# TOXICITY AND EFFECT OF A NEEM PRODUCT (RB-A) AND MESUROL ON TOTAL ESTERASE, OF A FRESHWATER SNAIL, *RADIX* SP.

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**ABSTRACT:** Toxicity and effect of a neem product, RB-a and a molluscicide, Mesurol on total esterase was evaluated against a freshwater snail, *Radix sp.* $LC_{20}$ and $LC_{50}$ of RB-a was found to be 2,250 ppm and 3,100 ppm whereas $LC_{20}$ and $LC_{50}$ of Mesurol was 17 ppm and 21 ppm, respectively. $LC_{20}$ treatment by both compounds caused inhibition of total esterase upto 30.21% by RB-a and 11.46% by Mesurol, whereas $LC_{50}$ treatment revealed 17% activation by RB-a and 21.87% by Mesurol.

## Introduction

Use of synthetic chemicals to kill undesirable fauna and flora, though very common, can affect the ecological balance and pollute the environment. At present more emphasis is being given to the use of natural products which are less hazardous to non-target fauna and flora. Extracts of the neem tree, *Azadirachta indica* have gained attention recently due to its pesticidal activity. However, its molluscicidal effect has hardly been reported. The present work was undertaken to check the effect of a crude ethanolic extract of the kernels of ripe neem berries RB-a on a freshwater snail. Previously the extract had been reported as an insect growth regulator and an insecticide (Naqvi *et al.* 1989, Nurulain *et al.* 1989, Naqvi *et al.* 1991, Naqvi *et al.* 1992a, b, Tabassum *et al.* 1992).

The efficacy of the neem product was compared with a molluscicide, Mesurol. The effect of the two toxicants on total esterase was checked to suggest a possible mode of action.

## Materials and Methods

RB-a was supplied by Dr. Beena Shaheen Siddiqui of H.E.J. Research Institute of Chemistry, University of Karachci, whereas Mesurol is a product of (Bayer) Chemdyes Pakistan Ltd., Karachi and the pellet contains 4% Mercaptodimethur as active ingredient. Mesurol was purchased from local market. Samples of the snail, *Radix* sp. were collected originally from a pond, at the University of Karachi and reared at the Zoological laboratory for a year.

### Toxicity determinations

Following a standard WHO method, a series of dilutions of the neem extract and Mesurol were prepared in 250ml beakers to each of which ten snails of uniform size were added. An untreated (control) and ethanol treated (check) were also kept. After 24 hours, mortality in each beaker was assessed. Percent mortality against each concentration was plotted on log-log graph and $LC_{20}$ and $LC_{50}$ were determined.

### Total esterase

Total esterase activity after the treatment with $LC_{20}$ and $LC_{50}$ of RB-a and Mesurol was determined according to Lewis and Madge's (1984) method. Two beakers for $LC_{20}$ and $LC_{50}$ of RB-a, two for Mesurol and one for control were taken for treatment. Then $LC_{20}$ and $LC_{50}$ dose of each compound was mixed, in repectively marked beakers, and twenty adult molluscs of uniform size were introduced. After 24 hours ten live molluscs (approximately 500mg) from each beaker were taken out for enzyme assay.

## Results

### Toxicity

$LC_{20}$ and $LC_{50}$ of RB-a were calculated as 2,250 ppm and 3,100 ppm, respectively, whereas for Mesurol, it was 17 and 21 ppm, respectively. The percent mortalities and range at 95% confidence limit was found to be directly proportional with applied concentrations (Tables 1 and 2: Fig. 1 and 2).

### To'al esterase activity

Table 3 shows 30.21% and 11.46% inhibition in total esterase acitivty by $LC_{20}$ treatment of RB-a and Mesurol, respectivley, whereas 17% and 21.87% increased activity was found by $LC_{50}$ treatment of RB-a and Mesurol.

