

Original Research Article

Attraction of umbelliferone in snail attractant pellets (SAP) against the snail *Lymnaea acuminata*: An intermediate host of *Fasciola gigantica*

Abstract

Snail control is one of the best ways to control the fasciolosis. Snail attractant pellets (bait) containing attractant and molluscicide could be an effective tool for selective control of the *L. acuminata*. The aim of the present study is to study the attraction of molluscicide umbelliferone in snail attractant pellets against the vector snail *Lymnaea acuminata* in different months of the year. Data shows that active molluscicidal component (umbelliferone) showed more attraction at lower concentration. On the other hand, 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). SAP containing 0.5 % umbelliferone + starch attract maximum snails (46.0 %); whereas SAP containing 0.5 % umbelliferone + proline, attract maximum snails (42.33%) in the month of June.

Keywords: Umbelliferone; Snail attractant pellets; fasciolosis; molluscicide

1. Introduction

Fasciolosis is a worldwide disease caused by the liver fluke *Fasciola* spp (Rokni, 2014). This food- and water-borne disease is a major public health and veterinary issue (Sabourin et al., 2018). Fasciolosis is found in all continents except Antarctica, in over 70 countries, especially where there are sheep or cattle. Fasciolosis in buffaloes is asymptomatic, subclinical, and/or chronic form of the disease, adversely affecting their reproductive cycle, weight gain, food conversion efficiency, and productivity (Pandya et al., 2015). Infection of fasciolosis in cattle across the India is between 1.69% and 94% (Singh et al., 2021). The host

suffers from unnoticed ill effects of the disease for a prolonged period before the disease is detected at a veterinary clinic and/or at the abattoir (Edith et al., 2010). Control of parasitic diseases is crucial to improve the productivity of the animals. In most fasciolosis endemic areas, the control of the intermediate snail host population offers a good opportunity for the reduction of transmission. One of the best ways to tackle the problem is to destroy the carrier snails and remove an essential link in the life cycle of the flukes. Bait formulation techniques containing an attractant and molluscicide is a best way to control the pest management (Agrahari et al., 2012). Molluscicide-containing bait is ingested by the snail and causes significant mortality without any adverse effect on other biota within the same habitat. Umbelliferone, also known as 7-hydroxycoumarin, is a phenolic metabolite found in many familiar plants. It is one of the components of asafoetida, the dried latex from the giant fennel (*Ferula communis*). *Ferula* spp. has a long history of medicinal use and their pharmacological effects are well documented in both the human and animal studies (Akaberi et al., 2015). It has broadly circulated inside the Rutaceae and Apiaceae (Umbelliferae) families and is extracted utilizing methanol. It is widely used as antibacterial and anti-fungal, 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 (Radha et al., 2019). Members of family **Umbelliferae** contain compound that are potential sources of molluscicides (Kumar et al., 2009; Agrahari and Singh, 2013). Therefore, umbelliferone bait containing an attractant is a best way to control the vector snail.

The aim of the present study is to study the attraction of molluscicide umbelliferone in snail attractant pellets against the vector snail *Lymnaea acuminata* in different months of the year.

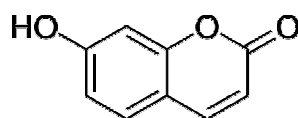
Materials and methods

Collection of animals

Adult *L. acuminata* (average length 2.25 ± 0.30 cm) were collected from Lake. Snails were acclimatized in dechlorinated tap water for 72 h at $25 \pm 18^\circ\text{C}$.

Test material

Umbelliferone (7-Hydroxycoumarin) ($\text{C}_9\text{H}_6\text{O}_3$; M.Wt. 162.14) is a yellowish, white crystalline solid, acetylthiocholine iodide (ATChI), and DTNB (5,50-dithio-bis-2-nitrobenzoic acid) were purchased from Sigma-Chemical Co. in the United States.



Umbelliferone ($\text{C}_9\text{H}_6\text{O}_3$)

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 (1992) as modified by Tiwari and Singh (2004a,b). Concentrations of carbohydrate and amino acid were based on the earlier reports of Tiwari and Singh (2004a,b). 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. These pellets were used for the attraction determination against *L. acuminata* in each month of the year 2021–2022.

Assay apparatus and procedure

The chemo-attraction studies of starch or proline with umbelliferone against *L. acuminata* were made in a clean circular glass aquarium having a diameter of 60 cm (Agrahari and Singh, 2010). 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

cm², 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 ± 18°C. At the start of the assay, 10 individually marked snails of uniform size were placed on the circumference of zone 0. The distance Toxicity of umbelliferone to snails 157 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 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 (Sokal and Rohlf, 1973). Control animals were kept in an equal volume of dechlorinated water under similar conditions without treatment.

