Bait Formulation of Molluscicides with Attractants Amino Acids Against he Snail *Indoplanorbis Exustus*

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Summary

The snail control is one of the important methods in the campaign to reduce the incidence of fascioliasis. In order to achieve this objective, bait formulation containing an attractant and molluscicides is an appropriate approach to lure the target snail population below threshold level. In the present study snails attractant food pellets (SAP) were prepared from 20 mM amino acid in 2% agar solution. Attraction of snails to different bait formulation (amino acid + molluscicides) was carried out in glass aquaria having diameter of 30 cm. Among all the bait formulations molluscicides ferulic acid + lysine containing bait attract 38.66% of snail after 2 hours. Bait containing eugenol as molluscicides with attractant amino acid tyrosine (96h LC_{50}-1.42 mg/l)/lysine (96h LC_{50}-1.56 mg/l) emerged as the strongest bait formulation against *Indoplanorbis exustus.*

Keyword: Bait formulation; Molluscicides; Amino acids; *Indoplanorbis exustus.*

Introduction

Fascioliasis is an important helminth disease caused by two trematodes *Fasciola hepatica* and *F. gigantica* of Africa and Asia [1]. The fresh water snails are an intermediate host of the *Fasciola* species [2]. Fascioliasis is a common worldwide disease among cattle [3]. Hunan fascioliasis is reported now in different parts of the world. According to WHO [3] 2.4 million human are infected with *Fasciola* and a further 180 million are at risk of infection. In northern India, *Indoplanorbis exustus* is the intermediate host of the *F. gigantica* [4]. Singh and Agarwal [5] reported that, 94% of buffaloes slaughtered in Gorakhpur district carry heavy infection of *F. gigantica*. One way to reduce the incidence of fascioliasis is to de-link their life cycle of fluke, by destroying the intermediate hosts [6, 7, 8, 9, 10]. Bait formulations of molluscicides with attractant would be an effective tool for selective control of the snail with minimal adverse effect on the non-target animal and environment. It is therefore, important to identify strong attractant compounds for preparing effective bait formulations with active molluscicidal components. These aquatic plants release different types of chemicals, such as carbohydrates and amino acids into the surrounding water [11, 12, 13, 14] which acts as attractant for snails. The present study assays the behavioral responses of *I. exustus* to different amino acids as attractant with active molluscicides ferulic acid (*Ferula asafoetida*), umbelliferone (*Ferula asafoetida*), eugenol (*Syzygium aromaticum*) and limonene (*Carum carvi*) [8,9] was used to kill snail *I. exustus.*

Material and methods

Collection of snails

Adult *Indoplanorbis exustus* (0.85±0.036 cm in length) were collected locally from lakes and low lying submerged fields of Gorakhpur. The snails were acclimatized for 72 hours in dechlorinated tap water at 26±1^{0}C. The pH of the water was 7.2-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.2 mg/l, 5.2-6.3 mg/l and 102.0-105.0 mg/l, respectively.

Formulation of snail-attractant food pellets (SAP)

Snail attractant food pellets (SAP) were prepared according to the method of Madsen [15] as modified by Tiwari and Singh [16, 17]. Agar-agar, amino acids (tyrosine, lysine), different active molluscicidal components (ferulic acid, umbelliferone, eugenol and limonene) were used in bait formulations. 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); were purchased from Sigma chemical Co. (USA). Binary combinations of (20 mM each) amino acids + molluscicides were prepared in 100 ml of 20% agar solution. After boiling, each of the active molluscicidal components was added to the solution in different concentrations (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 apparatus and procedure

The bioassay was performed by the method by Tiwari and Singh [16, 17]. 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), 2, 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 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 at a distance of 66 mm on the circumference of zone 0. Simultaneously, one of the prepared bait of different 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.

Mortality rate was observed after 24h up to 96h. 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 [18]. One–way ANOVA and product moment correlation coefficient was applied in between the different data to observe the significant mortality [19].

Results

Table 1 gives the distribution of *I. exustus* in the zone-3 around the snail attractant food pellets (SAP) of amino acids with various active molluscicidal components in zone-3 at different concentrations after 1 and 2 hours from the start of the experiment. Placement of SAP in center (Zone-3) affected the behavior of the snails. The effect of amino acids with various active molluscicidal components in SAP on the proportion of snail in zone-3 was analyzed by one-way ANOVA. Among all the bait formulations, lysine + 4% eugenol showed lowest attraction of the snails in zone-3 (Table 1). Bait containing 4% ferulic acid, umbelliferone, eugenol and limonene

with tyrosine attract 22.36%, 30.12%, 30.11% and 32.60%, of snails respectively, while with lysine they attract 28.15%, 28.76%, 21.02% and 25.99%, of snails respectively, after 2 hours (Table 1). Attraction of snails in bait containing molluscicide + attractant + agar was lower than control pellet containing agar + attractant (Tyrosine/ lysine). The attraction of the snails towards the SAP was significantly ($P<0.05$) reduced with increasing concentration of different molluscicides in bait.

