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TÜBİTAK

Toxicity of Crude Neem Leaf Extract Against Housefly *Musca domestica* L. Adults as Compared With DDVP, Dichlorvos

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Abstract: Toxicity of neem extract in comparison with DDVP (Dichlorvos) was determined against the housefly (*Musca domestica* L). Neem leaf extract was applied at 5, 10, 20, 40 and 80 microgram/fly and DDVP was treated in 0.1, 0.2, 0.4, 0.8 and 1.6 microgram/fly doses. Lethal dose values were calculated with probit analysis and LD_{50} and LD_{90} values were found to be 8.4 and 169.8 ug/fly of neem extract respectively, whereas, values of 0.44 and 3.58 ug/fly of DDVP were found for LD_{50} and LD_{90} against house flies.

Key Words: Neem (*Azadirachta indica*) extract, DDVP, LD50, House fly (*Musca domestica* L.).

Ham Neem Yaprağı Özütünün Yetişkin Karasineğe (*Musa domestica* L.) Karşı Toksisitesinin DDVP'nin (Dichlorvos) Toksisitesiyle Karşılaştırması

Özet: Ham neem yaprağı özütünün karasineğe (*Musca domestica* L.) karşı toksisitesi DDVP'nin (Dichlorvos) toksisitesiyle karşılaştırılmıştır. Neem yaprağı özütü 5; 10; 20; 40; 80µg/karasinek dozlarında, DDVP ise 0.1, 0.2, 0.4, 0.8, 1.6 µg/karasinek dozlarında uygulanmıştır. Probit analizi yöntemiyle hesaplanan öldürücü doz değerleri neem özütü için LD50= 8.4 µg/karasinek, LD90= 169.8 µg/karasinek; DDVP için LD50= 0.44, LD90= 3.58 µg/karasinek olarak tespit edilmiştir.

Anahtar Sözcükler: Neem (*Azadirachta indica*) özütü, DDVP, LD50, karasinek (*Musca domestica* L.).

ntroduction

Whenever a chemical is used to control the insect opulation, not only is the environment polluted but also ther desirable fauna is affected by the introduction of he toxicant in the ecosystem. Simultaneously, the target pecies is provoked to develop resistance against a wide ange of pesticides as well (1).

The high cost of chemical pesticides and the nvironmental hazards as a result of pesticide usage have ncouraged scientists to seek less hazardous and cheaper esticide groups. During the last few decades, the Neem ree (*Azadirachta indica* A. Juss) has been of great nterest to scientists all over the world. To date a number f reports have been published on neem extracts with esticidal activity (2-15).

Musca domestica causes a serious threat to human nd livestock health by transmitting many infectious iseases. In view of the severity of the problem, it is mperative that control of houseflies must be improved hrough the application of occupationally and nvironmentally safe natural pesticides. The aim of the resent study was to determine the toxicity of crude neem leaf extract against wild houseflies of the Karachi region as compared with DDVP (organo-phosphorus compound) for the safe control of *Musca domestica*.

Materials and Methods

The experimental wild insects (*Musca domestica*) were collected from the suburbs of Karachi East, brought to the laboratory and raised therein. The rearing method was that of Ashrafi et al. (16) with some modifications. All flies were housed in standard cages of 40cm x 30 cm x 30 cm. The sides of the cages had 20 mesh sieve and the bottom was made of hardboard. One side had a long sleeve muslin cloth for cleaning and feeding purposes. The roof of the cage was fitted with 25 watt bulbs for photoperiodicity (12.12 hours). The insects were reared at 30-32 °C and 60% ± 10 R.H. at the Pesticide Research Institute, Tropical Agricultural Research Centre, Karachi. One-day-old adults were subjected to toxicity studies.

Neem leaf extract was made from 100 g fresh spring neem leaves ground in 100 ml acetone and kept for 24 hours at room temperature and shaken at intervals.

hereafter the material was filtered and the residue was gain shaken twice with 100 ml acetone. All the extracts were combined and evaporated to almost dryness and edissolved in 1.0 ml acetone.

