Behavioural Responses of *Indoplanorbis exustus* Snails Against Different Amino Acids in Bait Formulation

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Abstract: Snail control is one of the most important tools in the campaign to reduce fascioliasis. Bait formulation using a strong attractant is of the important methods to control snail populations. The present study identifies certain amino acids namely arginine, proline, tryptophan, serine, citrulline, asparagine, glycine, cysteine, glutamine, glutamic acid, aspartic acid, alanine and ornithine for preparing such baits. These were tested on *Indoplanorbis exustus*, an intermediate host of digenean trematodes *Fasciola hepatica* and *Fasciola gigantica*. Significant variation in behavioural responses was observed in the snail even when all these amino acids were used in 50 mM concentration. In the present study proline emerged as the strongest attractant for *Indoplanorbis exustus*.
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1. Introduction

The endemic disease fasioliasis is caused by two digenean trematodes *Fasciola hepatica* and *F. gigantica* in cattle populations of eastern Uttar Pradesh (Singh and Agarwal, 1981). The snail *Indoplanorbis exustus* is the vector of these flukes. One way to reduce the incidence of Fascioliasis is to de-link the life cycle of flukes by destroying the intermediate hosts (Agarwal and Singh, 1988). The development of a selective and safe molluscicide should be realistic goal. Fresh water snails inhabit an environment containing macrophytes, algae and bacteria (Thomas, 1982). These organisms release copious amounts of chemicals such as carbohydrates and amino acids into the surrounding water (Thomas et al., 1989; Kpikpi and Thomas, 1992). It has been established that snails use these chemical signals in locating food sources (Kpikpi and Thomas, 1992). It has been observed that freshwater pulmonate snails are voracious feeders, grazing on epiphytic algae, decaying macrophytes and fine detritus (Madsen, 1992). In the present study a number of amino acid bioassay methods was screened to determine the behavioral responses of the snail *Indoplanorbis exustus* and which of them could preferably be used as a potent attractant for preparing bait along with molluscicide. Snails like other gastropod molluscs, use chemical cues to locate food sources (Uhazy et al., 1978; Croll, 1983; Audesirk and Audesirk, 1985; Masterson and Fried, 1992). Amino acids were identified in the snail modular system as chemical diffusing from aquatic organisms and are probably used by the snails as indicators to identify their food (Hamid, 1996). For this reason, the use of a combination of an attractant and toxicants has been seen as an effective tool for integrated pest management. The present study identifies certain amino acids namely arginine, proline, tryptophan, serine, citrulline, asparagine, glycine, cysteine, glutamine, glutamic acid, aspartic acid, alanine and ornithine as chemoattractive agents for preparing baits.

2. Materials and Methods

Adult *Indoplanorbis exustus* snails (mean length 2.25±0.20cm) were collected locally from lakes and low lying submerged fields and were used as test animals. The snails were acclimatized for 72 h in dechlorinated tap water at 25±1^{o} C.

2.1. Preparation of Snail Attractant Pellets (SAP)

The snail attractant pellets (SAP) containing different amino acids (arginine, proline, tryptophan, serine, citrulline, asparagine, glycine, cysteine, glutamine, glutamic acid, aspartic acid, alanine and ornithine) were prepared in agar-agar (Madsen, 19992; Tiwari and Singh, 2004 a b). These amino acids were prepared at the concentration of 10, 20, 30 and 40 mM and 50 mM each in 100 ml of 2% agar solution and were subsequently spread, after cooling, as small pieces of 5mm in diameter and at a uniform thickness of 5mm.

2.2. Assay Apparatus and Procedure

The chemoattraction study was done in circular glass aquarium of 30 cm diameter. Each aquarium was divided into four concentric zones; zone 3 (central zone), zone 2, 1 (middle zones) and zone 0 (outer zone) had diameter of 13, 18, 24 and 30 cm, respectively. Zone 0 had an area of 254 cm^2 on the periphery of aquarium. A small annular elevation of 9 mm and 1.5 cm diameter was made in the centre of

aquarium (zone 3). The aquaria were then filled with 500 ml dechlorinated tap water to a height of 8mm 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; SAP was added in the centre of zone 3. The position of each snail was recorded at 15 minutes interval for 2 hours. A clear glass aquarium containing agar alone was used as control for each experiment. Six replicates of the experiments were conducted. The chemoattraction behavior of the snails to SAP containing different amino acids 10, 20, 30 and 40 mM and 50 mM were observed and two way ANOVA was applied to determine significant alteration in behaviour (Sokal and Rohlf, 1973).

3. Results

The snail *Indoplanorbis exustus* showed a significant accumulation response after the placement of snail attractant pellets in zone 3. The accumulation of snails in zone 3 was not significant at low concentration especially 10 mM. When the concentration of different amino acids increases slowly, a significant increase in number of snails are found in zone 3. It is observed from the table 1 that the glutamine shows highest (56.3%) attraction towards the SAP after one hour in zone 1 and ornithine shows highest attraction (56.3%) after two hours from beginning of the experiment in zone 1. The highest attraction has been shown by SAP containing amino acid proline (53% in zone 3) after two hours from beginning of the experiment. There was no significant accumulation response of snails in different zones and different amino acids at 30 and 40 mM concentrations.

