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Toxicity of Neem Leaves Extract (NLX) Compared With Malathion (57 E.C.) Against Late 3rd Instar Larvae of *Culex fatigans* (Wild Strain) By WHO Method

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Abstract: Among the filariaris causing mosquitoes *Culex fatigans* is considered an important intermediate host in South-East Asia. Toxicity of ethanolic extract of neem leaves (NLX) is compared with an organophosphate pesticide malathion (57 E.C.). The LC_{50} value of malathion (Sisthion) determined presently is 0.45 ppm against late 3rd instar larvae of *Culex fatigans* (wild strain) and of neem fraction (NLX) is 390 ppm by standard WHO method.

Neem Yaprağı Özütünün (NLX) *Culex fatigans* (Yabanıl Suş) Geç 3. İnstar Kurtçuğunun Karşı WHO Yöntemiyle Tespit Edilen Toksisitesinin Malathionunkiyle Karşılaştırılması

Özet: Filariasis enfeksiyonuna sebep olan sivrisinekler arasında *Culex fatigans* Güneydoğu Asya'da önemli bir ara konakçı sayılmaktadır. Neem yaprağının etanolik özütünün (NLX) toksisitesi, organofosfat böcek zehiri olan malathionunki (57 E.C.) ile karşılaştırılmıştır. Malathionun (Sisthion) *Culex fatigans* (yabanıl suş) geç 3. instar kurtçuğuna karşı WHO yöntemiyle tespit edilen LC_{50} değeri 0,45 ppm, neem özütününki (NLX) ise 290 ppm'dir.

Introduction

Conventional pesticides such as malathion, DDT and pyrethroids are generally used for mosquito control but they cause the problems of environmental pollution, residual effect and resistane by their indiscriminate use.

In recent years malathion has been used for mosquito control in Pakistan, which has produced problem of resistance. Azmi et al. (1), Guneidy et al. (2), Kawakami (3) and Chen Wen-Mei (4) have reported resistance against malathion. Hussein and Fon (5), Rawlins and Singh (6) and Wesson (7) have reported susceptibility to OP compounds *Aedes albopictus*.

Due to the problem of pollution and resistance safe plant products are being tested round the globe as apest control agents. Qadri and Narsaiah (8), Chaven (9), Schumutterer and Zebitz (10), Rao et al, (11) Flint and Nancy (12), Islam et al. (13), Tanzubil and McCaffery (14), Alsharook et al (15), Khan et al. (16) and Nicol and Schmutterer (17) have tested various fractions of neem plant on different pests. We have, tested Locally prepared neem fraction (NLX) as a mosquito control agent, because it is safe and indigenous product.

Materials and Methods

Experimental insect

Culex fatigans (wild strain) was taken as experimental inscect becuse of its role as a vector of pathogens.

Rearing Tehnique

We have reared *Culex fatigans* (wild strain) according to technique of Ashrafi et al. (18) and used only late 3rd instar larvae in experiments. The larvae were collected from Gulshan-e-Iqbal, Karachi. The egg strips were immersed in water in plastic bowls of 6" diameter. The lst instar larvae which hatched within 48 hours were transferede in other bowl cantaining tap water or maintaining culture of similar ages and instars.

The male mosquitoes were fed on cotton pad soaked with 6% glucose solution, while the females were fed on blood diet twice a week by hand-feeding or by rat-feeding. During the rearing period the temperature was maintained at 28-31°C and relative humudiy 60-70%.

Pereparation of experimental concentration

Neem leaves extract (NLX)

10% stock solution was prepared by dissolving 9 ml of neem leaves extract in 1 ml of ethanol because neem leaves extract does not dissolve in water. This solution was used for making further dilutions. After preliminary experiments the final concenrations used in the experiments were 300, 350, 450 and 500 ppm.

Malathion (57 E.C)

1% stock solution was prepared and from the stock solution further dilutions were prepared and the concen-

trations used were 0.078, .0156. 0.312, 0.625 and 1.250 ppm. These concentrations were selected after a series of preliminary experiments.

Medhod of Treatment

Late 3rd instar larvae of *C. fatigans* (wild strain) were selected for the treatment following WHO immersion method. Five different concentrations of each compound were selected, for neem 300, 350, 400, 450 and 500 ppm and for malathion 0.078, 0.156, 0.3125, 0.625 and 1-25 ppm. respectively. Seven beakers of 250 ml were taken for neem and labelled with the above five cocentrations in addition to one for check and one for control.

