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RESEARCH ARTICLE

Toxicity of Plant Derived Molluscicides in Attractant Food Pellets against Snail, *Lymnaea Acuminata*

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ABSTRACT

Use of molluscicides in the attractant food pellet (AFP) is one of the effective methods of snail control. attractant food pellets containing starch and agar plus different concentrations of these molluscicides (Azadirachta indica bark powder, Allium sativum bulb powder, Polianthes tuberosa bulb powder, Annona squamosa seed powder, their active components azadirachtin, allicin, hecogenin, acetogenin; herbal molluscicide pestoban and a synthetic molluscicide, Snail Kill, were tested for molluscicidal activity for 144h against the snail, Lymnaea acuminata. Active components of all the plant derived molluscicides were highly toxic to L. acuminata compared with their crude forms. The stability of bait formulations was studied by storing the pellets up to 4 weeks. Storage of molluscicide baits caused higher reduction in their toxicity in comparison with synthetic molluscicides.

Keywords: *Attractant food pellets, Bait formulation, Fasciola, L. acuminata, Molluscicides, Starch*

Liver- flukes *Fasciola* hepatica Linnaeus and Fasciola gigantica Cobbold cause endemic fascioliasis in cattle population of eastern Uttar Pradesh [1-2]. The snail Lymnaea (Radix) acuminata Lamarck (Lymnaeidae) is the vector of these flukes. One way to reduce the incidence of fascioliasis is to de-link the life cycle of fluke by destroying the intermediate hosts [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 meshy vegetation, dilution in upwelling sewage water are two main causes of the failure of snail control programme. 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 [9-13]. Starch is the strongest attractant for L. acuminata [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 starch in bait formulation against *L. acuminata*.

MATERIALS AND METHODS

Agar, starch, different plant derived molluscicides such as *Allium sativum* and *Polianthes tuberosa* bulb powder, *Annona squamosa* seed powder and *Azadirachta indica* bark powder and their active components allicin, hecogenin, acetogenin and azadirachtin (Sigma Chemical Co. USA), Snail Kill (metaldehyde-Pesticide India) and herbal molluscicide, Pestoban (Liquid concentrate of *Cedrus deodara*, *Azadirachta indica* and seed powder of *Embelia ribes* in 90:2:1 ratio- Indian Herbs, Research and Supply Co. Pvt. Ltd., India) 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 ± 1^0 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.

Attractant food pellets (AFP) were prepared according to previous method [15] as modified by us [16]. 10 grams of starch (10 mM) was added to 2% agar solution. After boiling, each of the selective molluscicides were added to the solution in different concentrations (Table1), the mixture was stirred constantly for 30 minutes and spread to a uniform thickness (5 mm). After

Table 1. Mean number of snail 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				
	0.1%*	0.2%	0.5%	0.7%	1.0%
A. squamosa (SP)	1.16±0.16 (58.0) +	0.83±0.16 (43.0)	1.33±0.21 (53.4)	1.67±0.21 (45.6)	0.83±0.16 (50.0)
A. sativum (BP)	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 (47.0)
P. tuberosa (BP)	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)
A. indica (Ba P)	3.5±0.34 (60.0) +	1.83±0.16 (37.8)	3.0±0.22 (55.6)	2.33±0.42 (53.8)	0.83±0.30 (31.2)
Acetogenin	3.0±0.36 (50.0) +	2.0±0.25 (50.0)	1.16±0.3 (27.8)	1.5±0.22 (42.8)	0.5±0.22 (20.0)
Allicin	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)
Hecogenin	0.67±0.21 (50.3) +	0.33±0.21 (22.0)	0.5±0.22 (33.3)	0.83±0.30 (27.6)	0.33±0.21 (13.2)
Azadirachtin	1.16±0.16 (46.4) +	1.0±0.25 (31.5)	1.0±0.44 (33.3)	0.83±0.30 (33.3)	0.67±0.42 (21.2)
Snail Kill	1.67±0.21 (45.5) +	1.5±0.34 (42.8)	1.33±0.49 (39.9)	1.16±0.40 (36.7)	0.67±0.21 (40.2)
Pestoban	2.5±0.42 (57.7) +	1.5±0.34 (33.3)	1.33±0.5 (39.9)	1.33±0.42 (30.7)	0.83±0.40 (31.2)
Control (Agar)	4.33±0.21 (76.36)	3.83±0.16 (72.47)	4.5±0.34 (81.81)	4.16±0.16 (71.23)	3.5±0.34 (74.84)

