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In vitro Effect of LC₉₀ of albendazole and *Allium sativum* water extract on the fine structure of *Capillaria* sp. (Capillaridae: Nematoda)

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Abstract

Fish parasites are of economic and health importance where they cause serious problems in commercial fish farms and it can be transferred to human and animals. *Capillaria* species are nematodes of serious fish diseases which may be transferred to human. The present study was conducted to detect the in vitro effect of sublethal concentration (LC₉₀) of albendazole (ABZ) and *Allium sativum* (garlic) cloves water extract on ultra structure of *Capillaria* sp. collected from fresh water catfishes, *Bagrus docmac* and *B. bayad* using SEM and TEM. The mortality rates of the adult *Capillaria* sp. in vitro exposed to different concentrations of albendazole and *Allium sativum* water extract was recorded after 24 hours exposure time. *A. sativum* water extract had higher effect than albendazole where lower concentration of *A. sativum* extract (18×10^3 ppm) gave maximum mortality rate (100%), 24 hours post exposure. SEM studies of the cuticle of adult *Capillaria* sp. in vitro treated with LC₉₀ (17.161×10^3 ppm) of *A. sativum* water extract for 24 hours revealed that it induced severe changes in the cuticle integrity more than those treated with concentration LC₉₀ (5.543×10^6 ppm) of albendazole. TEM studies revealed deformation of the cuticle, hypodermis, muscles and digestive system in worms treated with LC₉₀ (17.161×10^3 ppm) of *Allium sativum* water extract.

Key words: *Capillaria*, Albendazole. In vitro, *Allium sativum*, SEM, TEM

1Introduction

Capillaria spp. are nematodes of many different species infecting a wide range of vertebrate hosts, some infect human hosts causing serious parasitic diseases. Capillariasis is a severe disease that may lead to death unless patients are treated (Saichua et al., 2008).

The use of chemotherapeutic agents in fish aquaculture has certain disadvantages. They may be toxic to fish or harmful to its surrounding environment which may lead to drug resistance. Nowadays, medicinal plants are gaining importance as alternative medical treatments. They were safe to non target organisms and to the environment (Abu Samak and Khidr, 1998).

It is proven that the naturally occurring products in plant extracts have potency against different pathogens and are mostly safe in use. (Mehlhorn, 2008; Abdel-Ghaffar et al., 2009; 2010, b and Bashtar et al., 2011). Albendazole belongs to the group benzimidazoles, it is a broad-spectrum anthelmintic drug used all over the world in human and veterinary medicine (Cheetham and Markus, 1991).

Mckellar and Scott (1990) and Benchaoui et al. (1993) revealed that, although several albendazole metabolites had been detected, the major metabolites were albendazole sulphoxide (ABZ-SO) and albendazole sulphone. The sulphoxide derivative was considered to be the active metabolite which had a significant anthelmintic effect.

Scanning electron microscopy (SEM) has been used as a powerful tool to study the topographical structures of helminth parasites (Abu Samak and Khidr, 2000 ,Nandi,2005&Naem,2007).Shalaby et al. (2012a) investigated in vitro the comparative morphological effects of ivermectin/*Nigella sativa* oil combination and each of them separately against adult helminth parasites include nematode, cestode and trematode (*Haemonchus contortus*, *Moniezia expansa* and *Fasciola gigantica*, respectively) using SEM.

The need for alternative, non chemical, control strategies in animal production systems has increased in the last decade due to development of antiparasitic resistant strains of parasites as well as toxicity problems (Waller and Prichard, 1985; Jackson and Coop, 2000; Sangster, 2008; Cezar et al., 2010 and Zaros, et al.2014).

