

Molluscicidal Activity of Bait formulation in attractant food pellets against vector snail, *Indoplanorbis exustus*

Farindra Tiwari

P. G. department of Zoology
M. G. P. G. College, Gorakhpur, 273 001, UP (INDIA)
drfarindra_mgpg@rediffmail.com

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 upto 144h against the snail, *Indoplanorbis exustus*. Active components of both the plant derived molluscicides were highly toxic to *Indoplanorbis exustus* compared with their crude forms.

[Farindra Tiwari. **Molluscicidal Activity of Bait formulation in attractant food pellets against vector snail, *Indoplanorbis exustus***. *Nat Sci* 2013;11(5):81-85]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 12

Keywords: Attractant food pellets, Bait formulation, *Fasciola*, *Indoplanorbis exustus*, Molluscicides, proline

1. Introduction

Certain freshwater snails are of great economic importance because they act as intermediate hosts for digenean trematodes. Two such flukes, *Fasciola hepatica* and *Fasciola gigantica*, are transmitted by the snail *Indoplanorbis exustus* which cause endemic disease fascioliasis in cattle population of eastern region of the state of Uttar Pradesh in India (Singh and Agarwal, 1981, Agarwal and Singh, 1988). An obvious solution is to reduce the incidence of fascioliasis is to de-link the life cycle of fluke by destroying the vector snails (Godan, 1983; Marston and Hostettmann, 1985, 1987; Ndamba, 1995; Singh et al., 1996; Singh and Singh, 2000). 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 programme. 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 (Thomas, 1982). 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 (Maclannis et al., 1974; Sterry et al., 1985; Thomas et al., 1989). Proline is the strongest attractant for *Lymnaea acuminata* and *Indoplanorbis exustus* at 20 mM concentration (Tiwari, 2011, 2013). 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 *Indoplanorbis exustus*.

2. 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 *Indoplanorbis exustus* (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±10 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.

2.1 Preparation of attractant food pellets (AFP)

Attractant food pellets (AFP) were prepared according to previous method (Madsen, 1992; Tiwari and Singh, 2004 a, b; Tiwari and Singh, 2007). 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 (Table1). 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.

2.2 Bioassay and apparatus

The bioassay was performed as reported earlier (Madsen, 1992; Tiwari and Singh, 2004 a, b). 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 cm² 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±10 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 or every molluscicide used in this study. The mortality of the snails was observed after every 24h up to 144h. Lethal concentration values (LC₅₀), lower and upper confidence limits (LCL and UCL), slope values, t- ratio, 'g' value and heterogeneity factor were calculated using POLO computer programme (Russel et al., 1977). Two way ANOVA and product moment correlation coefficient was applied between different data obtained in Tables 1-2 (Sokal and Rohlf, 1973).

3. 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 *Indoplanorbis exustus* followed a time and dose dependence relationship (Table 2). There was a significantly ($p<0.05$) negative correlation between exposure period and LC₅₀ in different molluscicides. AFP containing bioactive components of different plants were more toxic (acetogenin 24h LC₅₀-1.19%_n AFP; 144h LC₅₀-0.28% in AFP) than the crude preparations. The crude preparations of plant derived molluscicides containing AFP caused significant molluscicidal activity against *Indoplanorbis exustus* (Table 2).

The slope values given in Tables 2 were steep. Separate estimate of LC₅₀ 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).

4. Discussion

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 *Indoplanorbis exustus* followed a time and dose dependence relationship (Table 2). There was a significantly ($p<0.05$) negative correlation between exposure period and LC₅₀ in different molluscicides. AFP containing bioactive components of different plants were more toxic (acetogenin 24h LC₅₀-1.19%_n AFP; 144h LC₅₀-0.28% in AFP) than the crude preparations. The crude preparations of plant derived molluscicides containing AFP caused significant molluscicidal activity against *Indoplanorbis exustus* (Table 2).

