



ALTERATION IN ENZYMATIC ACTIVITY IN OVOTESTIS OF SNAIL *LYMNAEA ACUMINATA* FED TO BAIT CONTAINING RICIN (*RICINUS COMMUNIS*) AND ATROPINE (*DATURA STRAMONIUM*)

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ABSTRACT

Medical and veterinary trematodiasis, primarily fascioliasis, are spread by lymnaeid snails. The distribution and features of diseases are determined by the vector specificity of fasciolid parasites. By eliminating the Lymnaeid snails, fascioliosis can be controlled by severing the fluke's life cycle. *Lymnaea acuminata* snails were fed several bait formulations in transparent glass aquariums with a diameter of 30 cm. A variety of binary combinations of carbohydrates (Starch, Glucose, Maltose, Sucrose) and amino acids (Proline, Serine, Alanine, Glycine) 10 mM in 100 ml of 2% agar solution plus sub-lethal (20% and 60% of 24-hour LC₅₀) doses of ricin, atropine, and their binary combinations were used to create snail-containing bait formulations. Acid phosphatase and Alkaline phosphatase were significantly inhibited and protease was accelerated in the ovotestis of the vector snail *Lymnaea acuminata* when snails

were fed bait containing sub-lethal concentrations of molluscicides within the snail attractant pellets. The ovotestis of *Lymnaea acuminata* subjected to bait containing 60% of the 24-hour LC₅₀ binary combination of ricin and atropine for 96 hours showed the greatest inhibition in ACP (33.13% of control), ALP (40.15% of control), and acceleration in protease (299% of control).

KEYWORD: Molluscicide, Carbohydrate, Amino acids, Bait formulations, ACP, ALP, Protease, *Lymnaea acuminata*.

INTRODUCTION

Endemic fascioliasis is caused by *Fasciola gigantica* and *Fasciola hepatica* in many regions of the world. Fascioliosis is typically recorded in livestock animals, but more cases of fascioliasis in humans are being reported worldwide. Hypereosinophilia, stomach discomfort, and extremely severe pancreatitis are the hallmarks of human fascioliosis. Delinking the intermediate host's life cycle by eliminating the snails is one strategy to lower the incidence of fascioliosis. A bait formulation of several molluscicides would be a useful tool for killing snails selectively with the least amount of negative impact on the ecosystem and nontarget animals. For bait compositions to be effective, it is crucial to find potent attractant chemicals and molluscicides. An efficient method for controlling pests is to combine molluscicides and snail attractants when creating bait. Our previous research is continued in this paper. The current study aims to assess the impact of sublethal feeding of the molluscicides ricin, atropine, and their binary combination in bait formulations containing attractant carbohydrates (starch, glucose, maltose, sucrose 10 mM) and amino acids (proline, serine, alanine, glycine 10 mM) on various enzyme activities (acid phosphatase, alkaline phosphatase, and protease) in the ovotestis of *L. acuminata*, the intermediate host of the *Fasciola* species.

MATERIALS AND METHODS

Collection of Snails: In this work, test animals were adult *L. acuminata* (2.25 ± 0.20 cm in length), an intermediate host of the liver fluke *Fasciola gigantica*. The snails were gathered locally from low-lying submerged fields and lakes. These snails were acclimated to dechlorinated tap water at 25 ± 1 °C for 72 hours. The water had a pH of 7.1-7.3 and levels of dissolved oxygen, free carbon dioxide, and bicarbonate alkalinity were 6.5-7.2 mg/l, 5.2-6.3 mg/l, and 102.0-150.0 mg/l, respectively.

Plants Used: *Ricinus communis* and *Datura stramonium* were taken to perform the experiment. Both the plants were collected from the D.D.U. Gorakhpur University campus (India).

Pure Compounds: Sigma Chemical Co. (USA) supplied all necessary components, including ricin, atropine, carbohydrates, amino acids, and agar-agar.

Preparation of Bait Formulation: Using the Madsen (1992) approach, bait formulations containing binary combinations in a 1:1 ratio of amino acids Serine (10 mM) and carbohydrates Starch (10 mM) as well as sub-lethal (20% and 60% of 24h LC₅₀) molluscicides (ricin, atropine and their binary combination) were made in 100 ml of 2% agar solution. Amino acid and carbohydrate concentrations were derived from Tiwari and Singh's prior reports. The preceding study by Kumar *et al.* and Singh and Yadav (2025) served as the basis for sub-lethal doses of molluscicides in bait. A consistent 5 mm thickness was applied to these solutions. A 5 mm diameter corer was cut out of the bait that contained sub-lethal molluscicides after it had cooled. For every concentration, six duplicates were made. Bait devoid of molluscicides was supplied to control snails.