A number of medicinal plants had been investigated in vitro for their anthelmintic activity from different regions of the world (Akhtar et al., 2000; Tagboto and Townson, 2001; Athansiadou et al., 2007). Tandon et al. (2011) provided an overview of a large number of traditional medicinal plants that are used for curing intestinal helminthic infections in different regions of the world, with particular reference to north-east India. *Allium sativum* (garlic) had been used in herbal medicine for thousands of years as a remedy for intestinal disorders (Bolton et al., 1982). In Chinese herbal medicine, garlic was used to prevent influenza, relieve toxicities, and killed parasites such as roundworms and tapeworms (Bensky and Gamble, 1993). Garlic had been used medicinally worldwide for many centuries (Silagy and Neil, 1994 Petry, 1996). Aize et al. (2003) reported that extensive studies had shown that garlic had a beneficial effect on human health and therefore would possibly be a suitable remedy to increase the health of animals in organic production.

Pena et al. (1988) indicated that minced garlic at a concentration /dose of 200 mg/L showed maximum anthelmintic activity in carp infested with *Capillaria* spp. Soffar and Mokhtar (1991) referred that garlic extract was successfully used against *Hymenolepis nana*. A number of human immune functions were found to be enhanced in vitro by aqueous garlic extract (Burger et al., 1993).

Ankri et al. (1997) reported that garlic major component, allicin, had been known to have antibacterial, antifungal, antiviral and protozoan pathogenics for a long time. Krest and Kengen (1999) indicated that allicin was naturally produced when garlic was damaged or crushed, allowing the release of alliin which transformed to allicin by the action of allinase enzyme. Sutton and Haik (1999) reported that garlic has been used as anthelmintic against strongylid nematodes in donkeys by boiling 300 ml of water for every head of garlic until soft, the product was then mashed and administered orally with a syringe at the dosage of one head of garlic/ donkey and it had been found that garlic was effective in the control of intestinal parasites. Plants with anthelmintic activity have been reviewed by Akhtar et al. (2000). Allicin was suggested to produce much of the activity of garlic; it inhibits a wide variety of bacteria, moulds, yeasts and viruses in vitro

(Harris et al., 2001). Anthelmintic activity of some plants had also been reported by Iqbal et al. (2001a and b) for *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana* and *Ficus religiosa*, and by Iqbal et al. (2004) for *Artemisia brevifolia*. Anthony et al. (2005) highlighted the importance of plant essential oils as a novel antiparasitic agent using garlic oil which proved a broad-spectrum activity against *Trypanosoma*, *Plasmodium*, *Giardia* and *Leishmania*. Mansson (2006) revealed that garlic extracts has been used successfully against tapeworm, hookworm, *Capillaria* in humans and animals and against parasites that contaminate vegetables. Allicin is the source of garlic odour and it is heat- unstable. The allicin molecule produced has very short half-life. More than 200 other sulphur compounds can be formed when raw garlic crushed or disrupted, particularly by boiling. The allicin is categorized as the mother substance from which all others flow (Josling, 2005; Duka and Ardelean, 2010). The present study was conducted to detect the *in vitro* effect of sublethal concentration (LC₉₀) of albendazole (ABZ) and *Allium sativum* (garlic) cloves water extract on ultra structure of *Capillaria* sp. collected from fresh water catfishes, *Bagrus docmac* and *B. bayad* using SEM and TEM.

2 Materials and Methods

Albendazole, Yellow to grey suspension (trade name: Alzental, from Egyptian International Pharmaceutical Industries Company, E.I.P.I.Co). Chemical name: Methyl 5-propylthio-2-benzimidazolecarbamate. *Allium sativum* (garlic) cloves were bought from Menoufia Governorate markets and used in the present study.

A. Isolation of nematodes :

Adult *Capillaria* sp. was collected from the stomach of the fresh water fishes, *Bagrus docmac* and *B. bayad* from Menoufia and Kalyobia Governorates fish markets, Egypt. Fish was examined immediately after collection. The stomach was removed from the viscera and opened with a pair of scissors, placed in saline solution (0.7%) and examined using a binocular dissecting microscope (10X). Nematodes were isolated in separate Petri-dishes containing 0.7% saline solution.

