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# Toxicity of *Euphorbia tirucalli* Plant Against Freshwater Target and Non-target Organisms

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**Abstract:** The present paper deals with the molluscicidal activity of dried powder of latex and stem bark of *Euphorbia tirucalli* (Family- Euphorbiaceae) against fresh water snail *Lymnaea* (Radix) *acuminata* Lamarack and *Indoplanorbis exustus* (Deshyas) and anticholinesterase activity of *Euphorbia tirucalli* latex and stem bark in the nervous tissue of *Lymnaea acuminata*. Both the snails are the vector of liver fluke, *Fasciola hepatica* Linnaeus and *Fasciola gigantica* Cobbold, which causes endemic fascioliasis in cattle and livestock. The toxic effect of the latex and stem bark powder was both time as well as dose dependent. There was a significant negative correlation between $LC_{50}$ values and exposure periods thus increase in exposure time, the $LC_{50}$ values of *Euphorbia tirucalli* latex was decreases from 0.92 mg $DWL^{-1}$ (24 h) to 0.51 mg $DWL^{-1}$ (96 h) against *Lymnaea acuminata* and 1.42 mg $DWL^{-1}$ (24 h) to 0.55 mg $DWL^{-1}$ (96 h) against *Indoplanorbis exustus*. Similar trend was also observed in case of stem bark powder. Treatment of sub-lethal doses (40 and 80% of $LC_{50}$) of latex and stem bark powder of *E. tirucalli* also shows significant ($P<0.05$) time and dose dependent inhibition in the activity of enzyme acetylcholinesterase (AChE) in the nervous tissue of *Lymnaea acuminata* after 24 h or 96 h exposure periods. Withdrawal study also shows that there is a significant recovery in the acetylcholinesterase activity in the nervous tissue of snail after the 7th day of the withdrawal of treatment. Dried powders of latex and stem bark powder of *E. tirucalli* at higher doses were also lethal to fresh water fish *Channa punctatus* ($LC_{50}$ 24 h: 9.01 mg $DWL^{-1}$), which shares the habitat with these snails, but the doses $LC_{90}$ (24 h) of snail did not cause any mortality among fish in a mixed population of snails and fish. It is proposed that the latex and stem bark powder of *E. tirucalli* can be used as a molluscicide for controlling the snails

**Key words:** *Euphorbia tirucalli*, Molluscicidal and anticholinesterase activity, *Lymnaea acuminata* and *Indoplanorbis exustus*

## Introduction

Fascioliasis is a very common disease of cattle and livestock in northern part of India. The main causative agent of this disease is trematode *Fasciola hepatica* and *Fasciola gigantica* both are endoparasite in liver of cattle and livestock (Singh and Agarwal, 1981). Both are transmitted to their primary host with the help of their intermediate host freshwater snail *Lymnaea acuminata* and *Indoplanorbis exustus* (Hyman, 1970). In the eastern part of U.P. the population of these vectors *Lymnaea acuminata* and *Indoplanorbis exustus* is more pronounced so the occurrence of this disease is very common in this region.

For controlling the vector born diseases a worldwide vector controlling programme were carried out (WHO., 1992). Control of snail vector population through plant origin pesticides is a very effective and new tool of Integrated Vector Management programme. Botanical molluscicides can provide an ideal source of low cast, safe and effective molluscicides (Marston and Hostettmann, 1985; Singh *et al*., 1996).

Although plant products should be preferred to synthetic pesticides due to their biodegradability, the use of plant molluscicides would be justified only if it can be demonstrated that the effect of the doses needed as molluscicide is nontoxic to other non target aquatic animals especially fish, which shares the habitat with snails.

The plant of *Euphorbia tirucalli* belongs to family-Euphorbiaceae is commonly known as Barki-thohar. This plant is native of America but has become acclimatised and grows freely in all parts of India. This is a common medicinal plant of India; the plant parts used milky juice and stem bark. Milky juice in small doses is a purgative but in large doses it is acrid, counter-irritant and emetic (Satyavati and Gupta, 1987).

