Advances in Bioresearch
Adv. Biores., Vol 8 (6) November 2017: 135-139

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.6.135139

Advances in Bioresearch

ORIGINAL ARTICLE

Pulicaria crispa (Asteraceae) extract affects Survival and fecundity of *Bulinus truncatus* vector snails of Schistosomes

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ABSTRACT
Schistosomiasis is a serious disease in many parts of the world. Many approaches were examined to control the disease. Control of snail vectors by chemical molluscicides was discouraged because of the high cost and the undesirable effects on the environment. Recently, use of plant molluscicides has received increasing attention. The current study aimed to investigate effects of aqueous extract of Pulicaria crispa leaves on survival and fecundity of Bulinus truncatus snails. The results obtained were 206.06 ppm and 237.19 ppm for LC_{50} and LC_{90}, respectively. Effects of sub-lethal concentrations for 24, 48, 96, and 166 hours resulted in 40.00%, 53.33%, 53.33%, and 100% mortality, respectively; and significantly affected fecundity with 96 and 166 hours exposure periods. On conclusion, application of low concentrations of P. crispa extract could be used as one of the powerful approaches in schistosomiasis control programs.
Keywords: *Pulicaria crispa - Bulinus truncatus - plant molluscicide - schistosomiasis - survival – fecundity*

Received 24.05.2017 Revised 20.06.2017 Accepted 19.08.2017

How to cite this article:
E Abdelmageed, Hamid O. Bushara, Mohamed Y. A. Babiker, Mohanad Abdelgadir · *Pulicaria crispa* (Asteraceae) extract affects Survival and fecundity of *Bulinus truncatus* vector snails of Schistosomes. Adv. Biores., Vol 8 [6] November 2017.135-139.

INTRODUCTION

Schistosomiasis is a water-borne disease caused by parasites of the genus *Schistosoma*. Aquatic snails of several genera including *Biomphalaria*, *Bulinus*, and *Oncomelania* are known to be the intermediate hosts. Human schistosomiasis remains a major public health problem in many countries and is ranked second after malaria in terms of public health significance. About 70-78 countries are schistosomiasis-endemic, and almost 240 million people are infected and require preventive chemotherapy; while over half a billion others (approximately 10% of the world's population) are at risk of infection. The infection is prevalent in tropical and sub-tropical areas including Africa (with more than 90% of infection in sub-Saharan Africa), the Americas, the Eastern Mediterranean region, the Southeast Asian region, and the Western Pacific [1,2,3]. The rapid increase in human population, especially in developing countries and the ensuing demands for energy and food, has led to increased construction of hydroelectric and irrigation schemes[4]. Scarcity of pure water system in many developing countries force people to use raw water, thus increase the chance of getting infected [5]. Control methods of Schistosomiasis include: chemotherapy, focal and seasonal snail host control, environmental and sanitation improvement, clean water supply, vaccination, and health education [6 7, 3]. None of these methods is capable, on its own, of bringing an effective control of schistosomiasis, except in very small populations or under special conditions. However, compared to other methods, snail destruction is probably the most effective single control approach[7]. Recently, many countries avoided the use of chemical molluscicides in snail control programs and began exploiting indigenous plants as sources of molluscicides. Plant-derived molluscicides have many advantages over chemical ones. They are of low cost, less toxic to non-target organisms, and easily biodegradable along the food chain. Moreover, the use of indigenous molluscicides rather than imported ones is desirable, especially the strategies for schistosomiasis control programs should be based

on long-term operations [6,8,9,10]. *Pulicaria crispa* (Forssk.) Oliv, - with synonyms *Pulicaria undulata* (L.) C.A. Mey and *Francoeuria crispa* (Forssk.) Cass - is an annual herb or sometimes a perennial sub-shrub belonging to the family Asteraceae. The plant is found in Sudan, Saudi Arabia, Kuwait, Iran, Iraq, Egypt, Afghanistan, Pakistan, India and parts of north and west tropical Africa [11]. The current study was designed to investigate the effect of long-term exposure to sub-lethal concentrations of *Pulicaria crispa* aqueous extract on survival and fecundity of *Bulinus truncatus* snails.

MATERIALS AND METHODS

Animals

Laboratory-reared adult *B. truncatus* snails (5-7mm long) collected initially from Alsiliet Agricultural Scheme, Khartoum North (Sudan) were used in the experiments.

