World Journal of Zoology 5 (2): 143-147, 2010
ISSN 1817-3098

Binary Combination of Amino Acids and Plant Molluscicide in Bait Formulations Against *Lymnaea acuminata*

Pradeep Kumar, V.K. Singh and D.K. Singh

Department of Zoology, DDU Gorakhpur University, Gorakhpur, 273009, U.P. India

Abstract: Snail control is one of the important methods in the campaign to reduce the incidence of fascioliasis. However, in order to achieve this objective, the method of bait formulation containing an attractant and molluscicide is an appropriate approach to lure the target snail population to the molluscicide. In the present study snails attractant pellets (SAP) were prepared from binary combination (1:1 ratio) of 20mM amino acids in 2% agar solution. Attraction of snails to different combinations was studied in glass aquaria having diameter of 30 cm. Among all the binary combination of amino acids valine + aspartic acid, with molluscicide ferulic acid, attract 42.36% of snails after 2h which was not significantly different from their control a. SAP containing umbelliferone emerged as the strongest bait formulation (96h LC_{50} 1.09%) against *L. acuminata.*

Key word: Snail attractant pellets · Amino acids · Molluscicides · Fascioliosis · *Lymnaea acuminata*

INTRODUCTION

Fascioliasis is an important live-stock health problem in eastern Uttar Pradesh [1]. This disease is transmitted by the Liver- flukes *Fasciola gigantica* [2]. Snail *Lymnaea acuminata* is the vector of this fluke. One way to reduce the incidence of fascioliasis is to de-link the life cycle of fluke by destroying the intermediate hosts [3]. The development of a selective and safe molluscicide should always be a realistic goal. It must be effective at low concentration and minimal adverse effect on the other biota sharing the same habitat with snail. 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 [4]. Earlier, Abd El-Hamid [5], Tiwari and Singh [6,7], Singh and Singh [8] have reported the behavioral responses of snail *Biomphalaria alexandrina* and *L. acuminata* against different amino acid and carbohydrate. Consequently, the use of a combination of snail attractants and toxicants in bait formulation has been seen as an effective tool for the pest management. A large number of plant derived molluscicides have been identified [9]. Recently, it has been observed that ferulic acid, umbelliferone in *Ferula asafoetida* latex, eugenol *Syzygium aromaticum* bud and limonene in *Carum carvi* seed are potent molluscicides [3, 10].

The present study assayed the toxicity of bait formulation containing most preferred combination of amino acids attractants [11] and active molluscicidal component ferulic acid, umbelliferone, eugenol and limonene against *L. acuminata.*

MATERIALS AND METHODS

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 6.5-7.2 l, 5.2-6.3 and 102.0-105.0 mg/l, respectively.

Agar- agar, amino acids and active molluscicidal component such as eugenol, ferulic acid, umbelliferone and limonene [3,11] were used in the bait formulations of the present study. The pure active component ferulic acid (4-Hydroxy-3 methoxycinnamic), umbelliferone (7-Hydroxy coumarin; 7-hydroxy-2H-1-benzopyran-2-one), eugenol (2-Methoxy-4-(2-propenyl) phenol) and limonene ((R)-4-Isopropenyl-1-methyl-1-cyclohexene); from Sigma chemical Co. (USA) were procured and used in the bait formulations.

Correspondence Author: D.K. Singh, Malacology Laboratory, Department of Zoology, DDU Gorakhpur University,Gorakhpur-273009, U.P., India, Tel- +91-551-2202187(O), 2200509(R) E-mail: dksingh_gpu@yahoo.co.in; pkumar_gpu@yahoo.co.in

Preparation of Snail-Attractant Pellets (SAP): Snail-attractant food pellets (SAP) were prepared according to the method of Madsen [12]. Binary combinations of valine + aspartic acid / lysine amino acid (20mM) were prepared in 100ml of 2% agar solution. After boiling, each of the active molluscicidal components in different concentration was added to the solution. (Table 1). The mixture was stirred constantly for 30 minutes and spread to a uniform thickness (5 mm). After cooling, the pellets were cut out from the layer with a corer (5 mm diameter).

