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Effect of bait formulation containing binary mixture of carbohydrate and amino acids on the Behavioural Responses of snail *Lymnaea acuminata*

S.P. Singh, Ajay Singh* and R.P. Yadav
Dept. of Zoology, D.D.U. Gorakhpur University
Gorakhpur, U.P. India. 273009

Abstract

Different binary combination (1:1 ratio) of carbohydrates + amino acids was used as attractant in the bait formulation against *Lymnaea acuminata*. Bait containing 10mM concentration of carbohydrate + amino acids in 2% agar solution was prepared. Attraction of snails to different combinations was studied by using clear glass aquaria having diameter of 30cm. Each aquarium was divided into four concentric zones-Zone-3 (Central Zone), Zone 2 and 1 (middle Zone) and Zone -0 (outer Zone) had a diameter of 13, 18, 24 and 30cm, respectively. The fraction of snails that was in contact with the bait at different times was used as a measure of attraction. Among all the binary combinations Starch + Serine (48.36%) emerged as the strongest attractant, after 2h. The second most attractant is combination of Sucrose + Serine (46.34%).

Key Words: Bait; Carbohydrates; amino acids; snail; *Lymnaea acuminata*.

Introduction

The snail *Lymnaea acuminata* is the intermediate host of the liver fluke *Fasciola gigantica*, which cause endemic fascioliasis in the cattle population of Nothern part of India (Singh and Agrawal, 1981; Agrawal and Singh, 1988) .The snail *Lymnaea acuminata* is the vector of

these fluke. One way to reduce the incidence of this disease is to delink the life cycle of fluke by destroying the intermediate host (Marston and Hosttetman, 1985, 1987; Singh et al., 1996; Kumar and Singh 2006). Fresh water snail lives in an environment containing macrophytes , algae and bacteria (Thomas et al., 1982); These organism release copious amount of chemical, such as carbohydrates and amino acids into the surrounding water (Thomas et al. 1989; KpiKpi and Thomas 1992). It has been established that the snails use chemical signals in locating food sources (Thomas 1989; Kpikpi and Thomas 1992, 1993, Tiwari and Singh 2004 a and b). Starch and a variety of free sugars, namely glucose, fructose, maltose and sucrose are abundantly present in decaying animals and plants (Livingstone and Dezwann 1983). Use of a combination of attractants & toxicants has been observed as an effective tool for pest managements (Tiwari and Singh 2007). Bait formulation feeding by snails is safe as less amount of molluscicide will be diffuse in the aquatic environment. Only attracted snails feeding on bait will be killed. The present study assays the behavioural responses of *L. acuminata* towards baits containing different amino acids and carbohydrates combinations and identifies which of them could preferably be used as a potent attractant for preparing bait along with molluscicides so as to ensure good levels of contact between the molluscicide and the target snail population.

Materials & Methods:

Collection of Snails: Adult *L. acuminata* snails (Average length 2.6±0.3 cm) collected locally from pools, lakes and low-lying submerged fields near Ramgarh lake in Gorakhpur, were used as the test animal. The snails were acclimatized for 72 h in de- chlorinated tap water of 25± 1^0 C.

Chemicals: All required chemicals were purchased from sigma chemical co (St Louis, M O, U S A).

Preparation of Bait: The bait containing binary combination of Carbohydrates (Starch, Glucose, Sucrose, Maltose, 10mM) + amino acids (Proline, Serine, Alanine, Glycine, 10mM) in 1:1 ratio were prepared in 100ml 2% agar solution by the method of Madsen (1992), as modified by Tiwari and Singh (2004 a and b); Singh and Singh (2008). These solutions were spread at a uniform thickness of 5 mm. After cooling baits were cut in small pieces of 5mm in diameter.

Assay apparatus & Procedure: Chemoattraction studies of different binary combination of carbohydrates and amino acids against *Lymnaea acuminata* were made in a circular glass aquarium having a diameter of 30cm. Each aquarium was divided into four concentric Zone; Zone-3 (Central Zone), Zone-2 & Zone_1 (Middle Zone) and Zone-0 (Outer Zone)having diameter of 13, 18, 24 and 30 cm, respectively. Zone 0,1,2and 3 had an area of 254.35, 197.82, 121.62 and 132.66 cm^2, respectively. A small annular elevation of 9mm and 1.5 cm diameter was made in the center of the aquarium (Zone-3). The aquaria were then filled with 500ml of dechlorinated tap water to height

of 8mm, and maintained at 25 ± 1^0C. At the start of assay 10 individually marked snails of uniform size were placed on the circumference of Zone-0. The distance between two snail was 66 mm, simultaneously the attractant bait was placed inthe center of Zone-3. The location of each snail was noted every 15 min for 2h. For each combination six sets with 10 snails each at the required concentration were used.

