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Seasonal variation in toxicity of citral against *Fasciola larva*

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ABSTRACT

Objective: To test whether the larvicidal activity of citral against *Fasciola* varies by season.
Methods: Mortality of *Fasciola larva* in different month of year (2011-2012) in *in vitro* and *in vivo* condition were observed at 2 h, 4 h, 6 h and 8 h exposure of citral.
Results: *In vitro* toxicity of citral against redia was highest in between the June to August (8 h LC_{50}: 2.58-2.62 mg/L), whereas against cercaria 8 h LC_{50} was in between 3.44-2.62 mg/L. Highest *in vivo* toxicity against redia was noted in between June to August (8h LC_{50}: 4.20-5.09 mg/L). The lowest toxicity was observed from November to April. The highest temperature, free carbon dioxide, and lowest pH, dissolved oxygen were observed from June to August.
Conclusions: The present study conclusively shows that varying a biotic factor can significantly alter the *in vitro* and *in vivo* toxicity of citral against sporocyst redia and cercaria larva.

1. Introduction

Fasciolosis is one of the most debilitating zoonotic diseases. The World Health Organization has estimated that 2.4 million people are infected with *Fasciola*, and further 180 million are at risk of infection[1]. Two species *Fasciola hepatica* and *Fasciola gigantica* (*F. gigantica*) are found throughout the world with several outbreaks in humans from many countries[2]. The intermediate host of liver fluke *F. gigantica* is a hermaphroditic mollusc *Lymnaea acuminata* (*L. acuminata*) inhabiting freshwater ponds and ditches. Snail *L. acuminata* serves as intermediate host of *Fasciola* species[3]. Incidence of endemic fasciolosis is very common in the eastern region of the state of Uttar Pradesh in India[4-8]. Development of larval digenetic trematodes is complex process involving initial infection of the snail host by the free-swimming miracidium, its sequent transformation to a parasite primary sporocyst stage, followed by asexual reproduction and release of secondary, sporocyst or redia and finally the eventual formation and release of cercaria the next free-swimming s+tage in the life cycle. One of the possible approaches to control or eradicate fasciolosis is to interrupt the life cycle of the parasitic trematodes by eliminating the larva (sporocyst, redia and cercaria) inside the snail body or killing the host snail. These snails are an important component of aquatic ecosystem. Now it is realized that instead of killing the snails, it is better to kill the larvae inside the snail body with the help of certain plant products. Recently, Sunita

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and Singh have reported that the phytotherapy of snail by diverse chemicals found in different plant products have sufficient larvicidal activity[5]. Natural products are eco-friendly and easily biodegradable. It has been noted by us that active molluscicidal component of *Zingiber officinale* (citral) (*Z. officinale*) have sufficient larvicidal effect against *Fasciola* larva in Sunita and Singh's study[5]. The aim of the present study is to explore the possibility that seasonal change in abiotic factors such as temperature, pH, dissolved oxygen and free carbon dioxide in test water can influence *in vitro* and *in vivo* larvicidal activity of citral against different *Fasciola* larva in infected snails in each month of the year 2011-2012.

2. Materials and methods

2.1. Test materials

Citral (3, 7- dimethyl-2, 6-0 octadienal) (Figure 1) was supplied by Sigma USA. Temperature and pH of water were measured by thermometer and digital pH meters, respectively.

Figure 1. Citral (3, 7- dimethyl-2, 6-0 octadienal).

2.2. Animals

Adult *L. acuminata* [(2.60±0.20) cm in length] were collected locally. Cercaria shedding infected and uninfected snails were separated in two groups. The snails were allowed to acclimatize for 24 h in laboratory condition. Each infected snail was dissected in a glass Petri dish containing 10 mL of dechlorinated water at 22 °C-24 °C. The pH of the water was 7.1-7.3 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. After dissecting sporocyst, redia and cercaria were separated in different Petri dish containing 10 mL of dechlorinated water by the method of Sunita and Singh[5]. These larvae were kept in dechlorinated tap water where they survive up to 48 h in laboratory condition.

2.3. Toxicity determination

2.3.1. In vivo

In vivo toxicity of citral against larvae of *Fasciola* in infected *L. acuminata* was done by the method of Sunita and Singh[5]. Physical parameters of water such as temperature, pH, dissolve oxygen and free carbon dioxide were measured in each month of the year (2011-2012). Dissolved oxygen and CO_2 were estimated according to methods described by American Public Health Association[9]. After 2 h, 4 h, 6 h and 8 h of treatment, infected snails were dissected. Live and dead sporocyst, redia and cercaria were counted. Mortality percent of larvae at each concentration for 2 h, 4 h, 6 h and 8 h were used for determination of LC_{50}.

2.3.2. In vitro

In vitro toxicity of citral was performed in the Petri dish by the method of Sunita and Singh[5]. Ten sporocyst, redia and cercaria larva of *Fasciola* were separated in different Petri dish containing 10 mL dechlorinated tap water. Treatment of citral was made directly in the Petri dish containing 10 sporocyst/redia/cercaria. Mortality of sporocyst, redia and cercaria were observed after 2 h, 4 h, 6 h and 8 h of treatment. Counting of larvae was done with the help of microscope.

Lethal value (LC_{50}), lower and upper confidence limits (LCL and UCL), slop-values, t-ratio, g value and heterogeneity factors were calculated with the help of POLO computer programme of Robertson *et al*[10]. One-way ANOVA and product moment correlation coefficient were done by the method of Sokal and Rohlf[11].

3. Results

In *in vivo* and *in vitro* larvicidal activity of citral against the sporocyst, redia and cercaria larva of *F. gigantica* was time and concentration dependent in each month of year 2011-2012 (Tables 1 and 2). In *in vitro* treatment, highest toxicity of citral was noted against redia and cercaria larva in months of June, July and August (redia-8 h LC_{50}: 2.58, 3.12, and 2.62 mg/L; cercaria-8 h LC_{50}: 3.44, 0.006 and 2.62 mg/L, respectively) and lowest in between September to February (redia-8 h LC_{50}: 6.41, 6.89, 7.74, 7.43, 13.18 and 11.70 mg/L; cercaria-8 h LC_{50}: 4.79, 5.27, 4.64, 6.66, 10.12 and 7.34 mg/L, respectively) (Table 1). In *in vivo* treatment

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