Assay and Apparatus: The bioassay was performed by the method of Tiwari and Singh [6,7]. Chemoattraction studies of different binary combination of amino acids against *L. acuminata* were made in a circular glass aquarium having a diameter of 30 cm. Each aquarium was divided into four concentric zones; zone 3 (Central zone), 2, 1 (Middle zone) and zone 0 (Outer zone) had diameters of 13, 18, 24 and 30 cm, respectively. Zone, 1, 2 and 3 had an area of 254.35, 197.82, 121.68 and 132.66 cm^2, respectively. A small annular elevation of 9 mm height and 2.4 cm diameter was made in the centre of aquarium (Zone 3). 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 containing active molluscicidal component was added on the small annular elevation in the center (Zone 3). The location of each snail was noted after every 15 min for two hours. Six sets of experiments have been designed with ten snails each for all molluscicides used in this study.

The mortality rates were observed after every 24h up to 96h. 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 [13]. One–way ANOVA and product moment correlation coefficient was applied between the different data obtained in Table 1 [14].

RESULTS

Table 1 gives the distribution *L. acuminata* in the 3rd zone around the SAP of different combination of amino acid after 1 and 2h from the start of the experiment. Placement of SAP in zone 3 affected the behavior of the snail. Among all the bait formulations, lysine + valine +0.7% limonene showed lowest attraction (26.34%) of the snails in zone 3. (Table 1) 0.7% eugenol, ferulic acid,

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

		Concentration of molluscicides			
Molluscicides	Time (hrs)	0.7%	1.0%	2.0%	3.0%
Vali+Aspa+Eug	1	2.58±0.45 (40.31)+	1.91±0.28 (28.38)	3.49±0.16 (34.11)	2.41±0.45 (34.52)
	2	3.66±0.13 (35.53)+	3.66±0.13 (35.53)	2.66±0.56 (39.06)	3.91±0.21 (35.09)
Vali+Aspa+Feru	1	1.58±0.25 (26.42)+	1.66±0.35 (25.97)	2.16±0.28 (31.71)	2.24±0.49 (30.64)
	2	3.91±0.16 (42.36)+	3.57±0.08 (36.42)	3.99±0.27 (35.27)	3.99±0.49 (34.51)
Vali+Aspa+Umb	1	2.08±0.47 (32.09)+	1.91±0.34 (35.90)	2.24±0.37 (28.97)	1.99±0.13 (33.27)
	2	3.74±0.25 (38.43)+	3.91±0.08 (37.59)	3.08±0.16 (41.62)	2.74±0.34 (35.86)
Vali+Aspa+Lim	1	1.91±0.28 (27.05)+	2.41±0.31 (34.52)	1.24±0.44 (32.54)	1.16±0.41 (30.44)
	2	2.49±0.31 (38.96)+	2.16±0.28 (40.67)	1.91±0.62 (36.52)	2.66±0.19 (38.10)
Lysi+Vali+Eug	1	1.16±0.21 (27.95)+	1.24±0.15 (28.24)	1.24±0.37 (29.38)	1.16±0.41 (27.42)
	2	2.33±0.23 (33.38)+	2.33±0.33 (35.03)	2.58±0.25 (35.24)	2.24±0.41 (30.64)
Lysi+Vali +Feru	1	1.33±0.23 (30.22)+	1.41±0.15 (30.38)	1.58±0.20 (29.69)	1.74±0.28 (29.09)
	2	2.66±0.23 (35.56)+	2.99±0.13 (37.46)	2.99±0.13 (36.68)	2.58±0.31 (33.08)
Lysi+Vali +Umb	1	1.74±0.28 (30.74)+	1.08±0.31 (24.54)	1.08±0.20 (26.02)	1.16±0.28 (26.97)
	2	2.91±0.16 (39.80)+	3.08±0.04 (38.59)	2.83±0.16 (37.38)	1.66±0.60 (38.57)
Lysi+Vali +Lim	1	0.83±0.50 (26.34)+	1.41±0.44 (28.77)	1.16±0.39 (26.36)	1.33±0.30 (27.14)
	2	2.66±0.23 (37.20)+	2.83±0.16 (36.61)	2..74±0.36 (36.05)	2.74±0.08 (37.00)
Control (Agar)	1	0.82±0.08 (18.02)	1.07±0.12 (19.13)	1.33±0.04 (19.88)	1.30±0.20 (20.01)
	2	1.41±0.31 (25.11)	1.11±0.03 (26.01)	2.03±0.42 (25.99)	2.33±0.13 (25.98)
Control (a) (Vali+Aspa)	1	3.22±0.13 (40.12)	2.67±0.21 (41.44)	3.78±0.22 (42.99)	2.79±0.13 (45.33)
	2	2.92±0.33 (42.33)	3.77±0.52 (44.32)	2.97±0.15 (46.02)	3.25±0.23 (57.49)
Control (b) (Lysi+Vali)	1	2.81±0.21 (49.33)	2.89±0.33 (51.00)	3.24±0.17 (51.99)	3.27±0.56 (55.32)
	2	3.36±0.31 (50.11)	3.26±0.34 (52.11)	3.67±0.25 (53.67)	2.46±0.79 (60.98)

