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

R Yadav, A Singh

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

(U.P) India. The latex of the Euphorbia pulcherima was drained in glass tubes by cutting their stem apices, this latex was lyophilized at -40°C 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.

**EXPERIMENTAL CONDITIONS**

The experiment was conducted in two freshwater earthen cemented ponds, 29.28 m² in area and 9.19 m³ water volumes. Water analysis for various physico-chemical parameters, viz. temperature, pH and dissolved O₂ and alkalinity was observed. Water temperature ranged from 27.4-28.6°C. 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 LC₅₀ 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 LC₅₀ (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 LC₅₀ 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).

**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 in ponds, for the toxicity experiment mixed combinations binary and tertiary was prepared using the method of Yadav and Singh, 2006 (10).
Combinations of binary and tertiary toxic effects of extracts of Euphorbia pulcherima latex powder with other plant derived molluscicides against freshwater vector snails

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

Figure 3
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.

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\textsubscript{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).

Statistical analysis of the data on the toxicity brings several important points. The χ\textsuperscript{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\textsubscript{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.
Combinations of binary and tertiary toxic effects of extracts of Euphorbia pulcherima latex powder with other plant derived molluscicides against freshwater vector snails

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 scholars have the strong molluscicidal activity, the molluscicidal activity of aqueous latex extracts of Thevetia peruviana and Alstonia scholars 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 activity. Molluscicidal activity 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 acuminata 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.

ACKNOWLEDGEMENTS

One of the authors (Ram P. Yadav) is thankful to Council of Scientific and Industrial Research, Govt. of India, New Delhi (CSIR sanction no. 13 (8190-A)/Pool/2007 dated 4-12-2007) for award of the SRA under Pool Scientist scheme.

References

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

16,113-138.
Author Information

Ram P. Yadav
Senior Research Associate, Department of Zoology, Gorakhpur University

Ajay Singh
Reader, Natural Products Laboratory, Department of Zoology, Gorakhpur University