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Effects of Synthetic and Extracts of Plant on Reproduction of Snail *Lymnaea Acuminata*

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ABSTRACT

Effects of sublethal treatments (20% and 60% of 24h LC_{50}) ofextracts of plants and synthetic molluscicidesagainst snails *Lymnaea acuminate* will be studied. The synthetics molluscicides (Permethrin and Cypermethrin) caused a significant reduction in the fecundity, hatchability and survival of the young snails. Treatment with the plant extracts increased the hatching period (9-21)of snails with respect to control (7-9 days). Plant extract causes significantly ($p<0.05$) reduction in the fecundity, hatchability and survival of the young snail *Lymnaea acuminata.*A significant recovery was observed when the snails treated with plant extracts. Withdrawal of the snails from constituent treatments after 96h with movement of fresh water enabled a significant reproductive recovery in the snail.

Keywords- Plants molluscicides, reproduction, synthetic molluscicides, snail and fascioliasis.

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INTRODUCTION

Fascioliasis is one of the major causes of mortality and morbidity among livestock [1]. *Fasciola hepatica* and *F. gigantica* is the causative agent of this disease [2,3]. It has been reported that 94% buffaloes, slaughtered in local slaughter houses of Gorakhpur district are heavily infested with *Fasciolagigantica*[2]. In northern part of India the fresh water snails *Lymnaeaacuminata* and *Indoplanorbisexustus* act as the intermediate host of the *F. hepatica* and *F. gigantica*[2,4,5]. Snail *Lymnaea acuminata* breeds round the year and lays eggs on the lower surface of the aquatic vegetation [6]. Synthetic and natural molluscicides have played a significant role in decline the population of *L. acuminata*. As a group of synthetic of pyrethroids cause high mortality or lower dose [7] but high cost, possibility of snail resistance and toxic to non-target organism [8]. The evaluation of molluscicidal properties of plant extracts, when should be easier to handle, comparative safer for environment and non-target organism. The application of plant derived as well as synthetic molluscicide is to decrease the population of snails, thus transmission of diseases (Fascioliasis) would be minimizing [9]. In the present study, effect of sub-lethal exposure synthetic (Permethrin and Cypermethrin) and *Allium satium* bulb and *Abrusprecatorius* were studied on the reproduction of snail *Lymnaea acuminata* withdrawal experiment were also performed to study the reversibility of the effect.

MATERIALS AND METHODS

Snail collection

The adult snail *Lymnaea acuminata* (2.60±0.30 cm in length) were collected locally from ponds, pools, lakes and low-lying submerged areas located almost adjacent to DDU Gorakhpur University campus. These snails were attached on the ventral surface of the leaves of aquatic plants. The collected snails were acclimatized dechlorinated tap water in laboratory condition for 72h and the used as experimental animal. Maintenance of snails culture for the procurements of animal of same size and age which can be used for experiment.

Plants used

Plants used in this works were collected locally. These were identified at the herbarium of the Botany Department, DDD Gorakhpur University, Gorakhpur where voucher herbarium specimens (# No 3126 for *Allium satium* and 3712 for *Abrusprecatorius*) are on deposit.

Synthetic molluscicides

Permethrin (3-phenoxybenzyl (1R, 1S, cis, trans)-2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate was obtained from Bharat Pulverising Mills Pvt. Ltd. India. and Cypermethrin 10% [(RS)-alph-cyano-3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] was obtained from Bharti Minerals Ltd, Secunderabad, India.

Experiment

Groups of 20 snails in 5 l dechlorinated water were exposed to the sub-lethal concentration (20% and 60% of 24h LC_{50}) of Permethrin, Cypermethrin, *Allium* sativum bulb and *Abrusprecatorius*fruit. The total number of eggs laid by treated and control groups of snails were counted every 24h up to 96h. Since it is difficult to identify a particular spawn, capsule containing eggs from each treated group were incubated at 30^0C in covered petridishes containing the same concentration as given to adult snails. The development of embryos was observed under a binocular microscope at regular interval up to their hatching. Percent hatching was studies only with eggs laid after the 24h exposure periods. Dead embryo was removed to avoid any contamination. Survival of young snails was observed up to 72h.