Values in parentheses are percentages of snails in zone 3 (in contact with attractant food pellet) compared with snails in zone 1 and 2.
Statistically significant (p<0.05) when two way ANOVA was applied in between different molluscicides (+) and their different concentrations (*).
Abbreviations: SP- Seed powder, BP- Bulb powder, BaP- Bark powder

cooling, the pellets were cut out from the layer with a corer (5 mm diameter).

Assay and Apparatus

The bioassay was performed as reported earlier [14, 16]. 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 zone), zones 2 and 1 (middle zone) and zone 0 (outer zone) 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^2 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±1^0 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. 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 the test with every 24h up to 144h. Lethal 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 [17]. One/two–way ANOVA and product moment correlation coefficient was applied between the different data obtained in Tables 1-4 [18]. These experiments were repeated in stored pellets kept for 7, 14, 21 and 28 days under laboratory conditions.

RESULTS

Low attraction (45.5%) of the snails was observed by the Snail Kill compared to plant derived molluscicides in zone 3 at 0.1% concentration in AFP (Table 1). 0.1% AFP containing *A. squamosa* seed powder, *A. sativum*, *P. tuberosa* bulb powder, *A. indica* bark pow-

Table 2. Mean number of snail L. acuminata in zone three in contact with the stored attractant food pellets (AFP) containing 0.1% molluscicides.

Molluscicides	TIME OF STORAGE (IN DAYS)				
	0	7	14	21	28
Pestoban	2.5±0.42 (57.7) +	2.16±0.16 (50.0)	1.83±0.16 (61.1)	1.75±0.19 (43.2)	1.16±0.16 (41.2)
Snail Kill	1.67±0.21 (45.5) +	1.67±0.21 (44.2)	1.33±0.21 (36.3)	0.83±0.16 (38.2)	0.67±0.21 (34.5)
A.indica (Ba P)	3.5±0.34 (60.0) +	3.33±0.21 (55.5)	1.83±0.16 (47.7)	1.5±0.22 (40.9)	1.33±0.21 (34.5)
A.sativum (BP)	3.67±0.2 (78.0) +	4.0±0.36 (44.4)	3.16±0.47 (41.2)	2.72±0.51 (40.4)	2.16±0.16 (35.0)
P.tuberosa (BP)	3.0±0.25 (60.0) +	1.83±0.16 (31.3)	1.67±0.21 (35.7)	1.16±0.16 (27.8)	0.83±0.16 (31.2)
A.squamosa (SP)	1.16±0.16 (58.0) +	1.67±0.21 (35.7)	1.33±0.42 (30.7)	1.16±0.16 (27.8)	0.83±0.16 (31.2)
Acetogenin	3.0±0.36 (50.0) +	2.67±0.21 (47.0)	2.0±0.44 (40.0)	1.5±0.34 (33.3)	1.16±0.16 (27.8)
Azadirachtin	1.16±0.16 (46.4) +	0.83±0.16 (31.2)	0.83±0.16 (31.2)	0.67±0.42 (28.6)	0.5±0.22 (25.0)
Allicin	1.4±0.35 (46.7) +	1.33±0.42 (36.3)	1.16±0.16 (34.9)	0.83±0.3 (31.2)	0.83±0.3 (31.2)
Hecogenin	0.67±0.21 (50.3) +	0.67±0.21 (28.6)	0.67±0.21 (28.6)	0.5±0.22 (25.0)	0.33±0.21 (24.0)
Control (Agar)	4.5±0.34 (81.81)	4.66±0.21 (78.23)	5.5±0.16 (74.87)	4.33±0.21 (78.56)	5.33±0.47 (77.68)

Values in parentheses are percentages of snails in zone 3 (in contact with the stored attractant food pellet) compared with snails in zone 1 and 2.
+ Statistically significant (p<0.05) when one way ANOVA was applied in between the number of snails in different storage period of bait formulations.
Abbreviations as in table 1.