Collection and extraction of plant samples

Plant samples were collected from wild un-protected area in Shambat (Khartoum North - Sudan). Leaves were picked from flowering herbs, air-dried, and then ground with a pestle and mortar. The non-homogeneous ground leaf material was passed through a plastic mesh of 1 mm^2 pores. The un-sieved residue of the leaves was extracted. Briefly, 1 gram of un-sieved residue of leaves was immersed in 250 ml distilled water, allowed to stand for 3 hours then sieved through 1 mm^2 pores plastic meshes. The marc was again soaked in new 250 ml distilled water. The process of soaking and sieving was repeated 4 times during the 12-hours of the extraction period. The suspensions were mixed and filtered to obtain a stock solution. Working solutions, of different concentrations, were prepared and tested against adult snails.

Potency tests of *Pulicaria crispa* extracts on *Bulinus truncatus* snails

Molluscicidal potency test was conducted according to the standard guidelines of the World Health Organization [12] to determine the activity range of *P. crispa* extract on adult snails. The experiments were carried out in a range of temperature between 26-29°C. Ten mature adult *B. truncatus* snails (5-7 mm long) were immersed in a transparent plastic container containing 500 ml of the working solution (50 ml/snail). The following concentrations were used: 140, 160, 180, 200, 220, 230, and 240 ppm. Control groups were run in parallel. The snails were immersed in the extract for 24 hours, then for 24 hours recovery period in dechlorinated tap water; mortality counts were recorded thereafter.

Exposure of snails to sub-lethal concentration of *Pulicaria crispa* extract

For prolonged exposure experiments, four groups, each of twenty snails, were treated with 140 ppm of the extract, the concentration that did not kill any snail, (zero mortality). The exposure period was 24 hours for group one, 48 hours for group two, 96 hours for group, and 166 hours for group. After then, the snails were removed from the extract and kept in plastic containers containing dechlorinated tap water (200 ml/snail). Observation and recording of the results continues for three weeks post exposure to the sub-lethal concentration. Control groups of snails were run in parallel.

Survival and fecundity of snails exposed to sub-lethal concentration of *Pulicaria crispa* extract

Egg masses were collected every 48 hours from the small plastic sheets immersed in aquaria, walls of the aquaria, and shells of the snails. The eggs were observed and counted under a dissecting microscope. Furthermore, mortality of snails was recorded every 48 hours; dead snails were immediately removed from the aquaria.

Statistical analysis

The regression equation, lethal concentration that killed 50% and 90% of animal population (LC_{50} and LC_{90}, respectively), fiducial limits with 95% confidence limits, and regression coefficient (r^2) values were calculated by using probit analysis. Moreover, the percentage mortality of snails, the total number, and means of eggs laid were calculated. The student t-test (α=5) was used to show the significance of difference between control and treated groups.

RESULTS AND DISCUSSION

Potency of *Pulicaria crispa* extracts on *Bulinus truncatus* snails

The LC_{50} and LC_{90} values on adult snails were 206.06 ppm and 237.19 ppm, respectively. The lethal concentration that did not kill any snail (LC_0) was 140 ppm, as shown in table 1. *P. crispa* is characterized by its somewhat whitish color of stems and leaves that is because of the presence of wooly hairs. The potency of un-sieved residue of *P. crispa* leaf extract is apparently attributable to the hairs, which tended to aggregate together and resist passing through the plastic mesh pores used in the sieving process. studied the effect of The geographical variation in molluscicidal potency of *Apoytes dimidiata* in South

Africa was investigated by Brackenbury 13]. The results showed that the plant which appeared to be the most potent was different from other plants in having hairs on the abaxial surface of the leaf lamina.

Effect of sub-lethal concentration of *Pulicaria crispa* extract on survival and fecundity of *Bulinus truncatus* snails

One day exposure period resulted in 40.00% mortality of treated snails, both two and four days exposure period caused 53.33% mortality of the treated snails, and 100.00% mortality was achieved only by exposure of treated snail to six days (figure 1). One day and two days exposure periods revealed that the extract did not affect fecundity of the treated group, meanwhile four and six days exposure periods affected fecundity of treated group snails (figure 2 and figure 3).

