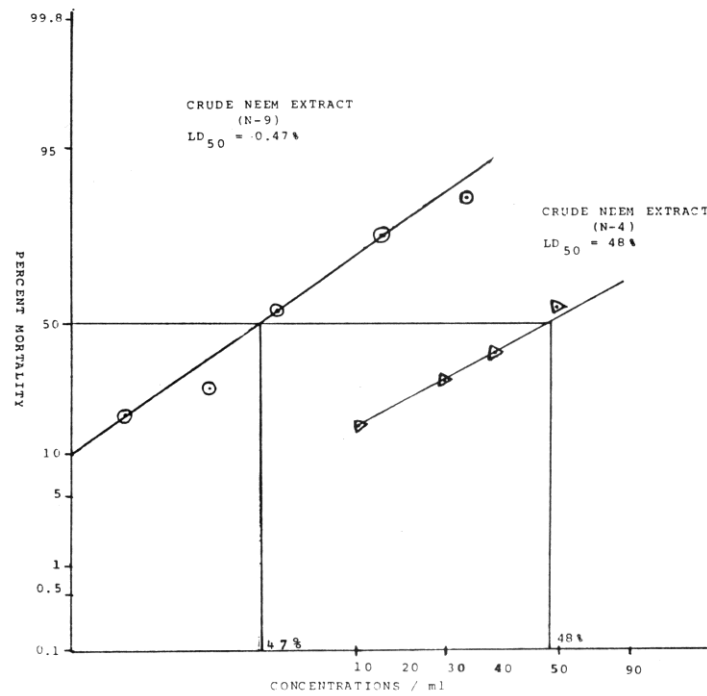
Statistical analysis of the toxicity data of (Neem Extract) N-4

Concentration	Mortality Mean %	S.D.	S.E.	$R = X \pm 1.96$ X S.E.
Control	0	+ 0	+ 0	0
Check	10%	+ 3.16	+ 1.41	7.24 - 12.76
1%	16	+ 5.4	+ 2.42	11.26 - 20.74
2%	28	+ 4.47	+ 1.38	25.3 - 30.7
3%	38	+ 19.49	+ 8.73	20.88 - 55.11
4%	44	+ 8.49	+ 4.008	36.15 - 51.85
5%	54	+ 11.4	+ 5.11	43.9 - 64.01

Table-2

Statistical Analysis of the Toxicity data of N-9 (Neem Extract).

Concent Ration	Mortality Mean %	S.D.	S.E.	R = + 1.96 X S.E.
Control	0	± 0	± 0	0
0.15625	18	± 13.03	± 5.84	6.65 - 29.44
0.3125	26	± 18.16	± 8.14	9.59 - 61.59
0.625	55	± 7.5	± 3.36	48.42 - 61.58
1.25	80	± 12.24	± 5.48	69.26 - 90.74
2.5	88	± 16.43	± 7.36	73.58 - 102.42



Discussion

Qadri and Narsaiah (1978) and Redfern *et al.* (1981) used 13 mg/g and 10.0 mg/insect of Azadirachtin against *Periplaneta americana* and 3rd and 4th instar larvae of *Spodoptera frugiperda* to obtain 50% and 83% mortality respectively. The present 4.80% and 0.470% LD_{50} values of two crude extracts (N-4) and (N-9) neem compounds probably show variation in the result because of variation in insects and because the exact amount of azadirachtin in these extracts is unknown. But 54% and 88% mortality presently obtained certainly confirms the above results and demonstrates the efficacy of the neem extracts used presently. Chavan's (1984) report of obtaining 100% mortality of *Culex pipiens fatigans* in 24 hours at 1% concentration of the neem leave extract and that of Hiram *et al.* (1984) of the efficacy of a crude neem seed extract against *Liriomyza saliva* and *L. trifolii* also confirm the above conclusion.

Arnason *et al.* (1985) also reported that azadirachtin used as a diet for 3rd instar larvae of european cornborer *Ostrinia nubilalis* (Hubner) with 1 and 10 ppm caused 100% mortality. The LD_{50} of 0.0392 mg/sq. cm obtained by Naqvi *et al.* (1989) of tetranortriterpenoids extracted from neem tree against one week old rice weevil *Sitophilus oryzae* and efficacy of NED (Neutral fraction of winter neem leaves containing nimocinolide and isonimocinolide as major components) also probably show promise in this direction.

Acknowledgement

We are grateful to Dr. (Miss.) Beena S. Siddiqui, Associate Professor of HEI Research Institute of Chemistry, University of Karachi for providing Crude Neem Extracts (N-4) and (N-9).

Special thanks and sincere gratitudes are also due to Mr. Habib Alam, Stenographer in the Office of the Dean, Faculty of Pharmacy, University of Karachi, for composing this technical manuscript.

References

- Ahmad, I. (1988). Shifting priorities of plant protection. *Pakistan J. Ent. Kar.* **3**(2): 145-157.
- Akhter, K., Naqvi, S.N.H. and Azmi, M.A. (1989). Determination of toxicity, fecundity and emergence inhibiting effects of Neem factor (NFD) against *Callosobruchus antis* C. Z. *Ang. Zool* (in press).
- Arnason J.T., Philogene, B.J.R., Donakov, N., Hudon, M., Mcdougall, C. and Nozzolillo, C. (1985). Antifeedant and insecticidal properties of azadirachtin to the European corn borer, *Ostrinia nubilalis*. *Entomological exp. appl.* **31**: 15-23.
- Ashrafi S.H., Muzaffar, S.A. and Anwarullah, M. (1966). Mass rearing of mosquitoes,

- houseflies and cockroaches in PCSIR laboratory for insecticide testing. *Sci. Ind.* **4**: 312-316.
- Chavan, S.R. (1984). Chemistry of alkanes separated from leaves of *Azadirachta indica* and their larvicidal / insecticidal activity against mosquitoes. *Proc. 2nd Int. Neem. Conf.* (Rauischholzhausen, 1983). Eschborn, pp.59-69.
- Hiram, G. L. and Ralph, E. W. (1984). Neem seed extract and agromyzid leaf miner. *Int. Cong. Entomol.* **17**: 864-865.
- Naqvi, S.N.H. (1987). Biological evaluation of fresh neem extracts and their components with reference to abnormalities and esterase activity in insects. *Proc. 3rd Int. Neon Conf.* (Nairobi, 1986). Eschborn, pp.315-330.
- Naqvi, S.N.H., Akhtar, K. and Azmi, M.A. (1989). Toxicity of NFD against *Sitophilus Oryzae* L. exposed to impregnated filter paper and its effects on phosphatases and protein metabolites *Z. Ang. Entomol.* (in press).
- Proceedings *1st. Int. Neem Conf. Rottach-Egem*, 1980 (1981). Eschborn, pp.297.
- Proceedings *2nd Int. Neem Conf. Rauischholzhausen*, 1983 (1984). Eschborn (1981). pp. 587.
- Proceedings *3rd Int. Neem Conf. Nairobi 1986* (1987). Eschborn. pp.703.
- Qadri, S.S.N. and Narsaiah, J. (1978). Effect of Azadirachtin on molting processes of last instar nymphs of *P. americana* *Indian J. Exp. Biol.* **16**(11): 1141-3.
- Redfern, R.E., Warthen, J.D, Uebel, E.C. and Mills, G.D. (1981). The antifeedant and growth disruption effects of azadirachtin on *Spodoptera frugiperda* and on *Oncoopeltus fasciatus*. *Proc. 1st Int. Neem Conf Rottach-Egern*, 1980. Eschborn, pp. 129-136.