In order to evaluate the toxicity of crude neem leaf xtract, 20 insects were used for each dose in three eplicates. In this way, 60 insects for one dose and 20 for olvent (acetone) treated controls were used. A total of 60 insects including solvent-treated controls were mployed. Insects were treated topically with selected oses of 5.0, 10.0, 20.0, 40.0 and 80.0 µg/adult using a microapplicator. Dose volume was kept constant and dose ariation was met with making various dilutions from tock solutionn of the extract in acetone. In order to avoid ny significant variation, the experiment was repeated ve times. In the case of DDVP, 10 insects were used for ach three replicates. So 30 insects for one dose were sed with the exception of the controls. A total of 150 nsects were used to determine the toxicity of DDVP in omparison with crude neem leaf extract. Insects were reated with doses of 0.1, 0.2, 0.4, 0.8 and 1.6 µg/adult f DDVP.

esults and Discussion

Six different doses of crude neem leaf extract i.e. 2.5, .0, 10.0, 20.0, 40.0 and 80.0 micrograms/adult were elected after preliminary experiments. Mortality count was made after 24 hours of treatment. LD_{50} and LD_{90} alues were calculated to be 8.415 and 169.833 ug/adult espectively, while the log probit regression line was alculated as Y= 5.015+0.981 (X-0.977) and eterogenity factor (Chi Square) 4.446 (Table 1). In the ase of DDVP five different doses i.e, 0.1, 0.2, 0.4, 0.8 and 1.6 micrograms per adult were selected after preliminary experiments. Mortality count was made after 24 hours of treatment. LD_{50}, LD_{90} values were calculated to be 0.441 and 3.581 µg/adult respectively while the log probit regression line was calculated as Y= 4.631+1.407 (X-(0.618) and heterogenity factor (Chi Square) 4.206 (Table 2).

Nurulain et al. (17) studied the toxic effect of crude neem seed kernel extract (RB-a) against *Musca domestica* L. (PCSIR Strain) and found it to be less effective than synergistic formulation, prepared with PBO, and Tx-100 was found to be 144 times more effective than RB-a alone. LD_{50} of RB-a formulation was determined as 5.5 ug/fly while LD50 of synergistic formulation of RB-a was about 800 µg/fly. The feeble killing action of neem extract RB-a when applied alone (i.e. without synergist) against a number of insect pests was reported by Naqvi et al. (18), Naqvi and Tabassum (19) and Azmi et al. (20, 21). They all found RB-a to be a less toxic killing agent than other tested compounds. However, Nurulain (22) found a neem compound to be better as an IGR (insect growth regulator) than as an acute poison. These reports are in agreement with the present findings i.e. 8.415 µg/fly and 0.44 µg/fly LD_{50} of neem extract and DDVP respectively. Toxicity and abnormalities caused by neem compounds, RBu-9, RB-b and Margosan-O™ determined bay Naqvi et al. (23) against 4th instar larvae of *Aedes aegypti* (PCSIR Strain) LC_{50} (24h) were found to be 380 ppm, 490 ppm and 340 ppm respectively. Most of the treated larvae had narrow abdomens and the surviving adults exhibited swollen abdomens and elongated, crumpled legs. Partially emerged adults were found with crumpled and entangled legs in the puparium. Jotwani and Srivastava (24) reported that when larvae of *Chilo partellus* were treated

Table 1. Toxicity of Neem leaf extract against *Musca domestica*.

ose g/adult	log dose	No. of insects	kill	%	E. Probit
2.5	0.40	20	6	30	4.48
5.0	0.70	20	8	40	4.75
10.0	1.00	20	9	45	4.87
20.0	1.30	20	14	70	5.52
40.0	1.60	20	15	75	5.67
80.0	1.90	20	17	85	6.04
ontrol	–	20	00	00	0.00

egression line: Y= 5.051+0.981 (X– (0.977))

D_{50}= 8.415 µg/adult

D_{90}= 169.833 µg/adult

eterogenity Factor: x^2= 4.446

Table 2. Toxicity of DDVP against *Musca domestica*.

Dose ug/adult	No. of insects	kill	%	E. Probit
0.100	10	2	20	4.15
0.200	10	3	30	4.48
0.400	10	4	40	4.75
0.800	10	7	70	5.52
1.600	10	8	80	5.84

Regression line: Y= 4.631±1.407 (X– (–0.618))
LD_{50}= 0.441 µg/adult
LD_{90}= 3.581 µg/adult
Heterogenity Factor: x^2= 4.206