**Table 1.** Toxicity data of RB-a against freshwater snail, *Radix* sp.

| Concentration (ppm) | Mean mortality X | S.D. | Range at 95% confidence limit |
|---|---|---|---|
| 1600 | 6% | 8.94 | 1.84-13.84 |
| 2000 | 12% | 10,95 | 2.37-21.62 |
| 2400 | 26% | 16.73 | 11.30-40.70 |
| 2800 | 46% | 11.40 | 35.98-56.01 |
| 3200 | 52% | 8.36 | 44.66-59.33 |
| Control | 0% | - | - |
| Check | 0% | - | - |

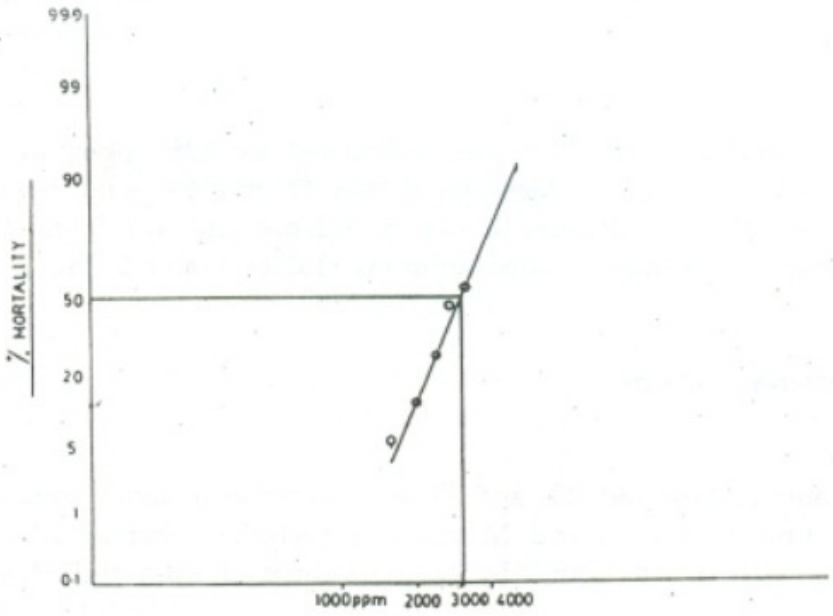


Fig- 1 TOXICITY CURVE OF RB-α AGAINST RADIX Sp.

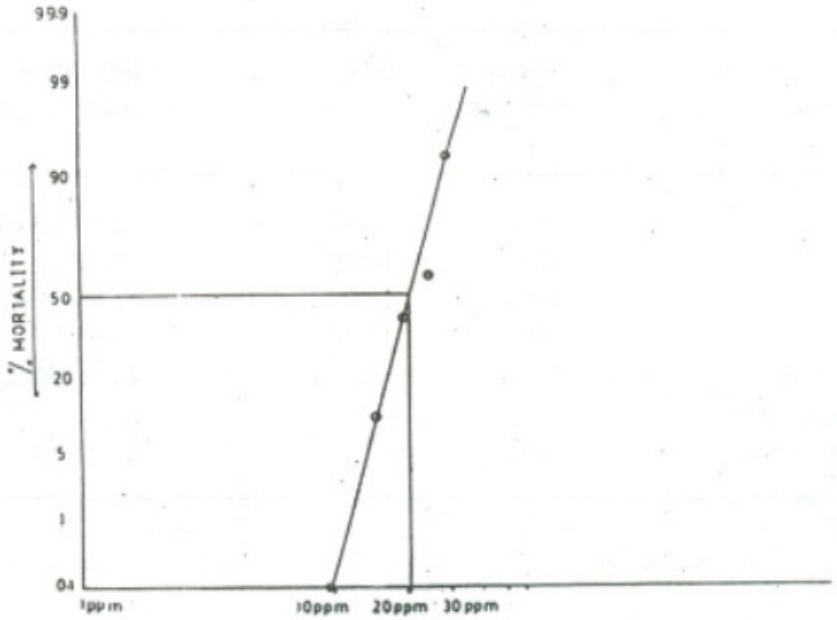


Fig - 2 TOXICITY CURVE OF MESEUROL AGAINST RADIX Sp.