Assay apparatus and Procedure: The bioassay was carried out using Tiwari and Singh's approach (2004 a, b). The bioassay chambers are made out of 30-cm-diameter, spotless glass aquaria. Each aquarium was separated into four concentric zones, with diameters of 13, 18, 24, and 30 cm for Zone 3 (Central zone), 2, 1 (Middle zone), and Zone 0 (Outer zone). In the middle of the aquarium, a little annular elevation of 2.4 cm in diameter and 9 mm in height was created (Zone 3). On the aquarium's perimeter, zones 0, 1, 2, and 3 had respective areas of 254.35, 197.82, 121.68, and 132.66 cm². After that, the aquaria were kept at 26 ± 1 °C and filled with 3L of dechlorinated tap water to a height of 8 mm. Ten uniformly sized, individually marked snails were positioned around zone 0 at the beginning of the experiment. Two snails were separated by 66 mm. On the small circular elevation in the centre (Zone 3), one of the prepared baits containing a separate active component—molluscicides—was added simultaneously. Ten snails are used in each of six sets of experiments.

Biochemical Estimations: Following a 24-hour and 96-hour feeding period, the snails were cleaned with water, and the ovotestis was removed and utilized to quantify the enzyme activity. Acid phosphatase, alkaline phosphatase, and protease were three different enzyme activities that were examined in both the feeding and control groups of snails.

Phosphatase: Phosphatase activity assays were performed using Bergmeyer's (1967) method, which Singh and Agarwal (1989) adapted. The ovotestis was centrifuged at $5000g \times 15\text{min}$ at 0°C after being homogenized (2% w/v) in ice-cold 0.9% NaCl. After being decanted, the supernatant was utilized as a source of enzymes. P-nitrophenol was used to create standard curves.

Acid phosphatase: 1.0 ml of acid buffer substrate solution that had been pre-incubated for 10 minutes (made by dissolving 0.41 g of citric acid, 1.125 g of sodium citrate, and 165 mg of disodium salt of p-nitrophenyl phosphate in 100 ml of double-distilled water) was combined with 0.2 ml of enzyme-containing supernatant. After properly mixing the mixture and letting it sit at 30°C for 30 minutes, 4.0 ml of 0.1 N NaOH was added. The production of p-nitrophenol caused the colour to turn yellow. At 420 nm, acid phosphatase activity was measured. The enzyme activity was measured in μ moles of hydrolysed substrate every 30 minutes per milligram of protein.

Alkaline phosphatase: Alkaline phosphatase's hydrolytic activity was measured by adding 0.1 ml of supernatant to 1 ml of an alkaline buffer substrate solution that had been pre-incubated for 10 minutes. This solution was made by dissolving 375 mg of glycine, 10 mg of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and 165 mg of disodium salt of p-nitrophenyl phosphate in 42 ml of 0.1 ml of NaOH and diluting it with double-distilled water to a volume of 100 ml. After fully mixing the mixture, it was incubated at 30°C for 30 minutes. Ten millilitres of 0.02 M NaOH were added to the mixture that was being incubated. An excess of NaOH solution was added to inhibit the reaction. Alkaline phosphatase activity was measured colorimetrically at 420 nm, which is a measure of the yellow colour of p-nitrophenol that is created when p-nitrophenyl phosphate is hydrolysed. The enzyme activity was measured in μ moles of hydrolysed substrate every 30 minutes per milligram of protein.

Protease: The Moore and Stein (1954) approach was used to quantify protease activity. Homogenates (50 mg/mL, w/v) were made in cold distilled water. The supernatant was kept for the enzyme test after they were centrifuged for 15 minutes at 1000 g. The assay mixture consisted of 100 μ mol of phosphate buffer (pH 7.0), 20 mg of heat-denatured haemoglobin (substrate), and 0.5 ml of homogenate supernatant. For thirty minutes, the contents were incubated at 37°C. Before adding the supernatant, 2.0 mL of TCA was added to halt the reaction. After filtering the contents of the samples—both incubated and unincubated—the amount of amino acids in the filtrates was calculated. After adding 2.0 mL of the ninhydrin reagent to 0.5 mL of the filtrate, the mixture was brought to a boil in a water bath for five minutes before being cooled. The colour was measured in a spectrophotometer at 570 nm in relation to a reagent blank after the capacity was filled to 10.0 mL with distilled water. For a total of zero controls, each sample was modified. The blank is processed in the same manner

as the samples and consists of 2.0 mL of ninhydrin reagent and 0.5 mL of distilled water. The enzyme activity was expressed as μ mole of tyrosine/mg protein/h.

