# Combinations of binary and tertiary toxic effects of extracts of Euphorbia pulcherima latex powder with other plant derived molluscicides against freshwater vector snails

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**Abstract**

Molluscicidal activity of different plant moieties in different combinations (binary or tertiary) were tested against freshwater vector snail Lymnaea acuminata and Indoplanorbis exustus in earthen cemented ponds. These snails are belonging to the family Lymnaeidae are known to act as intermediate host of liver fluke Fasciola hepatica cause endemic fascioliasis in cattle and live stock. Binary (1:1) and tertiary (1:1:1) combinations of the rutin, ellagic acid, betulin and taraxerol with Euphorbia pulcherima latex powder were studied molluscicidal activity against freshwater snails Lymnaea acuminata and Indoplanorbis exustus in earthen cemented pond. It was observed that the molluscicidal activity of E. pulcherima latex powder with other plant product in combinations of binary and tertiary against harmful snails Lymnaea acuminata and Indoplanorbis exustus was time as well as dose dependent. There was a significant negative correlation between $LC_{50}$ values and exposure periods thus increases in exposure time mixed in binary combination (1:1) of the E. pulcherima latex powder with rutin, ellagic acid and taraxerol the $LC_{50}$ values decreased from 3.19 mg/L (24h)> to 1.82 mg/L (96h); 6.75 mg/L (24h)> to 3.65 mg/L (96h) and 10.33 mg/L (24h)> to 7.37 mg/L (96h) respectively against snail Lymnaea acuminata and E. pulcherima latex powder with rutin, ellagic acid and taraxerol the $LC_{50}$ values is decreased from 5.91 mg/L (24h)> to 4.0 mg/L (96h); 8.12 mg/L (24h)> to 5.67 mg/L (96h) and 11.94 mg/L (24h)> to 9.48 mg/L (96h) against Indoplanorbis exustus. Same trend of the toxicity was also observed in the molluscicidal activity of E. pulcherima latex with rutin, ellagic acid, taraxerol and betulin in tertiary combinations (1:1:1) against the freshwater snail Lymnaea acuminata and Indoplanorbis exustus.

## INTRODUCTION

The freshwater snail Lymnaea acuminata and Indoplanorbis exustus is the intermediate hosts of the liver flukes Fasciola hepatica (1,2). Fascioliasis is very common in cattle population and live stock of northern part of India, It is caused by trematode Fasciola hepatica. This snail breeds year-round and lays eggs on the lower surface of the aquatic plants. One way to tackle the problem of fascioliasis is to decline the life cycle of the fluke by destroying the carrier snails (3,4,5). This can be achieved with the aid of synthetic product or alternatively, with molluscicides from plant sources (6,7).

Plant product molluscicides are a focus of attention as a suitable alternative to synthetic molluscicides to their low cost, easy availability, biodegradability and non-toxic to human beings (5,7,8,9). It has been observed that molluscicidal activity of latex, stem bark and leaf of Jatropha gossypifolia and binary, tertiary combinations of Jatropha gossypifolia latex powder with taraxerol, rutin, betulin and ellagic acid are potent molluscicides against freshwater snail Lymnaea acuminata and Indoplanorbis exustus in earthen cemented pond (10). Previously Yadav and Singh 2007, (11) reported the aqueous latex extracts of Euphorbia pulcherima have strong molluscicidal activity against Lymnaea acuminata in pond.

The present study deals with the use latex powder of Euphorbia pulcherima as molluscicidal agent in mixed with binary (1:1) and tertiary (1:1:1) combinations with the rutin, ellagic acid, taraxerol and betulin against freshwater snails Lymnaea acuminata and Indoplanorbis exustus in earthen cemented pond.

## MATERIALS AND METHODS

The euphorbious plant Euphorbia pulcherima was collected locally from their natural habitat and identified by the Botany Department, DDU, Gorakhpur University Gorakhpur

(U.P) India. The latex of the Euphorbia pulcherima was drained in glass tubes by cutting their stem apices, this latex was lyophilized at -40C and lyophilized powder was stored for further use. The freeze-dried powder was mixed with appropriate volume of distilled water to obtain the desired concentrations.