In the present study the molluscicidal and anticolinesterase activity of latex and stem bark of *Euphorbia tirucalli* was observed against target snails *Lymnaea acuminata* and *Indoplanorbis exustus* and toxicity of both was also studied against fresh water non target fish *Channa punctatus* and *Colisa fasciatus,* which shares the freshwater habitat with these snails.

## Materials and Methods

Adult *Lymnaea acuminata* (2.6±0.3 cm in shell height); *Indoplanorbis exustus* (0.85±0.03 cm in shell height) and fish *Colisa fasciatus* (6.0± 1.5 cm in total length, 7.0± 2.0 gm in weight) and *Channa punctatus* (13.5± 3.0 cm in length, 16.0± 4.0 gm in weight) were collected locally from the Ramgarh lake of Gorakhpur district and used as test animal. The collected animals were maintained in glass aquaria containing de-chlorinated tap water for acclimatization to laboratory conditions. The water in aquaria was aerated continuously and changed at every 24 h. The dead animals were removed as soon as possible from test container to prevent water fouling.

*Euphorbia tirucalli* was collected locally from botanical garden of D.D.U. Gorakhpur University Gorakhpur and identified by Prof. S.K. Singh, Plant Taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, India, where a voucher specimen is deposited.

### Preparation of dried powder of latex and stem bark

**Latex:** The white, milky latex produced by the plant was drained into glass tubes by cutting the stem apices. The latex was lyophilized at –40°C and lyophilized powder was stored in airtight desicator, for further use. The wet weight of one ml latex of *Euphorbia tirucalli* was 1.370 gm and dry weight (lyophilized at –40°C) was 0.315 gm.

**Stem bark:** The stem bark of *Euphorbia tirucalli* was dried in incubator at 37°C. With the help of mechanical device powdered the dried stem bark. The dried, powder was stored in airtight desiccator's, for further use. At the time of treatment the dried powder was dissolved in distilled water, centrifuged at 1000 g for 10 min, only supernatant was used for treatment.

Toxicity experiment was performed by using method of Singh and Agarwal (1988); experimental animals were kept in glass aquaria containing 3 L de-chlorinated tap water for the snails and 6 L for the fishes. The aquaria contained either ten snails or ten fishes. The snails were exposed for 96 h to four different concentrations of *Euphorbia tirucalli* latex powder (0.5 mg $DWL^{-1}$, 0.7 mg $DWL^{-1}$, 0.9 mg $DWL^{-1}$ and 1.1 mg $DWL^{-1}$) and stem bark powder (10 mg $DWL^{-1}$, 30 mg $DWL^{-1}$, 50 mg $DWL^{-1}$ and 70 mg $DWL^{-1}$). Fishes were exposed for four different concentrations (6.30 mg $DWL^{-1}$, 9.45 mg $DWL^{-1}$, 12.60 mg $DWL^{-1}$ and 15.75 mg $DWL^{-1}$) of latex powder only. Six aquaria were set up for each dose. Control animals were reared in similar condition without treatment. The toxic effect of dried powders of latex and stem bark was also studied in mixed population of fish and snails. In these experiments, a group of 10 snails and 10 fishes were put together in 8 litres of dechlorinated tap water.

Table 1: Experimental conditions of water determine by the method of APHA/WEF (1998)

| | |
|---|---|
| Atmospheric temperature, °C | 31.5± 1.0 |
| Water temperature, °C | 29.0± 1.5 |
| pH | 7.3 to 7.5 |
| Dissolved oxygen, mg $L^{-1}$ | 7.0 to 7.8 |
| Free carbon dioxide, mg $L^{-1}$ | 4.0 to 5.2 |
| Bicarbonate alkalinity, mg $L^{-1}$ | 103.0 to 106.0 |

These mixed populations were exposed to previously determined $LC_{90}$ (24 h) of snails for 24 h. Behavioural responses of snail were observed up to 2 h of the beginning of the treatment. Mortality was recorded at every 24 h, 48 h, 72 h or 96 h respectively. $LC_{10}$, $LC_{50}$ and $LC_{90}$ values, upper and lower confidence limits, slope value, t-ratio, g-factor and heterogeneity were calculated according to probit log method using POLO computer programme of Russel *et al.* (1977) (Table 1).