Statistical analysis

Analysis of variance in each of the 12 months, in order to observe any significant variation according to the method of Sokal and Rohlf (1973).

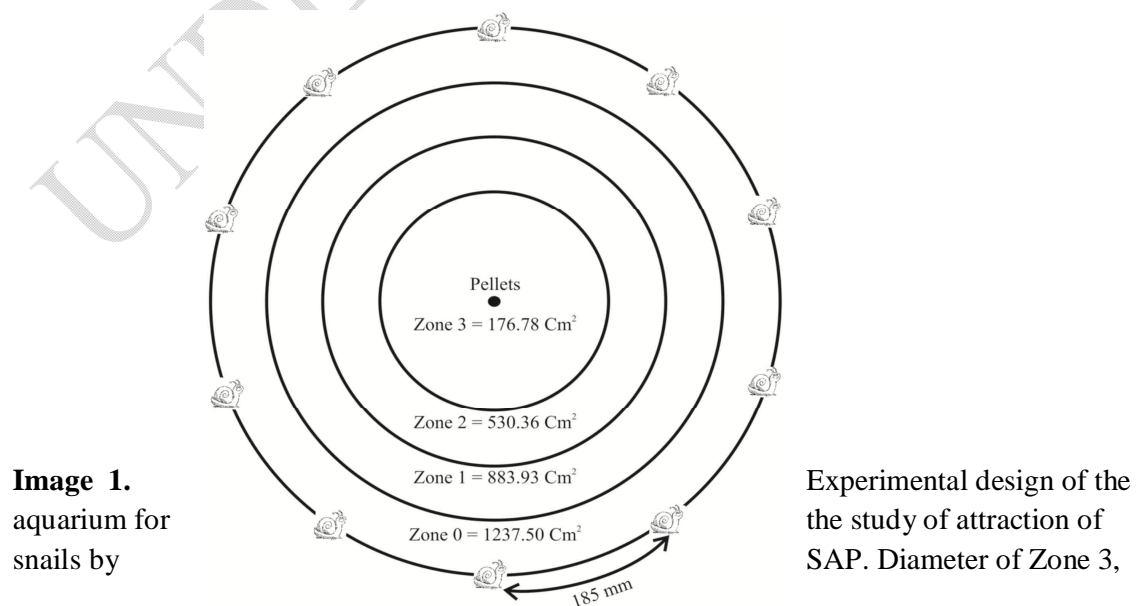


Image 1.
aquarium for
snails by

Experimental design of the
the study of attraction of
SAP. Diameter of Zone 3,

Zone 2, Zone 1 and Zone 0 were 176.78, 530.36, 883.93 and 1237.50 cm respectively. SAP was placed in the centre of Zone 3, whereas 10 marked snails were placed at periphery of Zone 0. The distance between the two snails was 185 mm.

Effect of seasonal variation on the attraction of the snail *Lymnaea acuminata*, towards the snail attractant pellets (SAP) containing 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 favoured attractant in SAP (Agrahari and Singh, 2010). Umbelliferone were incorporated inside the SAP containing starch or proline as attractant. Before studying the toxicity of these pellets, attraction of the snails on the desired SAP in the experiment was recorded after two hours from the beginning of the experiment in different months of the year at 60 cm diameter.

Data given in the figure is the mean number of snail *L. acuminata* in zone 3 with the SAP containing molluscicide at different concentrations 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 significant ($P < 0.01$) variation in the number of snails reaching zone-3 in different months of the year. The effect of seasonal variation and concentration of molluscicide in SAP on the proportion of snails in zone-3 was analyzed by two-way ANOVA. SAP containing 0.5 % umbelliferone + starch attract maximum snails (46.0 %) in the month of June. Minimum attraction of snails was observed in the month of January to March (6.23-8.1 %) when they were fed with SAP containing starch + 7.0 % umbelliferone in SAP (Figure-1). There was a significant variation ($F_{(4, 28)} = 267.02$, $F_{(7, 28)} = 29.52$; $P = 0.01$ and $F_{(4, 12)} = 164.13$, $F_{(3, 12)} = 27.48$; $P = 0.01$) between attraction in different months at different concentration of eugenol in SAP (Figure-1).

SAP containing 0.5 % umbelliferone + proline, attract maximum snails (42.33%) in the month of June. Minimum attraction was noted in the month of January to March (7.33-9.45 %) when snails were exposed to 3.0 % umbelliferone + proline in SAP (Figure-2). There was a significant 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 (Figure-2).

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.