Table 3. Toxicity in different bait formulations of molluscicides against the snail L. acuminata at different time exposure.

Expo-sure Period	Molluscicides	LC_{50} % in AFP	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
	A. sativum	1.57	1.07	4.38	1.80±0.45	3.92	0.24	0.28
	P. tuberosa	2.07	1.5	5.0	2.09±0.54	3.87	0.25	0.17
24h	Allicin	1.35	0.84	4.1	1.45±0.33	4.38	0.20	0.32
	Hecogenin	1.54	1.04	6.0	1.9±0.57	3.34	0.34	0.13
	Snail Kill	1.36	1.04	2.56	2.52±0.57	4.38	0.19	0.30
	A. sativum	1.53	0.93	11.29	1.12±0.37	3.03	0.41	0.15
	P. tuberosa	1.19	1.00	1.60	2.41±0.49	4.84	0.16	0.15
48h	Allicin	0.92	0.59	2.59	1.14±0.27	4.15	0.22	0.16
	Hecogenin	0.90	0.74	1.32	2.34±0.51	4.52	0.19	0.16
	Snail Kill	1.30	0.97	2.68	2.07±0.50	4.08	0.23	0.22
	A. sativum	0.86	0.61	2.0	1.16±0.34	3.35	0.34	0.22
	P. tuberosa	1.01	0.85	1.33	2.12±0.48	4.36	0.20	0.27
72h	Allicin	0.32	0.24	0.45	1.34±0.25	5.21	0.14	0.28
	Hecogenin	0.70	0.58	0.93	2.0±0.48	4.33	0.20	0.15
	Snail Kill	0.94	0.76	1.43	2.08±0.46	4.48	0.19	0.16
	A. sativum	0.44	0.28	0.61	1.19±0.33	3.55	0.30	0.21
	P. tuberosa	0.76	0.64	0.88	2.61±0.50	5.22	0.14	0.29
96h	Allicin	0.20	0.14	0.27	1.36±0.25	5.30	0.13	0.21
	Hecogenin	0.55	0.45	0.67	2.15±0.47	4.53	0.18	0.20
	Snail Kill	0.68	0.55	0.91	1.84±0.44	4.19	0.21	0.16
	A. sativum	0.28	0.08	0.42	0.99±0.33	2.99	0.42	0.28
	P. tuberosa	0.57	0.46	0.66	3.26±0.56	5.80	0.11	0.36
120h	Allicin	0.12	0.07	0.15	1.68±0.28	5.99	0.10	0.20
	Hecogenin	0.39	0.27	0.46	2.11±0.47	4.43	0.19	0.20
	Snail Kill	0.41	0.29	0.51	1.89±0.44	4.28	0.20	0.18
	A. sativum	0.18	0.07	0.26	1.46±0.35	4.15	0.22	0.34
	P. tuberosa	0.48	0.38	0.55	3.92±0.70	5.55	0.12	0.44
144h	Allicin	0.09	0.05	0.12	2.03±0.33	6.11	0.10	0.42
	Hecogenin	0.27	0.17	0.34	2.63±0.53	4.95	0.15	0.53
	Snail Kill	0.33	0.25	0.40	2.90±0.50	5.82	0.11	0.31

Product moment correlation showed significant ($p<0.05$); negative correlation in between the exposure period and LC_{50} of different molluscicides.
Abbreviations as in table 1.

der, their active components i.e. acetogenin, allicin, hecogenin, azadirachtin and pestoban caused more attraction (58.0%, 78.0%, 60.0%, 60.0% 50.0%, 46.7%, 50.3%, 46.4% and 57.7%, respectively) than the AFP containing 0.2% to 1.0% of the same molluscicides. The attraction of the snails was significantly ($p<0.05$) reduced with increasing concentration of different molluscicides in AFP. Lowest attraction (13.2%) of snails was observed for 1.0% hecogenin containing AFP. There was a significant ($p<0.05$) decrease in the number of the snails attracted by all other 0.1% stored AFP for 7, 14, 21 and 28 days except *A. sativum* containing AFP (Table2).