Similarly six beakers of 250 ml were taken for malathion labelled with five concentrations in addition to one for control. In ecah beaker respective doses of neem leaves extract (NLX) and malathion were pipetted. In case of control water and for check 1 ml of eathanol was added. The volume of each beaker was made upto 200 ml by adding distilled water. A duplicate set was arranged for each experiment.

Twenty late 3rd instar larvae of *C. fatigans* (wild strain) were introduced in each beaker and kept for 24 hours. After 24 hours the mortality in each beaker was noted separately. Each experiment was repeated 10 times. Moribuand larvae were counted as dead.

Average values were calculated and mortality curves were drawn on log-log graph paper to find out LC_{50} for each compound.

Calculation

Equations used for statistical calculations were as follows:

$$S.D. = \sqrt{\frac{\sum x^2 - n(\bar{x}^2)}{n - 1}}$$

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

Where,

$\sum x^2$ = The notation for varianceof variable x.

n = The total number of observations.

x = The avarage of variable x.

Results

Toxicity of neem leaves extract (NLX)

Mortalities after 24 hours were 8, 50, 68, 84 and 92% for the concentrations used (300, 350, 400, 450 and 500 ppm respectively, Table 1). The maximum range of mortality at 95% confidence limit was found to be 85.53-98.48 ppm. By plotting average values on log-log graph paper the LC_{50} was found to be 390 ppm (Fig. 1).

Table 1. Estimation of toxicity of neem leaves extract (NLX) after 24 hours of treatment showing mortality range at 95% confidence limit

S.No.	Concentration in ppm	Mean% mortality	S.D.	S.E.	Range at 95% confidence limit
1.	Control	-	-	-	-
2.	Check	-	-	-	-
3.	300	8	4.47	2.005	4.07-11.93
4.	350	50	14.14	6.340	37.57-62.42
5.	400	68	16.43	7.360	53.57-82.42
6.	450	84	8.94	4.008	76.14-91.85
7.	500	92	10.95	4.910	85.53-98.48

Toxicitly of Malathion (57 E.C.)

Mortalities after 24 hours were 14, 28, 40, 56 and 74% for concentraitons used (0.078, 0.156, 0.3125, 0.625 and 1.25 ppm respectively, Table 2). The maximum range of mortaltiy at 95% confidence limit was found to be 69.20-78.80 ppm. By plotting average values on log-log graph paper the LC_{50} was found to be 0.45 ppm (Fig. 2).

Table 2. Estimation of toxicity of malathion (25 E.C.) after 24 hours of treatment showing mortality range at 95% confidence limit

S.No.	Concentration in ppm	Mean% mortality	S.D.	S.E.	Range at 95% confidence limit
1.	Control	-	-	-	-
2.	0.078	14	5.47	2.44	9.20-18.80
3.	0.156	28	10.24	4.58	19.02-36.98
4.	0.3125	40	2.00	0.89	38.24-41.75
5.	0.625	56	8.94	4.00	48.16-63.84
6.	1.25	74	5.47	2.44	69.20-78.80

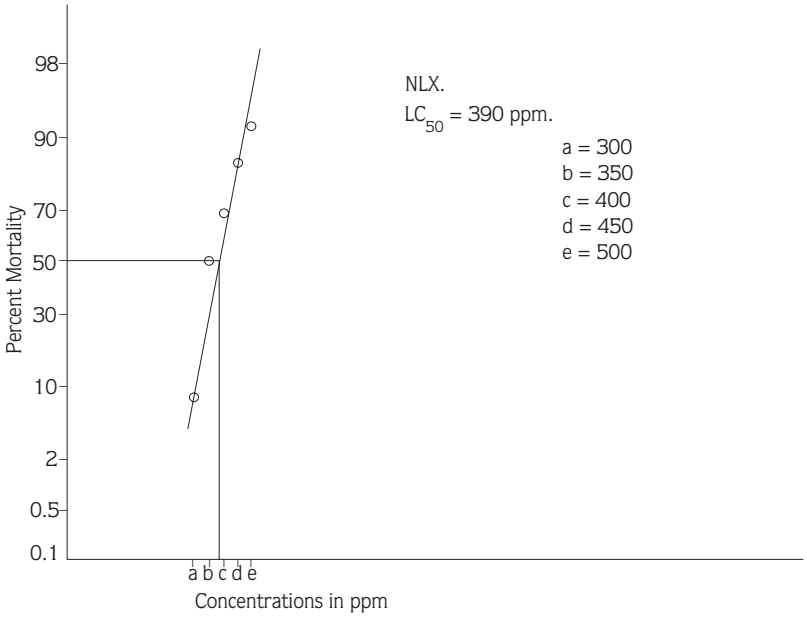

Figure 1. Mortality curve of *Culex fatigans* for the determination of LC_{50} of neem leaves extract (NLX).

