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Attractant Food Pellets Containing Molluscicides Against the Fresh Water Snail *Indoplanorbis exustus*

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

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

Abstract: Fascioliasis is an important helminth disease caused by *Fasciola gigantica*. The snail control is one of the important methods in the campaign to reduce the incidence of fascioliasis. The order to achieve this objective, using of bait formulations containing an attractant and molluscicides is an appropriate approach to lure the target snail population to the molluscicides. In the present study snails attractant food pellets (SAP) were prepared from 20 mM carbohydrate in 2% agar solution. Attraction of snails to different bait formulation (carbohydrate + molluscicides) was carried out in glass aquaria having diameter of 30 cm. Among all the bait formulations containing molluscicides, limonene, attract 34.04% of snail after 2 hours, which was significantly different from their control. Bait containing ferulic acid emerged as the strongest bait formulation (LC_{50} 96 h, 1.229 %) against *Indoplanorbis exustus.*

Key words: Bait formulation · Carbohydrate · Molluscicides · *Indoplanorbis exustus*

INTRODUCTION

The fresh water snails are an intermediate host of the *Fasciola* species [1]. Fascioliasis is a common worldwide disease among cattle. Hunan fascioliasis is reported now in different parts of the world. WHO [2] reported that, 2.4 million human are infected with *Fasciola* and a further 180 million at risk for infection. In northern India, *Indoplanorbis exustus* is the intermediate host of the *Fasciola* species [3]. Singh and Agarwal [4] reported that, 94% of buffaloes slaughtered in Gorakhpur district carry heavy infection of *F. gigantica*. *Fasciola hepatica* and *F. gigantica* are the causative agents of endemic fascioliasis in different parts of world [5]. One way to reduce the incidence of fascioliasis is to de-link their life cycle of fluke, by destroying the intermediate hosts [6-9]. Bait formulations of different molluscicides would be an effective tool for selective killing 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 molluscicides. Snails, like other gastropods mollusc use chemical clues to locate food sources [9-16]. The freshwater snails inhabit an environment containing macrophytes algae and bacteria [17]. These aquatic organisms release different types of chemicals, such as carbohydrates and amino acids into the surrounding water [18-21] which acts as attractant for snails. The present study assays the behavioral responses of *I. exustus* to different carbohydrate with active molluscicidal components which can be used against *I. exustus*. The active molluscicidal components ferulic acid (*Ferula asafoetida*), umbelliferone (*Ferula asafoetida*), eugenol (*Syzygium aromaticum*) and limonene (*Carum carvi*) [8, 9] will be used inside the preferred snails attractant food pellets (SAP) for the effective control of snail *I. exustus.*

MATERIALS 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°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.

Pure Compounds: Agar-agar, carbohydrates, different active molluscicidal components such as eugenol, ferulic acid, umbelliferone and limonene were used in bait formulations. The pure active component ferulic acid (4-Hydroxy-3 methoxycinnamic), umbelliferone (7-Hydroxy

Corresponding Author: D.K. Singh, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur-273009, U.P., India.

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).

Formulation of Snail-Attractant Food Pellets (SAP): Snail attractant food pellets (SAP) were prepared according to the method of Madsen [22] as modified by Tiwari and Singh [11, 12]. Binary combinations of (20 mM each) carbohydrates + 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 [10, 11]. 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°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 [23]. One-way ANOVA and product moment correlation coefficient was applied in between the different data to observe the significant mortality [24].

RESULTS

Table 1 illustrates the distribution of *I. exustus* in the zone-3 around the snail attractant food pellets (SAP) of carbohydrate 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 carbohydrate 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, starch + 3% ferulic acid showed lowest attraction (18.33%) of the snails in zone-3 (Table 1). Bait containing 3% ferulic acid, umbelliferone, eugenol and limonene with starch attract (28.0%, 31.7%, 27.1% and 34.0%, of snails respectively, while with maltose they attract 29.2%, 33.6%, 30.6% and 32.9%, respectively, after 2 hours (Table 1). Attraction of snails in bait containing molluscicide + attractant + agar was lower than control pellet containing agar + attractant (starch/ maltose). The attraction of the snails towards the SAP was significantly ($P<0.05$) reduced with increasing concentration of different molluscicides.

Molluscicidal activity of different SAP containing active component against *I. exustus* was time and dose dependant (Table 2 & 3). There was a significant ($P<0.05$) negative correlation between exposure period and LC_{50} of different molluscicides. The active component ferulic acid (LC_{50} 24 h, 3.358%) and umbelliferone (LC_{50} 24h, 1.974%) were more toxic than eugenol and limonene (Table 2 & 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 (Table 2 & 3).