Statistical analysis: Each experiment was replicated at least 6 times. Values were expressed as Mean $\pm$ S E. Students 't'-test was applied to determine the significant ($P < 0.5$) difference between bait feeded and control group of the animals.

RESULT

Sub-lethal feeding to 20% and 60% of 24h LC₅₀ of the ricin, atropine alone and their binary combination bait containing binary combination (1:1) of different four types of carbohydrates and amino acids attractant after 24h and 96h exposure caused a significant ($P < 0.05$) inhibition and acceleration in ovotestis of snail *L. acuminata*.

Acid Phosphatase Activity

- Table 1 shows that the control activity level was 1.69 μ moles substrate hydrolysed/30 min/mg protein. Acid phosphatase activity significantly decreased in all treatments, and this effect grew with concentration and time.
- **Maximum inhibition:** 33.13% of control (60% LC₅₀ binary combination, 96h).
- **Minimum inhibition:** 76.92% of control (20% LC₅₀ ricin, 24h).

Alkaline Phosphatase Activity

Alkaline phosphatase activity in control snails was 2.54 μ moles substrate hydrolysed/30 min/mg protein (Table 2). Enzyme activity was dramatically decreased by treatments in a dose- and time-dependent manner.

- The **Greatest inhibition** was 40.15% of the control (60% LC₅₀ binary combination, 96h).
- The **Lowest inhibition** was 63.38% of the control (20% LC₅₀ ricin, 24 hours).

Protease Activity

2.55 μ moles of tyrosine equivalents/mg protein/h was the baseline protease activity in the controls (Table 3). Unlike other enzymes, protease activity rose dramatically after exposure, and this increase was influenced by exposure duration and concentration.

- The **Highest increase** was 299.21% of the control (60% LC₅₀ binary combination, 96h).
- A **Minimum increase** of 144.70% of the control (20% LC₅₀ ricin, 24 hours).

Table 1: Effect of sub-lethal treatment (20% and 60% of 24h LC₅₀) of 24h and 96h exposure of ricin and atropine alone and their binary combination (1:1) on the activity of acid phosphatase (μ mole substrate hydrolyzed/30min/mg protein) in ovotestis of the snail *Lymnaea acuminata*.

Treatment	Acid Phosphatase (μ mole substrate hydrolyzed/30 min/mg protein)			
	After (24h) exposure		After (96h) exposure	
	20% of 24h LC ₅₀	60% of 24h LC ₅₀	20% of 24h LC ₅₀	60% of 24h LC ₅₀
Control	1.69 $\pm$ 0.09 (100)			
Ricin	1.30 $\pm$ 0.11* (76.92)	1.28 $\pm$ 0.02* (75.73)	1.25 $\pm$ 0.11* (73.96)	1.20 $\pm$ 0.40* (71.00)
Atropine	1.20 $\pm$ 0.01* (71.00)	1.15 $\pm$ 0.03* (68.04)	1.09 $\pm$ 0.21* (64.49)	1.02 $\pm$ 0.60* (60.35)
Binary combination	1.11 $\pm$ 0.02* (65.68)	1.03 $\pm$ 0.01* (60.94)	0.64 $\pm$ 0.00* (37.86)	0.56 $\pm$ 0.02* (33.13)

- Each value is mean $\pm$ SE of six replicates.
- Values in parentheses are percent change with control taken as 100%.
- (*) Significant ($P < 0.05$) when student's t- test was applied in between treated and control group.

Table 2: Effect of sub-lethal treatment (20% and 60% of 24h LC₅₀) of 24 h and 96 h exposure of ricin and atropine alone and their binary combination (1:1) on the activity of alkaline phosphatase (μ mole substrate hydrolyzed/30 min/mg protein) in ovotestis of the snail *Lymnaea acuminata*.