The effect of sub-lethal concentrations of the extract on mortality and fecundity of snails seemed to be dependent on prolongation of the exposure period. This suggests that *P. crispa* extracts exerted their action on some metabolic pathways of snails, which was reflected in inhibition of egg production capacity and longevity observed, especially during 4 and 6 days. Exposure of snails to sub-lethal concentrations of the extract for one and two days resulted in higher mortality in treated groups. However unexpectedly, the total number of eggs produced by the treated groups or per individual snails was more than those produced by control. This might suggest that the mode of action of the extract on survival of snails differs from that on egg production capacity. Moreover, it is believed that density of snail is one of the governing factors that affect growth and fecundity of snails. Faiza *et. al* [14] reported that five snails of *Biomphalaria Alexandrina* immersed in one liter laid more egg masses than populations of ten, twenty and thirty snails per liter. Adewunmi *et. al* [15] studied the effect of prolonged administration of sub-lethal concentrations of aridanin isolated from *Tetrapleura tetraptera* on the glycogen and protein content of *Biomphalaria glabrata*. They stated that the significant reduction in glycogen and protein content could be responsible for the reduction in egg production and growth rate. Sub-lethal concentrations of *Calendula micrantha officinalis* and *Ammi majus* affected fecundity, longevity, transaminase activity, transaminase activity, total protein content, and total lipid content in the hemolymph of *B. alexandrina* and *B. truncatus* snails [16]. They claimed that inhibition of egg production might arise from the action of the natural products on the steroid hormones or due to harmful effects on the male and female parts of the genital tract. The results obtained by Bode *et. al* [17] when studied the ultra-structural effects of low concentrations of saponins from *Tetrapleura tetraptera* on *Bulinus glabrata* snails, showed that the ratio of the digestive cells to the secretory cells was inverted in molluscicides-treated snails. In addition, the major ultra-structural effects were seen in the digestive gland with dose-dependent autolysis of the membranous structures such as the Golgi apparatus, mitochondria and endoplasmic reticulum.

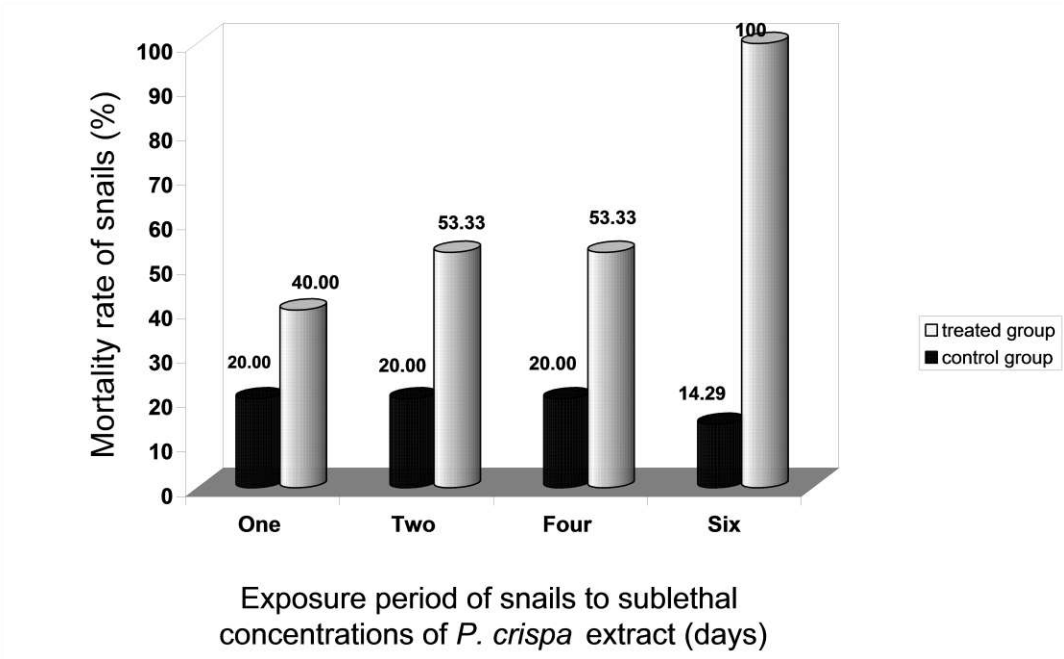

Figure 1; Effect of sub-lethal concentration of *Pulicaria crispa* extract on survival of *Bulinus truncatus* snails

