

## Bait Formulation Toxicity of Plant Derived Molluscicides in Attractant Food Pellets Against Vector Snail, *Lymnaea acuminata*

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**Abstract:** Snail control is one of the most important tools in the campaign to reduce the incidence of fascioliasis. Use of molluscicides in the attractant food pellet (AFP) is one of the effective methods of snail control. Attractant food pellets containing amino acid proline and agar plus different concentrations of the molluscicides *Azadirachta indica* bark powder, *Annona squamosa* seed powder and their bio-active components azadirachtin and acetogenin were tested for molluscicidal activity up to 144h against the snail, *Lymnaea acuminata*. Active molluscicidal components of both plants were highly toxic to *L. acuminata* compared with their crude forms.

**Key words:** Attractant Food Pellets • Bait Formulation • *Fasciola* • *L. acuminata* • Molluscicides • Proline

### INTRODUCTION

Certain freshwater snails are of great economic importance because they act as intermediate hosts for digenetic trematodes. Two such flukes, *Fasciola hepatica* Linnaeus and *Fasciola gigantica* Cobbold, are transmitted by the snail *Lymnaea acuminata* Lamarek (Lymnaeidae) which cause endemic fascioliasis in cattle population of eastern region of the state of Uttar Pradesh in India [1, 2]. An obvious solution to reduce the incidence of fascioliasis is to de-link the life cycle of fluke by destroying the vector snails [3-8]. The development of a selective and safe molluscicide should always be a realistic goal. It must be effective at low concentrations and exert minimal adverse effect on the other biota sharing the same habitat with snail. Lack of contact between molluscicides and target snail population due to mushy vegetation, dilution in upwelling sewage water are two main causes of the failure of snail control programmes. It has been reported earlier that the utilization of attractants, arrestants, phagostimulants and toxic factors in control release formulations or bait formulations designed to remove trematode host snails from the fresh water environment is cost effective and ecologically acceptable [9]. The snails use chemical signals for locating food sources. These signals are released from the dead and living aquatic organisms into the modular system of the snails [10-13]. Proline is the strongest attractant for *L. acuminata* at 20 mM concentration [14]. Bait

formulation containing attractant and a molluscicide is an expedient approach in order to lure the target snail population to the molluscicide. In the present study different plant derived molluscicides have been used along with amino acid proline in bait formulation against *L. acuminata*.

### MATERIALS AND METHODS

Agar-agar, proline, different plant derived molluscicides such as *Annona squamosa* seed powder, *Azadirachta indica* bark powder and their bio-active components, acetogenin and azadirachtin (Sigma Chemical Co. USA) were used in bait formulation. Adult *L. acuminata* (2.25±0.20 cm in length) were collected locally from lakes and low lying submerged fields in Gorakhpur. The snails were acclimatized for 72 hours in dechlorinated tap water at 25±1°C. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were set to 6.5-7.2 mg/l, 5.2-6.3 mg/l and 102.0-105.0 mg/l, respectively.

**Preparation of Attractant Food Pellets (AFP):** Attractant food pellets (AFP) were prepared according to previous method [15] as modified by us [14, 16, 17]. For the preparation of attractant food pellets, 20 mM proline was added to 2% agar solution. After boiling, each of the selective molluscicides were added to the solution in different concentrations (Table 1). The mixture was stirred

constantly for 30 minutes and spread to a uniform thickness (5 mm). After cooling, the pellets were cut in small pieces 5 mm in diameter

**Bioassay and Apparatus** The bioassay was performed as reported earlier [14, 16, 17]. The bioassay chamber consists of a clean glass aquarium having a diameter of 30 cm. Each aquarium was divided into four concentric zones; zone 3 (central one), zones 2 and 1 (middle zone) and zone 0 (outer one) had diameters of 13, 18, 24 and 30 cm, respectively. A small annular elevation of 9 mm

height and 2.4 cm in diameter was made in the centre of aquarium (zone 3). Zone 0 had an area of 254 cm<sup>2</sup> on the periphery of aquarium. The aquaria were then filled with 500 ml of dechlorinated tap water to a height of 8 mm and maintained at 25±1°C. At the start of the assay ten individually marked snails of uniform size were placed on the circumference of zone 0. The distance between two snails was 66 mm. Simultaneously, one of the prepared bait of different molluscicides was added on the small annular elevation in the centre (zone 3). The location of each snail was recorded every 15 min for two hours.