DISCUSSION

The present study clearly demonstrates that the snail *I. exustus* showed a significant behavioral response towards the different combinations of carbohydrates with molluscicides. Earlier, it has been observed that gastropods detect the carbohydrates as indicator of their food [9-13]. Significant variation in the number of snails in zone-3 attracted by different carbohydrate with molluscicides in snail attractant food pellets (SAP) clearly indicate that, snails are capable of differentiating type of carbohydrate in the SAP. It is obvious from the observations that among different carbohydrate tested, snails were most, responsive to the starch and limonene. Higher attraction towards the starch in the SAP is possibly due to fact that in nature starch is the major carbohydrate stored in aquatic plants [20, 22]. The current study clearly demonstrates that the different carbohydrate

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

		Concentration of molluscicides*		
Molluscicides	Time (hr)	3%	4%	5%
Starch+Feru	1	0.99±0.23 (18.33)+	1.33±0.04 (24.62)	3.08±0.01(57.03)
	2	2.49±0.34 (28.00)+	2.41±0.15 (27.10)	3.99±0.36 (44.88)
Starch+Umb	1	1.49±0.31 (25.64)+	1.33±0.30 (31.89)	2.99±0.27 (51.46)
	2	2.83±0.21 (31.79)+	2.91±0.08 (32.69)	3.16±0.21 (35.50)
Starch+Eug	1	1.74±0.499 (28.71)+	2.41±0.21 (39.76)	1.91±0.56 (31.51)
	2	3.07±0.25 (27.09)+	3.41±0.41 (30.09)	4.85±0.42 (42.80)
Starch+Lim	1	1.57±0.34 (27.83)+	1.74±0.75 (30.85)	2.33±0.23 (41.31)
	2	3.83±0.44 (34.04)+	3.57±0.34 (31.73)	3.85±0.28 (34.22)
Maltose+Feru	1	1.16±0.34 (26.91)+	1.49±0.44 (34.57)	1.66±0.52 (38.51)
	2	2.82±0.288 (29.25)+	3.16±0.25 (32.78)	3.66±0.13 (37.96)
Maltose+Umb	1	0.83±0.09 (25.15)+	0.98±0.31 (29.69)	1.49±0.51 (44.54)
	2	2.91±0.21 (33.64)+	2.83±0.28 (26.93)	3.41±0.15 (39.42)
Maltose+Eug	1	1.33±0.23 (28.60)+	1.41±0.37 (30.30)	1.91±0.58 (41.07)
	2	3.33±0.13 (30.63)+	3.16±0.48 (29.07)	4.33±0.36 (40.29)
Maltose+Lim	1	0.74±0.08 (24.18)+	1.33±0.13 (43.46)	0.99±0.38 (32.35)
	2	2.74±0.16 (32.97)+	2.66±0.36 (32.00)	2.91±0.21 (35.01)
Control (Agar)	1	0.82±0.16 (24.84)	0.99±0.13 (30.00)	1.49±0.21 (45.15)
	2	1.82±0.16 (25.49)	2.24±0.15 (31.37)	3.08±0.16 (43.13)
Control (Starch)	1	2.34±0.13 (24.81)	3.11±0.16 (32.97)	3.98±0.34 (42.20)
	2	3.84±0.13 (30.00)	4.67±0.17 (36.48)	4.28±0.13 (33.43)
Control (Maltose)	1	2.15±0.23 (24.26)	3.67±0.31 (41.42)	3.04±0. 13 (34.31)
	2	3.56±0.13 (31.81)	3.70±0.21 (33.06)	3.88±0.21 (34.67)

Values in parentheses are percentages of snails in zone 3 (in contact with attractant food pellet) with respect to snails 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

Table 2: Bait formulations of different molluscicides and their toxicity against *I. exustus* at different time exposure

Exposure period	Molluscicides	LC_{50}	LCL	UCL	Slope value	T-ratio	G-value	Heterogeneity
24h	Maltose +Feru	3.358	2.530	6.190	1.350±0.378	3.575	0.300	0.17
	Maltose+Umb	4.300	3.023	7.659	1.020±0.374	2.725	0.317	0.37
	Maltose +Eug	3.871	3.117	5.753	1.041±0.420	4.852	0.163	0.18
	Maltose +Lim	4.280	3.122	4.563	1.622±0.380	1.959	0.432	0.28
48h	Maltose +Feru	2.691	2.074	3.925	1.456±0.374	3.893	0.253	0.31
	Maltose+Umb	3.212	3.530	4.660	1.791±0.364	2.174	0.313	0.15
	Maltose +Eug	3.593	2.872	5.404	1.843±0.402	4.579	0.183	0.28
	Maltose +Lim	3.960	3.850	4.853	1.631±0.380	2.096	0.192	0.26
72h	Maltose +Feru	2.265	1.590	3.274	1.254±0.368	3.412	0.330	0.22
	Maltose+Umb	2.084	2.330	3.430	1.673±0.362	2.211	0.486	0.22
	Maltose +Eug	3.197	2.535	4.777	1.656±0.388	4.266	0.211	0.29
	Maltose +Lim	3.756	3.615	4.822	1.852±0.386	1.576	0.392	0.35
96h	Maltose +Feru	1.955	1.345	2.590	1.383±0.368	3.754	0.273	0.26
	Maltose+Umb	1.364	1.399	1.970	1.863±0.362	2.383	0.376	0.13
	Maltose +Eug	2.611	1.990	3.803	1.415±0.373	3.796	0.266	0.26
	Maltose +Lim	3.682	1.875	4.920	1.382±0.386	3.605	0.421	0.27

Abbreviations: Feru - ferulic acid, Umb - umbelliferone, Eug - eugenol, Lim- limonene, LCL - lower confidence limits, UCL - upper confidence limits.