**Treatment protocol for measuring Acetylcholinesterase enzyme activity:** Acetylcholinesterase inhibition was measured in the nervous tissue of *Lymnaea acuminata*, after exposure to 8.83 mg $L^{-1}$ (40% of 24 h $LC_{50}$) and 17.67 mg $L^{-1}$ (80% of 24 h $LC_{50}$) and 2.89 mg $L^{-1}$(40% of 96 h $LC_{50}$) and 5.78 mg $L^{-1}$(80% of 96 h $LC_{50}$) of *Euphorbia tirucalli* stem bark and 0.37 mg $L^{-1}$ and 0.74 mg $L^{-1}$ (40% and 80% of 24 h $LC_{50}$) and 0.20 mg $L^{-1}$and 0.41 mg $L^{-1}$ (40% and 80% of 96 h $LC_{50}$) of *Euphorbia tirucalli* latex. Control animals were kept in similar condition without any treatment. After 24 h or 96 h exposure period of 24 h or 96 h $LC_{50}$ the acetylcholinesterase (AChE) activity was measured in the nervous tissue of snails by the method of Ellman *et al.* (1961), as modified by Singh and Agarwal (1982). Homogenate (50 mg $ml^{-1}$, W $V^{-1}$) was prepared in 0.1 M-phosphate buffer, pH 8.0 for 5 min in an ice bath and centrifuged at 1000 g for 30 min at -4°C. The enzyme containing supernatant (0.05 ml) was pipetted to a cuvette. To this was added 10 µl ($5x10^{-4}$M) of freshly prepared acetylthiocholine iodide solution in distilled water as substrate, 1.45 ml of buffer (pH 8.0) and 0.05 ml of the chromogenic agent, 5:5 dithio-bis-nitrobenzoate (DTNB). The change in optical density at 412 nm, caused by the enzymatic reaction, was monitored for 3 min at 25°C. Protein estimation was done by the method of Lowry *et al.* (1951). Enzyme activity has been expressed as $\mu$ mol 'SH' hydrolyzed $min^{-1}mg^{-1}$ protein.

**Withdrawal experiment:** In order to see effect of 7th withdrawal, snail *Lymnaea acuminata*, were exposed for 24 h exposure period at sub-lethal dose of 17.67 mg $L^{-1}$(80% of the 24 h $LC_{50}$) of *E. tirucalli* stem bark and 0.74 mg $L^{-1}$(80% of the 24 h $LC_{50}$) of *E. tirucalli* latex. Similarly, for 96 h exposure period snail *L. acuminata*, were exposed to sub-lethal dose of 5.78 mg $L^{-1}$ (80% of

the 96 h $LC_{50}$) of *E. tirucalli* stem bark and 0.41 mg $L^{-1}$(80% of the 96 h $LC_{50}$) of *E. tirucalli* latex.. After termination of experiments, the one half of the animal was sacrificed and the activity of acetylcholinesterase enzyme was measured in nervous tissue. The other half was transferred to fresh water free from any treatment, which was changed every 24 h for the next 6 days. Following to this the activity of acetylcholinesterase enzyme was measured in nervous tissue of snail. Control animals were kept in similar condition without any treatment.

Each experiment was replicated at least six times and data have been expressed as mean± SE. Test of correlation, two-way analysis of variance and student's t-test were applied for locating significant differences (Sokal and Rohlf, 1973).

## Results

**Effect on behavioral Changes:** Exposure to the dried powder of latex and stem bark of *E. tirucalli* caused behavioural changes in the freshwater harmful snails *L. acuminata* and *I. exustus*. Behavioural changes appear with in 5 to 10 min of exposure. The initial 30-40 min. was a period of hyperactivity during which sluggish snails moved rapidly in the aquarium water. After some time they started crawling on each other. As the poison took over, there was a muscular twitching and the snails become spirally twisted. Prior to death, there was complete withdrawal of the body inside the shell. At the highest doses mortality took after about 90-120 min. of exposure period. Even with the weaker doses the sequences of events leading to death were the same.

**Toxicity:** LC values of dried powder of *E. tirucalli* latex and stem bark for periods ranging from 24 h to 96 h are given in Table 2 to 5 respectively.