B .Maintenance of *Capillaria* in vitro:

For in vitro studies, 10 ml of natural calf serum was put together with 2 ml of antiseptic solutions (1 ml streptomycin and 1 ml penicillin) in separate sterile Petri-dishes (5 cm in diameter) (Arias-Diaz et al., 2006). To each Petri-dish, 10 worms were added and incubated. Nematodes were examined at 24 hours.

C.Determination of LC₉₀ of albendazole and *Allium sativum* water extract:

LC₉₀ values of albendazole and *Allium sativum* water extract were calculated using Proban Software version 1.1, PB-program (Jedrychowski, 1999).

D. In vitro effect of LC₉₀ of albendazole and *Allium sativum* water extract on adult *Capillaria* sp.:

Three replicates, each of 10 worms for each treatment were transferred to sterile Petri-dishes containing 10 ml of the

maintenance medium that has been proved to be the optimal medium (natural calf serum) (Radwan,1999), 2ml of antiseptic solution and 10 ml of LC₉₀ of the drug (5.543 x 10⁶ ppm) or *Allium sativum* water extract (17.161 x10³ ppm) and kept in an incubator at optimal conditions (pH 7 and 20°C).

The control group containing 10 worms was also maintained in the same medium without drug or garlic extract and under the same conditions. Treated worms were examined and the mortality rate was calculated after 24 hours. Post –incubation, live worms were collected from the media, washed in 0.7% buffer saline solution and fixed for SEM and TEM studies.

E. Scanning electron microscopy (SEM) study :

Worms were washed several times in 0.7% phosphate buffered saline (PBS), and fixed for 24 hours in a mixture of formaldehyde and glutaraldehyde (4:1) at 4°C. Specimens were washed three times in PBS and post fixed in 1% osmium tetroxide in 0.1M phosphate buffer for two hours at 4°C. Worms were washed three times in PBS, dehydrated through a graded series of ethanol and critical point dried. Specimens were mounted on metal stubs and coated with gold. Examination and electron micrographs were made using a JEOL JSM 5300 scanning electron microscope at an accelerating voltage of 30 K.V at the electronic microscope unit, Alexandria University.

F. Transmission electron microscopy (TEM) study:

worms were cut into three equal parts, fixed and dehydrated as in SEM, then embedded in Epon-Araldite. Semithin sections (1µm thick) were cut using a LKB ultramicrotome and stained with 1% toluidine blue for light microscopic examination. For transmission electron microscopy, ultrathin sections (60-90 nm thick) were mounted on copper grids, and then stained with uranyl acetate followed by lead citrate. Examination and electron micrographs were made using a JEOL 1200CX electron microscope at 80 K.V at the electronic microscope unit, Alexandria University.

3 Results

The present description is based on 25 adult female specimens collected from the stomach of naturally infected *B. docmac* and *B. bayad* collected from Menoufia and Kaliobya Governorates. Only female specimens were found in 4800 out of 5760 examined fish. Morphometric measurements of described specimens are illustrated in table 1 & Fig. (1,A-D)

A- Effect of LC₉₀ of albendazole and *Allium sativum* water extract on the fine structure of *Capillaria sp.* in vitro:

1- Scanning electron microscopy examination :

Scanning electron micrographs of adult female worms treated with LC₉₀ of albendazole (5.543 x 10⁶ ppm) for 24 hours,(Figs.7-11) showed that the surface structures were clearly affected compared with the control worms (Figs.2-6) . The mouth opening is swollen and flaccid with loss of the normal structure of the lips (Fig. 7).

The drug had a severe effect on the worm integrity as observed by the deformation of the cuticular surface. The surface of the cuticular annulations appears corrugated especially in the somatic region (Fig. 8).

The bacillary bands transformed into swollen structures and the gland openings appeared blocked. The cuticular rim of the bacillary pore was swollen and inflated (Fig. 9).