Discussion

It is clear from result section that snail *L. acuminata* showed a significant ($P < 0.01$) change in attraction towards starch and proline in SAP in different months of the year. Abdel-Hamid and Madsen (1995) reported that the adult snail *B. alexandrina* and different developmental stages are attracted towards carbohydrates and amino acids. They found that the snails were highly attracted towards starch, maltose and glycogen. Abd El-Hamid (1996) reported that proline is most preferred amino acid for attracting the snail *Biomphalaria alexandriana* because it is released from the snails into the surrounding water as a signal (MacInnis et al., 1974). These amino acids attract the snails because of three reasons- (1) amino acids are present in both plant and bacteria, which are considered as essential dietary requirements for the snails, (2) amino acids are released from aquatic organisms into the surrounding water, and (3) snails use these amino acids as indicator for the presence of their preferable food. Additionally, these amino acids were detected in snail modular system as chemical diffusing out from aquatic organisms, and probably snails use them as indicator for the presence of their preferable food.

Many reports have been published on phytotoxic, fungitoxic, insecticide, antibacterial and, nematocidal activity of different coumarins (Razavi, 2011). When sweet potato is attacked by *Fusarium oxysprum*, Umbelliferone is produced in plant tissue, which can be regarded as phytoalexin and may well be considered as a defense tool for plants against pathogenic fungi (Brooker et al., 2007). They also have strong insecticidal activity and caused high percentage of mortality" on eggs and larvae of insects and regarded as an ovicide agent (Razavi, 2011). It has been reported that umbelliferone (*Ferula asafoetida*-Umbelliferae) significantly killed the sporocyst, redia and cercaria larva of *F. gigantica* inside the body of vector snail *L. acuminata* (Sunita et al., 2013). 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 repellent 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 more safe and economical in field.

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UNDER PEER REVIEW

Percentage of SAP (umbelliferone + starch) attracted snails in different months

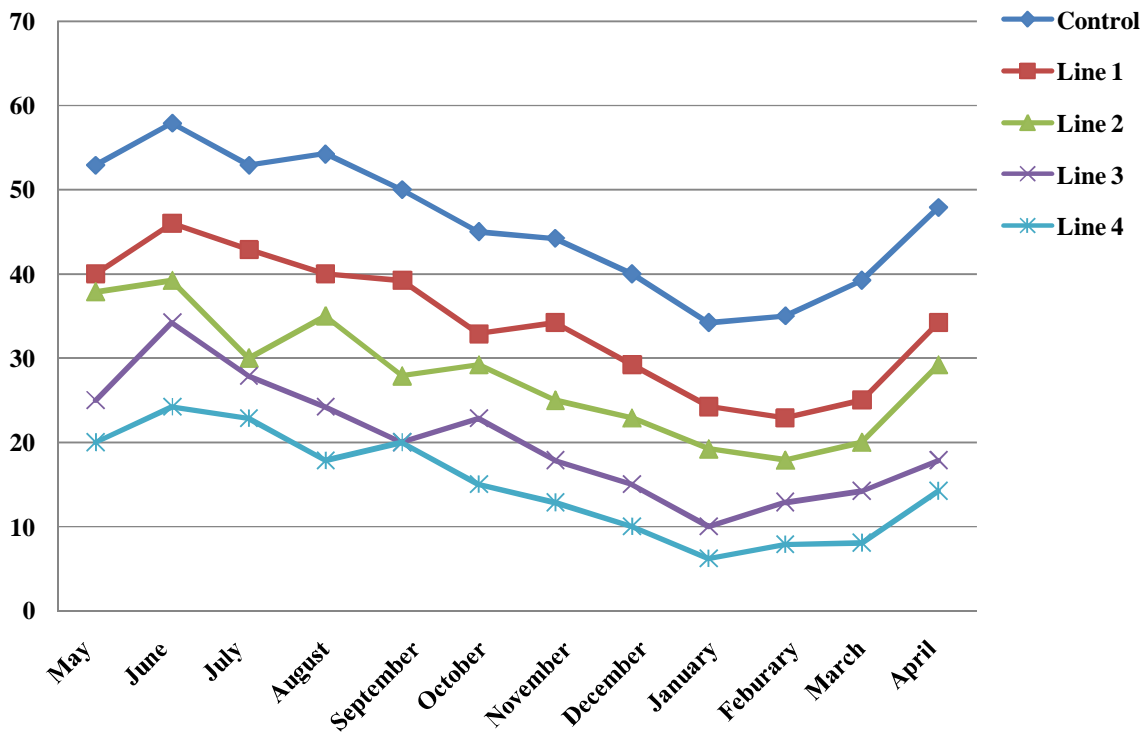


Figure 1: Figure showing percentage of snails in zone-3 in contact with snail attractant pellets (SAP) that contain umbelliferone + starch at different concentrations after two hours from beginning of the experiment in different months of the year 2020-2021. Control pellets contain agar-agar and starch (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 to April; **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.