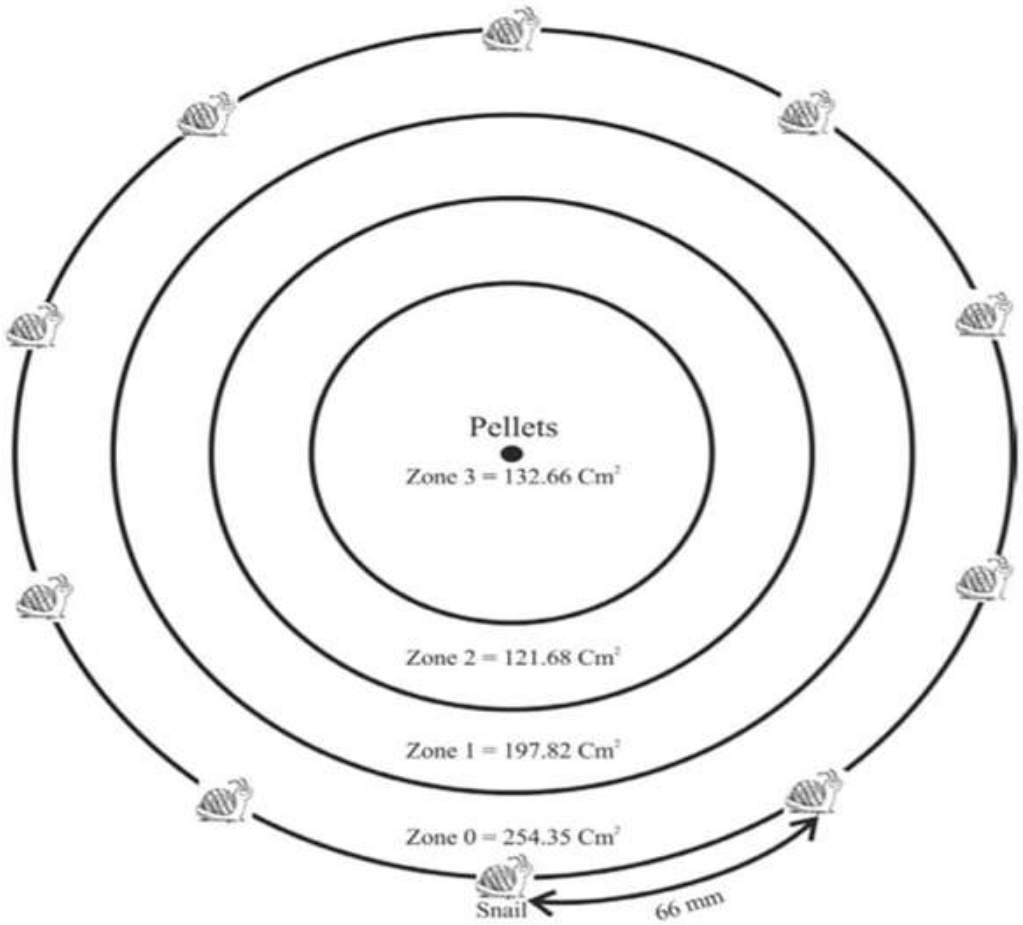

Figure: Experimental design of the aquarium for the study of attraction of snails by SAP. Diameter of Zone 3, Zone 2, Zone I and Zone 0 were 13,18,24 and 30 cm respectively. SAP was placed in the center of Zone 3, whereas 10 marked snails were placed a periphery of Zone 0. The distance between the two snails was 66 mm.

Data Analysis: Proportion of snails was arcsine transformed for each replicates in Zone-3. These proportions were compared between each concentration of the attractants for a given concentration separately using one way ANOVA (Sokal and Rohlf 1973).

Result:

The Table (1 and 2) gives the distribution of *Lymnaea acuminata* in the 3rd Zone around the bait of different

binary combination of 10mM carbohydrates & amino acids after 1 and 2 h from the start of the experiment. Placement of bait in center (Zone3) affected the behaviour of the snails.

Among all the binary combinations (1:1) ratio of carbohydrates and amino acids, Starch + Serine (48.36%) & Sucrose + Serine (46.34%) containing bait attracts highest percentage of snails after 2h in Zone3.Combination of carbohydrates (Starch, Glucose, Sucrose, Maltose) + amino acid (Proline, Serine, Alanine, Glycine) caused a significant variation ($P<0.05$) in number of snails in Zone-3 (Table 1 and 2).

Table 1. Distribution of snail *Lymnaea acuminata* in the third Zone around bait of different combination (1:1) of carbohydrates(10mM) and amino acid (10mM) after 1and2 hours from the beginning/start of the experiment.