The slope values given in Tables 2 were steep. Separate estimate of LC₅₀ 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).

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 *Indoplanorbis exustus* followed a time and dose dependence relationship (Table 2). There was a significantly ($p<0.05$) negative correlation between exposure period and LC₅₀ in different molluscicides. AFP containing bioactive components of different plants were more toxic (acetogenin 24h LC₅₀-1.19%_n AFP; 144h LC₅₀-0.28% in AFP) than the crude preparations. The crude preparations of plant derived molluscicides containing AFP caused significant molluscicidal activity against *Indoplanorbis exustus* (Table 2).

The slope values given in Tables 2 were steep. Separate estimate of LC₅₀ 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).

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 *Indoplanorbis exustus* 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 dependent as evident from the negative correlation between LC₅₀ 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 *Indoplanorbis exustus* (Singh and Singh, 1995; Singh and Singh, 2000). Toxicity of acetogenin (24h LC₅₀- 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 (Reyes and Santos, 1931) and their organic extracts have been

reported to possess insecticidal activity (Mukerja and Govind, 1958; Chatteraj and Tiwari, 1965). Molluscicidal activity of *A. indica* bark powder (24h LC₅₀ 1.35% in AFP) is lower than the bio-active component azadirachtin (24h LC₅₀ 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 (Singh and Singh, 1996).

It has been also reported that the snails are highly attracted towards the bait formulation of plant origin (Abd El Hamid, 1997; Tiwari and Singh, 2007). This behavioural 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 *Indoplanorbis exustus*. Use of these plant derived molluscicides inside attractant food pellets are ecologically sound, target specific and economic.

4. Conclusion

It can be concluded that by using attractant food pellets containing a strong attractant amino acid and a specific molluscicide of plant origin can effectively control the snail *Indoplanorbis exustus* population. By using the bait formulations the incidence of fascioliasis might be reduced.

Table 1. Mean number of snails *Indoplanorbis exustus* 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 Z₃ 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 *Indoplanorbis exustus* at different time exposure.

Exposure period	Molluscicides	LC ₅₀ % 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±0.45	3.92	0.24	0.28
	<i>A. indica</i> BP	1.35	0.84	4.1	1.45±0.33	4.38	0.20	0.32
	Acetogenin	1.19	1.0	1.6	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>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	2.0	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>A. 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 in between the exposure period and LC₅₀ of different molluscicides. Abbreviations as in table 1.

5. Acknowledgement

Author is thankful to the authorities of Mahatma Gandhi P. G. College, Gorakhpur for providing lab facilities.

References

1. Abd-El Hamid, A. Z. (1997) Development of bait formulation for control of intermediate hosts of African Schistosome species, *J. Appl. Toxicol.*, 17(6):391-395.
2. Agarwal, R. A. and Singh, D. K. (1988) Harmful gastropods and their control, *Acta Hydrochim Hydrobiol*, 16:113-138.
3. Chatteraj, A. N. and Tiwari, S. C. (1965) A note on the insecticidal property of *Annona squamosa* (Annonaceae), *Natl Acad Sci India Proc Sec B (Biol Sci)*, 35:351-353.
4. Godan, D. (1983) "Pests slugs and snails. Biology and control" (ed., Dora Godan) translated by Sheila Grouber, Springer Verlag. Berlin Heidelberg New York.
5. Kpikpi, J. E. K. and Thomas, J. D. (1992) A study of sugar chemoreception niches of two bulinid snail hosts of schistosomiasis, *Ann Trop Med Parasitol*, 86:181-198.
6. MacInnis, A. J., Bethal, W. M. and Cornford, E. M. (1974) Identification of chemicals of snail origin that attract *Schistosoma mansoni* miracidia, *Nature*, 248:361-363.
7. Madsen, H. (1992) A comparative study on the food locating ability of *Helisoma duryi*, *Biomphalaria camerunensis* and *Bulinus truncatus* (Pulmonata:Planorbidae), *J Appl Ecol*, 29:70-78.
8. Marston, A. and Hosttetmann, K. (1985) Plant molluscicides, *Phytochemistry*, 24:639-652.
9. Marston, A. and Hosttetmann, K. (1987) Antifungal, molluscicidal and cytotoxic compounds from plants used in traditional medicines. In: "Biologically Active Natural Products" (Ed. Hosttetmann, K and Lea, PJ), Clarendon Press Oxford, 65-83.