Values in parentheses are percentages of snails in zone 3 (in contact with attractant food pellet) with snail 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: Vali-valine, Aspa - aspartic acid, Lysi - lysine, Vali - valine, Eug - eugenol, Umb - umbelliferone, Feru - ferulic acid, Lim-limonene

Table 2: Bait formulation of different molluscicides and their toxicity against *Lymnaea acuminata* at different time exposure

Exposure Period	Molluscicides	LC_{50}% AFP	LCL	UCL	Slope Value	t-ratio	g-value	Heterogeneity
24h	Vali+Aspa+Eug	9.21	4.38	224.0	1.20±0.40	3.02	0.42	0.32
	Vali+Aspa+Feru	3.39	2.41	7.69	1.45±0.35	4.06	0.23	0.19
	Vali+Aspa+Umb	2.91	2.16	5.46	1.53±0.35	4.33	0.20	0.18
	Vali+Aspa+Lim	4.77	2.76	50.72	1.03±0.35	2.94	0.44	0.26
48h	Vali+Aspa+Eug	3.29	2.36	7.11	1.45±0.35	4.13	0.22	0.20
	Vali+Aspa+Feru	2.69	1.85	8.13	1.07±0.33	3.18	0.37	0.21
	Vali+Aspo+Umb	2.29	1.63	5.20	1.11±0.33	3.32	0.34	0.11
	Vali+Aspa+Lim	3.92	2.45	21.19	0.90±0.34	2.64	0.54	0.26
72h	Vali+Aspa+Eug	2.29	1.70	4.20	1.30±0.34	3.83	0.26	0.29
	Vali+Aspo+Feru	1.78	1.28	3.01	1.15±0.33	3.44	0.32	0.28
	Vali+Aspa+Umb	1.36	0.83	2.10	1.03±0.33	3.09	0.40	0.22
	Vali+Aspa+Lim	2.38	1.74	4.77	1.24±0.34	3.67	0.28	0.14
96h	Vali+Aspa+Eug	1.31	1.04	1.61	1.89±0.34	5.45	0.12	0.16
	Vali+Aspa+Feru	1.27	0.87	1.72	1.29±0.33	3.86	0.25	0.23
	Vali+Aspa+Umb	1.09	0.76	1.39	1.54±0.34	4.51	0.18	0.22
	Vali+Aspa+ Lim	1.90	1.40	3.16	1.24±0.33	3.69	0.28	0.24

Abbreviations: Vali - valine, Aspa - aspartic acid, Eug - eugenol, Umb - umbelliferone, Lim-limonene, LCL - lower confidence limits, UCL - upper confidence limits.

Six batches of ten snails were exposed different concentration of the above molluscicides inside the attractant food pellets (AFP). Mortality was determined after every 24h.

Significant negative regression (P<0.05) was observed between exposure time and LC_{50} of treatments. Ts - testing significant of the regression coefficient – Vali + Aspa + Eug – 4.87++; Vali + Aspa + Feru – 12.19+; Vali + Aspa + Umb – 13.49+; Vali + Aspa +Lim – 1.84++.

+: linear regression between *x* and *y*; ++: non – linear regression between log *x* and log *y*.