The vulva was deformed with swollen cuticle (Fig.10). The cuticle around the anal opening appeared swollen and deformed. The posterior end of tail was severely damaged and lost its natural configuration with disappearance of the anal papillae (Fig. 11).

Incubation of adult worms in LC₉₀ of *Allium sativum* water extract (17.161 x10³ ppm) showed that the surface structures were severely affected as compared with specimens treated with albendazole at the same exposure time. Scanning electron micrographs (Figs.12-17) showed that the cuticle surrounding the mouth was deformed and retracted with contraction of the cuticle annulations around the cephalic end (Fig. 12).

Cuticular annulations were more wrinkled and corrugated (Fig.13). The bacillary bands were extensively swollen with gland openings deeply embedded in characteristic cuticular grooves (Figs. 14 , 15). The vulva was severely damaged and the cuticular edge deformed (Fig. 16). The cuticle around anal opening became swollen and corrugated. The posterior end of tail appeared deformed but less than that treated with albendazole (Fig. 17).

2- Transmission electron microscopy examination:

1- Body wall:

a- Cuticle:

Transmission electron micrographs of females treated with LC₉₀ of albendazole (Figs. 27-34) showed that the cuticle was ridged and more opaque compared to the untreated control. The triple-layered epicuticle became dense and granular (Fig. 27). Fibrillar layer decreased in size and became less electron dense .No change was detected in the basal layer (Fig. 28).

Transmission electron micrographs of females treated with LC₉₀ (17.161 x10³ ppm) of *Allium sativum* (Figs. 35-41) revealed the deformation of the cuticle in the form of ridges which was increased and changes in the density of the cuticular layers, where the cuticular layers became more electron dense (Fig. 35).

b- Hypodermis:

The hypodermis in albendazole treated worms appears more electron dense (Fig. 29) and vacuolated (Figs. 29-30). It appeared detached from the cuticle in many places (Fig. 29). Bacillary band gland cells lost its characteristic lamella and showed chromatin fragmentation of the nuclei (Fig. 31).

In LC₉₀ *Allium sativum* treated worms, the hypodermis appeared more electron dense and enclosed electron dense deposited (Fig. 35). The bacillary band gland cells showed disrupted lamella and nucleus show dispersed fragmented chromatin (Fig. 36) as a possible indication of apoptosis in both treatments.

Table (1): Morphometric dimensions (in mm) of *Capillaria* sp. :-

Parameter	Length	Mean \pm SD
Body length	3.30 -7.40	5.85 \pm 1.441835
Body width	0.035- 0.08	0.05275 \pm 0.011574
Mouth aperture	0.01- 0.03	0.0185 \pm 0.007826
Distance of nerve ring from the anterior extremity	0.0775- 0.3	0.15425 \pm 0.076058
Total esophagus	2.04-4.59	3.016786 \pm 0.897892
Muscular esophagus	1- 2.60	1.44 \pm 1.077281
Stichosome	1- 3.24	1.633125 \pm 0.413858
Wide of bacillary bands at stichocyte region	0.01-0.005	0.01125 \pm 0.004432
Number of stichocytes (lxw)	23-36	28.55556 \pm 4.126473
	0.075-0.11	0.094286 \pm 0.011965
	0.035-0.02	X 0.029375 \pm 0.005132
Egg	0.0625-0.045	0.0535 \pm 0.005798 X
	0.0225-0.03	0.02575 \pm 0.005898
Ovary	0.5-0.1125	0.239444 \pm 0.112478
Tail	0.2-0.6	0.39275 \pm 0.188924
Rectum	0.1 - 0.34	0.22275 \pm 0.104718
Vulva	0.075 - 0.0175	0.088611 \pm 0.01469 X
		0.029167 \pm 0.007806
End of stichosome to oesphagointestinal- junction	1 - 2.15	1.592143 \pm 0.374632
Area filled with egg	0.9 - 2.5	1.7125 \pm 0.589403