Withdrawal experiment

In order to study the effect of withdrawal from treatment, the snails were exposed to the (20% and 60% of 24h LC_{50}) of synthetic and plant molluscicides following which they are transferred to fresh water. This water was changed every 24h for the next 72h after which hatchability and survival of the young one well be studies.

Statistical analysis

Each experiment was replicate at least six times. The values of fecundity are express as mean ±SE of six replicate. T-test and product moment correlation coefficient was applied to determine significant ($p< 0.05$) negative correlation in between exposure period and fecundity[10].

RESULTS

A significant reduction ($p<0.05$) in the fecundity, hatchability and survival of snail *L. acuminata* exposed to Permethrin, Cypermethrin, *Allium sativum*and *Abrusprecatorius*. In control group of 20 snails laid 213 to 196 eggs/day. Treatment of 20% and 60% of the 24h LC_{50} of Permethrin caused significantly reduction in the egg laying capacity compare than the other treatment (Table-1). The hatching period was prolonged in treated group (9-21 days) with respect to control group (7-9 days) Table-2, while the control snail eggs laid in two row in gelatinous strings with each, equal sized eggs floating equal distance from one another in albuminous fluids bounded externally by a membrane, the eggs in treated snail groups were decrease in number and of variable in shape and size. The sublethal concentration of Permethrin and Cypermethrin(20% and 60% 24h LC_{50}) was more effective in the fecundity of snail *L. acuminata*with the *Allium sativum*and *Abrusprecatorius* (Table-1,2).

The newly hatched snails from eggs exposed to different concentration of plant and synthetic molluscicides were mostly attached to the walls of the container and had thin shells in comparison to the control groups. In addition, movement of the newly hatched snails in the treated group was slow, and the snail had smaller tentacles as compared with those in control groups. A significant reduction ($p<0.05$) negative correlation between the survival period and

survival of young snails, and exposure period and survival of snail *L. acuminata* was also observed. Withdrawal of the snails after 96h treatment to fresh water for the next 72h caused a significant ($p<0.05$) recovery in the fecundity and survival of snails compared with that of snails after 96h of treatment.

Table 1 Effect of sublethal concentration (20% and 60% of 24h LC_{50}) of permethrin, cypermithrine, *Allium sativum*and *Abrusprecatorius* on the fecundity of snail *L. acuminata*.

Treatment	Fecundity24h	Fecundity 48h	Fecundity 72h	Fecundity 96h	Withdrawal
Control	213.34±0.76	207.66±0.55	200.98±0.36	196.45±0.22	210.56±0.22
Permethrin	96.00±0.36*+	58.66±0.92*	35.33±0.36*	29.33±0.36*	88.66±0.34
	62.16±0.64*+	32.98±0.08*	26.00±0.96*	23.00±0.96*	54.68±0.22
Cypermethrin	110.52±0.44*+	80.66±0.84*	68.24±0.21*	41.39±0.92*	91.33±0.36
	83.33±0.66*+	69.00±0.79*	55.19±0.22*	32.34±0.46*	79.68±0.34
A. sativum	119.00±0.73*+	105.66±0.34*	61.84±0.22*	31.00±0.55*	195.60±0.55
	82.33±0.92*+	67.00±0.84*	52.33±0.92*	26.54±0.66*	180.97±0.56
A.precatorius	126.67±0.45*+	110.33±0.96*	80.66±0.71*	46.67±0.62*	204.54±0.34
	103.66±0.54*+	89.16±0.42*	67.50±0.22*	39.33±0.23*	198.00±0.22

Each value is mean ± SC of six replicates- Each replicates represent the egg laid by the group of 20 snails. (*) Significant ($p>0.05$) When student t test was applied to treated and control groups. (+) Products moment correlation coefficient showed that there was significant ($p>0.05$) negative correlation in between exposure period and fecundity of snail *L. acuminata*.
Abbreviation; *A- Allium sativum, A- Abrusprecatorius*

DISCUSSION

Sub-lethal concentration of (20% and 60% of 24h LC_{50}) of Permethrin, Cypermethrin, *Allium sativum*bulb and *Abrusprecatorius*fruit reduced the fecundity of snail *L. acuminata.* The time dependent reduction in fecundity was evidenced by the negative correlation between the fecundity and exposure periods. The effect of *A. sativum, A. indica*oil and *Zingiberofficinale*on the reproduction of the snail *L. acuminata* and their active molluscicidal component allicin, azadirachtin and [6]-gingerol caused a significant reduction in the fecundity, eggs viability and survival of young snail *L. acuminata* [11].