Table3: Bait formulation of different molluscicides and their toxicity against *Lymnaea acuminata* at different time exposure

Exposure Period	Molluscicides	LC_{50}% AFP	LCL	UCL	Slope Value	t-ratio	g-value	Heterogeneity
24h	Lysi +Vali+Eug	3.72	2.52	10.77	1.32±0.35	3.73	0.27	0.19
	Lysi +Vali +Feru	24.8	4.98	11.34	1.45±0.35	4.06	0.23	0.19
	Lysi+Vali +Umb	7.59	3.76	171.43	0.89±0.35	2.51	0.60	0.18
	Lysi +Vali +Lim	6.15	3.47	47.06	1.24±0.37	3.31	0.35	0.14
48h	Lysi +Vali+Eug	2.04	1.55	3.38	1.37±0.33	4.03	0.23	0.16
	Lysi +Vali +Feru	9.78	4.37	475.8	0.89±0.36	2.44	0.64	0.34
	Lysi+Vali +Umb	5.52	3.07	60.47	0.89±0.34	2.56	0.58	0.20
	Lysi +Vali +Lim	5.25	3.07	36.93	1.17±0.36	3.23	0.36	0.28
72h	Lysi +Vali+Eug	2.10	1.60	3.37	1.40±0.34	3.13	0.22	0.20
	Lysi+Vali +Feru	5.48	2.97	116.8	0.99±0.35	2.80	0.48	0.24
	Lysi+Vali +Umb	3.91	2.44	20.86	0.90±0.34	2.65	0.54	0.24
	Lysi +Vali +Lim	3.50	2.38	10.02	1.28±0.35	3.66	0.28	0.28
96h	Lysi +Vali+Eug	1.57	1.29	1.97	1.93±0.34	5.59	0.12	0.25
	Lysi +Vali +Feru	2.09	1.68	2.89	1.83±0.35	5.21	0.14	0.72
	Lysi+Vali +Umb	2.15	1.61	3.79	1.30±0.33	3.83	0.26	0.31
	Lysi +Vali +Lim	2.61	1.91	5.20	1.33±0.34	3.87	0.25	0.36

Abbreviations: Lysi - lysine, Vali - valine, Eug - eugenol, Umb - umbelliferone, Lim-limonene, LCL - lower confidence limits, UCL - upper confidence limits.

Six batches of ten snails were exposed different concentration of the above molluscicides inside the attractant food pellets (AFP). Mortality was determined after every 24h.

Significant negative regression (P<0.05) was observed between exposure time and LC_{50} of treatments. Ts - testing significant of the regression coefficient – Lysi + Vali+ Eug – 4.43++; Lysi + Vali + Feru – 6.56++; Lysi + Vali + Umb – 27.46+; Lysi + Vali +Lim – 10.15+.

+: linear regression between *x* and *y*; ++: non – linear regression between log *x* and log *y*.

umbelliferone and limonene in bait containing valine + aspartic acid (35.53, 42.36, 38.43 and 38.96%, respectively) and lysine + valine (33.3, 35.56, 39.80 and 37.20%, respectively), caused higher attraction after 2h (Table 1). However, attraction of snails in bait containing molluscicide + attractant was lower than control pellet containing only agar or agar + attractant (valine + aspartic acid / lysine + valine). The attraction of the snails was significantly (P<0.05) reduced with increasing concentration of different molluscicides in SAP.

Molluscicidal activity of different SAP containing active molluscicidal component against *L. acuminata* was time and dose dependant (Tables 2 and 3). There was a significant ($P<0.05$) negative correlation between exposure period and LC_{50} of different molluscicides. The active component umbelliferone (24h LC_{50}-2.91%) and eugenol (24h LC_{50}-3.72%) were more toxic in SAP containing valine + aspartic acid and lysine + valine, respectively than ferulic acid and limonene (Table 2 and 3). The 96h LC_{50} of SAP containing limonene was higher than that of ferulic acid (Tables 2 and 3).

The slope values given in tables 2 and 3 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 and 99, respectively) (Tables 2 and 3).

DISCUSSION

The present study clearly demonstrated that the snail *L. acuminata* showed a significant behavioral response towards the different binary combinations of amino acids. It has been shown that gastropods detect the amino acids/carbohydrates as indicator of their food [8]. Significant variation in the number of snails in zone 3 attracted by different binary combinations of amino acid in SAP clearly indicate that snails are capable of differentiating type of amino acid in the SAP. It is obvious from the observations that among different binary combinations tested, snails were most, responsive to the valine + aspartic acid with molluscicide ferulic acid. Molecular weight of aspartic acid (133.10) is lower than lysine (182.65) and valine (117.15) is common in both. The present study also indicated that the binary combination of different amino acids are recognized more rapidly by the snails than bait containing single amino acid [6 – 8].

