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TOXICITY AND IGR EFFECT OF TWO NEW NEEM PRODUCTS AGAINST AEDES AEGYPTI (PCSIR STRAIN)

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ABSTRACT

Crude extract and isolated compounds from neem, *Azadirachta indica* and *Melia azedarach* (Meliaceae) are being tested in various countries. The present study deals with the effect of two new compounds and their parent fraction from the fresh winter neem leaves, isolated by Siddiqui et. al., 1986. The LCso of NFD is 0.58 ppm, nimocinolide 0.625 ppm and isonimocinolide 0.47 ppm. Abnormal larvae, pupae and intermediates were also found.

Introduction

Due to various problems created by the use of synthetic pesticides, entomologists have diverted their attention towards natural plant products. Among these, extracts and isolated compounds from neem, *Azadirachta indica* and *Melia azedarach* (Meliaceae) are being tested in various countries. This work has been reviewed by various workers (Chiu, 1984; Jacobson, 1986; Schmutterer, 1984). In addition Ahmed et al., (1984) have summarized the effect of a number of plant products. On the chemistry of these products Siddiqui (1942), Butterworth and Morgan (1971), Nakanishi (1975), Chavan (1984), Kraus et al., (1981, 1986), Rembold and Foster (1986), Morgan (1981) and Broughton et. al., (1988) have done a lot of work.

The present paper deals with the effect of two new compounds and their parent fraction from the fresh winter neem leaves, isolated by Siddiqui et al., (1986) against yellow fever mosquito, *Aedes aegypli* (PCSIR strain). Naqvi (1986) has briefly mentioned about it in the Third International Neem Conference and the work on other aspects is in progress at the Toxicology Lab. of Zoology Department, University of Karachi.

Materials and Methods

Samples were obtained from Professor Dr. Salimuzzaman Siddiqui, FRS, Director, H.EJ. Research Institute of Chemistry, University of Karachi.

1. NFD: Neutral fraction of winter neem leaves extract, which contained mostly Nimocinolide and iso-Nimocinolide and some other tetranortriterpenoids in small quantity.

2. NP1: Nimocinolide with molecular formula as $C_{28}H_{36}O_7$ and melting point, 160°C.

3. Iso: Iso-Nimocinolide with molecular formula as above but melting point, 165°C. (Structural formula- Fig. 1.)

One percent stock solutions of the above samples were prepared in acetone (analar grade) and five different concentrations, (0.5%, 0.25%,0.125%,0.0625% and 0.03125%) were prepared by diluting the respective stock solution. These dilutions were used during experiments, in such a way that final concentrations were 2.5 ppm, 1.25ppm, 0.625ppm, 0.312ppm and O.156ppm, for treatment.

Rearing of *Aedes aegypti* (PCSIR strain) was done according to Ashrafi et. al., (1966) and 4th instar larvae of about 12 hrs age were treated with different concentrations according to WHO method. For this purpose beakers of 250 ml capacity were taken and labelled with respective concentration, in duplicate. Control and check (with acetone only) were also kept simultaneously. In each beaker different concentrations of the samples were added and the volume was made upto 200 ml. Then 20 4th instar larvae were taken in small beaker with 50 ml water, in each case. This water with larvae was poured in the treated beaker, respectively, to make the total volume as 250 ml. These beakers were kept in a room at 28±2°C, andd after 24 hrs readings were taken for mortality. Each experiment was repeated 7 times and the data were analysed statistically. Probit-mortality curves (Fig. 1) were made and observations for growth abnormalities were also taken for one week

Table-1.

Percent abnormality caused by NfD and its components, in *Aedes aegypti*.

Dose in ppm	NfD	Nimocinolide	Iso-nimocinoide
0.156	10 ± 2.23	6 ± 2.10	8 ± 1.82
0.312	28 ± 3.05	29 ± 3.12	18 ± 2.27
0.625	54 ± 4.00	50 ± 3.02	40 ± 2.92
1.250	88 ± 1.82	77 ± 4.22	72 ± 3.20
2.500	97 ± 3.05	93 ± 3.20	87 ± 2.12

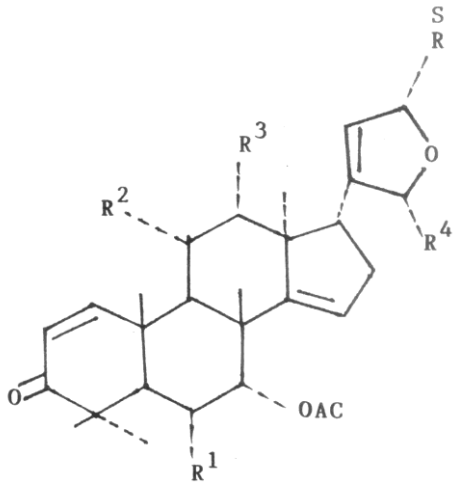

Figure 1. R^1 = OH, R^2 = R^3H, R^4 = O, R^5 = OH = Nimocinolide R^1 = OH, R^2 = R^3 = H, R^4 = OH, R^5 = O = Iso-nimocinolide.

