

## EFFICIENCY OF CUMIN BLACK SEED (*NIGELLA SATIVA*) AND *CINCHONA* SP. ON *POLYONCHOBOTHRIMUM CLARIAS* (CESTODE) *PARACAMALLANUS CYATHOPHARYNX* (NEMATODE) IN VITRO

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### ABSTRACT

Effect of both cumin black seed *Nigella sativa* and *Cinchona* species aqueous extracts on both the cestode *Polyonchobothrium clarias* and the nematode *Paracamallanus cyathopharynx* were investigated in vitro. Results showed that cumin black seed extract changes in morphology of the anterior region of worms, immature and gravid proglottids beside their eggs. After 3 hours of treatment by black seed extract the adult cestode and nematode were died. In conclusion, this study recommends by using cumin black seed as a new antihelminthic medicament against these worms of fish while *Cinchona* species affected only on the larvae of nematode worms.

### 1. INTRODUCTION

Catfish *Clarias gariepinus* infected with the cestode *Polyonchobothrium clarias* and the nematode *Paracamallanus cyathopharynx*. These helminthes may harm their hosts especially in massive infestation. They may cause injury of intestine or other ducts. Their presence may lead to certain changes in the activity of blood enzymes or hormones of the fish and they may produce toxic metabolic by-products (Williams, 1967). The histopathological alterations due to infection of freshwater fishes with helminth parasites were variable and almost potentially pathogenic in heavy infection (Nassef, 1988; El-Mansy, 1992; Taraschewski 2000; Matter, 2005).

*Nigella sativa* is an annual herbaceous plant and believed to be indigenous to the Mediterranean region. It has been cultivated in other parts of the world (e.g. Saudia Arabia, Northern Africa and parts of Asia). Historically as well it was discovered in

Tutankhamen's tomb that was played an important role in ancient Egyptian practices. Ibn Sina regarded by many as the most famous book in the history of medicine, East or West, refers to black seed that stimulate the body's energy and helps recovery from fatigue. Recent researches have indicated that black seed contained ability to significantly the human system if taken over time. A Greek physician of the 1<sup>st</sup> century recorded that black seed as taken to treat headaches, nasal congestion, toothache, and intestinal worms and others. (<http://www.kingtutshop.com/>).

One of the most commonly cited rainforest medicinal plants is the *Cinchona* tree. This plant's bark is the source of quinine, a highly effective drug for treatment of malaria. *Cinchona* is a genus of about 25 species, native to tropical South America. They are large shrubs or small trees with evergreen foliage. The fruit is a small capsule containing numerous seeds. The medicinally active bark, which is stripped from the tree

dried and powdered, includes other alkaloids that are closely related to quinine but react differently in treating malaria. The main reason for using is to treat malaria but it is rarely used today as many people thinking it is dangerous as it can kill in case of large amounts. (<http://encyclopedia.thefreedictionary.com/cinchona>).

Therefore, the main objective of this study is to use two medicinal plants *Nigella sativa* L. (black seed) and *Cinchona* L. in vitro and recorded their effect on adult, eggs and larvae compared with control specimens.

## 2. MATERIALS AND METHODS

Out of 45 specimens of catfish *Clarias gariepinus* (length 18-37cm and weight of 45-300g) were obtained from the River Nile at El-Kanater and some markets. Fish were transported alive immediately after collection to the laboratory. The catfish were dissected and examined one by one searching for helminth parasites. About 24 mature worms were isolated from stomach and intestine of the catfish. They were examined freshly and identified by comparison with other previously recorded parasites as the cestode *Polyonchobothrium clarias* and the nematode *Paracamallanus cyathopharynx*.

The collected worms were placed into cell well plates within aqueous extracts of black seed and cinchona sp. those obtained from commercial shops. In order to prepare the used extracts we use 20 g from black seed that were boiling in 15 ml water for about 5 minutes. However we put 150 ml boiling water onto 20 g of dried seed from *Cinchona* sp. for about half an hour we monitored the treat worms for any modification or up normal behavior. The observations were

registered each half an hour, photographed and compared with control cestode and nematode.