Molluscicidal activity of different SAP containing active component against *I. exustus* was time and dose dependant (Fig-1 to 2). There was a significant ($P<0.05$) negative correlation between exposure period and LC_{50} of different molluscicides. The bait formulation containing eugenol + lysine (96h LC_{50}- 1.50 mg/l) and eugenol + tyrosine (96h LC_{50}- 1.42 mg/l) were more toxic than ferulic acid, umbelliferone and limonene (Fig-1 to 2).

The slope values were steep and separate estimation of LC_{50} based on each of the six replicates, were found to be within 95% confidence limits of LC_{50} (Fig-1 to 2). The t- ratio was higher than 1.96 and the heterogeneity factor was less than 1.0. The g value was less than 0.5 at all probability levels; 90, 95 and 99. There was significant negative regression ($p<0.05$) between exposure time and LC_{50} of the treatments (Fig-1 to 2).

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

Molluscicides	Time (hr)	Concentration of molluscicides*		
		6%	5%	4%
Tyrosine +Feru	1	1.12±0.33 (21.21)+	1.35±0.04 (23.86)	2.09±0.01(32.21)
	2	1.66±0.24 (22.36)+	1.75±0.18 (25.92)	2.25±0.30 (35.38)
Tyrosine +Umb	1	1.21±0.11 (24.63)+	1.34±0.32 (25.03)	1.98±0.27 (28.68)
	2	2.35±0.20 (30.12)+	2.50±0.18 (32.56)	2.78±0.23 (35.88)
Tyrosine +Eug	1	1.88±0.19 (27.10)+	2.33±0.11 (28.36)	2.52±0.75 (30.76)
	2	2.77±0.24 (30.11)+	2.95±0.61 (32.05)	3.22±0.33 (35.13)
Tyrosine +Lim	1	1.66±0.13(28.19)+	1.87±0.70 (30.89)	2.15±0.20 (32.62)
	2	2.83±0.40 (32.60)+	3.33±0.21 (33.17)	3.75±0.16 (35.00)
Lysine +Feru	1	1.19±0.66 (25.91)+	1.59±0.44 (35.55)	1.75±0.45 (37.32)
	2	2.88±0.63 (28.15)+	3.11±0.22 (31.76)	3.67±0.54 (38.66)
Lysine +Umb	1	1.66±0.12 (25.15)+	1.75±0.34 (28.71)	1.98±0.75 (35.60)
	2	2.88±0.66 (28.76)+	2.99±0.18 (30.11)	3.43±0.12 (36.50)
Lysine +Eug	1	1.43±0.25 (20.32)+	1.70±0.23 (22.67)	1.99±0.25 (25.32)
	2	2.30±0.15 (21.02)+	2.67±0.31 (23.86)	3.26±0.21 (27.82)
Lysine +Lim	1	1.89±0.23 (24.17)+	2.01±0.17 (27.67)	2.33±0.27 (32.38)
	2	2.21±0.61 (25.99)+	2.51±0.42 (28.00)	2.98±0.28 (33.72)
Control (Agar)	1	0.84±0.19 (26.89)	0.99±0.23 (32.12)	1.72±0.20 (47.11)
	2	1.80±0.31 (27.90)	2.20±0.15 (32.38)	2.66±0.10 (50.13)
Control (Tyrosine)	1	2.36±0.16 (24.72)	3.10±0.19 (32.33)	3.88±0.35 (41.19)
	2	2.89±0.13 (26.38)	3.77±0.10 (35.45)	4.20±0.18 (42.00)
Control (Lysine)	1	2.20±0.20 (28.16)	3.77±0.41 (25.66)	3.11±0. 15 (30.32)
	2	3.57±0.19 (25.82)	3.75±0.20 (28.92)	3.89±0.25 (35.32)

Values in parentheses are percentages of snails in zone 3 (in contact with attractant food pellet) with respect to 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: Feru - ferulic acid, Umb - umbelliferone, Eug - eugenol, Lim - limonene