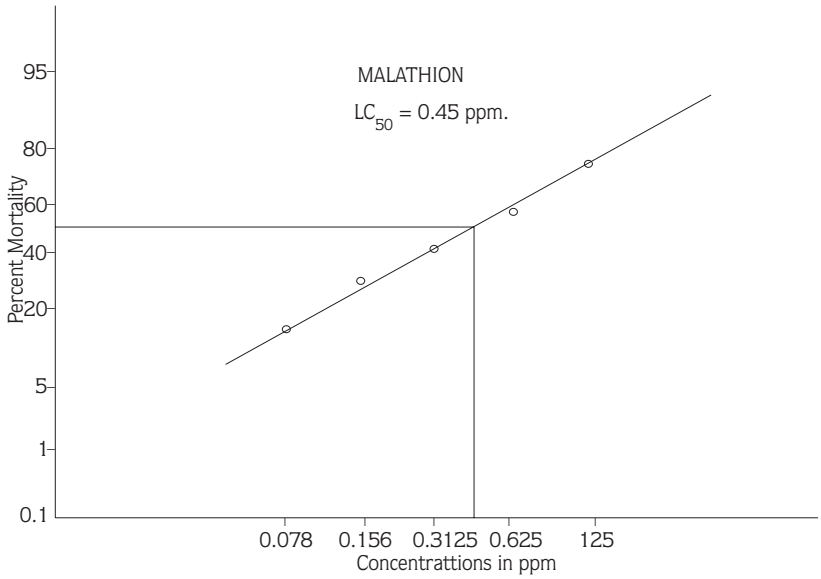

Figure 2. Mortality curve of *Culex fatigans* for the determination of LC_{50} of malathion.

Discussion

On the basis of the result obtained presently malathion was found to be much more toxic than neem leavs extract (NLX) due to the fact that it is a neurotoxic poison, that kills the larvae as well as the adults by rapidly disturbing the normal functions of the nervous system.

Qadri and Narsaiah (8) reported LD_{50} value of azadirachtin against *Periplaneta americana* as 1.5 mg/g after 24 hours. In the peresent finding the LC_{50} of NLX was found to be 390 ppm against *C. fatigans* (wild strain). Present findings are in accordance with Qadri and Narsaiah (8) although there is a difference in insect species as well as a difference in mode fo treatment. It could be concluded that azadirachtin is equally effective to both types of inscet.

Khalid (19) reported the effect of Margosan-O™, RbU-9 and a RBb against the 4th instar larvae of *C. fatigans* (wild strain) and *Aedes aegypti* (PCSIR strain). LC_{50} values of different neem fractions were 154.1, 502 and 545 ppm respectively against *C. fatigans* and 148, 475 and 575 ppm against *A. aegypti*, respectievely. In the present results the LC_{50} of neem fraction (NLX) was found to be 390 ppm against *C. fatigans* (wild strain). The present result therefore is in accordance with that of Khalid (of. cit.) and shows that neem fraction (NLX) is effective against *C. fatigans*.

Mwangi and Rembold (20) reported LC_{50} values as 50 µg)ml of *Melia volkensii* against *A. aegypti* larvae. In the present work the LC_{50} of neem leavs extract (NLX) was found to be 390 ppm against *C. fatigans* (wild strain).In view of Mwangi and Rembold (20) report neem extract (NLX) was found less effective in comparison with Melia sp. extract. The difference in LC_{50} values between the two reports could be due to dufference in atcive ingredients contained in the two plant species, or method of extraction and difference in insect species as well.

Naqvi et al. (21) reported the effect of different neem fractions i.e. Margosan-0, RBa, RBA and OP malathion against white flies *Aleurolobus barodensis* after 48 and 96 hours treatment and found that neem fractions were effective and toxic even after 96 hours in comparison to malathion. In the present findings neem fraction (NLX) was also found effetive against C. fatigans but the residual effect was not noted.