## 3. RESULTS

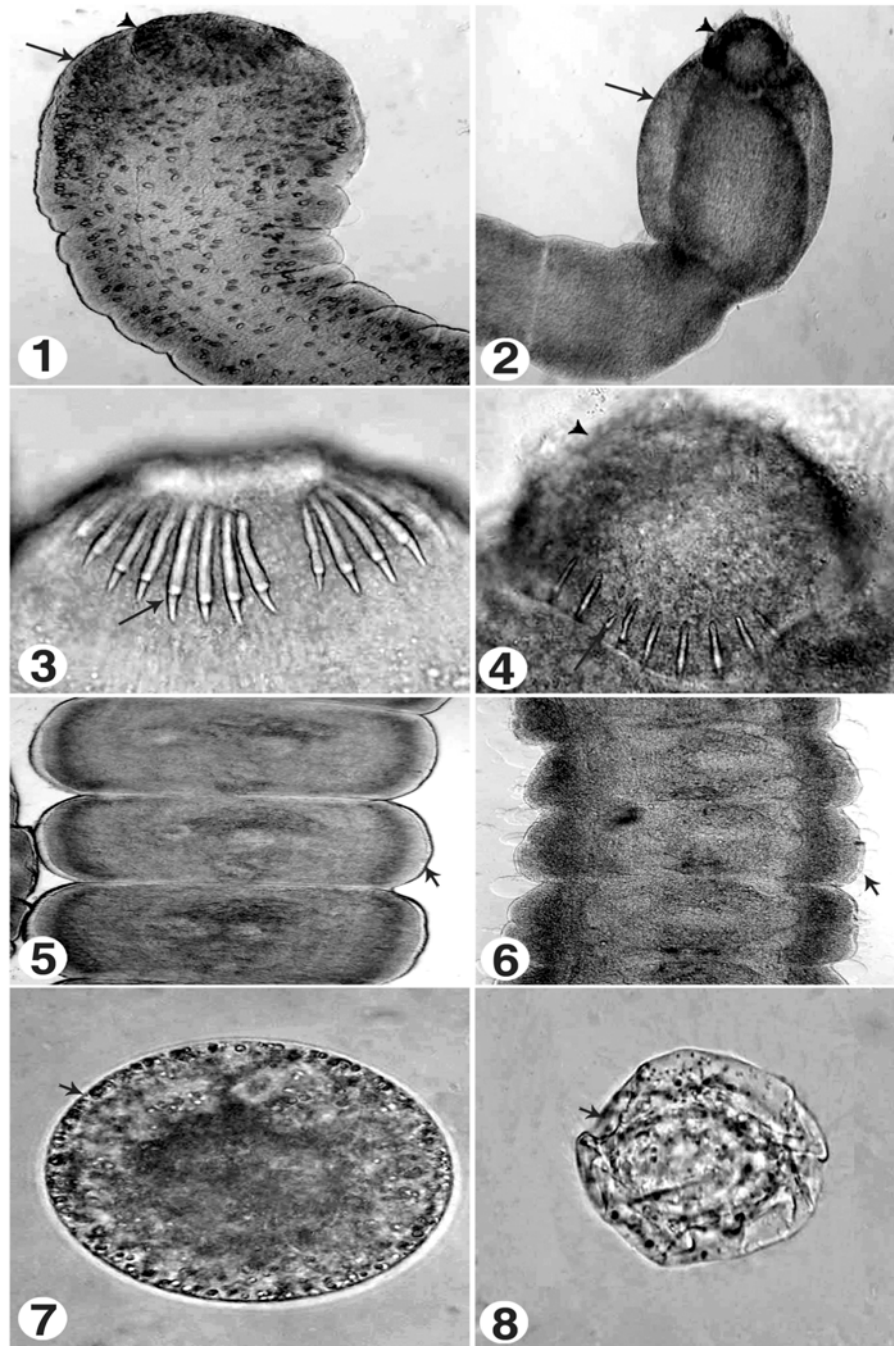
By comparison control cestode (Figs. 1, 3, 5, 7) and nematode (Figs. 9, 11, 13). *Nigella sativa* (black seed) affected the adult and egg of the cestode *Plyonchbothrium clarias* (Figs. 2, 4, 6, 8) and adult and somewhat larvae of the nematode *Paracamallanus cyathopharynx* (Figs. 10, 12, 14).

