Original Research Article

Attraction of umbelliferone in snail attractant pellets (SAP) against the snail *Lymnaeaacuminata*: An intermediate host of *Fasciolagigantica*

Abstract

Snail control is one of the best ways to control the fasciolosis. Snail attractant pellets (bait) containing attractant and molluscicidecould be an effective tool for selective control of the *Lymnaeaacuminate*which is a known intermediate host of *Fasciolagigantica*. The aim of the present study is to investigatethe attraction of molluscicideumbelliferone in snail attractant pellets (SAP)against the vector snail *Lymnaeaacuminata*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 throughthe 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 thesnail and causes significant mortality without any adverseeffect 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-fungalagent, 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, umbelliferonebait considered a best way to control the vector snail.

The aim of the present study is to investigatethe attractionability of molluscicideumbelliferoneto the vector snail *Lymnaeaacuminata*through different months of the year.

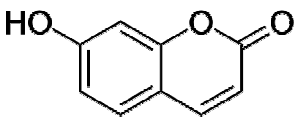
Materials and methods

Collection of animals

Adults of*Lymnaea**acuminata*(average length 2.25 ± 0.30 cm) were collected from Lake. Snails were acclimatized in dechlorinated tap water for 72 h at 25 ± 18ºC.

Tested materials

Umbelliferone (7-Hydroxycoumarin) ($C_9H_6O_3$; 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 ($C_9H_6O_3$)

Preparation of SAP with molluscicides

SAP containing attractant starch or proline and molluscicideumbelliferone 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. The attraction of these pellets to*L. acuminata*snails was determinedin each month of the year 2021–2022.

Assay apparatus and procedure

The chemo-attraction studies of starch or proline with umbelliferonetoL. *acuminata* were made in a clean circular glass aquarium witha 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 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 ± 18ºC. At the beginning of the assay, 10 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 through the yearwere 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 variancethrough the 12 months was performed in order to determine any significant variation according to the method of Sokal and Rohlf (1973).

Results

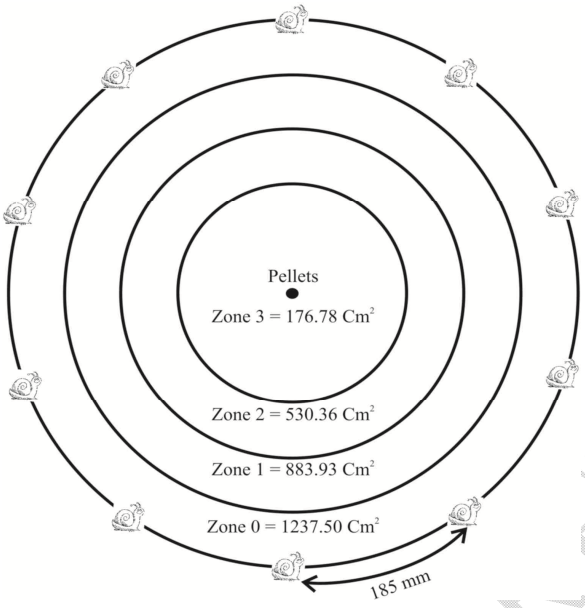


Figure 1. Experimental design of the aquarium for the study of attraction of snails by SAP. Diameter of Zone 3, Zone 2, Zone 1 and Zone 0 were 176.78, 530.36, 883.93 and 1237.50 cm respectively. SAP was placed in the centre of Zone 3, whereas 10 marked snails were placed at periphery of Zone 0. The distance between the two snails was 185 mm.

Effect of seasonal variation on the attraction of the snail *Lymnaeaacuminata*, towards (SAP)baitsthat containsplant 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 (Agrahari and Singh, 2010). Umbelliferone were incorporated inside the SAP 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 the beginning of the experiment in different months of the year at 60 cm diameter.

Data given in the figure 1.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 % umbelliferonein 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 umbelliferonein 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) showed that proline is themost preferred amino acid for attracting the snail *Biomphalariaalexandriana*because it is released from the snails into the surrounding water as a signal (Maclnnis*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 studies have been published on phytotoxic, fungitoxic, insecticide. antibacterial and, nematociadal activity of different coumarins (Razavi, 2011). When sweet potato is attacked by *Fusariumoxysprum,*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).On the other hand, 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 molluscicideumbelliferone 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 more safe and economical in field.

