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Laboratory evaluation of *Limnatis nilotica* leech (Annelida: Hirudinea) as a biocontrol agent for the schistosome-vector snail, *Bulinus truncatus*

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ABSTRACT

Objective: To investigate the predation efficacy of the freshwater leech, *Limnatis nilotica* (*L. nilotica*) as a potential biological control agent against different stages of the *Bulinus truncatus* (*B. truncatus*), the intermediate host of human *Schistosoma haematobium*, under laboratory conditions.

Methods: The leech *L. nilotica* and the snail *B. truncatus* were collected from El Kiryab Agriculture Scheme, Sudan. Thereafter, the predatory activity of the leech was evaluated against eggs, neonates and adults of the snail in a series of different experiments under laboratory conditions.

Results: The findings showed that the *L. nilotica* leech was a voracious predator towards the eggs and neonates of *B. truncatus* snails with a shell length of up to 3 mm, as well as of adult snails with a shell length greater than 3 mm.

Conclusions: The results showed that *L. nilotica* had a significant impact on *B. truncatus* populations. However, long term studies under natural field conditions are needed to support these results.

1. Introduction

Schistosomiasis is a prevalent disease in developing countries, with a huge impact on public health and socioeconomic development[1-3]. Eliminating or decreasing the intermediate snail host species of schistosomiasis is an appropriate method for controlling outbreaks of the disease, and this can be accomplished by using chemical molluscicides, environmental management methods and biological control agents[3,4]. However, efforts to control snail populations in the past through the use of chemicals have resulted in environmental pollution and have impacted local faunas[5]. Therefore, utilizing locally available biological control agents seems to be a relatively safe and more economical approach.

Among the potential biological control agents, many invertebrate and vertebrate predator species have been evaluated, including microbial agents, parasites, predators and competitors[6-9]. Moreover, many leech species such as *Helobdella triserialis lineate*, *Glossiphonia complanata* and *Helobdella punctatolineata* have been found to be good snail predators[10-12].

The Hirudinea or leeches are a quite small group of macrophagous or blood-sucking annelids, including about 680 species[13,14]. They are present in the sea, and in terrestrial habitats, but they mainly inhabit freshwater ponds, lakes, small streams, ditches and reservoirs[14,15]. Among them, *Limnatis nilotica* (*L. nilotica*) is a blood-sucking parasite that lives in stagnant and semi-stagnant freshwater[16]. This species is commonly found in Southern Europe, North Africa, and the Middle East[17].

The objective of the present study was to investigate the predation rate of the above mentioned, freshwater leech, *L. nilotica*, with a view to employing it as a potential biological control agent against different stages (eggs, neonates and adults) of the snail, *Bulinus truncatus* (*B. truncatus*), the intermediate host of human *Schistosoma haematobium*, under laboratory conditions.

2. Materials and methods

2.1. Leech samples

Specimens of the freshwater leech, *L. nilotica*, with mean body length of 29 mm, were collected from the water canals of El Kiryab Agriculture Scheme, Khartoum State, Sudan, by using a fine wire-mesh scoop. The leeches were transported in a plastic bowl provided with canal water to the Schistosomiasis Research Laboratory,

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University of Khartoum and maintained in $30 \times 20 \times 20$ cm aquaria, provided with dechlorinated water and maintained at $25\text{--}27^\circ\text{C}$. The leeches were allowed to feed on *Physa* snails until a week prior to the experiment, and thereafter they were starved until the beginning of the experiment.

2.2. Snail samples

Specimens of the snail, *B. truncatus* were also collected from water canals of El Kiryab Scheme, using a deep wire-mesh scoop. The snails were transported to the laboratory in plastic bowls provided with canal water and lined with vegetation. In the laboratory, the snails were screened for natural trematode infections and those found to be free from infection were kept in $30 \times 20 \times 20$ cm aquaria equipped with an aerator, provided with dechlorinated water maintained at $25\text{--}27^\circ\text{C}$, and left under artificial illumination during daytime hours. The snails were fed with lettuce when needed and the water in the aquaria was also regularly changed. Small pieces cut from plastic sheets were put onto the surface of the aquaria, to provide a suitable substrate for the snails to lay eggs on. When a considerable number of egg masses were observed on the sheets, they were transferred to other aquaria containing aerated water to enable them to hatch into neonates.

Three stages of the laboratory bred, *B. truncatus* snails were prepared to be used in the experiments. Two of these were classified into groups, based on their shell length (SL): group (1), neonates had an SL of up to 3 mm, while group (2), adults had an SL greater than 3 mm. In addition a third group, group (3) used in the experiments was made up of the egg mass of the snail.