The toxicity was both time as well as dose dependent. There was a significant negative correlation between LC values and exposure periods. Thus with an increase in exposure time, the $LC_{50}$ values of latex powder decreased from 0.92 mg $DWL^{-1}$ (24 h) >0.63 mg $DWL^{-1}$ (48 h) > 0.54 mg $DWL^{-1}$ (72 h) >to 0.51 mg $DWL^{-1}$ (96 h) in case of *L. acuminata* (Table 2). $LC_{50}$ values were also decreased from 1.42 mg $DWL^{-1}$ (24 h) >0.83 mg $DWL^{-1}$ (48 h) > 0.71 mg $DWL^{-1}$ (72 h) > to 0.55 mg $DWL^{-1}$ (96 h) in case of *I. exustus* (Table 3).

Similar trend was also observed in dried powder of *E. tirucalli* stem bark. In this treatment $LC_{50}$ decreased from 22.09 mg $DWL^{-1}$ (24 h) > 12.78 mg $DWL^{-1}$ (48 h) > 8.89 mg $DWL^{-1}$ (72 h) > to 7.22 mg $DWL^{-1}$ (96 h) against *L. acuminata* (Table 4). In case of *I. exustus* $LC_{50}$ were decreased from 22.91 mg $DWL^{-1}$ (24 h) > 16.37 mg $DWL^{-1}$ (48 h) > 7.56 mg $DWL^{-1}$ (72 h) > to 7.46 mg $DWL^{-1}$ (96 h) (Table 5).

The slope values given in (Tables 2 to 5) were steep and the separate estimation of LC based on each of the six replicates was found to be within the 95% confidence limits of LC doses. The't' ratio was greater than 1.96 and heterogeneity factor was less than 1.0. The 'g' value was less than 0.5 at all the probability levels.

**Effect on Acetylcholinesterase activity:** Table 8, shows that treatment of snail *L. acuminata* with sub lethal doses of aqueous extract of latex and stem bark of *E. tirucalli* for the exposure period of 24 h or 96 h, which caused significant ($P<0.05$) inhibition in acetylcholinesterase activity in the nervous tissue. Thus 24 h exposure to 8.83 mg $L^{-1}$ and 17.67 mg $L^{-1}$ of *E. tirucalli* stem bark reduced the AChE activity up to 64 and 33% of controls in nervous tissue of *L. acuminata*. Similarly, 96 h exposure to 2.89 mg $L^{-1}$ and 5.78 mg $L^{-1}$ of *E. tirucalli* stem bark reduced the AChE activity up to 56 and 20% of controls in nervous tissue of *L. acuminata* (Table 8). While 24 h exposures to 0.37 mg $L^{-1}$ and 0.74 mg $L^{-1}$ of *E. tirucalli* latex inhibits the AChE activity up to 55 and 25% of controls in nervous tissue of *L. acuminata*. Similarly, 96 h exposure to 0.20 mg $L^{-1}$ and 0.41 mg $L^{-1}$ of *E. tirucalli* latex inhibit the AChE activity up to 44 and 16% of controls in nervous tissue of *L. acuminata* (Table 8). Analysis of variance demonstrated that the inhibition of AChE was both time and dose dependent ($P<0.05$).

After seven days withdrawal experiment of after exposure to sub-lethal doses of 17.67 mg $L^{-1}$of stem bark and 0.74 mg $L^{-1}$ of latex for 24 h exposure period and for 96 h exposure period at sub-lethal doses of 5.78 mg $L^{-1}$of stem bark and 0.41 mg $L^{-1}$ of latex (Table 8) shows, there was highly significant ($P<0.05$) recovery in the acetylcholinesterase enzyme activity in the nervous tissue of snail *L. acuminata*.

## Discussion

Data of present study shows that *E. tirucalli* stem bark and latex caused significant behavioral changes in both the snails. The most obvious sign of distress in the treated snails were muscular twitching and spiral twisting of body, followed by crawling on each other. The nature and rapid onset of these behavioral responses indicates both stem bark and latex perhaps contain some neurotoxins, which amongst other think, might be active at neuromuscular system of exposed animals. Similar behavioral response was also observed by Singh and Agarwal (1990) in case of *Euphorbia royleana*, *Euphorbia antisyphliatica* and *Jatropha gossypifolia* on snail *L. acuminata*. The behavioral changes are indeed reminiscent to the response of snails to organophosphorus and carbamate pesticides (Singh and Agarwal, 1981).