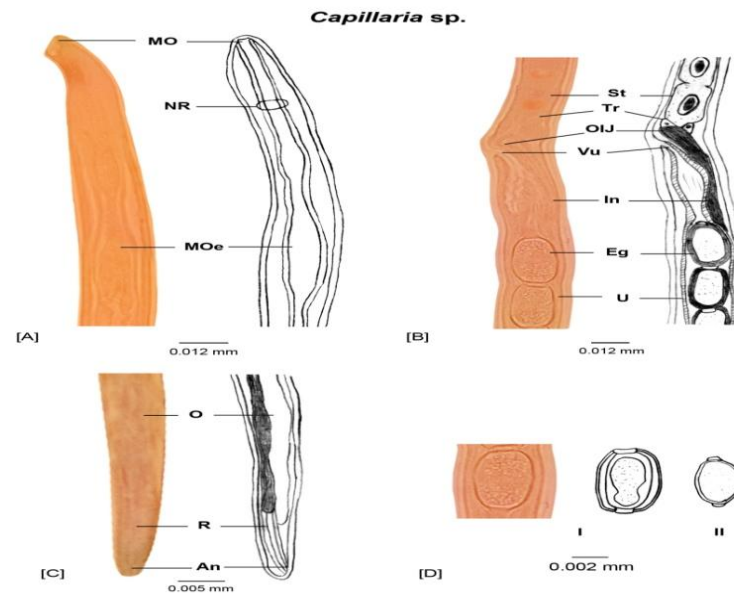


Fig. (1, A-D): Diagrammatic drawing of whole mount of female *Capillaria* sp. A, Anterior end showing mouth opening (MO), nerve ring (NR), and muscular oesophagus (MOe); B, Somatic region showing uterus (U) filled with egg (Eg) arranged in one row at its distal part before vulva (Vu) and showing two triangular glandular cells (Tr) at the oesophago-intestinal junction (OIJ) between the intestine (In) and stichosome (St); C, Posterior end of the body showing ovary (O), extends posterior to about one third of the rectum (R) and terminal anus (An); D, Eggs with polar plugs (I) Mature egg, (II) Immature egg; Hypothetical position of nerve ring (NR).

c- Muscular layer:

Muscular layer in albendazole treated worms showed deformation of the shape and orientation of the muscle fibers (Fig. 29) which was disrupted by electron lucent vacuoles enclosing electron opaque material (Fig. 30). Mitochondria appeared swallowed and decreased in number (Fig. 29). Electron dense bodies were detected between the muscle bundles (Fig. 28). The cytoplasmic part of muscle cells showed degenerated nuclei with irregular shape (Fig. 30).

Allium sativum treated worms show that the contractile part revealed the disorientation of muscle fibers with hardly distinguishable muscle septa (Fig. 38). The cytoplasmic part of muscle cells enclosed electron dense deposited (Fig. 35). Body wall was detached from the internal organs by wide spaces filled with electron dense granules (Fig. 36).

2- Digestive system:**a- Oesophagus:**

In worms treated with albendazole, the oesophageal cuticular lining appears slightly thicker with heterogeneous appearance in its layers (Fig. 32). The oesophageal wall enclosed distorted nerve elements, dense bodies and large vacuoles (Fig. 33).

In worms treated with *Allium sativum*, the cuticle lining the oesophagus was electron dense, the layers were poorly differentiated and enclosed dense deposits (Figs. 39-40).

b- Intestine:

The intestinal wall of worms treated with albendazole showed deformation of cytoplasmic organelles, swallowing of mitochondria, formation of electron lucent vacuoles, where some enclosed electron opaque material and degeneration of nuclei which enclosed heterogeneous chromatin. Intestinal microvilli appeared disrupted (Fig. 34).

The intestinal wall of worms treated with *Allium sativum* showed dilation of basal lamina, swallowing of mitochondria and accumulation of vacuoles enclosing electron opaque material (Fig. 41).