Nurulain *et al*. (22) worked on different neem extracts i.e. RBa, RBA and Margoson-O™ and also malathion against *Oxycarenus lugubris* Motsch. in laboratory. The LD_{50} values of compounds after 24 hours were 5.8, 9.2, 0.17 and 0.0039% respetcively. They reported that percent mortality was directly proportional to the concentration of the compound. In the present investigation the LC_{50} for NLX and malathion was found to be 390 and 0.45 ppm against *C. fatigans*. Toxicity data indicated that with increase in concentration there was a significant increase in percent mortality. Thus Nurulain *et al.'s* (22) results appear comparable with the present results.

Tanzubil and McCaffery (14) reported that high dose upto 10µg/larvae of azadirachtin resulted in 100% mortality of african army worm. They reported that at lower dose abnormal pupae were produced. In the present finding as shown in mortality curve (Fig. 17 neem extract (NLX) containing azadirachtin also was more effective at relatively high dose against *C. fatigans*. By comparing the two results it could be said that there is silght difference between the two reports which may be due to difference in inset species.

Al-Sharook *et al*. (15) reported that *Melia volkensii* was equally toxic for larvae and puapae and *Melia azaderach* was found toxic for larvae only, having LD_{50} of 30 µg/ml and 40 µg/ml respectively against *C. pipiens* molestus. They reported that pure azadirachtin was aqually toxic for both larva and pupae. In the peresent work it was observed that neem leaves extract (NLX) was toxic for larvae with LC_{50} of 390 ppm but inhibiting effect was not noted in pupae of *C. fatigans*. When the two results were compared it was noted that azadirachtin is equally toxic for the larvae of two *Culex spp*. Some difference between the Al-Sharook et al. (15) reports and the present one could be due to the difference in plant extract and *Culex* spp.

Freisewinkle and Schumetterer (23) discussed the contact effect of neem oil ranging from 0.25-1.0 ml/m^2 against five nymphal instars of Locusta migratoria migratorioides and found increased mortality and morphogenetic effect on different parts of body. In the present results there was also an increased mortality with the increase of dose of NLX against *C. fatigans* (late 3rd instar larvae) Table 1, Fig. 1). Both results therefore support each other and confirm that neem is toxic to all kinds of inscets giving increased mortality with the increase of dose.

Khan *et al* (16) investigated the LD_{50} of crude neem extracts (N-4) and (N-9) as 4.80% and 0.47% against late 2nd instar of houseflies, Musca domestica (PCSIR strain). Similarly in the present work as the concentration of neem leaves extract (NLX) increased an increase in percent mortality of late 3rd instar larvae of *C. fatigans* (wild strain) was observed. There is, however, a silight difference in Khan et al. (16) result and the present one probably due to difference in insect species. A great deal of difference was also in the experiment, the flies were laboratory reared and in the present case the mosquitoes were fields collected.

Naqvi *et al*. (24) determined the toxiciy of crude neem extract (NfD) against *A. aegypti* (PCSIR strain) and found the LC_{50} as 0.58 ppm of NfD, nimocinolide 0.625 ppm and isonimocinolide 0.47 ppm and also abnormal larvae, pupae and intermediate stages were reported. In the present results the LC_{50} of neem fraction (NLX) was found to be 390 ppm against *C. fatigans* (wild strain). By comparing both results it was observed that neem compound they, used were more effective than the neem fraction used in the present study. However, even the neem leave's fraction was found effective against present *Culex* sp. The

difference may also be due to the fact that *Aedes aegypti* (PCSIR strain) are laboratory reared while *C. fatigans* are field collected which may have higher tolerance level as reported by Azmi et al (25) and Naqvi *et al*. (26)

Rovesti and Deseo (27) described that neem seed kernel extract was more effective at low dose of 1.25 g/L and gave 80-100% mortality of *Leucoptera malifoliella* Coast. In the present finding treated larvae of *C. fatigans* (wild strain) with neem leaves extract (NLX) showed 80% mortality. Both results are almsot identical with slight difference that colud be due to difference in insect species.

Fenally it may be concluded that neem extracts are safer and effective and therefore can be used as indigenous pest control agent. Moreover, no reistance has been reported against them so far (28).

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