Molluscicidal activity of different AFP containing products/compounds against *L. acuminata* followed a time and dose dependence relationship (Table 3-4). 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}-1.02% in AFP; 144h LC_{50}-0.12% in AFP) than synthetic ones. The molluscicidal activity of garlic crude bulb powder (144h LC_{50}-0.18% in AFP) was higher than Snail Kill (144h LC_{50}-0.33% in AFP). Allicin was more toxic (144h LC_{50}-0.09% in AFP) than the crude bulb powder of *A. sativum* (144h LC_{50}-0.18% in AFP). The bioactive components hecogenin, acetogenin and azadirachtin were more toxic (24h LC_{50}- 1.54%, 1.35% and 1.10% in AFP, respectively) than their crude preparations (Table 3 and 4). The crude preparations of plant derived molluscicides and AFP containing pestoban caused significant molluscicidal activity against *L. acuminata* (Table 3 and 4).

The slope values given in Tables 3 and 4 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 less than 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 0.1% plant derived molluscicides compared with Snail Kill, 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 than corresponding concentration of Snail Kill. It indicates that when higher titer of active components of plant derived molluscicides was used in AFP, snails were less attracted. Higher concentration (1.0%) of allicin, hecogenin and azadirachtin [19-21] attracted less snails than Snail Kill. 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 mollus-

Table 4. Toxicity in different bait formulations of molluscicides against the snail L. acuminata at different time exposure.

Exposure Period	Molluscicides	LC_{50} % in AFP	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
	A. indica BaP	1.48	0.86	6.98	1.24±0.33	3.76	0.27	0.18
	A. squamosa	1.53	1.23	2.57	3.18±0.74	4.27	0.21	0.26
24h	Azadirachtin	1.10	0.86	1.99	2.26±0.54	4.15	0.22	0.12
	Acetogenin	1.02	0.72	2.16	1.68±0.36	4.62	0.18	0.29
	Pestoban	2.82	2.24	5.71	3.14±0.86	3.62	0.29	0.23
	A. indica BaP	1.01	0.62	3.90	1.03±0.28	3.61	0.29	0.17
	A. squamosa	1.25	1.08	1.88	2.75±0.65	4.24	0.21	0.14
48h	Azadirachtin	1.03	0.78	2.14	1.82±0.50	3.64	0.28	0.13
	Acetogenin	0.78	0.54	1.75	1.23±0.29	4.22	0.21	0.19
	Pestoban	2.46	1.98	4.94	2.43±0.71	3.40	0.33	0.23
	A. indica BaP	0.53	0.37	1.09	1.05±0.27	3.88	0.25	0.15
	A. squamosa	0.98	0.83	1.32	2.38±0.60	3.95	0.24	0.11
72h	Azadirachtin	0.73	0.59	1.11	1.78±0.47	3.75	0.27	0.13
	Acetogenin	0.39	0.29	0.58	1.23±0.27	4.57	0.18	0.21
	Pestoban	1.73	1.48	2.27	2.44±0.66	4.14	0.28	0.21
	A. indica BaP	0.25	0.17	0.34	1.23±0.26	4.66	0.17	0.22
	A. squamosa	0.63	0.50	0.73	2.71±0.61	4.45	0.19	0.11
96h	Azadirachtin	0.41	0.25	0.52	1.60±0.46	3.45	0.32	0.14
	Acetogenin	0.32	0.24	0.42	1.47±0.27	5.42	0.13	0.52
	Pestoban	1.33	0.99	1.60	2.07±0.63	3.26	0.36	0.28
	A. indica BaP	0.15	0.70	0.23	1.10±0.26	4.20	0.21	0.23
	A. squamosa	0.53	0.45	0.59	4.75±0.74	6.37	0.09	0.37
120h	Azadirachtin	-	-	-	-	-	-	-
	Acetogenin	0.19	0.10	0.27	1.09±0.26	4.18	0.22	0.49
	Pestoban	1.06	0.75	1.25	2.58±0.64	4.00	0.24	0.28
	A. indica BaP	0.09	0.03	0.14	1.27±0.27	4.57	0.84	0.36
	A. squamosa	0.48	0.40	0.54	5.32±0.88	6.04	0.10	0.37
144h	Azadirachtin	-	-	-	-	-	-	-
	Acetogenin	0.12	0.06	0.16	1.40±0.27	5.10	0.14	0.55
	Pestoban	0.96	0.75	1.10	3.65±0.68	5.32	0.13	0.86