The caudodorsal cells (CDC) in the brain of the test snails release on ovulation hormones that control the reproduction activity of snail *L. acuminata* [12,13]. In the present study it may be possible that the molluscicides affected the caudodorsal cells, reducing the release of the ovulation hormone that resulted in a decrease in the fecundity of the treated snail. The reduction of hatchability of *L. acuminata* exposed to the different molluscicidal component may be due to the interference with the embryonic growth and development of the snails. In treated groups, young larvae were weak unable to break the egg capsule and died owing to starvation. Young snails hatched from the treated egg masses showed delay in attaining maturity in the comparison with control groups. In general the egg shells were thinner and the hatchings had shorter tentacles and slower movement and were smaller in size as compared with untreated groups. Lower reproduction in the treated snails suggest the molluscicidal

activity of the test plant as well as synthetic molluscicides was able to control the population of snail *L. acuminata* by inhibiting development at any stage of growth. Mello-Silva et al., [14] have reported *Euphorbia splendens*latex is a potent molluscicidal activity against the snail *Biomphalariaglabrata*as a sublethal treatment with 1 mg/l of latex significantly reduced the reproductive capacity of the *Biomphalariaglabrata*. A number of different plants products have been effectively used for control of snail reproduction [14-16]. Gomot, [17] have been reported that the treatment of 100 to 200 µg/CD L^{-1} blocked various stages of embryogenesis in *Lymnaeastagnalis*, leading to cessation of egg laying in the snail exposed to 400 µg/CD L^{-1}. The prolong hatching period of 7 to 9 days in control to 09 to 21 days in treated eggs indicates a retardaion in embryonic growth and development in the snails. In the treated group egg masses swelled and become viscous and the egg capsule turned white from a dark cream colour owing to the toxicity of treatments [15]. The reduction in survival of newly hatched snails, with respect to time may be due to the penetration of treated material in young hatched snails as the exposure periods increased from 24 to 72h. A significant negative correlation was observed between the survival of the young snail and the exposure period.

Table-2 Effect of sublethal concentration (20% and 60% of 24h LC_{50}) of permethrin, cypermithrine, *Allium sativum*and *Abrusprecatorius* on the hatchability and survival of the snail *Lymnaea acuminata*.

Treatment	Fecundity 24h	Hatchability% (hatching period)	Percent survival after 24h	Percent survival after 48h	Percent survival after 72h	Withdrawal
Control	213.34±0.76	100 (7-9)	100	100	100	100
Permethrin	96.00±0.36	87.66±0.56 (10-18)	64.00±0.37*+	57.96±0.24	38.72±0.92	49.16±0.66
	62.16±0.64	52.76±0.34 (12-18)	53.56±0.24*+	39.56±0.55	29.33±0.55	41.56±0.34
Cypermethrin	110.52±0.44	94.98±0.22 (10-16)	65.78±0.46*+	51.00±0.96	34.19±0.38	57.00±0.33
	83.33±0.66	67.50±0.12 (9-16)	55.33±0.22*+	42.16±0.34	31.00±0.55	44.96±0.46
A. sativum	119.00±0.73	81.33±0.34 (10-16)	73.56±0.66*+	61.33±0.22	40.56±0.96	92.66±0.22
	82.33±0.92	70.58±0.22 (10-20)	66.19±0.52*+	49.00±0.36	32.46±0.30	89.52±0.36
A.precatorius	126.67±0.45	91.67±0.37 (9-16)	79.16±0.55*+	68.54±0.22	47.19±0.46	95.16±0.52
	103.66±0.54	78.00±0.56 (10-21)	62.96±0.24*+	53.00±0.38	30.84±0.34	90.19±0.92

Each value is mean ± SC of six replicates- Each replicates represent the egg laid by the group of 20 snails. (*) Significant ($p>0.05$) When student t test was applied to treated and control groups. (+) Products moment correlation coefficient showed that there was significant ($p>0.05$) negative correlation in between exposure period and fecundity of snail *L. acuminata*.

Abbreviation; *A- Allium sativum, A- Abrusprecatorius*

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