Table 2: Toxicity ($LC_{10}$, $LC_{50}$ and $LC_{90}$) of dried powder of *Euphorbia tirucalli* latex against *Lymnaea acuminata* at different time intervals

| Exposure periods | Effective dose (mg $L^{-1}$) | Limits (mg $L^{-1}$) LCL | UCL | Slope value | 'g' factor | 't' rating | Heterogeneity |
|---|---|---|---|---|---|---|---|
| 24 h | $LC_{10}$ = 0.45 | 0.33 | 0.53 | | | | |
| | $LC_{50}$ = 0.92 | 0.84 | 1.06 | 4.10±7.20 | 0.12 | 5.72 | 0.14 |
| | $LC_{90}$ = 1.90 | 1.50 | 3.03 | | | | |
| 48 h | $LC_{10}$ = 0.32 | 0.23 | 0.38 | | | | |
| | $LC_{50}$ = 0.63 | 0.53 | 0.71 | 3.26±0.67 | 0.16 | 4.87 | 0.05 |
| | $LC_{90}$ = 1.56 | 1.24 | 2.62 | | | | |
| 72 h | $LC_{10}$ = 0.31 | 0.24 | 0.32 | | | | |
| | $LC_{50}$ = 0.54 | 0.48 | 0.59 | 5.46±0.77 | 0.08 | 7.10 | 0.58 |
| | $LC_{90}$ = 0.93 | 0.85 | 1.09 | | | | |
| 96 h | $LC_{10}$ = 0.26 | 0.12 | 0.35 | | | | |
| | $LC_{50}$ = 0.51 | 0.45 | 0.55 | 6.35±0.91 | 0.08 | 6.96 | 0.45 |
| | $LC_{90}$ = 0.81 | 0.74 | 0.91 | | | | |

Table 3: Toxicity ($LC_{10}$, $LC_{50}$ and $LC_{90}$) of dried powder of *Euphorbia tirucalli* latex against *Indoplanorbis exustus* at different time intervals

| Exposure periods | Effective dose (mg $L^{-1}$) | Limits (mg $L^{-1}$) LCL | UCL | Slope value | 'g' factor | 't' rating | Heterogeneity |
|---|---|---|---|---|---|---|---|
| 24 h | $LC_{10}$ = 0.51 | 0.29 | 0.62 | | | | |
| | $LC_{50}$ = 1.42 | 1.13 | 2.65 | 2.87±0.76 | 0.27 | 3.78 | 0.06 |
| | $LC_{90}$ = 3.95 | 2.27 | 21.96 | | | | |
| 48 h | $LC_{10}$ = 0.39 | 0.28 | 0.48 | | | | |
| | $LC_{50}$ = 0.83 | 0.76 | 0.94 | 3.99±0.69 | 0.15 | 5.75 | 0.24 |
| | $LC_{90}$ = 2.03 | 1.46 | 4.75 | | | | |
| 72 h | $LC_{10}$ = 0.25 | 0.09 | 0.36 | | | | |
| | $LC_{50}$ = 0.71 | 0.59 | 0.81 | 2.80±0.66 | 0.21 | 4.26 | 0.17 |
| | $LC_{90}$ = 1.75 | 1.40 | 2.69 | | | | |
| 96 h | $LC_{10}$ = 0.21 | 0.08 | 0.32 | | | | |
| | $LC_{50}$ = 0.55 | 0.42 | 0.63 | 3.13±0.68 | 0.18 | 4.62 | 0.09 |
| | $LC_{90}$ = 1.42 | 1.14 | 2.34 | | | | |