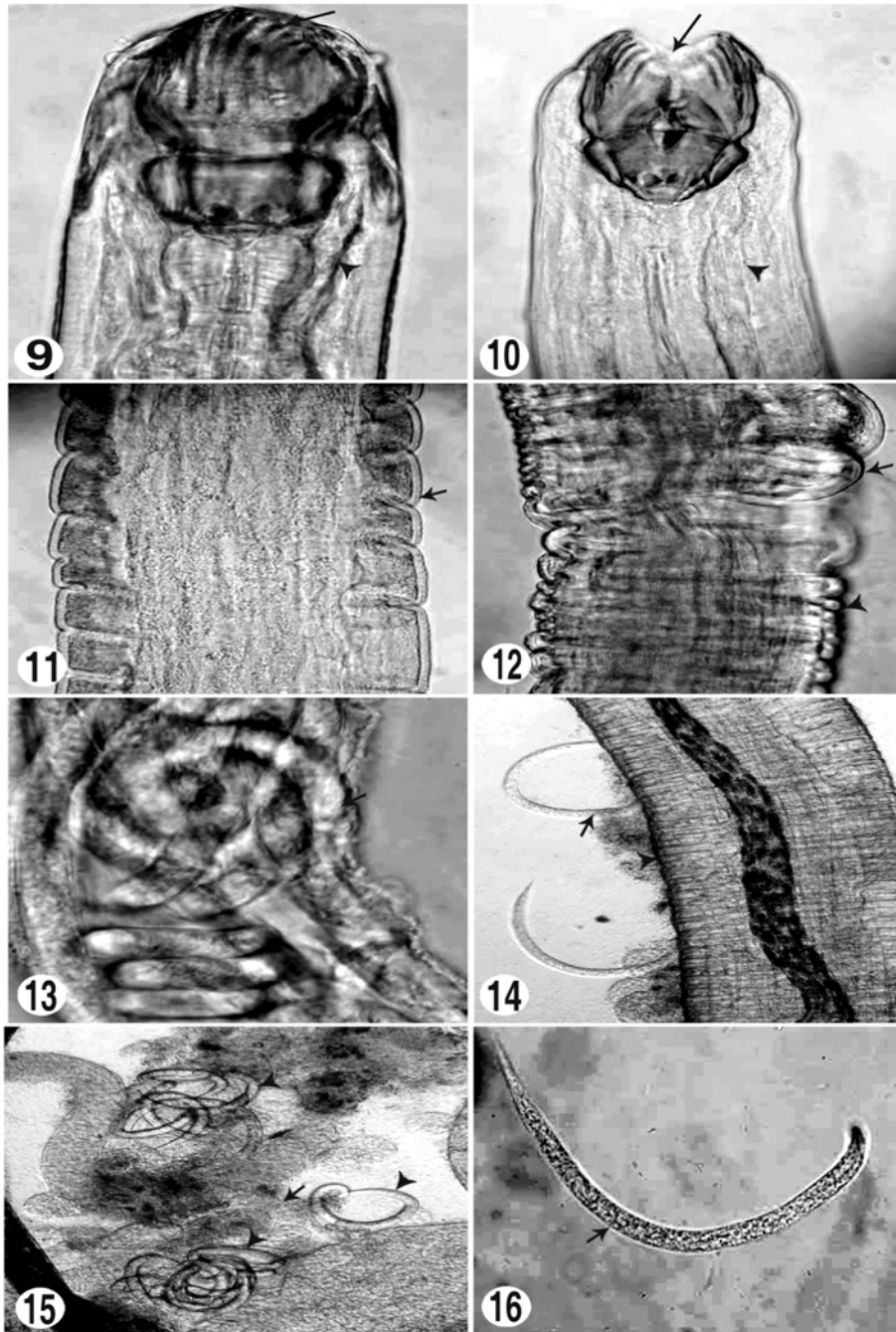
Adult cestode incubated at room temperature with about 5ml of aqueous extract of black seed in vitro medium was moved abnormally with evaginated scolex and a retractable rostellum. The neck was long slender and proglottids were wider than long. Thereafter about 3 hours of incubation the adult cestode in black seed dead with severe swelling scolex and botheria (Fig. 2). In addition, damage of rostellum teeth (Fig. 4) shrinkage of skin (Fig. 6) and evacuated eggs on the worm surface were also noticed with severely shrinkage and reduced size (Fig. 8).

