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Influence of Abiotic Factors and Umbelliferone Toxicity in Snail Attractant Pellets Against the Vector Snail *Lymnaea acuminata*

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Abstract

Background and Objective: Fasciolosis is a zoonotic disease, which is transmitted by *Lymnaea acuminata* snails of the Lymnaidae family and causes the disease in human and herbivorous mammals in all over the world. One of the suitable approaches for the control of fasciolosis is the snail control. The snail control by snail attractant pellets is a new technique because there is no any side effect on other biota/human/natural environment. **Material and Methods:** The influence of various abiotic factors and umbelliferone toxicity in bait against the vector snail *Lymnaea acuminata* was checked in different months of the years 2018-2019. **Result:** After 24h of molluscicide exposure, it was found that LC_{50} values 4.37, 4.22 and 5.66 % in SAP containing starch + umbelliferone and 4.02, 4.33, and 4.76 % in SAP containing proline + umbelliferone during May, June and July, respectively, were most effective treatments in killing the snails. While SAP containing starch + umbelliferone was 9.84% and proline + umbelliferone was 8.94% were minimum effective in January in 24h toxicity. **Conclusion:** This study indicates that various abiotic factors significantly changed the toxicity of umbelliferone; so, the snails can be controlled by snail attractant pellets containing umbelliferone will be applied in a particular month rather than the whole month of the year.

Keywords: Abiotic Factor, Bait Formulation, *Lymnaea acuminata,* Snail Attractant Pellets, Toxicity, Umbelliferone

1. Introduction

The genus *Fasciola* (liver fluke) caused fasciolosis, which has been recognized because of its high veterinary impact but it has been the most neglected disease for a long time ago with respect to human infection[1,2]. The World Health Organization (WHO) in 2021 has also identified fasciolosis in human population as a re-emerging disease associated with endemic and epidemic outbreaks of disease[3]. It is a zoonotic disease, infecting herbivorous mammals and humans transmitted by *Lymnaea acuminata* snails of the Lymnaeidae family. Fasciolosis is emerging in numerous parts of the world.

Members of family Umbelliferae contain compound that are potential sources of molluscicides. Singh *et al.*, studied the molluscicidal properties of some common spice plants[4]. The most common medicinal and spice plants viz. *Ferula asafoetida* (Umbelliferae) and *Carum carvi* (Umbelliferae) are intoxicating molluscicides[5]. Ferulic acid, umbelliferone and limonene have enzyme inhibition activity against *L. acuminata*. Kumar *et al.*, reported that umbelliferone and ferulic acid are competitive, inhibitors of acetylcholinesterase[6].

The snail *Lymnaea*[7] acts as the intermediate host of liver flukes[8]. For the control of fasciolosis, required to control the vector (snails) and break the life cycle of parasites. For the control and management of snails, molluscicide delivery is improved by containing an attractant and molluscicides in bait formulations is a best process. The snails and other aquatic animals are attracted towards food source, to escape predators and to locate mates by water-borne "chemical signals". Baits are also recommended for controlling the terrestrial snail and slugs in horticulture and agriculture[9]. Baits are the best method for long-lasting effect on slugs and snails[10]. Bait formulation is a very unique technology because in bait a balanced concentration of attractants viz., fat, protein and carbohydrates and a plant-derived toxicant is present[11-13]. This is an alternative process for control of snail in place of synthetic pesticides that cause no any effect on humans or natural environment.

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2. Materials and Methods

2.1 Study Area

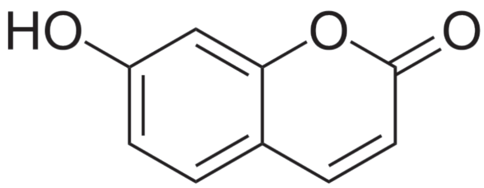
The research work was completed in, Department of Zoology. D.D.U. Gorakhpur University, from May 2018 to April 2019.

2.2 Collection of Snails

Snail of equal size (average length 2.25 ± 0.30 cm) were collected from a Lake named Ramgarh, near the Gorakhpur University. *L. acuminata* were acclimatized in normal condition of lab in dechlorinated tap water at 25°C for 48-72 hr.

2.3 Test Material

Umbelliferone (7-Hydroxyoomarin) ($C_9H_6O_3$; M.Wt. 162.14) was used as molluscicides. Thermometer, digital pH, and conductivity meters, respectively measured temperature, pH, and electrical conductivity of water. Dissolved oxygen and carbon-di-oxide levels in water were checked by APHA[14].