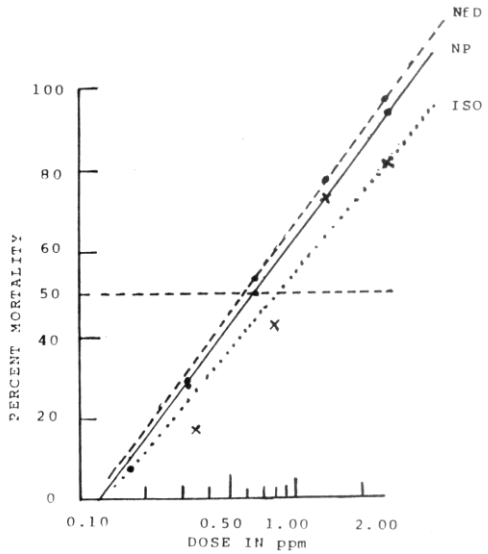

Fig. 2. Toxicity curve showing percent mortality of *Aedes aegyoti* larvae.

Results and Discussion

Experiments indicated that the parent fraction (NFD) is slightly more toxic with LCso as 0.58 ppm in comparison to the individual components, i.e. nimocinolide with LCso as 0.625 ppm and iso-nimocinolide with LCso 0.47 ppm (Fig.2). The slightly higher toxicity of parent fraction may be either due to some minor component present in it or the joint action of the components. However, the difference is very little. The difference may be due to change in the Rt, R2 etc. and 02 positions in the two isomers.

Although much work has been done on the neem fractions and isolated compounds (Scbmutterer, 1984; Chavan, 1984; Rembold and Foster, 1986 and Schwinger et al., 1984), but mostly on the agriculture pests and few reports are available on their effect on mosquitoes. In this connection Chavan (1984) has reported the effect against *Culex pipiens fatigans*. He reported that 1% petroleum extract produced 100% larval mortality and 0.2% solution had residual effect up to 6 days. According to him benzene eluted sample gave 100% larval mortality at 10 and 100 ppm and the treatment was effective for 8 days. These doses are much higher as compared with the doses of the present samples. It indicates that either the present samples are more effective due to extraction from fresh winter neem leaves or the *Culex pipiens fatigans* is more tolerant as compared to *Aedes aegypti*. The work on the comparison among two genera and residual effect is in progress and will be reported soon. Similarly, Schmutterer and Zebitz (1986) reported LCso of various neem extract samples, from seed kernel, ranging from 54.9 mg/kg to 89.09 mg/kg against *Aedes aegypti*. According to calculations it also comes to 50-90 ppm approximately. This is again much higher dose than that of the present samples. However, Zebitz (1986) reported the LC_{50} of an enriched extract (AZT-VR-KE) 1.19 ppm against *Aedes togoi* and 18.1 ppm against *Aedes aegypti*. The dose in the former case is quite close to the present ones but it is perhaps related with the higher susceptibility of the species (*A. togoi*). It may persumably be concluded that the fresh leaves have more active ingredient (a.i.) as compared to the dried leaves used by Chavan (1984) and the 6 month old seed kernel used by Scbmutterer and Zebitz (1986).

As far as the abnormalities are concerned they were similar to the reports of the above workers. Most of the larvae died as intermediates and sometimes highly melanized pupae. There was general contraction of the larval body and highly reduced abdomen of pupae was also observed. The adults could not emerge and died as pharate adult and if they were able to emerge, had abnormal crippled legs, reduced wings and abdomen. These abnormalities are similar to the effect of methoprene as pointed out by Zebitz (1986). Similar abnormalities were reported by Naqvi et al., (1976) using methoprene against the same strain. This indicates that meliacins have also IGR effect.

Thus it may be concluded that neem extracts may prove useful, simple, Inomic and safe agents in controlling the population of mosquitoes as compared 11 malathion or other OP compounds and synthetic pyrethroids against which mosquitoes develop

resistance. It may be pointed out here that Vollinger (1986) has tested neem fraction (NSKE) and deltamethrin for resistance. He reported that *Plutella xylostella* did not develop resistance against NSKE up to 35 generations whereas it developed 20-35 times resistance against deltamethrin, during the same period. However, much work and effort is required in this connection.

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