### Figure 1

Table 1 Concentration used for the toxicity testing of latex powder with combinations of binary (1:1) latex powder with rutin, ellagic acid and taraxerol and in tertiary combinations (1:1:1) latex powder with rutin, ellagic acid, taraxerol and betulin against and .

| Treatments | Concentration used (mg/L) | |
|---|---|---|
| | *Lymnaea acuminata* | *Indoplanorbis exustus* |
| *E. pulcherima* latex powder+rutin | 1.5, 2.0, 3.0, 4.0 | 3.5, 4.5, 5.0, 6.0 |
| *E. pulcherima* latex powder+ellagic acid | 3.0, 4.0, 5.0, 6.0 | 5.0, 6.0, 7.0, 8.0 |
| *E. pulcherima* latex powder+taraxerol | 6.5, 7.5, 8.5, 9.5 | 8.5, 10.0, 11.0, 12.0 |
| *E. pulcherima* latex powder+Rutin+betulin | 1.5, 2.5, 3.5, 4.5 | 3.5, 4.5, 5.5, 6.5 |
| *E.pulcherima* latex powder+ellagic acid+betulin | 2.0, 3.0, 4.0, 5.0 | 4.0, 5.5, 6.5, 7.5 |
| *E. pulcherima* latex powder+taraxerol+betulin | 3.0, 4.0, 5.0, 6.0 | 5.0, 5.5, 6.5, 7.5 |

Rutin ($C_{27}H_{30}O_{16}$) (EC NO-205-814-1), ellagic acid ($C_{14}O_6O_8$) (4,4,5,5,6,6-Hexahydroxydiphenic acid, 2,6,2,6-dilactone) (EC NO-207-508-3), betulin ($C_{30}H_{50}O_2$) (Lup-20 (2a)-ene-3-28-diol) (EC NO-207-475-6) supplied by Sigma Chemical Co. P.O. Box 14508 St. Louis. Mo. 63178 USA 314-771-5750, taraxerol extracted from the stem bark of Codiaeum variegatum (12). Different mixed combinations of binary (1:1) and tertiary (1:1:1) were prepared using for the toxicity experiment latex powder of E. pulcherima with rutin, ellagic acid, taraxerol and betulin against freshwater snails Lymnaea acuminata and Indoplanorbis exustus in ponds, for the toxicity experiment mixed combinations binary and tertiary was prepared using the method of Yadav and Singh, 2006 (10).

## TEST ANIMALS

Lymnaea acuminata (2.6±0.3 cm in shell height) and Indoplanorbis exustus (0.87±0.035 cm in shell height) were collected from Ramgarh Lake of Gorakhpur district and acclimatized to laboratory conditions for 72h. Hundred experimental animals were kept in glass aquaria, containing 30L dechlorinated tap water for the freshwater snails Lymnaea acuminata and Indoplanorbis exustus. Toxicity experiments were performed using the method of (10). The experimental animals freshwater snails Lymnaea acuminata and Indoplanorbis exustus were exposed continuously 24h up to 96h to four different concentrations of the doses in earthen cemented ponds (Table 1). Experimental animals of the control group were kept under similar conditions without any treatment.

## EXPERIMENTAL CONDITIONS

The experiment was conducted in two freshwater earthen cemented ponds, 29.28 $m^2$ in area and 9.19 $m^3$ water volumes. Water analysis for various physico-chemical parameters, viz. temperature, pH and dissolved $O_2$ and alkalinity was observed. Water temperature ranged from 27.4-28.6C. The other parameters were within the following range total alkalinity 43-62 ppm, pH 6.8-7.7, and dissolved oxygen 7.8-10.3 mg/L (13).

Mortality was recorded at 24h intervals up to 96h. Lethal concentrations $LC_{50}$ values, upper and lower confidence limits (UCL, LCL) and slope values were calculated by computer programme for analysis of bioassay data POLO computer programme of (14). The regression coefficient was determined between exposure time and different values of $LC_{50}$ (15).

## RESULTS

Toxicity of latex powder of Euphorbia pulcherima with rutin, ellagic acid, taraxerol and betulin in combinations of binary and tertiary against the freshwater snail Lymnaea acuminata and Indoplanorbis exustus was time and dose dependent. Behavioural changes appear with 5 to 10 min of exposure, the initial 30-45 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 enters in the snail body, a musculature twitching and the snails become spirally twisted, which resulted ataxia, convulsion, paralysis and finally death of snails. Prior to death, there was complete withdrawal of the body inside the shell that indicates nerve poisoning.