10. Mukerja, T. D. and Govind, R. (1958) Indigenous insecticidal plants:II *Annona squamosa*, *J Indian Res* (India), 17C:9-15.
11. Ndamba, J. (1995) Response of the molluscicidal berry plant *Phytolacca dodecandra* to different climatic and edaphic conditions, *Trop Agric*, 72:135-140.
12. Reyes, F. R. and Santos, A. C. (1931) Isolation of Anonaine from *Annona squamosa* Linn., *Philippines J of Sci*, 44:409-410.
13. Russel, R. M., Robertson, J. L. and Savin, N. E. (1977) POLO: A new computer programme for probit log analysis, *Bull Entomol Soc Amer*, 23:209-213.
14. Singh, A., Singh, D. K., Mishra, T. N. and Agarwal, R. A. (1996) Molluscicides of plant origin, *Biol Agri Horti*, 13:205-252.
15. Singh, K., Singh, A. and Singh, D. K. (1996) Molluscicidal activity of neem (*Azadirachta indica* A. Juss), *J of Ethnopharmacol*, 52:35-40.
16. Singh, K. and Singh, D. K. (2000) Effect of different combinations of MGK-264 and piperonyl butoxide with plant derived molluscicides on snail reproduction, *Arch EnvironContam Toxicol*, 38:182-190.
17. Singh, O. and Agarwal, R. A. (1981) Toxicity of certain pesticides to two economic species of snails in northern India, *J Econ Entomol*, 74:568-571.
18. Sterry, P. R., Thomas, J. D. and Patience, R. L.(1985) Changes in the concentrations of short-chain carboxylic acids and gases during decomposition of the aquatic macrophytes *Lemna paucicostata* and *Ceratophyllum demersum*, *Freshw Biol*, 15:139-153.
19. Sokal, R. R. and Rohlf, F. J. (1973) *Introduction to Biostatistics*, W H Freeman, San Francisco, 368pp.
20. Thomas, J. D. (1982) Chemical ecology of the snail hosts of schistosomiasis: snail-snail and snail- plant interactions, *Malacol*, 33:81-91.
21. Thomas, J. D., Kowalczyk, C. and Somsundaram, B. (1989) The biochemical ecology of *Biomphalaria glabrata*, a snail host of *Schistosoma mansoni*; short chain carboxylic and amino acids as phagostimulants, *Comp Biochem Physiol*, 93A:899-911.
22. Tiwari, F. and Singh, D. K. (2004a) Behavior responses of the snail *Lymnaea acuminata* to carbohydrates in snail attractant pellets, *Naturewissenschaften*, 91:378-380.
23. Tiwari, F. and Singh, D. K.(2004b) Attraction to amino acids by *Lymnaea acuminata*, the snail host of *Fasciola* species, *Braz J Med Biol Res*, 37:587-590.
24. Tiwari, F. (2011) Chemoattractive effect of amino acids against *Lymnaea acuminata* snails, *World Journal of Zoology*, 6(2):117-119.
25. Tiwari, F. and Singh, D. K. (2007) Toxicity of plant derived molluscicides in attractant food pellets against snail, *Lymnaea acuminata*. *Iranian J of Pharmacol Therapeutics*, 6:103-107.
26. Tiwari, F. (2013) Behavioural responses of *Indoplanorbis exustus* snails against different amino acids in bait formulation. *Researcher*, 5(4):16-18.