Umbelliferone ($C_9H_6O_3$)

2.4 SAP Preparation with Umbelliferone

By the method of Madsen[15] SAP were prepared which contained attractant (starch/proline) and umbelliferone. Tiwari and Singh modify this method[16,17]. The most preferable concentration of starch and proline was taken from the report of Agrahari and Singh[18]. Umbelliferone was also added in SAP in 2% agar solution in 100ml. The thickness of SAP was maintained at 5mm and the diameter of SAP is also cut in 5mm. These SAP were prepared in each month and toxicity was determined by SAP in the months of 2018-2019.

2.5 Assay Apparatus and Procedure

Clean glass aquariums of 60cm diameter were made to study the chemo-attraction of bait containing starch/proline and umbelliferone against *L. acuminata*[18]. The aquaria were divided into four different zones by the method of Agrahari and Singh[18]. The Zone 3 (central zone) of the aquarium having a center area of 1.5 cm diameter, where the bait was applied. The aquaria were filling by dechlorinated tap water to 8 mm height. Ten snails of equal size were placed in zone 0. After every 15 min, the position of *L. acuminata* was noted for two hours in each month and each combination. Ten snails with six sets are required for each combination (umbelliferone + starch or proline). Snail's proportions were transformed into arcsine. The proportions of snails were compared between different concentrations of umbelliferone + attractant and different months by two-way ANOVA[19]. At different concentrations, the mortality of snails was noted at 24h to 96h in each month. The untreated animals were also kept in dechlorinated water under normal conditions. Different abiotic factors of pond and control water were checked simultaneously with toxicity text. All lethal parameters were calculated by Robertson *et al*[20].

2.6 Statistical Analysis

The LC_{50} and exposure period was determined by Regression coefficient. The LC_{50} and different abiotic factors was determined by product-moment correlation coefficient to check any significant correlation by the method of Sokal and Rohlf[19].

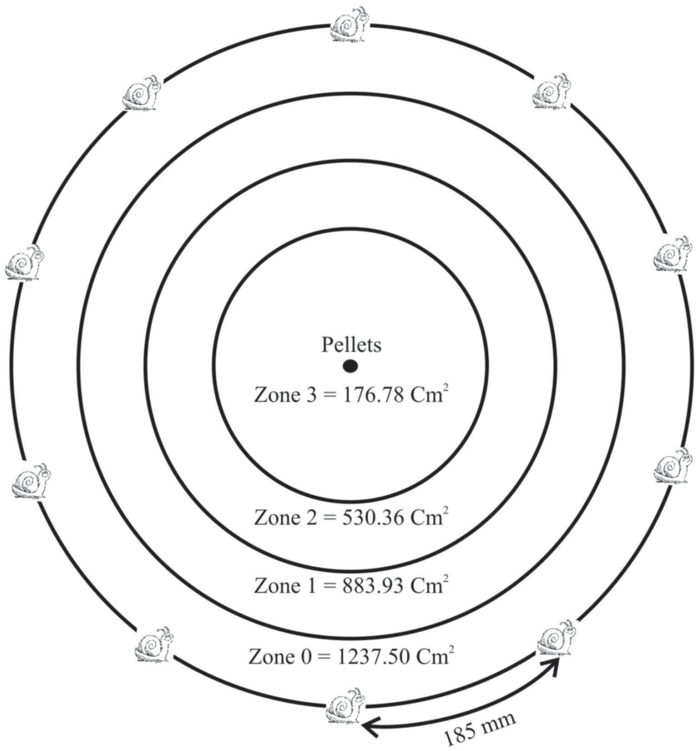


Figure 1. The aquarium was designed in Zone 0, 1, 2, and 3 for attraction of snail. Bait was placed in the centre of Zone 3, and 10 equal snails were placed at periphery of Zone 0.

3. Results

It was found that maximum attraction was recorded in May to August month and minimum was in January to March month in Zone-3. There was a marked variation ($P < 0.01$) between different months and concentrations of umbelliferone. It was also recorded significantly (P < 0.05) time-dependent variation in the toxicity in different months. (Table 1). The 24h toxicity was highest in months of May to July of umbelliferone + starch are 4.22–5.66 % and umbelliferone + proline 4.02–4.76 % in SAP whereas the lowest toxicity in the months of December to February of umbelliferone + starch 8.65–9.84 % and umbelliferone + proline 6.76–8.94 % in SAP). A significant (*P* < 0.05) negative correlation was observed between LC_{50} and CO_2 concentration/temperature of water, whereas a significant (*P* < 0.05) positive correlation was observed between LC_{50} and dissolved oxygen/pH of water (Table 1). There is no any correlation between LC_{50} and conductivity of water. The high toxicity of umbelliferone was observed when the water temperature is high (35-37 °C) whereas pH (7.25-7.48) and dissolved O_2 (1.3-1.4 ppm) was low in water. The slope values were found within the 95% confidence limits of LC_{50}. The g-value was < 0.5 at all probability levels. The t-ratio was >1.96, and the heterogeneity factor was <1.0.