Table 1: Mean number of snails *L. acuminata* in zone three in contact with the attractant food pellets (AFP) that contain different molluscicides after two hours from beginning of experiment

Molluscicides	Concentration of Molluscicides				
	0.15%*	0.25%	0.5%	0.75%	1.0%
<i>Azadirachta indica</i> (BaP)	3.67±0.2 (78.0)+	3.5±0.96 (53.8)	3.5±0.22 (61.8)	3.0±0.44 (50.0)	2.67±0.42
<i>Annona squamosa</i> (SP)	2.5±0.42 (57.7)+	1.5±0.34 (33.3)	1.33±0.5 (39.9)	1.33±0.42 (39.9)	0.83±0.46 (31.2)
Azadirachtin	1.4±0.35 (46.7)+	1.33±0.21 (46.9)	1.0±0.63 (33.3)	1.5±0.34 (42.8)	0.83±0.16 (27.6)
Acetogenin	3.0±0.25 (60.0)+	2.67±0.21 (57.2)	2.33±0.21 (53.8)	1.83±0.40 (52.2)	1.67±0.21 (45.3)
Control (Agar)	4.5±0.34 (81.81)+	4.66±0.21 (78.23)	5.5±0.16 (74.8)	4.33±0.21 (78.56)	5.33±0.47 (77.68)

Values in parentheses are percentages of snails successfully locating AFP i.e. snails in Z<sub>3</sub> compared with that of failed in their location

Statistically significant (p<0.05) when two way ANOVA was applied in between different molluscicides (+) and their different concentrations (\*)

Abbreviation: BaP- Bark powder, SP- Seed powder

Table 2: Toxicity of bait formulations of molluscicides against the snail *L. acuminata* at different time exposure

Exposure period	Molluscicides	LC <sub>50</sub> % in AFP	LCL	UCL	Slope Value	t-ratio	G-value	Heterogeneity
24h	<i>A. squamosa</i> SP	1.57	1.07	4.38	1.8±0.450	3.92	0.24	0.28
	<i>A. indica</i> BP	1.35	0.84	4.10	1.45±0.33	4.38	0.20	0.32
	Acetogenin	1.19	1.00	1.60	2.41±0.49	4.84	0.16	0.15
	Azadirachtin	1.25	1.08	1.88	2.75±0.65	4.24	0.21	0.19
48h	<i>A. squamosa</i> SP	1.53	0.93	11.29	1.12±0.37	3.03	0.41	0.15
	<i>A. indica</i> BP	0.92	0.59	2.59	1.14±0.27	4.15	0.22	0.16
	Acetogenin	1.01	0.85	1.33	2.12±0.48	4.36	0.20	0.27
	Azadirachtin	0.98	0.83	1.32	2.38±0.60	3.95	0.24	0.11
72h	<i>A. squamosa</i> SP	0.86	0.61	2.00	1.16±0.34	3.35	0.34	0.22
	<i>A. indica</i> BP	0.32	0.24	0.45	1.34±0.25	5.21	0.14	0.28
	Acetogenin	0.76	0.64	0.88	2.61±0.50	5.22	0.14	0.29
	Azadirachtin	0.63	0.50	0.73	2.71±0.61	4.45	0.19	0.11
96h	<i>A. squamosa</i> SP	0.44	0.28	0.61	1.19±0.33	3.55	0.30	0.21
	<i>A. indica</i> BP	0.20	0.14	0.27	1.36±0.25	5.30	0.13	0.21
	Acetogenin	0.57	0.46	0.66	3.26±0.56	5.80	0.11	0.36
	Azadirachtin	0.53	0.45	0.59	4.75±0.74	6.37	0.09	0.37
120h	<i>A. squamosa</i> SP	0.28	0.08	0.42	0.99±0.33	2.99	0.42	0.28
	<i>A. indica</i> BP	0.12	0.07	0.15	1.68±0.28	5.99	0.10	0.20
	Acetogenin	0.48	0.38	0.55	3.92±0.70	5.55	0.12	0.44
	Azadirachtin	-	-	-	-	-	-	-
144h	<i>A. squamosa</i> SP	0.18	0.07	0.26	1.46±0.35	4.15	0.22	0.34
	<i>M. indica</i> BP	0.09	0.05	0.12	2.03±0.33	6.11	0.10	0.42
	Acetogenin	0.28	0.08	0.42	0.99±0.33	2.99	0.42	0.28
	Azadirachtin	-	-	-	-	-	-	-

Product moment correlation showed significant (p<0.05); negative correlation between the exposure period and LC<sub>50</sub> of different molluscicides. Abbreviations as in Table 1

Six sets of experiments were carried out with ten snails each for every molluscicide used in this study. The mortality of the snails was observed after every 24h up to 144h. Lethal concentration values ( $LC_{50}$ ), lower and upper confidence limits (LCL and UCL), slope values, t-ratio, 'g' value and heterogeneity factor were calculated using POLO computer programme [18]. Two way ANOVA and product moment correlation coefficient was applied between different data obtained in Tables 1-2 [19].