Toxicity against both the freshwater snails L. acuminata and I. exustus was time as well as dose dependent. There was a significant correlation between $LC_{50}$ values of latex powder of E. pulcherima in binary combinations with latex powder+rutin is decreases from 3.19 mg/L (24h);>2.67 mg/L (48h);> 2.22 mg/L (72h) and 1.82 mg/L (96h); latex powder+ellagic acid is decreases 6.75 mg/L (24h);> 5.59 mg/L (48h);> 4.51 mg/L (72h);> to 3.65 mg/L (96h) respectively and latex powder+taraxerol is decreases from 10.33 mg/L (24h);> 8.88 mg/L (48h);> 7.86 mg/L (72h);> to 7.37 mg/L (96h) respectively against Lymnaea acuminata (Table 2). Same trend of toxicity was observed in the binary combinations of latex powder of E. pulcherima with rutin, ellagic acid and taraxerol against the freshwater snail Indoplanorbis exustus (Fig 1).

### Figure 2

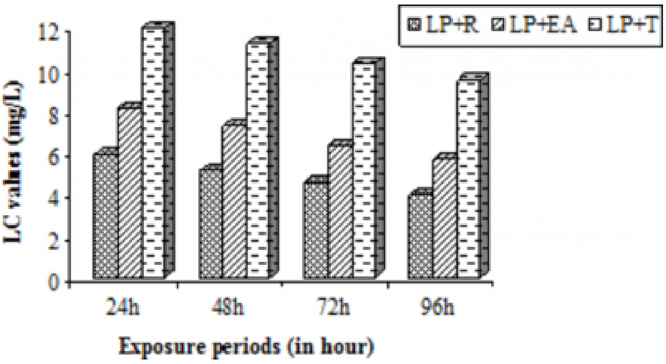
Table 2 Toxicity of binary combinations (1:1) of latex powder with rutin, ellagic acids and taraxerol against freshwater snail at different time exposure periods

| Hours | Combinations | LC values (95%confidence limits) | Slope values |
|---|---|---|---|
| 24h | Latex powder+rutin | $LC_{50}$=3.19 (2.91-3.53) | 0.490±0.105 |
| | Latex powder+ellagic acids | $LC_{50}$=6.75 (6.09-7.95) | 0.169±0.241 |
| | Latex powder+taraxerol | $LC_{50}$=10.33 (9.771-11.266) | 0.728±0.777 |
| 48h | Latex powder+rutin | $LC_{50}$=2.67 (2.20-3.16) | 0.487±0.109 |
| | Latex powder+ellagic acids | $LC_{50}$=5.59 (5.12-6.26) | 0.148±0.214 |
| | Latex powder+taraxerol | $LC_{50}$=8.88 (8.543-9.305) | 0.637±0.686 |
| 72h | Latex powder+rutin | $LC_{50}$=2.22 (2.024-2.422) | 0.478±0.112 |
| | Latex powder+ellagic acids | $LC_{50}$=4.51 (4.153-4.868) | 0.144±0.212 |
| | Latex powder+taraxerol | $LC_{50}$=7.86 (7.501-8.180) | 0.619±0.673 |
| 96h | Latex powder+rutin | $LC_{50}$=1.82 (1.183-2.260) | 0.488±0.120 |
| | Latex powder+ellagic acids | $LC_{50}$=3.65 (3.143-4.041) | 0.182±0.283 |
| | Latex powder+taraxerol | $LC_{50}$=7.37 (6.823-7.785) | 0.766±0.844 |

Concentrations given are the final concentration (mg/L) in earthen cemented pond. 't' ratio was more than 1.96. The heterogeneity factor was less than 1.0. The g-values were less than 0.5. Significant negative regression ($P<0.05$) was observed between exposure time and $LC_{50}$ of treatments, testing significance of the regression coefficient latex powder+rutin, latex powder+ellagic acid and latex powder+taraxerol, 0.8821**; -0.89714*; 0.84392* (*, Linear regression between x and y, **, non-linear regression between log x and y).

### Figure 3

Fig 1. Histogram showing the toxicity (LC) of (LP+R= Latex powder+ rutin, LP+EA= Latex powder+ ellagic acid, LP+T= Latex powder+ taraxerol) against freshwater snail at different exposure periods.