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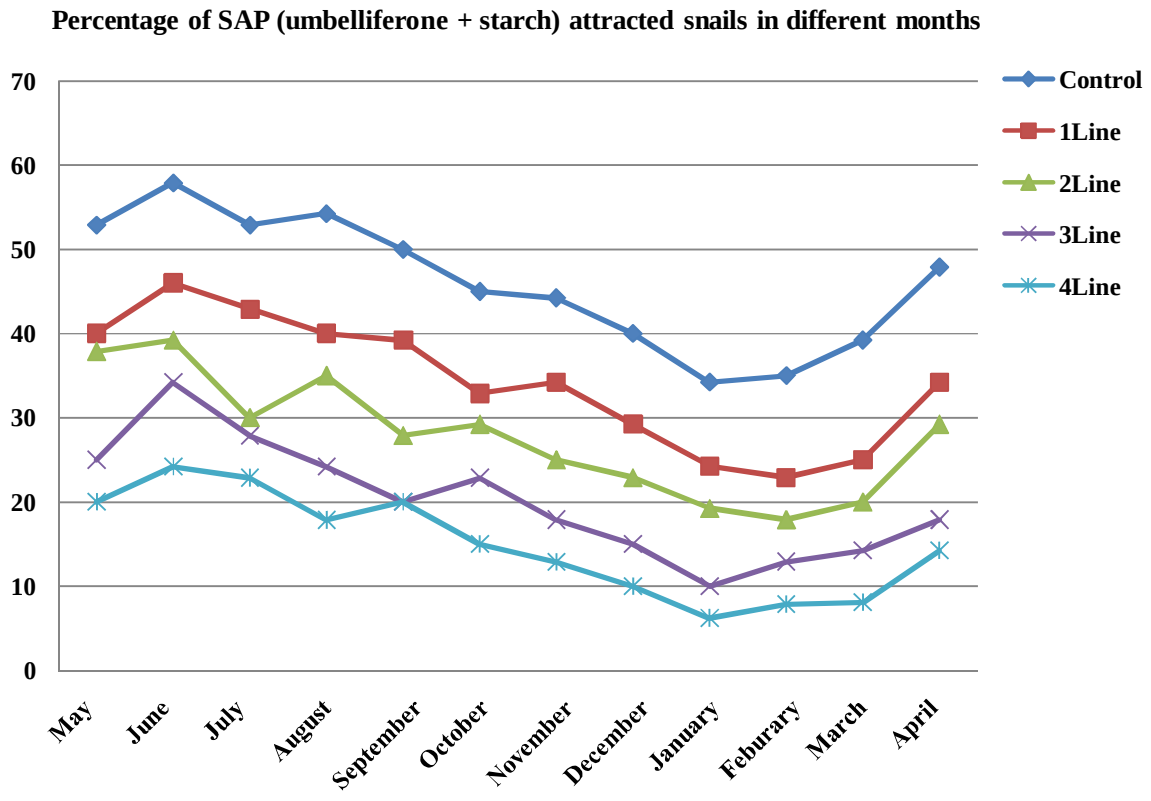

Figure 1: Figure showing percentage of snails in zone-3 in contact with snail attractant pellets (SAP) that contain umbelliferone + starch at different concentrations after two hours from beginning of the experiment in different months of the year 2020-2021. Control pellets contain agar-agar and starch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

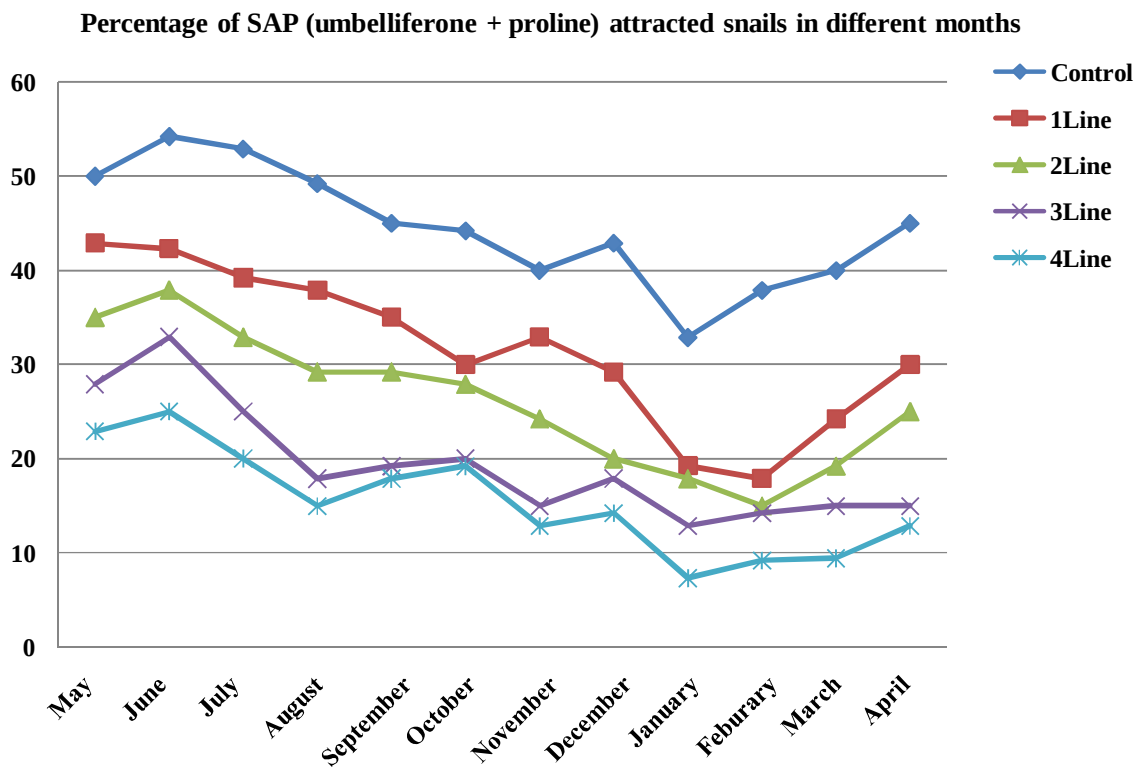

Figure 2: Figure showing percentage of snails in zone-3 in contact with snail attractant pellets (SAP) that contain umbelliferone + proline at different concentrations after two hours from beginning of the experiment in different months of the year 2020-2021. Control pellets contain agar-agar and proline (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.