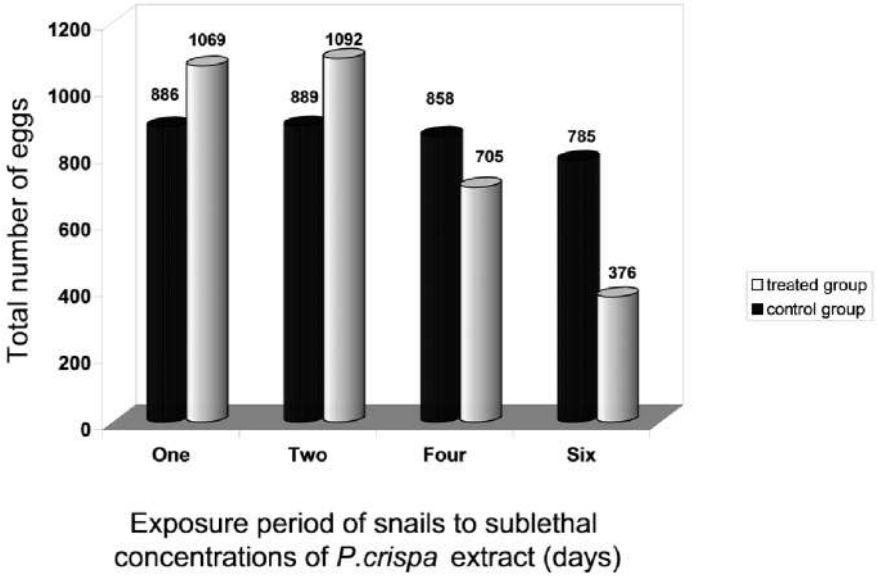

Figure 2: Effect of sub-lethal concentration of *Pulicaria crispa* extract on fecundity of *Bulinus truncatus* snails (total number of eggs). Results of total number of eggs laid are significant at P=0.05 for one-day and four-day exposure periods, not significant at P=0.05 for two-day exposure period, and significant at P=0.01 for six- days exposure period.

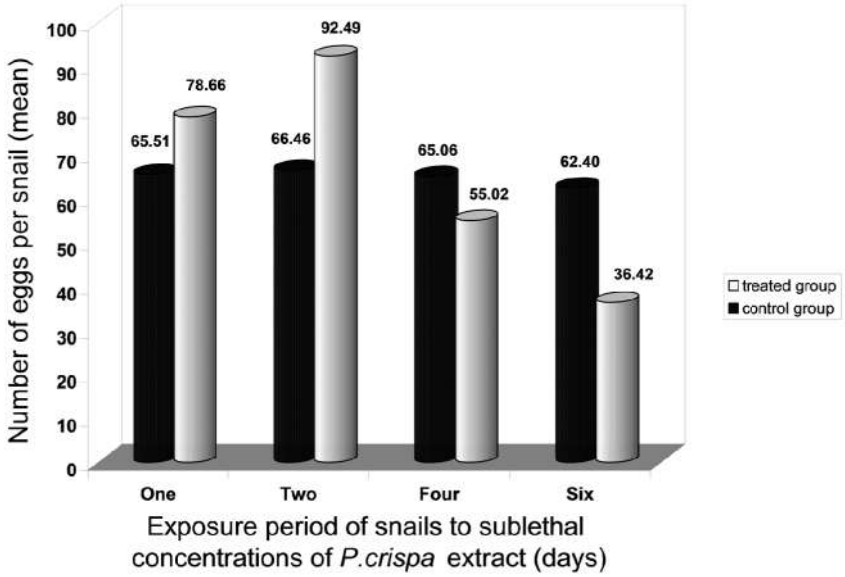

Figure 3: Effect of sub-lethal concentration of *Pulicaria crispa* extract on fecundity of *Bulinus truncatus* snails (number of eggs per a snail). Results of total number of eggs laid per a snail are significant at P=0.05 for one-day and four-day exposure periods, not significant at P=0.05 for two-day exposure period, and significant at P=0.01 for six- days exposure period.

Table I. Molluscicidal activity of aqueous extract of *Pulicaria crispa* extract *on Bulinus truncatus* snails

Concentration (logarithm)	2.380	2.362	2.342	2.301	2.255	2.204	2.146	control
Mortality (%)	100	93.33	73.33	16.67	6.67	3.33	0.00	0.00
regression equation	Y = 20.947 X - 43.471							
LC_{50}	206.06 ppm							
LC_{90}	237.19 ppm							
Fiducial limits with 95% confidence limits	±12.52							
Regression coefficient (r^2)	0.887							
Slope (in degree)	87.27							

CONCLUSION

On conclusion**,** the reason behind using sub-lethal concentrations rather than lethal concentrations is that this should reduce the hazards for non-target organisms and thereby lessen any possible ecological damage. On the other hand, prolonged application of low molluscicide concentration may be effective in eliminating the juvenile snails as they emerge from the eggs, as well as the possible adverse effect on their fecundity, or the susceptibility to miracidial infection of resident population of young and adult snails. *Pulicaria crispa* could be considered as one of the promising plant molluscicides in the control of schistosomiasis. The use of low concentrations, instead of high ones, produced significant effects on survival and fecundity of *Bulinus truncatus* snails.

COMPETING INTERESTS

The authors have declared that no competing interest exists.

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