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Attraction of Umbelliferone in Snail Attractant Pellets (SAP) against the Snail *Lymnaea acuminata*: An Intermediate Host of *Fasciola gigantica*

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Snail control method is one of the best ways to management of the fasciolosis. Snail attractant pellets (bait) containing attractant and molluscicide is the good alternative method for control of the *Lymnaea acuminata, which* is a known intermediate host of *Fasciola gigantica*. The result of the present study is to find the attraction of molluscicide umbelliferone in snail attractant pellets (SAP) against the *Lymnaea acuminata,* a vector of snail in different months of the year. Data shows that active molluscicidal component (umbelliferone) showed more attraction at lower concentration. On the contrary, at higher concentration, attraction of snail decreases significantly throughout the year. The attraction of snails declined significantly ($P < 0.01$) as the concentrations of molluscicide incorporated inside the pellets were increased and water temperature is low (i.e., in winter season). SAP containing 0.5% umbelliferone + starch attract maximum snails (46.0%) and 0.5% umbelliferone + proline, attract maximum snails (42.33%), in the month of June, whereas minimum attraction was observed in the month of January to March when SAP containing 7.0% umbelliferone + starch (6.23-8.1%) and when SAP containing 3.0 % umbelliferone + proline (7.33-9.45%).

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Keywords: Umbelliferone; snail attractant pellets; fasciolosis; molluscicide.

1. INTRODUCTION

Fasciolosis is an infectious disease caused by *Fasciola* parasites referred as the liver fluke [1]. This food- and water-borne disease is a major public health and veterinary issue [2]. Fasciolosis is found in tropical climates and temperate climates except Antarctica, over the 70 countries, especially where there are cattle or sheep population are found. It is asymptomatic, subclinical, chronic disease in buffaloes that adversely affect the reproductive cycle, weight, food conversion efficiency and productivity [3]. Infection of fasciolosis ranges between 1.69 to 94 per cent cattle population in India [4]. During this infection the host suffers from unnoticed ill effects continuing for a long time before the detection of disease is by veterinary clinician [5]. Control of parasitic diseases is animal aimed to increase the productivity of the animals. Therefore, the control of the *(Lymnaea acuminata*) an intermediate snail offers a good opportunity to reduce the transmission in host population. One of the best methods to tackle the problem is to dissipate the carrier snails and break the life cycle of the flukes. For control and management of this bait formulation techniques containing an attractant and molluscicide is a best process [6]. Molluscicide-containing bait is ingested by the vector that causes significant effect without any adverse effect on other biotic component within the same habitat. Umbelliferone, is 7-hydroxycoumarin that is pharmacologically active compound extracted by methanol found in many plants of Umbelliferae. It is one of the components of *asafoetida*, the dried latex from the giant fennel (*Ferula communis*). *Ferula* spp. has medicinal values and used by different researcher for their pharmacological effects for both the human and animal [7-10]. “It has broadly circulated inside the Rutaceae and Apiaceae families. It is widely used as antibacterial and anti-fungal agent, for the treatment of diabetes, cancer, hepatocellular carcinoma, has antioxidant property, in the treatment of cerebral ischemia, Parkinson’s disease, and in the treatment of bronchial asthma” [11]. Members of family Umbelliferae contain compound that are potential sources of molluscicides [12,13]. Therefore, umbelliferone bait considered a best way to control the vector snail.

Therefore, the aim of the present study is to investigate the attraction ability of molluscicide umbelliferone to the *Lymnaea acuminata* in different months of the year.

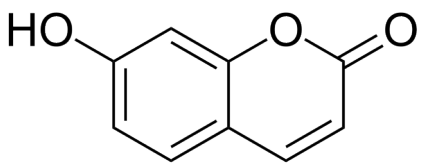
2. MATERIALS AND METHODS

2.1 Study Area

This work was carried out at Malacology Laboratory, in Zoology Department of D.D.U. Gorakhpur University, from 2018 to 2019.

2.2 Tested Materials

Umbelliferone (7-Hydroxycoumarin) ($C_9H_6O_3$; M.Wt.162.14) is a yellowish, white crystalline solid, acetylthiocholine iodide, and DTNB were purchased from Sigma-Chemical Co. in the United States.



Umbelliferone ($C_9H_6O_3$)

2.3 Collection of Animals

Adults of *Lymnaea acuminata* collected from collection sites of Gorakhpur and snail were acclimatized in tap water at suitable temperature for 72 h.

2.4 Preparation of SAP with Molluscicides

SAP containing attractant starch or proline and molluscicide umbelliferone were prepared in 100 ml of 2% agar solution by the method of Madsen [14] as modified by Tiwari and Singh [15,16]. Concentrations of carbohydrate and amino acid were based on the earlier reports of Tiwari and Singh [15,16]. Umbelliferone was added to the attractant food pellets simultaneously with starch or proline in 100 ml of 2% agar solution. These solutions were subsequently spread at a uniform thickness of 5 mm. After cooling, the SAP containing molluscicides were cut out using a corer, measuring 5 mm in diameter. The attraction of these pellets to *L. acuminata* snails was determined in each month of the year.