4. Discussion

After 24h toxicity assay, it was found that molluscicide were more effective during May to July, while January was the less effective month for killing the snails. These findings support

Table 1. Alterations in toxicity (LC_{50} % in SAP) of SAP containing Umbelliferone + starch / proline against *Lymnaea acuminata* and abiotic environmental factors in different month of the year 2018-2019

A (Control water)													
Parameters		**May**	**June**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
Temp (°C) *		36 ± 0.33	37 ± 0.30	37 ± 0.60	33 ± 0.46	34 ± 0.32	32 ± 0.67	26 ± 0.35	21 ± 0.40	16 ± 0.54	18 ± 0.50	22 ± 0.35	32 ± 0.66
pH +		7.44 ± 0.03	7.40 ± 0.06	7.37 ± 0.02	7.33 ± 0.09	7.66 ± 0.04	8.01 ± 0.02	8.75 ± 0.01	8.77 ± 0.04	8.89 ± 0.05	8.45 ± 0.05	8.33 ± 0.03	8.21 ± 0.04
DO (ppm) +		1.2 ± 0.04	1.5 ± 0.05	1.4 ± 0.02	1.6 ± 0.07	1.8 ± 0.04	2.0 ± 0.08	2.1 ± 0.02	2.5 ± 0.05	3.0 ± 0.04	3.0 ± 0.07	2.4 ± 0.03	1.7 ± 0.06
DCO_2 (ppm) *		22.2 ± 0.33	24.3 ± 0.66	23.0 ± 0.23	23.4 ± 0.56	19.4 ± 0.28	18.9 ± 0.87	17.3 ± 0.50	14.5 ± 0.33	12.2 ± 0.66	13.4 ± 0.46	14.4 ± 0.76	19.1 ± 0.56
Conductivity (µmhos/cm)		31.2 ± 0.47	31.3 ± 0.66	30.5 ± 0.24	31.7 ± 0.75	29.0 ± 0.56	26.5 ± 0.43	25.1 ± 0.62	36.5 ± 0.33	33.8 ± 0.22	29.2 ± 0.70	32.4 ± 0.54	32.7 ± 0.55
B (Pond water)													
Temp (°C)		36 ± 0.66	39 ± 0.24	35 ± 0.36	31 ± 0.22	34 ± 0.32	30 ± 0.57	26 ± 0.76	18 ± 0.67	15 ± 0.55	19 ± 0.66	21 ± 0.40	33 ± 0.30
pH		7.3 3 ± 0.02	7.22 ± 0.06	7.48 ± 0.05	7.56 ± 0.05	7.66 ± 0.02	8.04 ± 0.05	8.33 ± 0.07	8.48 ± 0.08	8.92 ± 0.01	8.56 ± 0.05	8.25 ± 0.07	7.88 ± 0.06
DO (ppm)		1.7 ± 0.07	1.6 ± 0.02	1.7 ± 0.03	1.8 ± 0.05	1.6 ± 0.03	2.2 ± 0.03	2.0 ± 0.06	2.5 ± 0.04	3.4 ± 0.06	3.2 ± 0.08	2.6 ± 0.02	1.8 ± 0.09
DCO_2 (ppm)		26.2 ± 0.41	25.2 ± 0.33	25.1 ± 0.33	24.5 ± 0.65	23.3 ± 0.92	18.4 ± 0.24	18.1 ± 0.53	15.7 ± 0.43	14.2 ± 0.39	14.1 ± 0.28	15.9 ± 0.73	21.3 ± 0.87
Conductivity (µmhos/cm)		31.8 ± 0.12	32.4 ± 0.13	30.4 ± 0.33	35.5 ± 0.54	30.3 ± 0.20	31.6 ± 0.43	38.0 ± 0.55	39.6 ± 0.45	40.3 ± 0.37	28.4 ± 0.25	28.6 ± 0.65	33.4 ± 0.42
Treatment	**Exposure Period**												
Umbelliferone + Starch (LC_{50} % in SAP)	24 h	4.37	4.22	5.66	5.89	6.50	7.35	7.66	8.81	9.84	8.65	8.43	7.22
	48 h	3.06	3.48	3.93	3.34	3.84	4.26	5.88	6.76	7.33	7.21	6.75	5.65
	72 h	1.66	1.87	2.56	2.44	2.56	2.83	2.33	3.77	3.96	4.88	4.23	2.88
	96 h	0.95	0.92	1.22	1.66	1.77	1.87	1.42	2.38	2.67	2.34	2.45	2.33
Umbelliferone + Proline (LC_{50} % in SAP)	24 h	4.02	4.33	4.76	4.89	5.02	4.92	5.72	6.76	8.94	7.47	6.02	5.38
	48 h	2.38	2.42	2.89	2.62	2.69	2.99	2.99	3.21	4.98	3.77	3.72	2.66
	72 h	1.23	1.12	1.35	1.66	1.52	1.88	1.52	1.84	2.88	1.78	1.57	1.32
	96 h	0.88	0.70	0.85	0.95	0.94	0.97	0.94	0.98	1.64	1.33	1.06	1.02