Table 4: Toxicity ($LC_{10}$, $LC_{50}$ and $LC_{90}$) of dried powder of stem bark of *Euphorbia tirucalli* against *Lymnaea acuminata* at different time intervals

| Exposure periods | Effective dose (mg $L^{-1}$) | Limits (mg $L^{-1}$) LCL | UCL | Slope value | 'g' factor | 't' rating | Heterogeneity |
|---|---|---|---|---|---|---|---|
| 24 h | $LC_{10}$ = 6.34 | 3.68 | 8.95 | | | | |
| | $LC_{50}$ = 22.09 | 17.80 | 26.38 | 2.37±0.30 | 0.06 | 7.94 | 0.43 |
| | $LC_{90}$ = 76.94 | 60.12 | 111.42 | | | | |
| 48 h | $LC_{10}$ = 3.22 | 1.41 | 5.22 | | | | |
| | $LC_{50}$ = 12.78 | 8.98 | 16.24 | 2.14±0.30 | 0.08 | 7.02 | 0.34 |
| | $LC_{90}$ = 50.68 | 39.69 | 72.98 | | | | |
| 72 h | $LC_{10}$ = 2.25 | 0.79 | 3.98 | | | | |
| | $LC_{50}$ = 8.89 | 5.49 | 11.91 | 2.15±0.34 | 0.10 | 6.33 | 0.33 |
| | $LC_{90}$ = 35.09 | 27.60 | 49.45 | | | | |
| 96 h | $LC_{10}$ = 1.95 | 0.59 | 3.59 | | | | |
| | $LC_{50}$ = 7.22 | 4.07 | 9.95 | 2.25±0.39 | 0.11 | 5.82 | 0.40 |
| | $LC_{90}$ = 26.76 | 21.02 | 37.17 | | | | |

Table 5: Toxicity ($LC_{10}$, $LC_{50}$ and $LC_{90}$) of dried powder of stem bark of *Euphorbia tirucalli* against *Indoplanorbis exustus* at different time intervals

| Exposure periods | Effective dose (mg $L^{-1}$) | Limits (mg $L^{-1}$) LCL | UCL | Slope value | 'g' factor | 't' rating | Heterogeneity |
|---|---|---|---|---|---|---|---|
| 24 h | $LC_{10}$ = 5.42 | 2.71 | 8.18 | | | | |
| | $LC_{50}$ = 22.91 | 17.92 | 27.91 | 2.05±0.29 | 0.08 | 7.16 | 0.19 |
| | $LC_{90}$ = 96.90 | 71.62 | 157.81 | | | | |
| 48 h | $LC_{10}$ = 4.21 | 2.07 | 6.47 | | | | |
| | $LC_{50}$ = 16.37 | 12.29 | 20.20 | 2.17±0.30 | 0.07 | 7.36 | 0.38 |
| | $LC_{90}$ = 63.62 | 49.45 | 93.23 | | | | |
| 72 h | $LC_{10}$ = 1.87 | 0.55 | 3.52 | | | | |
| | $LC_{50}$ = 7.56 | 3.41 | 11.35 | 1.60±0.31 | 0.14 | 5.21 | 0.45 |
| | $LC_{90}$ = 47.77 | 35.02 | 80.93 | | | | |
| 96 h | $LC_{10}$ = 1.20 | 0.19 | 2.83 | | | | |
| | $LC_{50}$ = 7.46 | 4.18 | 10.37 | 2.14±0.36 | 0.11 | 5.88 | 0.41 |
| | $LC_{90}$ = 29.68 | 23.20 | 41.78 | | | | |

- Batches of ten snails were exposed to four different concentrations of *Euphorbia tirucalli* latex
- Concentrations given are the final concentrations ($WV^{-1}$) in aquarium water
- Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values
- LCL = Lower confidence limit and UCL = Upper confidence limit
- There was no mortality in control groups

Table 6: LC values ($LC_{10}$, $LC_{50}$ and $LC_{90}$) of dried powder of *Euphorbia tirucalli* latex against fishes at different exposure period

| Experimental animal | Effective Doses (mg $L^{-1}$) | 24 h | 48 h | 72 h | 96 h |
|---|---|---|---|---|---|
| *Colisa fasciatus* | $LC_{10}$ | 5.56 (4.79-6.15) | - | - | - |
| | $LC_{50}$ | 8.14 (7.58-8.66) | - | - | - |
| | $LC_{90}$ | 11.91 (11.01-13.26) | - | - | - |
| *Channa punctatus* | $LC_{10}$ | 6.19 (5.43-6.80) | - | - | - |
| | $LC_{50}$ | 9.01 (8.44-9.57) | - | - | - |
| | $LC_{90}$ | 13.12 (12.15-14.57) | - | - | - |