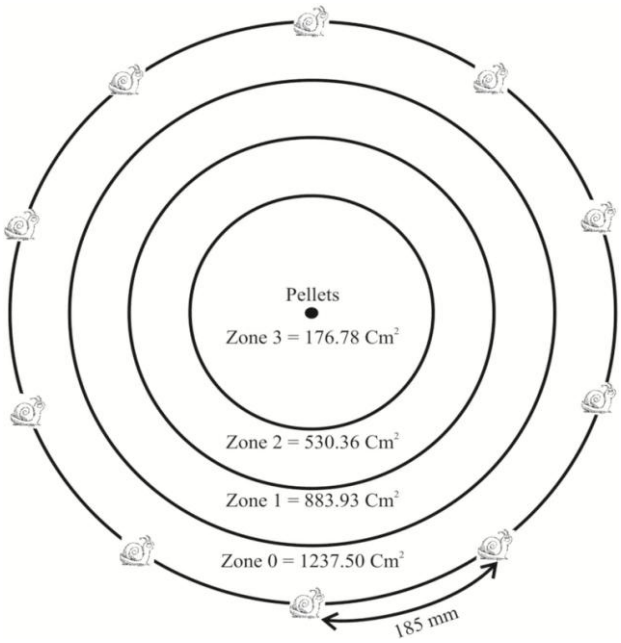


Fig. 1. For the study of attraction of snails by SAP aquarium is design in three zones. SAP was placed in centre and snails were placed at aquarium periphery (Zone-3)

2.5 Assay Apparatus and Procedure

"The chemo-attraction studies of starch or proline with umbelliferone to *L. acuminata* were made in a clean circular glass aquarium with a diameter of 60 cm" [17]. "Each aquarium was divided into four concentric zones: zone 3 (central zone), zones 2 and 1 (middle zone), and zone 0 (outer zone) had a diameter of 15, 30, 45, and 60 cm, respectively. Zones 3, 2, 1, and 0 had an area of 176.78, 530.36, 883.93, and 1237.50 cm^2, respectively. A small annular elevation of 9 mm height and 1.5 cm in diameter was made in the centre of each aquarium (zone 3). The aquaria were then filled with 2262 ml of dechlorinated tap water to a height of 8 mm and maintained at 25 ± 1ºC (Fig. 1). At the beginning of the assay, 10 marked snails of uniform size were placed on the circumference of zone 0. The distance between two snails was 185 mm; simultaneously, the SAP containing umbelliferone was added on the small annular elevation in the centre (zone 3). The position of every snail was noted every 15 min for 2 h and the attraction of snails in each month through the year were determined. For each combination (umbelliferone + starch or proline), six sets with 10 snails each at the required concentrations were used. Proportions of snails were arcsine transformed. These proportions were compared between each combination of the attractant for different concentrations of molluscicide and different months using a two-way analysis of variance" [18]. Control animals were kept in an equal volume of dechlorinated water under similar conditions without treatment.

2.6 Statistical Analysis

Analysis of variance through the 12 months was performed in order to determine any significant variation according to the method of Sokal and Rohlf [18].

3. RESULTS

3.1 Effect of Seasonal Variation on the Attraction towards (SAP) Baits that Contains Plant Derived Molluscicides in Different Months of the Year

Behavioural responses of carbohydrates or amino acids at different diameter demonstrate that starch and proline are the most favorite attractant in SAP [17]. Umbelliferone were incorporated inside the SAP that containing starch or proline as attractant. Before exploring the toxicity of these pellets, attraction of the snails on the desired SAP in the experiment was recorded after two hours from starting of the work in different months at 60 cm diameter.

Data given in the Fig. 2 is the mean number of snail *L. acuminata* at different concentrations in zone 3 with the SAP containing molluscicide

throughout the year. Active molluscicidal component (umbelliferone) showed more attraction at lower concentration. At higher concentration, attraction of snail decreases significantly in different months of the year. The attraction of snails declined significantly (P < 0.01) as the concentrations of molluscicide incorporated inside the pellets were increased and water temperature is low (i.e., in winter season). There was a considerable (P < 0.01) change in the number of snails reaching zone-3 in various months during a year. The effect of seasonal variation and concentration of molluscicide in SAP on the proportion of snails in zone-3 was analyzed by using two-way ANOVA. SAP containing 0.5 % umbelliferone + starch attract maximum snails (46.0 %) in the month of June. Minimum attraction in the population of snails was found in the month of January-March (6.23-8.1 %) when fed with SAP containing starch + 7.0 % umbelliferone in SAP (Fig. 2). There was a significant variation ($F_{(4, 28)}$ =267.02, $F_{(7, 28)}$ =29.52; P =0.01 and $F_{(4, 12)}$ =164.13, $F_{(3, 12)}$ =27.48; P = 0.01) was seen between attraction of snails in different months at different concentration of umbelliferone in SAP (Fig. 2).