## RESULTS

Low attraction (27.6%) of the snails was observed in zone 3 in AFP containing 1.0% of azadirachtin. The maximum attraction (60.0%) was observed in zone 3 in AFP containing 0.15% of acetogenin. The attraction of the snails was significantly ( $p < 0.05$ ) reduced with increasing concentration of different molluscicides in AFP. Molluscicidal activity of different AFP containing molluscicides against *L. acuminata* is time and dose independent (Table 2). There was a significantly ( $p < 0.05$ ) negative correlation between exposure period and  $LC_{50}$  in different molluscicides. AFP containing bioactive components of different plants were more toxic (acetogenin 24h  $LC_{50}$ -1.19% in AFP; 144h  $LC_{50}$ -0.28% in AFP) than the crude preparations. The crude preparations of plant derived molluscicides containing AFP caused significant molluscicidal activity against *L. acuminata* (Table 2).

The slope values given in Tables 2 were steep. Separate estimate of  $LC_{50}$  based on each of the six replicates was found to be within 95% confidence limits. The t-ratio was greater than 1.96 and the heterogeneity was 1.0. The 'g' value was less than 0.5 at all probability levels (90, 95, 99).

## DISCUSSION

Higher attraction of the snails towards AFP containing 0.15% plant derived molluscicides appears to be due to the slower release of molluscicidal compounds in comparison with synthetic ones. Higher concentration of plant derived molluscicides and their active components in AFP caused less attraction. It indicates that when higher titer of active components of plant derived molluscicides was used in AFP, snails were less attracted. There was a significant decrease in the attraction of *L. acuminata* towards AFP containing

molluscicides compared with AFP alone with a significant variation in mean number of snails in zone 3 containing different concentrations of molluscicides after two hours of exposure. AFP containing acetogenin attracted more snails at lower concentrations than *A. squamosa* seed powder. It indicates that *A. squamosa* seed powder; instead of acetogenin contain some other compounds which reduce the attraction of snails towards AFP. In contrast AFP containing *A. indica* bark powder attracted more snails than their pure compound azadirachtin. It seems that these plant derived molluscicides either contain some other compounds which attract the snails or the concentrations of active molluscicidal components are less in crude preparations. However, toxicity of these AFP containing molluscicides was time and dose independent as evident from the negative correlation between  $LC_{50}$  in different molluscicides and exposure period.

Treatment of seed powder of *A. squamosa*, bark powder of *A. indica* and their bioactive components such as acetogenin and azadirachtin in aquatic environment are highly toxic to *L. acuminata* [19-21]. Toxicity of acetogenin (24h  $LC_{50}$ - 1.19% in AFP) extracted from the seeds of *A. squamosa* is higher than other plant derived molluscicides. Seeds of *A. squamosa* were used to kill human lice [22] and their organic extracts have been reported to possess insecticidal activity [23-25]. Molluscicidal activity of *A. indica* bark powder (24h  $LC_{50}$  1.35% in AFP) is lower than the bio-active component azadirachtin (24h  $LC_{50}$  1.25% in AFP). Toxicity of AFP containing azadirachtin was effective only up to 96h. It indicates that it is less stable in water or it is metabolized in snail body [20].

It has been also reported that the snails are highly attracted towards the bait formulation of plant origin [26, 27]. This behavioral response of the snails towards plant origin was because of the presence of some compounds that may be essential dietary requirements for the snails in their habitat. Use of plant derived molluscicides in aquatic environments requires large amounts of molluscicides for effective control of snails. Using attractant food pellets like this study will be beneficial since it requires small quantities of molluscicides while killing the target pest specifically. The present study shows that the use of AFPs containing plant derived molluscicides is very effective in killing the snail *L. acuminata*. Use of these plant derived molluscicides inside attractant food pellets are ecologically sound, target specific and economic.

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