Regarding the tertiary combinations (1:1:1) of E. pulcherima latex powder with rutin, ellagic acid, taraxerol and betulin against snail L. acuminata. There was significant correlation between $LC_{50}$ values and exposure periods. The LC values decrease from latex powder+rutin+betulin is 4.05 mg/L (24h);> 2.73 mg/L (48h);> 2.25 mg/L (72h);> 1.90 mg/L (96h) respectively against L. acuminata. In case of latex powder+ellagic acid+betulin decreases the LC values 5.88 mg/L (24h);> 4.39 mg/L (48h);> 3.17 mg/L (72h);> 2.50 mg/L (96h) respectively against L. acuminata. The treatment of the latex powder+taraxerol+betulin the LC values decreases from 6.16 mg/L (24h);> 4.52 mg/L (48h);> 3.89 mg/L (72h);> 3.39 mg/L (96h) against L. acuminata (Table 3). Similar trend of the toxicity was observed in tertiary combinations of E. pulcherima latex powder with rutin, ellagic acid, taraxerol and betulin against the snail Indoplanorbis exustus (Fig 2).

### Figure 4

Table 3 Toxicity of tertiary combinations (1:1:1) of latex powder with rutin, ellagic acids and taraxerol with betulin against freshwater snail at different time exposure periods.

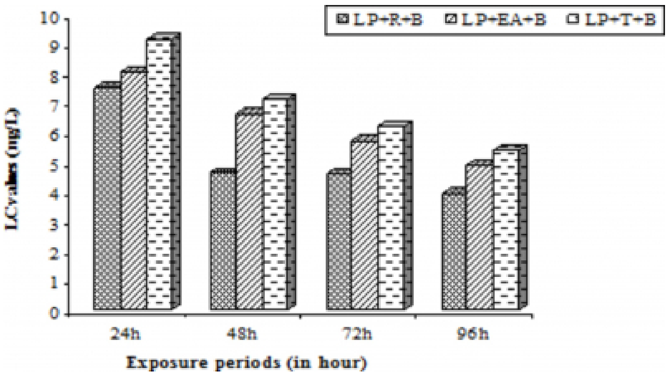
| Hours | Combinations | LC values (95%confidence limits) | Slope values |
|---|---|---|---|
| 24h | Latex powder+rutin+betulin | $LC_{50}$=4.05 (3.607-4.693) | 0.517±0.985 |
| | Latex powder+ellagic acids+betulin | $LC_{50}$=5.88 (15.165-7.215) | 0.891±0.148 |
| | Latex powder+taraxerol+betulin | $LC_{50}$=6.16 (5.632-6.852) | 0.162±0.232 |
| 48h | Latex powder+rutin+betulin | $LC_{50}$=2.73 (2.394-3.073) | 0.450±0.902 |
| | Latex powder+ellagic acids+betulin | $LC_{50}$=4.39 (3.784-5.377) | 0.842±0.142 |
| | Latex powder+taraxerol+betulin | $LC_{50}$=4.52 (4.234-4.821) | 0.152±0.224 |
| 72h | Latex powder+rutin+betulin | $LC_{50}$=2.25 (1.939-2.523) | 0.454±0.942 |
| | Latex powder+ellagic acids+betulin | $LC_{50}$=3.17 (2.323-3.887) | 0.731±0.130 |
| | Latex powder+taraxerol+betulin | $LC_{50}$=3.89 (3.244-4.387) | 0.163±0.248 |
| 96h | Latex powder+rutin+betulin | $LC_{50}$=1.90 (1.168-2.244) | 0.510±0.115 |
| | Latex powder+ellagic acids+betulin | $LC_{50}$=2.50 (1.508-3.115) | 0.804±0.151 |
| | Latex powder+taraxerol+betulin | $LC_{50}$=3.39 (3.005-3.698) | 0.155±0.237 |

Concentrations given are the final concentration (mg/L) in earthen cemented pond. 't' ratio was more than 1.96. The heterogeneity factor was less than 1.0. The g-values were less than 0.5. Significant negative regression ($P<0.05$) was observed between exposure time and $LC_{50}$ of treatments, testing significance of the regression coefficient latex powder+rutin+betulin, latex powder+ellagic acid+betulin and latex powder+taraxerol+betulin, -0.96314**; -0.96528*; 0.87862* (*, Linear regression between x and y, **, non-linear regression between log x and y).