SAP with 0.5 % umbelliferone + proline, attract maximum snails (42.33%) in the month of June. Minimum attraction was found in the month of January to March (7.33-9.45%) when snails were exposed to 3.0 % umbelliferone + proline in SAP (Fig. 3). There was a marked variation ($F_{(4, 28)}$ =343.47, $F_{(7, 28)}$ =35.26; P =0.01 and $F_{(4, 12)}$ =68.22, $F_{(3, 12)}$ =8.58; P = 0.01) in the number of snails reaching zone-3 in different months and concentrations of umbelliferone in SAP (Fig. 3).

Attraction of snails towards the SAP without molluscicide i.e., in control group was 56.67 % or 53.33% in the month of June when starch and proline were used as an attractant, respectively.

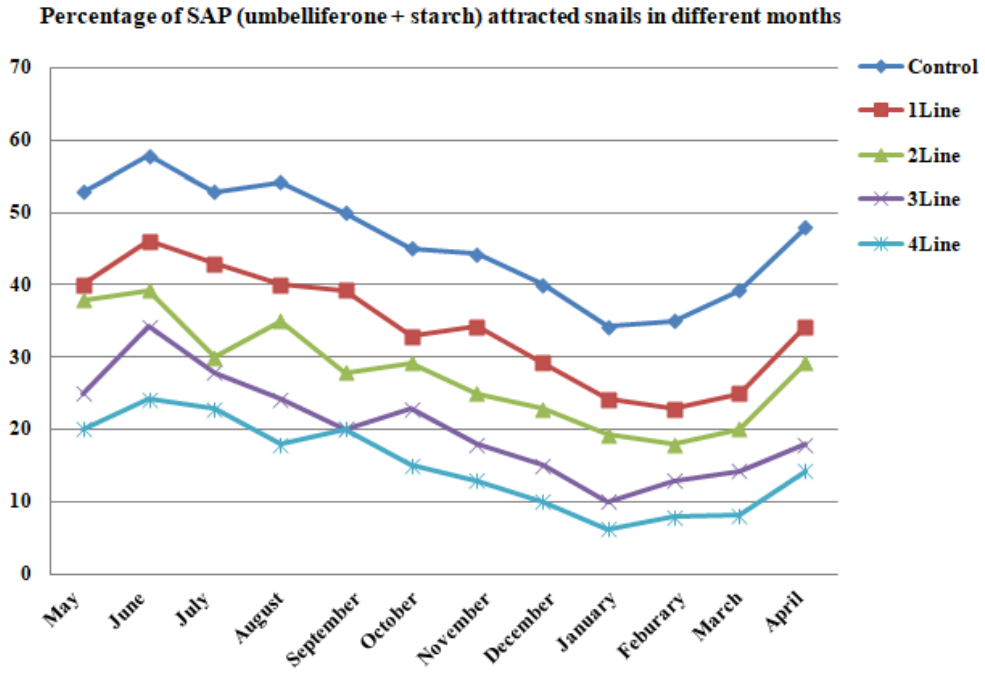


Fig. 2. Figure showing percentage of snails in zone-3 in contact with snail attractant pellets (SAP) that contain umbelliferone + starch at different concentrations after two hours from beginning of the experiment in different months of the year 2020-2021. Control pellets contain agar-agar and starch (10 mM). Line 1 represent mixing of 0.5 % umbelliferone in SAP given in between May to December and 2.0 % from January to April; Line 2 represent mixing of 0.7 % umbelliferone in SAP given in between May to December and 3.0 % from January toApril; Line 3 represent mixing of 1.0 % umbelliferone in SAP given in between May to December and 5.0 % from January to April; Line 4 represent mixing of 2.0 % umbelliferone in SAP given in between May to December and 7.0 % from January to April