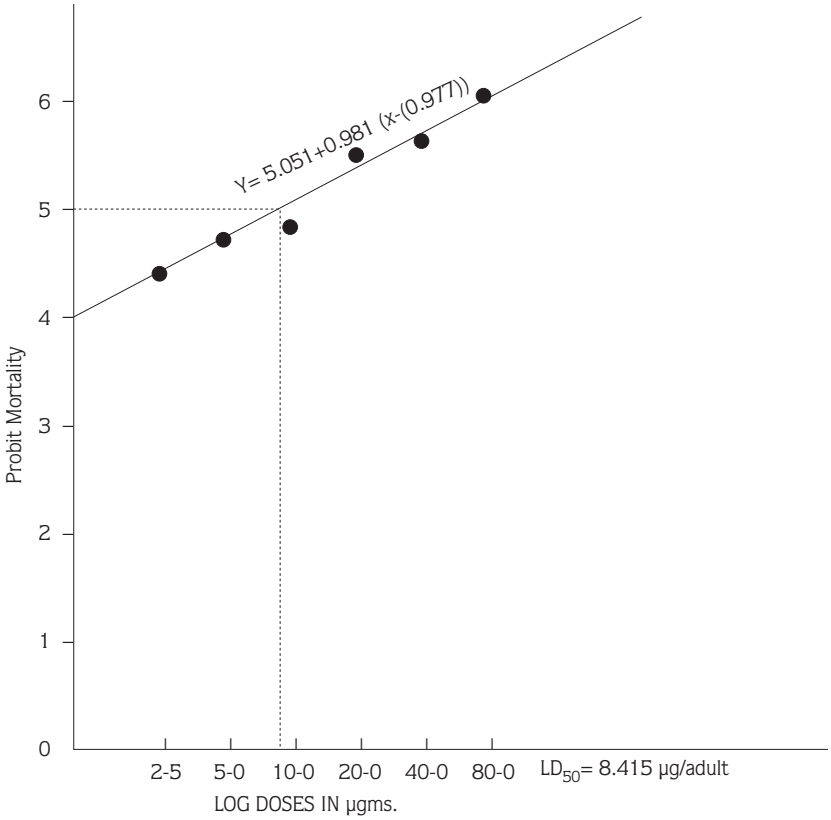

Figure 1. Toxicity curve of Neem leaf extract against *Musca domestica*.

with neem seed kernel extract, the larvae either died during the process of development or the adults emerging from the surviving larvae were abnormal. Haasler (25) reported the larval-pupal intermediates in *Manduca sexta* after using methanolic neem seed kernel extracts. Rao and Subrahmanyam (26) found that Azadirachtin at 1.66 µg/g of final instar larvae of *S. gregaria* caused 50% adult moult inhibition. Yasmin et al. (27) determined the toxic dose of RBa (Neem extract) against adults of *Drosophila melanogaster* by the contact method and calculated LC_{50} as 0.01% at 24 hours of post treatment from the mean values on log probit graph paper. Similar reports are given by Akhtar et al. (5), Bidmon et al. (6), Islam (28), Naqvi (7), Jehan et al. (29) and Naqvi et al. (30). These findings are generally in accordance with the present report and we can conclude

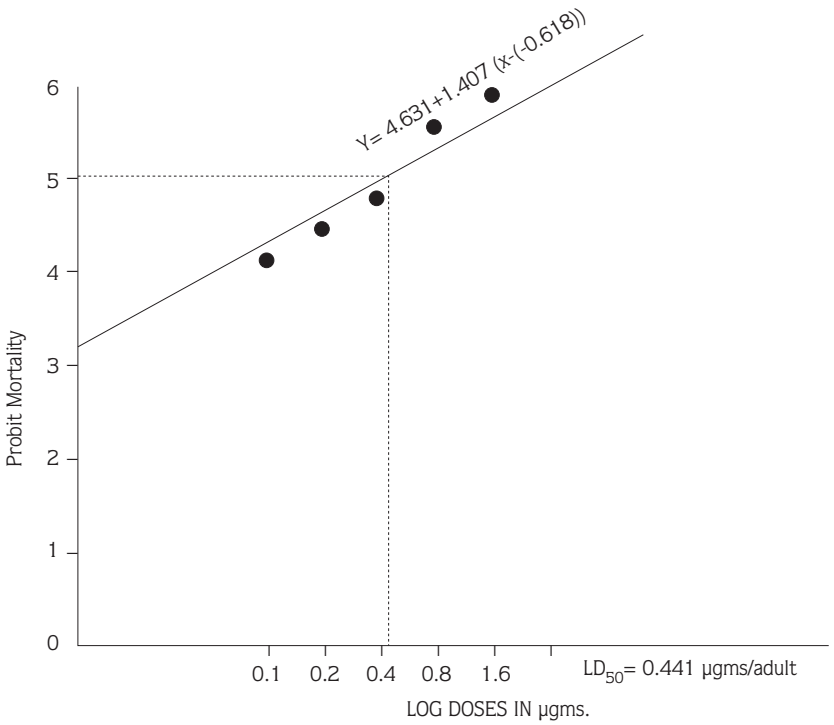

Figure 2. Toxicity curve of DDVP against *Musca domestica*.

that neem products may be used as population controlling agents for *Musca domestica* as they are cheaper and biodegradable, producing minimal pollution. Moreover, resistance does not develop in insects against neem products as reported by Naqvi and Tabassum (19), whereas against organo-phosphates insecticide resistance has been widely known for years (31). In the present report 0.441 µg/fly shows 33.9 fold resistance in comparison with LD_{50} of WHO housefly strains against DDVP (32).

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