Statistical analysis of the data on the toxicity brings 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, for example, resistance, did not significantly affect the $LC_{50}$ values, as these were within the 95% confidence limits. The dose mortality graphs exhibited steep slope values. The steepness of the slope line indicated a large increase in the mortality of snails with a relatively small increase in the concentration of the toxicant.

### Figure 5

Fig 2. Histogram showing the toxicity (LC) of (LP+R+B= Latex powder+ rutin+betulin, LP+EA+B= Latex powder+ellagic acid+betulin, LP+T+B= Latex powder+ taraxerol+betulin) against freshwater snail at different exposure periods.




## DISCUSSION

It is evident from the results shows that Euphorbia pulcherima latex powder is toxic in mixed binary (1:1) and tertiary (1:1:1) combinations of rutin, ellagic acid, taraxerol and betulin against both the freshwater snails Lymnaea acuminata and Indoplanorbis exustus in earthen cemented pond.

The increased in mortality with increased in exposure periods could be affected by several factors, which may be acting separately or conjointly. For example, uptake of active moiety is time dependent, which leads progressive increase the entrance of the drug and its effects in the snail body (16,17). Singh and Singh 2005 (18) was reported that aqueous latex extract of the Thevetia peruviana and Alstonia scholaris have the strong molluscicidal activity, the molluscicidal activity of aqueous latex extracts of Thevetia peruviana and Alstonia scholaris the $LC_{50}$ decreases 0.43 mg/L (24h) to 0.17 mg/L (96h) and 4.76 mg/L (24h) to 1.76 mg/L (96h) against freshwater snail Lymnaea acuminata. The latex of Jatropha gossypifolia can be used as potential source of molluscicides as the preparation of the latex has sufficient time dependent molluscicidal activitry. Molluscicidal acivity can be increased several times when mixed in binary and tertiary combinations of Jatropha gossypifolia latex powder with other plant derived molluscicides i.e. rutin, ellagic acid, taraxerol and betulin against the snails Lymnaea acuninata and Indoplanorbis exustus (10). The $LC_{50}$ values decreases in binary combination treated with J. gossypifolia latex powder+rutin is 1.36 mg/L (24h) to 0.73 mg/L (96h) against L. acuminata and 4.57 mg/L (24h) to 2.24 mg/L (96h) against I. exustus. In tertiary combination J. gossypifolia latex powder+rutin+betulin the $LC_{50}$ values decreases 6.15 mg/L (24h) to 5.01 mg/L (96h) against L. acuminata (10).

The increase in toxicity of $LC_{50}$ values in single treatment of the aqueous extracts of latex of Euphorbia pulcherima is 3.79 mg/L (24h) to 1.56 mg/L (96h) against Lymnaea acuminata in earthen cemented ponds was reported (11). The highest increase in the toxicity $LC_{50}$ 1.82 mg/L was observed after 96h treatment with E. pulcherima latex powder+rutin in binary (1:1) combinations was tested against freshwater snail Lymnaea acuminata (Table 2), compared to the tertiary (1:1:1) combinations treatment with E. pulcherima latex powder in combination of rutin ellagic acid, taraxerol and betulin against snail Lymnaea acuminata (Table 3).

Obviously natural conditions of the toxicity of tested plants were reduced. The reason for reduced toxicity could be soil particle adsorption or acceleration of the toxicant degradation process by temperature (19). A similar trend was reported by (20), in which the toxicity persistence of plant Masea ramentacea and tea seed cake was short and fish could be stocked in to ponds 4 days after applying the plant pesticides. The potential for using plant Masea ramentacea as a substitute for tea seed cake for killing the predatory fish in freshwater has been shown, however the effective concentration must be determined against the predatory air-breathing fish, such as Clarias sp., Ophicephalus striatus and Anabas testudineus that are generally more tolerant than other fishes (20)

The LC values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep slope values. The steepness of the slope line indicates that there is a large increase in mortality of snails 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 molluscicide used. A steep slope is also indicative of rapid absorption and onset of effects. Even though the slope alone is not a very reliable indicator of toxicological mechanism, yet it is a useful parameter, for such a study (21).

In conclusion it may be stated that binary and tertiary combinations of Euphorbia pulcherima latex powder with other common plant products can be used alternative of other plant origin molluscicides in the earthen cemented ponds to control the population of vector snails in aquatic medium. These binary and tertiary combinations can potentiate the efficacy and reduce the doses of plant derived molluscicides, that the areas of treated water will be environmentally safe.

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