4. Discussion

Except alanine and ornithine, all amino acids attracted the snail *Indoplanorbis exustus* significantly. Among all the amino acids the proline showed highest attraction for the snail *Indoplanorbis exustus*. It has been already reported by Abd El-Hamid (1996) that the proline is the preferred attractant for *Biomphalaria alexandrina* and suggested that this high attraction is due to the fact that it is released from the snails in the surrounding water as a signal. It has been earlier reported that the snail *Lymnaea acuminata* is highly attracted towards SAP containing proline at 20 mM concentrations (Tiwari, 2011). However in the present study the snail *Indoplanorbis exustus* is also attracted towards the SAP containing amino acid proline at 50mM. It has been already reported that at 50 mM concentration of amino acids, *Lymnaea acuminata* snails highly attracted towards the SAP containing non polar R group amino acid serine (Tiwari and Singh, 2004 b). The gastropods are able to detect their food sources sensing amino acids as indicators of the presence of their food (Tiwari and Singh, 2004 a,b; Tiwari, 2011). Amino acids are released by aquatic plants and some algae. It is possible that amino acid to which each species is sensitive relates to the detection of the extent of decay in the plant material on which it feeds. So the present study concludes that the snail *Indoplanorbis exustus*, an intermediate host of *Fasciola* species, are attracted to bait formulations containing highly attractive compounds at specific concentration. This response could be utilized to control snails by adding specific molluscicides to SAP.

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Table:1- Distribution of *Indoplanorbis exustus* snails in the various zones around SAP containing 50 mM amino acids after 1 and 2 hours from beginning of experiment.

Attractants	Time (hrs)	Mean no. of snails around SAP		
		Z_1	Z_2	Z_3
Agar-Agar (Control)	1	0.33±0.40 (45.6)	0.66±0.33 (36.2)	0.33±0.33 (18.1)
	2*	1.00±0.63 (27.3)	1.66±0.33 (45.3)	1.00±0.63 (27.3)
Arginine	1	1.5±0.40 (56.3)	0.5±0.22 (18.2)	0.66±0.21 (24.8)
	2	2.83±0.30 (53.3)	0.5±0.21 (9.3)	2.00±0.36 (37.5)
Proline	1	1.83±0.60 (55.2)	0.83±0.16 (25)	0.66±0.21 (20.4)
	2	1.33±0.21 (20.4)	1.83±0.30 (28.1)	3.5±0.63 (51)
Tryptophan	1	1.0±0.25 (31.6)	1.0±0.25 (31.6)	1.16±0.30 (36.5)
	2	1.83±0.47 (29.7)	2.16±0.16 (35.1)	2.16±0.16 (35.1)
Serine	1	1.66±0.33 (43.4)	1.16±0.30 (30.3)	1.0±0.32 (26.1)
	2	2.66±0.42 (32.5)	2.0±0.25 (24.5)	1.0±0.42 (42.4)
Citrulline	1	0.5±0.22 (12.0)	1.83±0.54 (43.9)	1.83±0.54 (43.9)
	2	1.66±0.21 (23.7)	2.16±0.65 (30.9)	3.16±0.91 (45.2)
Asparagine	1	1.5±0.34 (33.4)	1.16±0.40 (25.4)	1.83±0.54 (46.7)
	2	2.66±0.33 (36.3)	1.83±0.47 (25.0)	2.83±0.60 (34.6)
Glycine	1	1.0±0.25 (31.6)	1.0±0.25 (31.6)	1.16±0.30 (36.5)
	2	2.66±0.42 (32.5)	2.0±0.25 (24.5)	3.5±0.42 (42.4)
Cysteine	1	1.0±0.25 (31.6)	1.0±0.25 (31.6)	1.16±0.30 (36.5)
	2	1.83±0.47 (29.7)	2.16±0.16 (35.1)	2.16±0.16 (35.1)
Glutamine	1	1.5±0.40 (56.3)	0.5±0.22 (18.2)	0.66±0.21 (24.8)

	2	2.83±0.30 (53.3)	0.5±0.21 (9.3)	2.00±0.36 (37.5)
Glutamic Acid	1	1.83±0.60 (55.2)	0.83±0.16 (25)	0.66±0.21 (20.4)
	2	1.33±0.21 (20.4)	1.83±0.30 (28.1)	3.33±0.63 (51)
Aspartic Acid	1	1.5±0.40 (56.3)	0.5±0.22 (18.2)	0.66±0.21 (24.8)
	2	2.83±0.30 (53.3)	0.5±0.21 (9.3)	2.00±0.36 (37.5)
Alanine	1	1.83±0.60 (55.2)	0.83±0.16 (25)	0.66±0.21 (20.4)
	2	1.33±0.21 (20.4)	1.83±0.30 (28.1)	3.33±0.63 (51)
Ornithine	1	1.00±0.63 (27.3)	1.66±0.33 (45.3)	1.00±0.63 (27.3)
	2	1.5±0.40 (56.3)	0.5±0.22 (18.2)	0.66±0.21 (24.8)

Z_3-Zone three (center of container where SAP are found), Z_1, Z_2-The outer zones. Number between parentheses-percentage of snails successfully locating SAP i.e. snails in Z_3 compared with that of failed snails in their location. *Statistically significant ($P<0.05$). When two way ANOVA was applied between different amino acids and different zones.

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