•-,Values are not calculated because there was no further mortality after 24 h

Table 7: Percent mortality (Mean±SE) of snail and fish caused by dried powder of latex and stem bark ($LC_{90\ (24\ h)}$ of snail) of *Euphorbia tirucalli* after 24 h exposure period

| Euphorbia tirucalli | Dose (mg $L^{-1}$, $DWV^{-1}$) | Experimental animals | % Mortality |
|---|---|---|---|
| Latex | 1.90 | *Lymnaea acuminata* | 95.00±2.46 |
| | | *Channa punctatus* | - |
| Stem bark | 76.94 | *Lymnaea acuminata* | 91.67±1.83 |
| | | *Channa punctatus* | - |
| Latex | 3.95 | *Indoplanorbis exustus* | 96.67±2.32 |
| | | *Channa punctatus* | - |
| Stem bark | 96.90 | *Indoplanorbis exustus* | 93.33±2.32 |
| | | *Channa punctatus* | - |

•-, There is no mortality

No such behavioral symptoms and death occurred in control groups which indicating that no factor other than plant moieties was responsible for altered behavior and mortality.

Mortality caused by stem bark and latex showed a clear significant positive correlation between dose and mortality. The positive correlation between dose and mortality in stem bark and latex was noted because increase concentration of pesticides in aquarium water resulted in more intake or entry of pesticides in the body of animals. This trend is also independent upon several factors such as, rate of penetration, nature of slope, variability and maximal effects of active moieties (Goodmann *et al*., 1985). *E. tirucalli* stem bark and latex showed a significant negative correlation between LC values and exposure periods. e. g. $LC_{50}$ of *E. tirucalli* latex were decreased from 0.92 mg $DWL^{-1}$ (24 h); > 0.63 mg $DWL^{-1}$ (48 h); >0.54 mg $DWL^{-1}$ (72 h); > to 0.51 mg $DWL^{-1}$ (96 h) in the case of *Lymnaea acuminata* (Table 2).

Increased in mortality with increased in exposure periods could be effect by several factors, which may be acting separately or conjointly. For example, uptake of active moiety is time dependant, which leads progressive increase the entrance of drug and its effect on snail body (Singh and Agarwal, 1988; 1993 a; 1993 b). Stability (life span) of active moiety of pesticides in environment and the rate of their detoxification in animal body also alter the mortality and exposure period relationship (Matsumura, 1985). This possibility cannot be over ruled in case of plant origin pesticides also. More important is the fact that the latex of *E. tirucalli* is much more toxic than synthetic pesticides. The present study demonstrates that latex of *E. tirucalli* have higher mollscicidal activity than any of the prevalent synthetic pyrethroids. Thus, the 24 h $LC_{50}$ of mexacarbamate (3.5 ppm), aldicarb (30.0 ppm), farmothion (27.0 ppm), cypermethrin (2.5 ppm) and fenavalerate (2.5 ppm) against *Lymnaea acuminata* (Singh and Agarwal, 1981; Singh and Agarwal, 1986; 1987 a; 1988 and 1991; Sahay *et al*., 1991) is higher than that of *E. tirucalli* latex (0.92 ppm). Which is about 121 times stronger than standard molluscicides niclosamide ($LC_{50}$ 11.8 ppm) (Singh and Agarwal, 1984 a).

Toxicity data also indicates that the 24 h $LC_{50}$ of latex of *E. tirucalli* against the fresh water fish *Channa punctatus* and *Colisa fasciatus* is 10 and 9 times higher than the $LC_{50}$ of snail *L. acuminata*. Thus the doses that can be used for killing the snails are safe for the fishes (Table 6). This is further supported by our observations on a mixed population of snails and fish, in which even $LC_{90\ (24h)}$ doses of snail was found to be safe for fish (Table 7). From our study it is also clear that anticholinesterase activity of *E. tirucalli* extracts are reversible in their action, which is an advantageous factor for using this extract as effective molluscicides.