Product moment correlation showed significant (p<0.05); negative correlation between the exposure period and LC_{50} of different molluscicides.
Abbreviations as in table 1.

cicides 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 contains some other compounds which reduce the attraction of snails towards AFP. In contrast AFP containing *A. sativum* and *P. tuberosa* bulb powder and *A. indica* bark powder attracted more snails than their pure compounds viz. allicin, hecogenin and 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. The storage of attractant food pellets for up to 28 days caused significant decrease in the attraction of snails. Thus, it seems logical to assume that AFPs containing plant derived molluscicides are less effective in attracting snails, when stored up to 28 days. However, toxicity of these AFP containing molluscicides was time and dose dependent as evident from the negative correlation between LC_{50} in different molluscicides and exposure period.

Treatment of bulb powder of *A. sativum* and *P. tuberosa*, seed powder of *A. squamosa*, bark powder of *A. indica* and their active components such as allicin, hecogenin, acetogenin and azadirachtin in aquatic environment are highly toxic to *L. acuminata* [19-21]. Toxicity of acetogenin (24h LC_{50}- 1.02% in AFP) extracted from the seeds of *A. squamosa* is higher than other plant derived molluscicides and Snail Kill (24h LC_{50}- 1.36% in AFP). Seeds of *A. squamosa* were used to kill human lice [22] and their organic extracts have been reported to possess insecticidal activity [23-24]. Molluscicidal activity of *A. indica* bark powder (24h LC_{50} 1.48% in AFP) is lower than the Snail Kill. However, its active component azadirachtin (24h LC_{50} 1.10% in AFP) is more toxic than Snail Kill. 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]. AFP containing hecogenin in AFP is 1.5 time more toxic than the crude bulb powder of *P. tuberosa*. It has been reported that treatment of *P. tuberosa* bulb powder and hecogenin caused significant reduction in the reproduction of the *L. acuminata* [20]. It has been reported that the allicin caused an uncompetitive inhibition of acetylcholinesterase and competitive inhibition of lactic dehydrogenase and alkaline phosphatase activity in the nervous tissue of *L. acuminata* [25]. The toxicity of molluscicide Pestoban is due to the presence of *Cedrus deodara*, *A. indica* and *Embelia ribes* in liquid concentrate form [26]. The toxicity of Pestoban to the snail is lower (24h LC_{50} 2.82% in AFP) than the synthetic molluscicide Snail Kill (24h LC_{50} 1.36% in AFP).

The steep slope values indicate that a small increase in the concentration in different molluscicides cause a

significant mortality in the snail. t- ratio value greater than 1.96 indicates that the regression is significant. Values of heterogeneity less than 1.0 denote that in the replicates the concentration response line would fall within 95% confidence limit and thus the model fit the data adequately. The value of 'g' is less than 0.5 indicates the index of significance of potency estimation.

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 the baits (Attractant food pellets) are ecologically sound, target specific and economic.

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