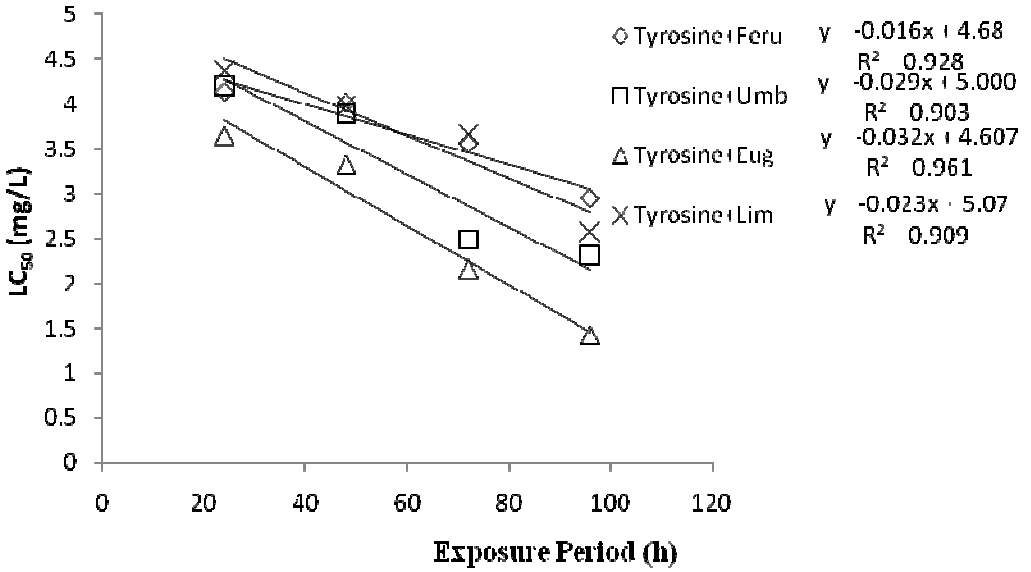

Figure -1: Regression analysis in between LC_{50} of different bait formulations of Tyrosine + molluscicides and exposure time

Abbreviations: Feru - ferulic acid, Umb - umbelliferone, Eug - eugenol, Lim - limonene

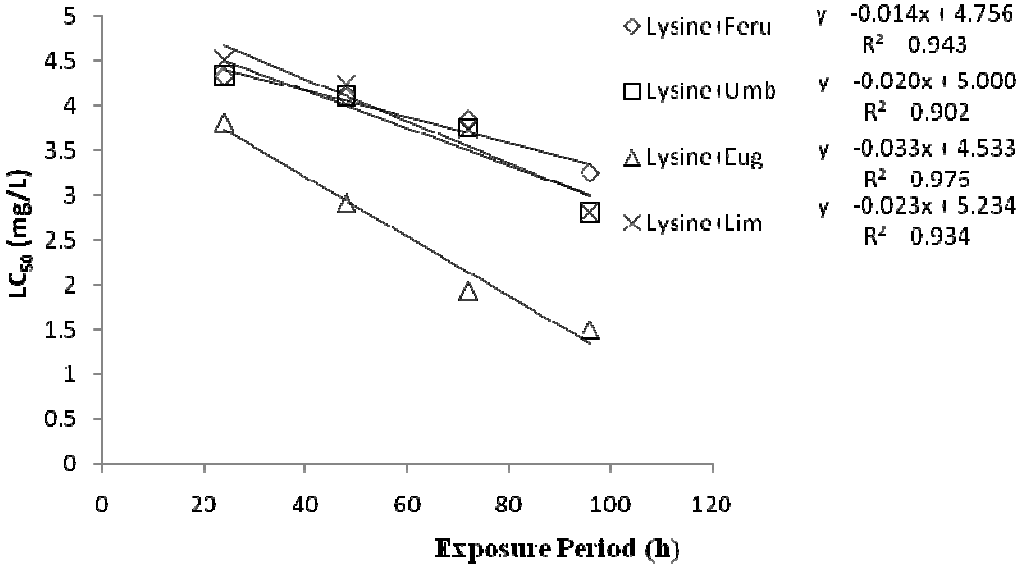

Figure- 2: Regression analysis in between LC_{50} of different bait formulations of Lysine + molluscicides and exposure time

Abbreviations: Feru - ferulic acid, Umb - umbelliferone, Eug - eugenol, Lim – limonene

Discussion

The present study clearly demonstrates that the snail *I. exustus* showed a significant behavioral response towards the different combinations of amino acid with molluscicides. Earlier, it has been observed that gastropods detect the amino acid as indicator of their food [9, 10, 16, 17, 20, 21]. Significant variation in the number of snails in zone-3 attracted by different amino acid with molluscicides in bait formulation clearly indicate that, snails are capable of differentiating type of amino acid in the bait. *I. exustus* was more attracted towards the lower concentration (3%) of molluscicide containing bait formulations.

Molluscicide in bait has some repellant action. Active components ferulic acid, umbelliferone (*Ferula asafoetida*), eugenol (*Syzygium aromaticum*), and limonene (*Carum carvi*) are very effective molluscicides when release directly in aquatic environment [7, 8]. Kumar et al., [22, 23, 24] has been reported that the toxicity of active components of ferulic acid, umbelliferone, eugenol and limonene were more effective in the bait formulations with binary combinations of amino acid as an attractant against the fresh water snail *I. exustus*. The bait formulation in the present study is very effective in the snail control programme as they use less amounts of molluscicides than their direct release in water. Among all the SAP containing molluscicides eugenol is more effective in killing the snail *I. exustus*.

The steep slope 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) since it is less than 0.5.

The present study clearly indicates that molluscicides of plant origin and their active components could be used with varying degrees of success in bait formulation. This concept is a new approach and technique for the control/management of fascioliasis by the use of fewer amounts of molluscicides in bait for control of harmful snails.

Acknowledgments

One of the authors (Dr. Pradeep Kumar- Post Doctoral Fellow) is thankful to University Grants Commission, New Delhi, for financial assistance.

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