Abd-El H amid [5] and Singh and Singh [8] have studied the behavioral responses of all stages of snail *B. alexandrina* and *L. acuminata* against the different amino acids, respectively. They suggested that the *B. alexandrina, L. acuminata* snails like other gastropods, are able to detect their food sources by using chemical sense of amino acid as sign for the presence of their food. Snails were more attracted towards the lower concentration (0.7%) of molluscicide containing bait formulations. It indicated that molluscicide in bait formulations has some repellant action. Among all the bait containing molluscicides umbelliferone was very effective in killing the snails.

The steep lope value indicates that a small increase in the concentration of different molluscicides caused higher snail mortality. A t-ratio value greater than 1.96 indicates that the regression is significant. Heterogeneity factor values less than 1.0 denote that in the replicate tests of random sample the concentration response curve would fall within the 95% confidence limits and thus the model fits the data adequately. The index of significance of the potency estimation g indicates that the value of the mean is within the limit at all probability level (90, 95 and 99, respectively) since it is less than 0.5.

Conclusively, it can be stated that molluscicides of plant origin could be used with varying degrees of success in bait formulation. This concept is a new approach for the control of harmful snails, without releasing more active molluscicide directly in the water and attracting specifically the particular target snail. In this way specific killing of snail by the use of fewer amounts of molluscicides in bait is a biotechnological tool for effective control of snail.

ACKNOWLEDGMENTS

One of the authors (Dr. Pradeep Kumar) is thankful to University Grants Commission, New Delhi (Post Doctoral Fellow) for UGC letter No. F. 31- 3(Sc)/2006(SA-II), for financial assistance.

REFERENCES

1. Agarwal, R.A. and D.K. Singh, 1988. Harmful gastropods and their control, Acta Hydrochim. Hydrobiol., 16: 118-138.
2. Singh, O. and R.A. Agarwal, 1981. Toxicity of certain pesticides to two economic species of snails in northern India, J. Econ. Entomol., 74: 568-571.
3. Kumar, P., V.K. Singh and D.K. Singh, 2009. Kinetics of enzyme inhibition by active molluscicidal agents ferulic acid, umbelliferone, eugenol and limonene in the nervous tissue of snail *Lymnaea acuminata,* Phytother. Res., 23: 172-177.
4. Kpikpi, J.E.K. and J.D. Thomas, 1992. A study of the sugar chemoreception niches of the two bulinid snail hosts of schistosomiasis, Ann. Trop. Med. Parasitol., 86: 181-198.
5. Abd El-Hamid, A.Z., 1996. Amino acids as attractant to *Biomphalaria alexandrina* snails intermediate hosts for attractant *Schistosoma mansoni,* Egyptian J. Bilharziasis, 18: 13-26.

6. Tiwari, F. and D.K. Singh, 2004a. Attraction of amino acids by *Lymnaea acuminata*, snail host of *Fasciola* species, Brazilian J. Medic. Biol. Res., 37: 587-590.
7. Tiwari, F. and D.K. Singh, 2004b. Behavioural responses of the snail *Lymnaea acuminata* to carbohydrates in snail attractant pellets, Naturwissenschaften, 91: 378-380.
8. Singh, P. and D.K. Singh, 2008. Binary combination of carbohydrates and amino acids as snail attractant in pellets containing molluscicides against the snail *Lymnaea acuminata,* Pesti Biochem. Physiol., 92: 120-124.
9. Singh, A., D.K. Singh, T.N. Misra and R.A. Agarwal, 1996. Molluscicide of plant origin, Biol. Agric. Horticu., 13: 205-252.
10. Kumar, P. and D.K. Singh, 2006. Molluscicidal activity of *Ferula asafetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata,* Chemosphere, 63: 1568-1574.
11. Kumar, P. and D.K. Singh, 2009. Amino acids and carbohydrates binary combinations as an attractant in bait formulations against the snail *Lymnaea acuminata.* Malays. Appl. Biol. (In Press).
12. Madsen, H., 1992. A comparative study on the food locating ability of *Helisoma duryi*, *Biomphalaria comerunensis and Bulinus truncates (*Pulmonate planorbidae), App. Ecol., 29: 70-78.
13. Robertson, J.L., R.M. Russell, H.K. Preisler and N.E. Savin, 2007. Bioassay with arthropods POLO. Computer programme for analysis of bioassay data 2^{nd} Eds. (Talar and Francis, CRC Press), pp: 1-224.
14. Sokal, R.R. and F.J. Rohlf, 1973. Introduction of biostatistics. W. H. Freeman San, Francisco, pp: 185-207.