Attractant	Time(hrs)	Mean Number of entries of snail around bait in Zone three
Agar (Control)	1	0.24±0.12 (10.6)
	2	0.49±0.16(14.28)
Glucose+Alanine	1	0.83±0 .07 (26.3)
	2	1.08± 0.12 (36.1)
Maltose+Alanine	1	0.91±0.06 (34.4)
	2	1.08.±0.28 (38.3)
Starch + Alanine	1	1.33±0.18 (23.8)
	2	1.24±0.24 (37.3)
Sucrose+Alanine	1	1.75±0.25 (38.8)
	2	1.75 ±0.48 (41.2)
Glucose + Serine	1	2.33 ±0.21(28.8)
	2	1.75± 0.48 (41.2)
Maltose + Serine	1	1.00 ±0.57 (30.8)
	2	1.25 ±0.48 (33.3)
Starch + Serine	1	3.5±0.34 (35.0)
	2	5.66 ±0.21 **(48.36)**
Sucrose + Serine	1	2.33 ±0.76 (28.26)
	2	5.33 ±0.55 **(46.34)**

Number given in parentheses, indicates the percentage of snails successfully locating Bait (i.e. snails in Zone-3 compared with that failed in their location).

Table.2 Distribution of snail *Lymnaea acuminata* in the third Zone around Bait of different combinations (1:1) of carbohydrates(10mM) and amino acid (10mM) after 1 and 2 hour from the start of experiment.

Attractant	Time (hrs)	Mean Number of entries of snail around attractant i n Zone three.
Control (Agar)	1 2	0.24±0.12 (10.6) 0.49±0.16 (14.2)
Glucose + Proline	1 2	0.58±0.12 (22.6) 3.33±0.21 (35.24)
Maltose + Proline	1 2	3.33±0.33 (34.22) 4.00±0 (40.0)
Starch + Proline	1 2	1.00±0.41 (26.7) 1.25±0.25 (29.4)
Sucrose + Proline	1 2	3.5±0.34 (35.0) 4.33± 0.21 (44.22)
Glucose + Glycine	1 2	2.66 ± 0.49 (27.89) 3.33±0.49 (34.22)
Maltose +Glycine	1 2	0.74±0.12 (32.5) 1.34±0.13 (41.0)
Starch + Glycine	1 2	0.91±0.12 (34.5) 0.99±0.35 (42.9)
Sucrose + Glycine	1 2	1.33±.42 (21.38) 3.33±.21 (35.24)

Number given in parenthesis, indicate the percentage of snails successfully locating Bait (ie snails in Zone-3 compared with that of failed in their location.)

Discussion:

The present study clearly demonstrate that the *L. acuminata* snails were attracted by baits. There was a significant variation in behavioural response towards the bait containing different carbohydrates and amino acids .It has been reported that gastropods detects the amino acids, carbohydrates as indicator of their food (Kpikpi and Thamos. 1992; Abd El. Hamid and Madsen 1995; Tiwari and Singh 2004 a-b). Among all the combination of carbohydrates + amino acid Starch + Serine (10mM) and Sucrose + Serine (10mM) show highest attraction towards the snail *L. acuminata*. This indicate that combination of different carbohydrate+amino acids is recognized rapidly by the snails. Abd El-Hamid (1996) and Tiwari and Singh (2004 a,b) reported the behavioural responses of the snail *Biompholaria alexandrina, Lymnaea acuminata* to carbohydrates and amino acids in bait. Diffusion of carbohydrate and amino into different Zones, results the orientation of snails towards their choice of

a specific carbohydrates &/ amino acids. It may be possible that differences in behavioural responses between *L.acuminata* and other snails may be due to difference in the feeding behaviour and metabolism of different species or it may be due to variation in receptor that detect the attractants. Combination of amino acids and carbohydrates are more effective than their singly use in bait as reported by Tiwari and Singh (2004 a,b) against *L.acuminata*. Significant variation is the number of snails in Zone-3 attracted by different combinations carbohydrates + amino acids in bait clearly demonstrates that snails are capable of differentiating type of carbohydrates and amino acid in bait . It is obvious from observations that among different combination tested, snails were respective to Starch+Serine and Sucrose+ Serine, as these combinations attract more snail than any other combinations of carbohydrates + amino acids. Abd El-Hamid (1996) revealed that the behavioural responses of all stage of snails *Biompholaria alexandrina* cause significant attraction to different amino acids. They suggested that the *Biomphalaria alexandrina* snails like other gastropods are able to detect their food sources by using chemical sense of amino acids as a sign for the presence of their food .All these suggestion are in agreement with the hypothesis that gastropod molluscs are attracted to some of the chemical diffusion out from dead and living aquatic organisms into the modular system of snails (Thomas,1982, Sterry et al., 1985, Thomas et al. 1989, KpiKpi and Thomas 1992: Abd El Hamid and Madsen,1995).

It is clear from the above study that the freshwater snail *L.acuminata* are able to detect their food sources sensing to combinations of carbohydrates + amino acid as indicator of the presence of their food (Tiwari and Singh 2004 a,b). A combination of amino acid with carbohydrate in bait is more effective in snails and these combinations might be used with molluscicide in bait formulations to kill the vector snail.

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