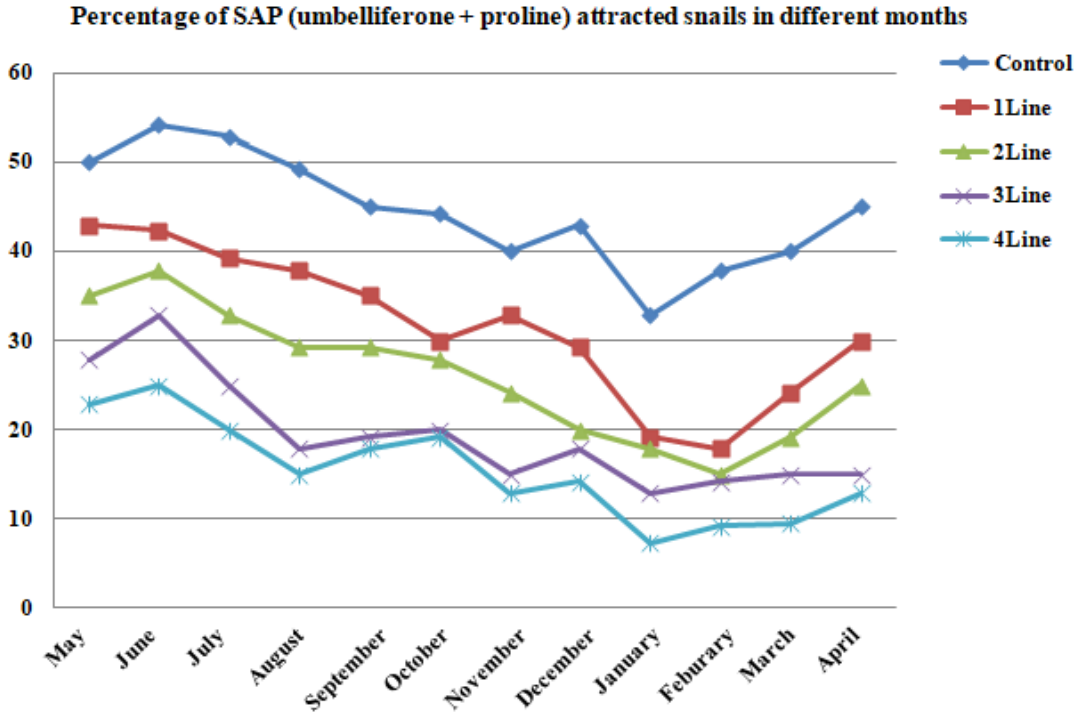


Fig. 3. Figure showing percentage of snails in zone-3 in contact with snail attractant pellets (SAP) that contain umbelliferone + proline at different concentrations after two hours from beginning of the experiment in different months of the year 2020-2021. Control pellets contain agar-agar and proline (20 mM). Line 1 represent mixing of 0.5 % umbelliferone in SAP given in between May to December and 0.7 % from January to April; Line 2 represent mixing of 0.7 % umbelliferone in SAP given in between May to December and 1.0 % from January toApril; Line 3 represent mixing of 1.0 % eugenol in SAP given in between May to December and 2.0 % from January to April; Line 4 represent mixing of 2.0 % umbelliferone in SAP given in between May to December and 3.0 % from January to April

4. DISCUSSION AND CONCLUSION

We have observed that *L. acuminata* showed high affinity (P<0.01) towards starch and proline in SAP in various seasons of the year. Besides that, *Biomphalaria alexendrina* and other developmental stages are also showing marked affinity towards carbohydrates and amino acids [19]. In addition to it, they observed that the snails are highly attracted towards starch, maltose and glycogen.

Abd El-Hamid [20] showed that proline is the most preferred amino acid for attracting the snail *B. alexandriana* because its release by the snails into the surrounding water as a signaling substance molecule. These amino acids attract the snails because of three reasons- firstly the presence of amino acids in both plant and bacteria, which are considered as crucial diet for the snails, secondly, it is released by the aquatic organisms into the surrounding water, and thirdly, snails can be use these amino acids as most preferable food indicator. Additionally, the snail modular system probably detect these amino acids as chemical, secreted out from aquatic organisms and use them as preferable food indicator.

Many studies have been published on insecticidal, antibacterial, nematociadal, and phytotoxic, fungitoxic activity of different coumarins [21]. It has been observed that sweet potato plant tissue produces umbelliferone (phytoalexin), and use it as defense tool against pathogenic fungi *Fusarium oxysprum* [22]. Besides that, it have substantially high insecticidal activity, leading to high percentage of eggs and larval mortality [21]. On the other hand, umbelliferone (*Ferula asafoetida*- Umbelliferae) significantly considered as by kill the sporocyst, redia and cercaria larva of *F. gigantica* in the body of *L. acuminata* [23]. In the result section, the molluscicide umbelliferone showed more

attraction at lower concentration. The attraction of snails declined significantly ($P < 0.01$) as the concentrations of different molluscicides incorporated inside the pellets were increased and water temperature is low (i.e., in winter season). SAP containing lowest percentage of molluscicide (umbelliferone) attracts fewer snails than SAP without molluscicide i.e., in control group. It indicates that the molluscicide in bait formulation have some repellant action against snail *L. acuminata*. Treatment of the molluscicide directly in aquatic medium require higher quantity to be released, which would affect the other animals living along with snails. Use of snail attractant pellets containing different molluscicides is taken selectively by the target snails, so that it will be safer and more economical in field.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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