Six batches of ten snails were exposed to different concentrations of the above molluscicides inside the snail attractant food pellets (SAP). Mortality rate 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 - Maltose+Feru-1.63++; Maltose+Umb-12.34+; Maltose+Eug-4.33+; Maltose+Lim-13.22++.

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

Table 3: Bait formulations of different molluscicides and their toxicity against *I. exustus* at different time exposure

Exposure period	Molluscicides	LC_{50}	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24h	Starch+Feru	2.303	1.686	3.223	1.358±0.369	3.679	0.284	0.15
	Starch+Umb	1.974	1.101	2.930	1.064±0.364	2.272	0.344	0.13
	Starch+Eug	3.629	2.883	5.585	1.797±0.401	4.478	0.192	0.13
	Starch+Lim	3.820	2.610	3.330	1.630±0.321	3.852	0.173	0.18
48h	Starch+Feru	1.971	1.415	2.557	1.489±0.370	4.024	0.237	0.31
	Starch+Umb	2.022	1.172	3.020	1.073±0.364	2.946	0.443	0.14
	Starch+Eug	3.219	2.565	4.750	1.702±0.390	4.368	0.201	0.23
	Starch+Lim	3.712	2.583	3.650	1.762±0.290	4.560	0.32	0.22
72h	Starch+Feru	1.844	1.112	2.529	1.21±0.365	3.325	0.347	0.42
	Starch+Umb	1.453	0.589	2.033	0.934±0.363	2.577	0.378	0.17
	Starch+Eug	2.782	2.204	3.901	1.616±0.380	4.253	0.212	0.32
	Starch+Lim	3.480	1.882	2.631	1.920±0.210	3.682	0.382	0.21
96h	Starch+Feru	1.229	0.420	1.747	1.152±0.367	3.138	0.390	0.44
	Starch+Umb	1.854	0.093	1.381	0.865±0.365	2.368	0.485	0.24
	Starch+Eug	2.123	1.347	3.152	1.130±0.365	3.095	0.401	0.42
	Starch+Lim	3.110	1.873	4.120	1.360±0.824	4.233	0.501	0.35

Abbreviations: Feru - ferulic acid, Umb - umbelliferone, Eug - eugenol, 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 snail attractant food pellets (SAP). 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 - Starch+Feru-3.34+; Starch+Umb-12.52++; Starch+Eug-5.11+; Starch+Lim-1.48++.

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

+ molluscicides are recognized rapidly by the snails. Abd EL-Hamid [25], Tiwari and Singh [10, 11], Singh and Singh [12], Kumar and Singh [13] and Kumar *et al* [15] reported the behavioral responses of the snail *Biomphalaria alexandrina* and *Lymnaea acuminata* to carbohydrates and amino acids in snail-attractant pellets. They suggested that the *B. alexandrina* and *L. acuminata* snails like other gastropods are able to detect their food sources by using chemical sense of carbohydrate as sign for the presence of their food. *I. exustus* were more attracted towards the lower concentration (3%) of molluscicide containing bait formulations. Starch is recognized more rapidly by the chemoreceptor present in the snails [12]. It may be possible that differences in behavioral responses between *I. exustus* and other snails may be due to differences in the feeding behavior and metabolism of different species or it may be due to variation in receptors that detect the attractants. Significant variation in the number of snails in zone-3 attracted by different carbohydrate + molluscicides in SAP clearly demonstrates that, snails are capable of differentiating type of carbohydrate in the SAP. Tiwari and Singh [11], Singh and Singh [12] and Kumar and Singh [9] revealed that, the behavioral responses of all stages of snails *L. acuminata* caused significant attraction to different binary combination of carbohydrate and amino acids, respectively. Among all the combination of carbohydrate (starch) + molluscicides (limonene) after 2 hours (34.04%) shows maximum attraction to the snails. Significant variation in the number of snails in zone-3 following the addition of the different bait formulations demonstrate that snails are capable of differentiating the different types of carbohydrate in the SAP.

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]. The bait formulation in the present study is very effective in the snail control programme as they use less amount of molluscicide than their direct release in water. Among all the SAP containing molluscicides ferulic acid 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 components could be used with varying degrees of success in bait formulation. This concept is a new approach and technique for the control of fascioliasis by the use of fewer amounts of molluscicides in bait for control of harmful snails.

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