**Table 2.** Toxicity data of Mesurol against freshwater snail, *Radix* sp.

| Concentration (ppm) | Mean mortality X | S.D. | Range at 95% confidence limit |
|---|---|---|---|
| 10 | 0.0% | | |
| 15 | 10.0% | | |
| 20 | 40.0% | 6.32 | 34.45-45.55 |
| 25 | 56.6% | 12.02 | 46.03-67.16 |
| 30 | 93.0% | 11:24 | 83.12-102.87 |
| Control | 0.0% | | |

**Table 3.** Total esterase activity on *Radix* sp. after treatment with $LC_{20}$ and $LC_{50}$ of RB-a and Mesurol.

| S.No. | Compound | Mean enzyme unit | | | % Activity | | | % Inhibition(-) activation(+) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | $LC_{20}$ | $LC_{50}$ | Control | $LC_{20}$ | $LC_{50}$ | $LC_{20}$ | $LC_{50}$ |
| 1. | RB-a | 288 | 201 | 337 | 100% | 69.79% | 117.00% | -30.21% | +17.00% |
| 2. | Mesurol | 288 | 255 | 351 | 100% | 88.54% | 121.87% | -11.46% | +21.87% |

## Discussion

### Toxicity

The $LC_{20}$ and $LC_{50}$ values of RB-a (2250, 3100 ppm) is considerably higher than Mesurol (17, 21 ppm). Obviously it is due to the crude nature of extract with no definite knowledge about the number, types and amount of active ingredient (triterpenoids) which may be present in it, and whether it does or does not contain any significant amount of toxic component that could kill or

check the population of molluscs. It was the first study of this compound (RB-a) as a molluscicide which was found positive though not comparable with mercaptodimethur, a well proven molluscicide. However, insecticidal and insect growth regulator effect of RB-a is reported by Naqvi *et. al.* (1989), Nurulain *et al.* (1989), Naqvi *et al.* (1991), Naqvi *et al.* (1991a,b) and Tabassum *et al.* (1992). These workers have also reported higher $LC_{50}$ value for various insects as compared to conventional pesticides. $LC_{20}$ and $LC_{50}$ of Mesurol was 17 and 21 ppm for 24-h exposure. $LC_{50}$ of same compound against another freshwater snail of Lymnaeidae family was reported as 13 ppm by Mohammad *et al.* (1994). The difference may be due to difference in mollusc species, nature of the compound and physico-chemical characteristics of the aquatic environment (Mane and Karte 1988).

**Total esterase activity**

$LC_{50}$ treatment revealed an activation of 17% and 21.87% of this enzyme by RB-a and Mesurol, respectively, whereas $LC_{20}$ treatment caused 30.21% and 11.46% inhibition of this enzyme. Such behaviour of various enzymes including acetylcholinesterase was also reported by Shakoori and Saleem (1989) in *Tribolium castaneum* larvae, after 200 ppm and 400 ppm dose treatments of malathion. At 400 ppm they reported that the activity of enzymes was significantly increased whereas at 200 ppm no increased activity was reported. Similar behaviour has been exhibited in the present case also where we found that at $LC_{50}$ of RB-a and Mesurol (molluscicide) the activity of total esterase increased but at $LC_{20}$ inhibition occurred. According to a recent study, much higher doses (1000 and 2000 ppm of pyrethroid) significantly increased or decreased the activity of enzymes, respectivley, in the case of *Tribolium castaneum* larvae (Shakoori *et al.* 1994). However a correlation can be made by this report with that of the present report on the basis of enzyme alteration by pesticide treatment. Decreased activity at low dose and increased activity of enzyme at high dose indicates that the animal may be resistant to that particular toxic compound, or under stress condition (higher dose), the protein and carbohydrate metabolism is enhanced resulting in more production of enzyme to combat pesticide.

Lower toxic activity of RB-a and other neem compounds against insect is reported by a number of workers like Naqvi *et al.* (1989), Naqvi *et al.* (1990) and Tabassum *et al.* (1992). Neem compounds are reported to be IGR or phagodeterrent rather than toxic (Ruscoe 1972, Redfern *et al.* 1981, Rembold *et al.* 1982), so their major effect may be on enzymes related to moulting or juvenile processes.

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