2.3. Experimental design

Four sets of experiments were designed for the assessment of the proposed bio-control agent, *L. nilotica*, against the three groups (egg masses, neonates and adults) of *B. truncatus*. The first three sets consisted of eight aquaria (four control aquaria and four experimental for each stage), respectively, with the fourth set, the combination set, consisting of six aquaria (three control aquaria and three experimental aquaria). In the first set, 50 egg masses [(500 ± 2) eggs] of the snail were placed in each aquarium of the set while in the second set, 50 neonate snails were placed in each aquarium and in the third set 40 adult snails were placed in each aquarium. Three *L. nilotica* leeches were added to each of the 12 experimental aquaria with no leech being placed in the 12 control aquaria. The frequency of the experimental agents uses was based on their observed field frequency. For the combination set, each aquarium was stocked with 50 egg masses, 50 neonates and 40 adult snails. Thereafter, nine *L. nilotica* leeches were added to the experimental aquaria with none being added to the control aquaria. Twenty pieces of gravel (~ 10 mm diameter) were added to each experimental aquarium as a place of rest or refuge for the leeches.

In each experimental set, the number of eggs remaining or the number of surviving neonates or adult snails were counted every four days, for eight similar intervals. The water was changed after each 4-day period and counting was conducted by passing the water through a small-mesh sieve to avoid washing-out the target organisms. Snail food (fresh lettuce leaves) was added to each experimental aquarium whenever needed.

2.4. Data analysis

Data analysis was performed by using independent sample *t*-tests.

The SPSS 16.0 statistical software program, was used to conduct the data analysis and values were considered significant when $P < 0.05$.

3. Results

3.1. Predation rate of the *L. nilotica* leech against *B. truncatus* snail eggs

The egg masses of the snail, were completely consumed by the leech in the experimental sets consisting only of eggs, by the end of the final (eighth) period of observation (Figure 1), and the consumption values were significantly different when compared with the corresponding controls ($t = 80.6$, $df = 62$, $P < 0.001$). Similarly, in the combination sets, the results showed that the number of snail eggs declined and all had been consumed by the end of the seventh period of observation (Figure 2) and this was significantly different when compared with the results from the corresponding control aquaria ($t = 54.2$, $df = 46$, $P < 0.001$).

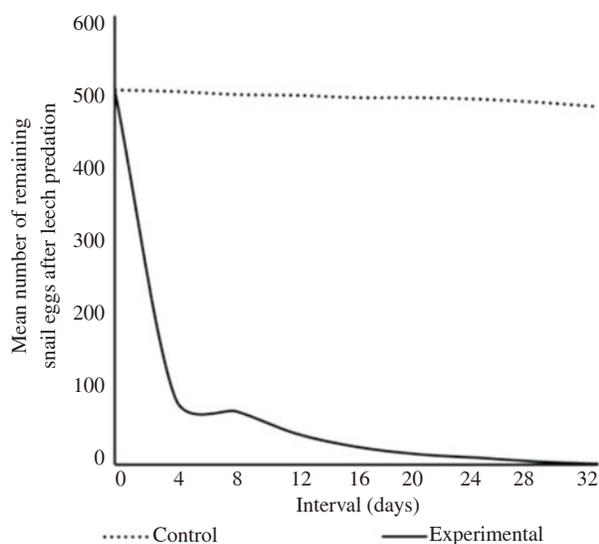


Figure 1. Mean number of *B. truncatus* eggs ($n = 500$ per set) collected after predation by *L. nilotica* ($n = 3$ per set) in four experimental sets (eggs alone) compared with the control groups.

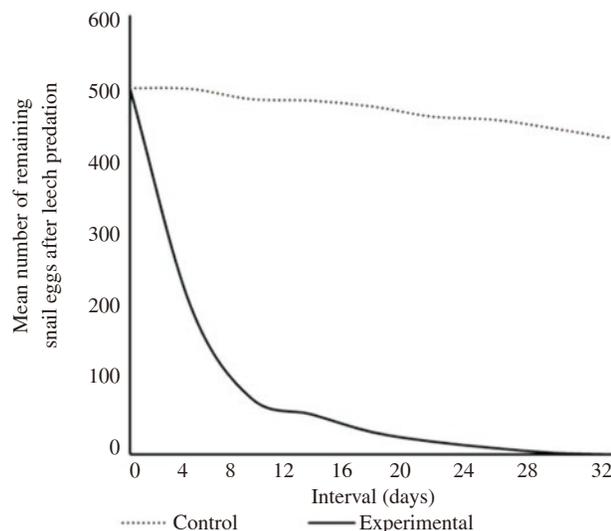


Figure 2. Mean number of eggs of *B. truncatus* ($n = 500$ per set) collected after predation by *L. nilotica* ($n = 9$ per set) in three combined sets (eggs, neonates and adults) compared with control groups.