Treatment	Alkaline Phosphatase (μ mole substrate hydrolyzed/30min/mg protein)			
	After(24h)exposure		After(96h)exposure	
	20% of 24h LC ₅₀	60% of 24h LC ₅₀	20% of 24h LC ₅₀	60%of 24h LC ₅₀
Control	2.54 $\pm$ 0.06 (100)			
Ricin	1.61 $\pm$ 0.30* (63.38)	1.59 $\pm$ 0.11* (62.59)	1.51 $\pm$ 0.12* (59.44)	1.47 $\pm$ 0.12* (57.87)
Atropine	1.51 $\pm$ 0.01* (59.44)	1.46 $\pm$ 0.06* (57.48)	1.38 $\pm$ 0.11* (54.33)	1.32 $\pm$ 0.09* (51.96)
Binary combination	1.40 $\pm$ 0.02* (55.11)	1.31 $\pm$ 0.01* (51.57)	1.16 $\pm$ 0.03* (45.66)	1.02 $\pm$ 0.04* (40.15)

- Each value is mean $\pm$ SE of six replicates.
- Values in parentheses are percent change with control taken as 100%.
- (*) Significant ($P < 0.05$) when student's t- test was applied in between treated and control group.

Table 3: Effect of sub lethal treatment (20% and 60% of 24h LC₅₀) of 24h and 96h exposure of ricin and atropine alone and their binary combinations (1:1) on the activity of enzyme protease (μmoles of tyrosine equivalents/h/mg protein) in ovotestis of the snail *Lymnaea acuminata*.

Name of molluscicides	Protease (μmoles of tyrosine equivalents/h/mg protein)			
	After(24h) exposure		After(96h)exposure	
	20% of24hLC ₅₀	60% of24hLC ₅₀	20% of24hLC ₅₀	60% of24hLC ₅₀
Control	2.55±0.08 (100)			
Ricin	3.69±0.07* (144.70)	3.93±0.05* (154.11)	4.45±0.04* (174.50)	4.94±0.07* (193.72)
Atropine	4.44±0.06* (174.11)	4.97±0.05* (194.90)	5.20±0.07* (203.92)	5.85±0.04* (229.41)
Binary combination	5.04±0.03* (197.64)	6.06±0.05* (237.64)	6.09±0.02* (238.82)	7.63±0.03* (299.21)

- Each value is mean ± SE of six replicates.
- Values in parentheses are percent change with control taken as 100%.
- (*) Significant (P<0.05) when student's 't'-test was applied in between treated and control group.

DISCUSSION

The results section makes clear that the active ingredients in the bait formulation, ricin and atropine from *Ricinus communis* and *Datura stramonium*, were more successful in killing *Lymnaea acuminata*. Ricin and atropine have been shown to have strong molluscicidal effects on *Lymnaea acuminata* when directly released into aquarium water (Singh and Yadav 2025). These bioactive components of plant-origin molluscicides also inhibited acid phosphatase (ACP), a lysosomal enzyme that is crucial for catabolism, pathological necrosis, autolysis, and phagocytosis. Protein levels in gastropods may decrease if alkaline phosphatase (ALP), which is essential for protein synthesis, shell building, and other secretory processes, is inhibited. It is crucial for the movement of metabolites across the membrane. The direct interference of these active molluscicidal components with the enzyme may be the cause of the suppression of ACP and ALP activity. These active molluscicidal ingredients in bait formulations have been shown by Singh and Yadav (2025) to function as powerful molluscicides when fed to snails. In the current investigation, molluscicides enter the snail body through the digestive system since they were utilized as bait.

In a previous investigation, molluscicides were delivered directly into water through the body's surface. Both approaches are equally successful in killing the snails, despite the differing ways that molluscicide enters the body. Protease is an enzyme that hydrolyses proteins to release tiny peptides and amino acids. Increased protease activity in the ovotestis demonstrated that proteins had undergone proteolysis and were used for enhanced energy consumption. Numerous researchers have documented similar protease tendencies in a variety of animals, including *Pila globosa* and *Tilapia mossambica*.

The current study demonstrates that the addition of ricin and atropine to the bait significantly inhibited the activity of the enzymes ALP and ACP over time and in a dose-dependent manner, while increasing the activity of protease in the snails *L. acuminata*. The fatal concentrations of molluscicide in bait formulations (2.1, 1.25, 0.61 mg/L 24h LC50) and direct treatment ricin/atropine (10.15, 9.66, 8.48 mg/L 24h LC50) varied. According to the current study, bait formulations are a better way to kill snails than directly releasing molluscicides into an aquatic environment. Because the effects of molluscicides are more than ten times greater in bait form than when they are directly disposed of in aquarium water, it is safer and more cost-effective. When these potent molluscicidal chemicals were administered to snails in bait formulations, Singh and Yadav (2025) found that the ovotestis of *Lymnaea acuminata* showed a decrease in protein and nucleic acid levels and a depletion of amino acids.

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