that abiotic factors alter the umbelliferone toxicity in snails. Miller *et al.*, found that loss of toxicity and attractiveness of metaldehyde baits after exposure to environmental conditions such as sunlight, soil pH, and moisture has been eliminated to some degree in formulation[21]. It is evident from result section that water temperature plays an important role that alter the umbelliferone toxicity in different months. During the summer season, when temperature is high, umbelliferone shows maximum toxicity while in winter season when water temperature is low, they show minimum toxicity (higher LC_{50} value). The bait pellets are also affected by the temperature of water. Hata *et al.*, conclude that in moist conditions the effectiveness of bait was very low[22]. The metabolic activity was affected due to change in abiotic and biotic factors that totally depend on the season in lower organisms. The snail's metabolic activity requires dissolved oxygen, which is present in water. When water temperature is high, the metabolic rate of snails is also high, it causes high concentration of CO_2 in water, which affect the pH range of water. This was proved from the high amount of CO_2 and low pH of water in the summer season[23]. It has been observed by Srivastava *et al.*, that molluscicides and abiotic factors affect the reproduction of *L. acuminata*[24]. The pH of water (pond and control) increases after summer season and becomes highest in the month of winter (January) and lowest in the May-June. The median lethal concentration of umbelliferone was lowest in summer. It is may be due to at high pH, it may molluscicides undergo hydrolysis (a process that degrades the active ingredient, reducing its effectiveness) and change into less toxic compound, which influence the mortality of snail *L. acuminata*. Glass and Darby reported previously that apple snail growth was limited at low pH[25]. Vasconcellos *et al.*, also conclude that the molluscicidal activity of plant latex against *Biomphalaria tenagophila* was maximum at low pH and minimum at high pH[26]. Oxygen is more soluble in cold water than in warm water[27]. The dissolved oxygen and temperature show inversely correlation. When the temperature of water is increasing, the O_2 dissolving capacity of the pond will be reduced, thus when the sufficient dissolve oxygen will not provide to snails; the snails appeared to be more susceptible against the umbelliferone molluscicide. The plant-derived molluscicids are neurotoxic and activate the CDC cells. Acetylcholinesterase (AChE) plays an important role at nerve ending of muscle tissue in nerve conduction process. AChE activity was inhibited by plant-derived molluscicides against snails[13,28]. Treatment of these molluscicides directly in aquatic medium require higher quantity to be released, which would affect the other animals living along with snails. Use of SAP containing different molluscicides will be taken selectively by the snails. It has been reported earlier that umbelliferone is highly toxic to the snails, when they are mix directly in aquatic habitat of snails[5]. Therefore, the result of the present study shows that umbelliferone toxicity was affected by various abiotic factors against the *L. acuminata*. The month of May to July was the promptest duration for the snail's control.

5. Conclusion

From result, it can be concluded that the toxicity of SAP is affected by every month's seasonal changes. The abiotic factors of water were altering in different month, which significantly affect the toxicity of SAP. Thus, summer season are suitable for snail control by bait formulation which is not harmful to another biota.

6. Significant Statement

This study discovers the control of fasciolosis by plant-based natural product in the form of bait, which help people to increase the information about most suitable months for control of snail and ultimately reduce the incidence of fasciolosis.

7. Authors' Contribution

The author is involved in designing of work, data collection, data interpretation, drafting, and approving the research paper.

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