Statistical analysis of the data on toxicity brings out several important points. The $\chi^2$ test for goodness of fit (heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance etc. do not significantly affect the LC values as these were found to lie within 95% confidence limits. The steepness of the slope line indicates that there is a large increase in the mortality of snail with relatively small increase in the concentration of the toxicant. The slope is thus an index of the susceptibility of the target animal to the pesticide used. A steep slope is also indicative of rapid absorption and onset of effects. Event though the slope alone is not a very reliable indicator of toxicological mechanism yet, it is a useful parameter (Rand and Petrocelli, 1988) for such a study. Since the LC values lay within 95% confidence limits, it is obvious that in replicate test of random samples, the concentration response lines would fall in the same range (Rand and Petrocelli, 1988).

The present study demonstrate that the latex and stem bark of *Euphorbia tirucalli* have high molluscicidal activity. Singh and Agarwal (1984 a, b and 1987 b) have reported that the latex of *Euphorbia royleana* and *Euphorbia antisyphilitice* not only cause inhibition of acetylcholinesterase but also reduces the endogenous level of 5-hydroxy tryptamine, epinephrine and dopamine in the nervous tissue of fresh water snail *L. acuminata*. Plants of family Euphorbiaceae thus effect all the know

Table 8: Inhibition of AChE activity in nervous tissue of fresh water harmful snail *Lymnaea acuminata* exposed to 24 h or 96 h to 40 and 80% $LC_{50}$ of aqueous extract of *E. tirucalli* stem bark, latex and recovery of AChE activity after 7th day withdrawal experiment of treatment

*E. tirucalli* stem bark

| Exposure period | Control | 40% of $LC_{50}$ (24h) (mg $L^{-1}$) | 80% of $LC_{50}$ (24h) (mg $L^{-1}$) | 7th day after withdrawal (mg $L^{-1}$) |
|---|---|---|---|---|
| 4h | 0.075±0.002(100) | 0.048±0.005*(64) | 0.025±0.004*(33) | 0.071±0.003**(95) |
| 96h | 0.075±0.002(100) | 0.042±0.005*(56) | 0.015±0.003*(20) | 0.069±0.004**(92) |

*E. tirucalli* latex

| Exposure period | Control | 40% of $LC_{50}$(96h) (mg $L^{-1}$) | 80% of $LC_{50}$(96h) (mg $L^{-1}$) | 7th day after withdrawal (mg $L^{-1}$) |
|---|---|---|---|---|
| 24h | 0.075±0.002(100) | 0.041±0.006*(55) | 0.019±0.004*(25) | 0.073±0.003**(97) |
| 96h | 0.075±0.002(100) | 0.033±0.007*(44) | 0.012±0.005*(16) | 0.072±0.002**(96) |

• Values are mean±SE of six replicates. • Values in parentheses are % change with control taken as 100%. • Data were analysed through student's test.
• *,Significant (P<0.05), when treated groups were compared with controls.
• **,Significant (P<0.05), when withdrawal groups were compared with treated groups

neurotransmitter mechanism of the snails nervous system. This might explain the quick knock down action of these plants.

Kinghorn and Evans (1975) reported that the pharmacological action of Euphorbious plants is due to presence of a group of diterpen phorbal esters, which are reported to promote activity of the enzyme protein kinase C (Aitken, 1987; Evans and Edwards, 1987), which specifically phosphorylates serine and threonine residues in proteins (Aitken, 1987). Since the active site of enzyme acetylcholinesterase contains a serine residue (Koelle, 1975), it is possible that the inhibition of this enzyme is due to the phosphorylation of the active site. Kamat and Muthe (1995) reported that the active compound present in *Euphorbia tirucalli* is also a 4- Deoxy phorbol, so the snail's mortality in present study is due to the inhibition of enzyme acetylcholinesterase activity in the nervous tissue of snail.

Thus we can conclude that *E. tirucalli* latex and stem bark are highly toxic to both the snail *L. acuminata* and *I. exustus* and the doses which can be used for killing the snails are safe for the fish. The reversibility of the action of both extracts despite of the high toxicity would be an added advantage in their use.

We thus believe that the latex and stem bark of *E. tirucalli* plant exhibit potential for biological control of snails. The doses found in the study can be extrapolated for use in field trails in larger aquatic environments without harming other non- target aquatic organisms.

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