3.2. Predation rate of the *L. nilotica* leech against neonate *B. truncatus* snails

In this part of the experiment, the neonate snails with an SL of up to 3 mm were completely killed by the leeches by the end of the fifth period of observation (Figure 3), with a significant difference when compared to the values for the control groups ($t = 15.3$, $df = 62$, $P < 0.001$). Similarly, the neonate snails in the combination sets were completely killed by the leeches by the end of the seventh period of intervention (Figure 4) and this was also significantly different when compared to the values for the control groups ($t = 9.7$, $df = 46$, $P < 0.001$).

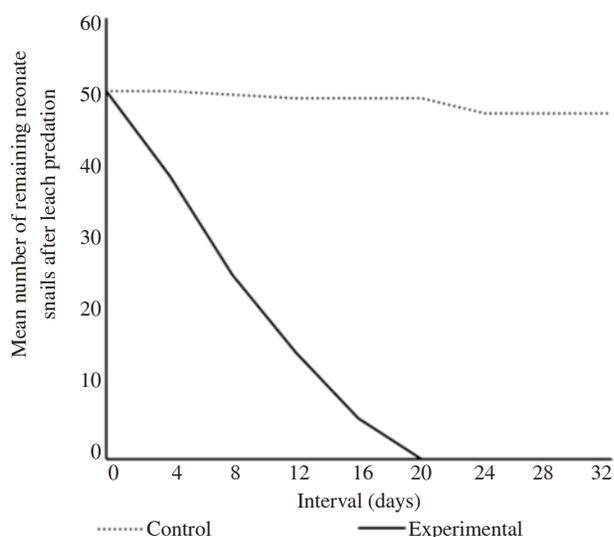


Figure 3. Mean number of live neonates of *B. truncatus* ($n = 50$ per set) collected after predation by *L. nilotica* ($n = 3$ per set) in four experimental sets (neonates alone) compared with control groups.

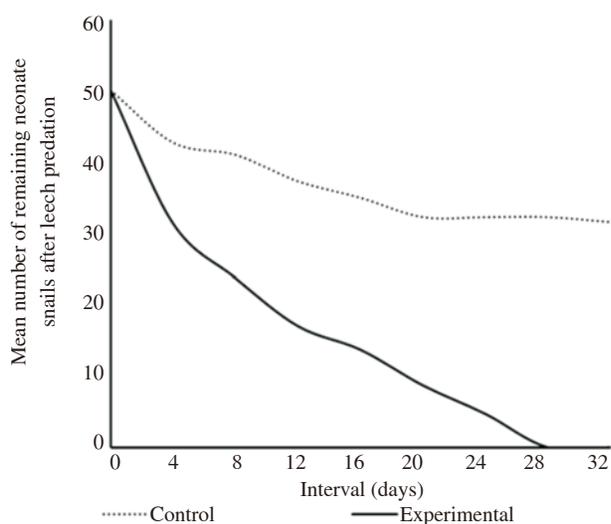


Figure 4. Mean number of live neonates of *B. truncatus* ($n = 50$ per set) collected after predation by *L. nilotica* ($n = 9$ per set) in three combined sets (eggs, neonates and adults) compared with control groups.

3.3. Predation rate of the *L. nilotica* leech against adult *B. truncatus* snails

The adult snails with an SL greater than 3 mm were reduced to their lowest number by the end of the last period of observation (Figure 5), with a significant difference compared with the control groups ($t = 15.9$, $df = 62$, $P < 0.001$). Likewise, the adult snails in

the combination sets were also reduced by the end of the last period of observation (Figure 6), with a significant difference compared with the control groups ($t = 5.7$, $df = 46$, $P < 0.001$).