Buccal capsule of the nematode worms become opened (Fig. 10). The skin severely shrinkage (Fig. 12) comparing with control (Fig. 11). About 5ml of black seeds affected and lead to death of the nematode. Also somewhat black seed fast-excreted larvae from the adult worm (Fig. 14).

However, about 5ml of aqueous extract of *Cinchona* sp. affected only larvae of the nematode that first released from the damage worm (Fig. 15) and thereafter about 2-3 hours in vitro were died (Fig. 16).

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#### 4. DISCUSSION

In the present study, aqueous extracts of two medicinal plants used to treat parasites of the catfish cestode and nematode. It is worthwhile to mention that most medicinal plants may be safe for fish than chemical preservatives. For example El-Mansy and Mohamed (2003) reported that aqueous extract of *Allium cepa* bulb is not toxic except in very large doses.

Black seed *Nigella sativa* was more effective in eradication these parasites particularly adult of both worms, eggs of cestode and somewhat larvae of the nematode. Akhtar and Riffat (1991) reported that anticestodal effect of *N. sativa* seeds were studied in children naturally infected with the respective worm. Korshom *et al.* (1998) investigated the antitreumatodal activity of *N. sativa* seeds against a ruminant fluke (*Paramphistomum*) in sheep.

Herein, the seeds of *Cinchona* sp. was only affected on the larvae of the nematode although *Cinchona* has been used for a number of medical reasons such as treats malaria, kills parasites, reduces fever, regulates heartbeat, calms nerves and stimulates digestion, kills germs, reduces spasms, kills insects, kills bacteria and fungi. The main reason for using is to treat malaria but it is rarely used today as many people thinking it is dangerous if taken in large amounts (<http://encyclopedia.thefreedictionary.com/cinchona>).

Morphologically, these plants affected the worm shape these observations were similar with that of El-Sayed and Lotfy (2002) who reported that the worms of the cestode *Vampirolepis nana* in vitro were recorded and the topographic tegumental changes were studied.

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## LEGEND OF FIGURES

**Figs. 1, 3, 5, 7:** Control cestode of untreated worm.

**Figs. 2, 4, 6, 8:** Treated cestode with black seed.

**Figs. 9, 11, 13:** Control nematode.

**Figs. 10, 12, 14:** Treated nematode with black seed.

**Figs. 15, 16:** Treated nematode larvae with *Cinchona* species.

**Fig. 1:** Anterior part of the cestode *Polyonchobothrium clarias* (arrow) and scolex (arrowhead) as a control, x135.

**Fig. 2:** In vitro treated worm with the medicinal plant black seed *Nigella sativa* shows swelling bothria (arrow) and scolex region (arrowhead), x100.

**Fig. 3:** Tip of the anterior portion of the cestode worm (arrow) shows healthy normal teeth, x150.

**Fig. 4:** Anterior portion of treated teeth (arrowhead) shows deformity of them (arrow) after treatment with the medicinal plant black seed, x150.

**Fig. 5:** Gravid proglottid as a control (arrow), x150.

**Fig. 6:** Gravid proglottid treated with black seed shows dislodge and shrinkage of segments (arrow), x115.

**Fig. 7:** An egg released from the worm show normal wall and constituents (arrow), x600.

**Fig. 8:** Treated egg shows severe shrinkage of egg wall (arrow) and damage of internal structure, x600.

**Fig. 9:** Anterior portion of the nematode shows normal hooks of the worm (arrow) and note internal walls (arrowhead), x340.

**Fig. 10:** Treated worm shows loosely opened hooks (arrow) and severe degenerated internal walls (arrowhead), x260.

**Fig. 11:** Untreated portion of the nematode shows somewhat normal body Wall (arrow), x503.

**Fig. 12:** Treated portion of the worm shows swelling (arrow) and severe Shrinkage (arrowhead), x260.

**Fig. 13:** A normal portion of the worm contains larvae (arrow), x260.

**Fig. 14:** Treated worm shows released larvae out (arrow). Note thick wall of the worm (arrowhead), x260.

**Fig. 15:** Treated worm shows complete releasing larvae (arrowheads) Surrounding with uterine remaining (arrow), x340.

**Fig. 16:** A dead unmovable larva (arrow) exposed to *Cinchona* sp. shows degenerate internal constituents, x370.