

**PATHOLOGICAL ALTERATIONS IN THE LAND SNAIL
EOBANIA VERMICULATA (MÜLLER) INFECTED WITH THE
NEMATODE *Rhabditis* sp.**

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ABSTRACT

The symptomatological and histopathological studies on the terrestrial snail *Eobania vermiculata* Müller infected with the parasitic nematode *Rhabditis* sp. showed that there is a great evidence of a direct effect of the nematodes on its movement, behavior and tissues of the snail before death. These symptoms were summarized in slow slipping, turning of the headfoot direction while slipping and suppressive feeding. Histological investigation revealed that focal cellular reaction was formed around the developing larvae in the snail tissues particularly the headfoot region. Capsules of different sizes were also developed around the nematode larvae.

Keywords: *Rhabditis*, Nematode, *Eobania vermiculata*, Snail, Pathology, histology

INTRODUCTION

Terrestrial molluscs represent an important economic problem in the world (Godan, 1983 and South, 1992). In Egypt, they infest ornamental plants, vegetables, orchard and field crops causing sever damage (El-Okda, 1979, 1980, 1984). *E. vermiculata* is one of these molluscs that is prevalent in different Governorates of the country (Azzam, 1995). It has been almost universally agreed that the biological control is the best possible method for molluscs. Many authors recorded the role of different nematodes as biological control of snails. *Phasmarhabditis hermaphrodita* (Schneider) was recorded as a successful biological control of slugs (Glen and Wilson, 1997). The aquatic nematode *Rhabditis onchomelaniae* (Jokko and Okabe) was used successfully against *Onchomelania nosophora* (Robson) the intermediate host of *Schistosoma japonicum* (Okabe and Shiraishi, 1971). The nematode *Rhabditis* sp. was first recorded in Egypt by Azzam (1998). The same authoress, (1999) recorded that this nematode successfully infects the slug *Limax flavus* (L.) and *Lehmania marginata*, the snails *Eobania vermiculata*, *Monacha obstructa*, *Helix aspersa*, and *Biomphalaria alexandrina* and 80-100% of the insect larvae and pupae of *Galleria mellonella*, *Spodoptera littoralis* and *Agrotis ipsilon*. the combination Azzam and Belal (1999a, b) also found that the combination between the nematode *Rhabditis* sp. and the bacterial exotoxin Victoback₁₂As caused 100% mortality to the snails *Biomphalaria alexandrina* and *Lymnaea cailliaudi* within 24 hours. Therefore, the present contribution deals with the pathological alterations in the land snail *E. vermiculata* caused by infection with the parasitic nematode *Rhabditis* sp.

MATERIALS AND METHODS

Parasitic nematode was provided from progeny of the original colony which was isolated for the first time in Egypt from *E. vermiculata* snail by Azzam in September, 1996, using the same technique previously described by Azzam, 1998 and 1999. Snails were infected by 40-50 or 80 I.S of the parasitic nematode *Rhabditis* sp. Infected snails were maintained at $26\pm 2^{\circ}\text{C}$ and 70 ± 5 RH%. After 24-36 hours, some of experimentally infected snails were fixed without anesthesia. The soft parts of the snail were obtained by gently crushing the shell and removing its fragments. Then they were immediately fixed in Bouin's solution for 18 hours. The sample was then washed in 70% ethanol, dehydrated through ascending grades of ethanol, cleared in terpineol and embedded in paraplast. Serial sections of 5 μm thick were obtained from snails and stained with Mallory Triple stain (Pantin, 1946). The sections were then mounted permanently for microscopic investigation.

RESULTS AND DISCUSSION

The illness symptoms began to appear after 6 hours post infection in the snail which died after 48 hours, Figs (1-8). These symptoms began with slow in slipping; and contraction of the optical tentacles. After 12 hours, the snails exhibited a very weak and sluggish movement with some bias on the lateral edge of the foot (always with the shell apex directed upwards), Eighteen to twenty four hours post infection the snail began to refrain from movement and feeding for a long time, stay with same bias. Thirty six hours post infection the snails showed no movement but delayed response to a thin stainless steel needle. This snail became moribund and died within 48 hours. The time of symptoms appearance was differed according to the time needed for snail death which in turns depend on the dose of nematode infection.

This phenomenon may be attributed either to the aggregation of the nematode inside the visceral mass of the snail which lead to burdensome or to direct damage of the foot muscles. Robson and Williams (1973) reported that the endo-parasites of snails caused disturbances in growth, leading to gigantism. They may also cause histopathological changes in several organs and also abnormalities in the shell (Johnnessen, 1973, Dix, 1973).

Histological examination of infected *E. vermiculata* snails revealed that focal cellular reaction was formed around the developing larvae in the snail tissues. Also tissue capsules of different sizes were formed around the larvae in the headfoot region (Figs. 9-10). These capsules were formed mainly of amoebocytes, which later became surrounded by several layers of fibroblast-like cells. The fibroblast-like cells were largely similar to those previously described in *Marisa cornuarietis* and *Biomphalaria glabrata* infected with *Angiostrongylus cantonensis* (Yousif et al., 1980 and Soliman, 1998). The larvae of the nematode *Mullerius capilaris* (Müller) were also found in the foot muscles of the slugs *Agriolimax agrestis* and *A. reticulatus* (Rose, 1957).

Fig. (1): Control snail

Fig. (2): 6hr. post-infection

Fig. (3): 12 hr. post-infection

Fig. (4): 18 hr. post-infection

Fig. (5): 24 hr. post-infection

Fig. (6): 36 hr. post-infection

Fig. (7): 36-48 hr. post-infection

Fig. (8): Died snail as a result of infection with *Rhabditis* sp.

Figs. 1-8: Photographs showing the effect of infection with *Rhabditis* sp. on the land snail *Eobania vermiculata*

Fig. (9): Photomicrograph of section of the experimentally infected *E. vermiculata* showing the ensheathed larva of *Rhabditis* sp. (X: 630)

Fig. (10): The same as above but (X: 1260)

It was concluded that, since *E. vermiculata* is considered a serious pest of the cultivated crops in Egypt, the role of the nematode *Rhabditis* sp. as a biological control agent to this snail should be taken into consideration.

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التغيرات الباثولوجية في القواقع الأرضية *أيوبانيا فرميكولاتا* المصاب بالنيماتودا من جنس رهابديتيس
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قد أظهرت هذه الدراسة أن إصابة القواقع الأرضية *أيوبانيا فرميكولاتا* بالنيماتودا من جنس *رهابديتيس* قد أحدثت تغييراً ملحوظاً على الحركة والتغذية، حيث تباطأت حركة القواقع أثناء انزلاقه وارتخت عضلات منطقة الرأس-قدم، ومالت على أحد جانبيها كما قلت قدرة القواقع على التغذية. وقد تم متابعة التغيرات التي حدثت في القواقع بدءاً من تعريضه للعدوى حتى موته والذي استغرق 48 ساعة بعد تعريضه للعدوى بالنيماتودا تحت الدراسة.

وتم أيضاً عمل قطاعات هستولوجية في الأجزاء الرخوة من القواقع المصاب حيث لوحظ تكون تفاعلات مناعية في صورة حويصلات نسيجية تحيط بيرقات نيماتودا *الرهابديتيس*.

وبذلك يظهر هذا البحث إمكانية استخدام النيماتودا تحت الدراسة من جنس *رهابديتيس* في المقاومة الحيوية لمتل هذه الآفات من القواقع الضارة بالمحاصيل الزراعية.