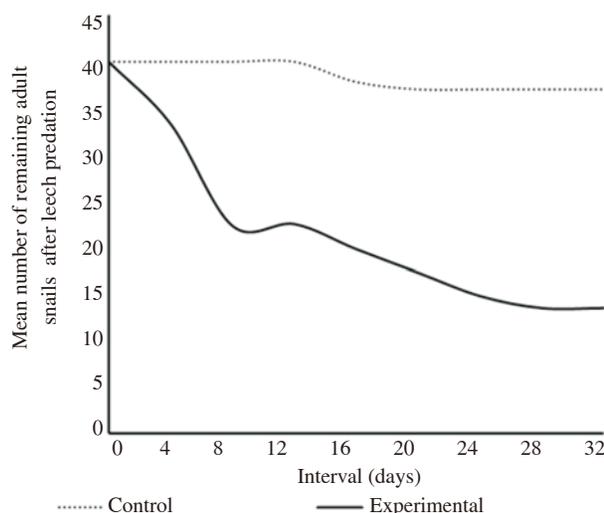


Figure 5. Mean number of live adults of *B. truncatus* ($n = 40$ per set) collected after predation by *L. nilotica* ($n = 3$ per set) in four experimental sets (adults alone) compared with control groups.

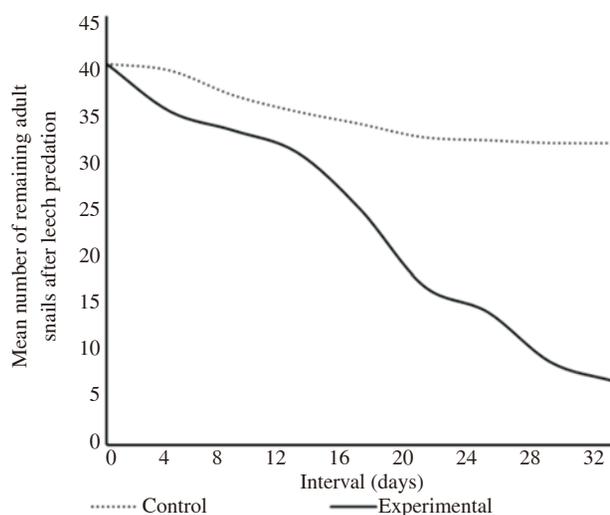


Figure 6. Mean number of live adults of *B. truncatus* ($n = 40$ per set) collected after predation by *L. nilotica* ($n = 9$ per set) in three combined sets (eggs, neonates and adults) compared with control groups.

4. Discussion

Over the last few decades, biological control has been considered as an alternative approach to chemical molluscicides for controlling the snails responsible for the spread of diseases, and several types of biological agents have been studied. Among those studied, shell-invading predators, such as leeches and belostomatid bugs, may be important sources of mortality in snails[11,18].

The results of the present study showed that the eggs of the snail *B. truncatus* were completely killed by the leech *L. nilotica* in the experimental aquaria containing eggs alone, by the end of the eighth and final period of observation. Similarly, in the combination sets, where the three snail stages were placed together, the results showed the disappearance of the snail eggs by the end of the eighth period of observation. This finding can be explained by the eggs of the snail being soft since they lack a protective shell and are therefore, easily

consumed by the leech. Previously, it has been reported that the fish predator, *Gambusia affinis* preferred to consume the egg masses and juveniles of the snail, *B. truncatus*[19].

Further, the results of the present study showed the complete predation of the neonate snails by the leech by the end of the fifth period of observation for the neonates-alone sets, while, the neonate snails in the combination sets were also completely killed by the leech by the end of the seventh period of observation. This might be because the body of the neonate snails is relatively soft and easier to invade. Previously, it has been shown that the predation rates of the leech *Glossiphonia complanata* and *Nepheleopsis obscura* increased with decreasing snail size[11,20]. Moreover, the strong jaws of this leech species may enable it to easily pierce and invade the skin of the prey and to ingest all their body fluids and tissues. In addition, this leech, *L. nilotica* has strong muscular suckers which enable it to attach firmly on the prey. Previously, it has been reported that smaller snails are easier prey and can be attacked and crushed by predators such as species of crayfish[7,21].

In the present study, the adult snails were reduced to their lowest number by the end of the last period of observation in both the individual and combined set experiments. However, when comparing these results with that of the neonates, it seems that adult snails with thicker shells are often less susceptible to the leech. In general, the choice of snails as prey strategy adopted by predators may be influenced by many factors such as the presence or absence of an operculum, prey movement and speed as well as the hunger level of the predators[22]. Previously, it has been reported that, the fish, *Gambusia affinis* is able to consume the flesh of the adult snail *B. truncatus* in a size range of 3 to 6 mm in the absence of other food material and the nymph of the Odonata, *Hemianax ephippiger* is able to consume even larger sizes of the snail *Lymnaea natalensis*[9,19].

In conclusion, the present laboratory results showed that the leech *L. nilotica* has a significant impact on populations of the snail, *B. truncatus*. However, long term studies under natural field conditions are needed to support these results.

Conflict of interest statement

We declare that we have no conflict of interest.

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