4. Discussion

The present results revealed the presence of deformed cuticle with the presence of vacuoles, dense bodies and deformation of muscle fibers of *Capillaria sp.* treated with sub lethal dose (LC₉₀) of ABZ.

The principle mode of action of albendazole is by its inhibitory effect on tubulin polymerization which results in the loss of cytoplasmic microtubules (Cook, 1990 and 1991). Albendazole is very effective anthelmintic which acts by preventing worms from utilizing glucose. Glucose is not absorbed and the parasite's glycogen becomes depleted and the worms are not able to synthesize adenosine triphosphate (A.T.P.), which is essential for survival.

Solana et al. (2001) characterized the sulfoxidative metabolism of ABZ by three different helminth species, *Fasciola hepatica*, *Moniezia expansa* and *Ascaris suum*. Their study indicated that helminthes have the capacity to biotransform benzimidazole compounds and this metabolic activity differed qualitatively and quantitatively among

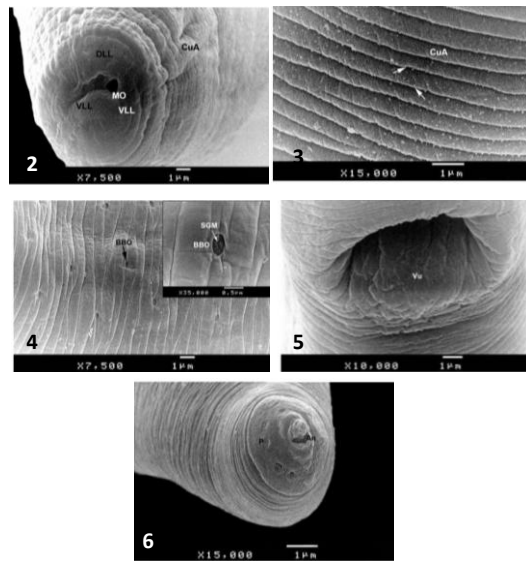
helminth species. Residue depletion of ABZ and its main metabolites after oral administration in rainbow trout, tilapia, Atlantic salmon and channel catfish has been studied by Shaikh et al. (2003 a, b, 2006). Gonzalez et al. (2009) reported that albendazole was transformed by hepatic microsomes to a single metabolite, ABZ sulfoxide (ABZ-SO) in channel catfish (*Ictalurus punctatus*), tilapia (*Oreochromis sp.*), rainbow trout (*Oncorhynchus mykiss*). Generally, anthelmintic drugs treat parasitic infestations either by: destroying the worm on contact or by paralyzing them or by altering the permeability of their plasma membrane (Mckellar and Scott, 1990; Reynoldson et al., 1992). Suitable targets in anthelmintics drug include process such as muscular activity and neuromuscular coordination, sensory process, feeding and the regulation of coleomic pressure. Some potential chemotherapeutic targets affect several groups: these include energy metabolism, nutrient uptake, and nucleic acid metabolism. Cellular integrity is important too, as attested by the success of the benzimidazoles (Jenkins and Bryant, 1996).

The current results disagree with Kim et al. (1986) who reported the disappearance of electron dense granules from muscles of *Metagonimus yokogawai* treated with ABZ.

In contrast, the results are in agreement with those of Hrcova et al. (1993) that ABZ induced damage to *T. spiralis* larvae in the muscle phase of infection, Kaur and Sood (1995) who indicated that absorption was affected most seriously by ABZ as a result of destruction of the intestine; the motility of the parasite was also affected, due to the effect of ABZ on muscle cells. The major alterations observed in *H. contortus* and *T. globulosa* after incubation with ABZ was the vacuolation and disruption of the epithelium and with those of Sukontason et al. (2000) on *Gnathostoma spinigerum* advanced third stage larvae. Where the cuticular surface was swollen and overlaid with fuzzy material and the spines on the posterior part of the body were removed after exposure to albendazole sulfoxide in vitro.

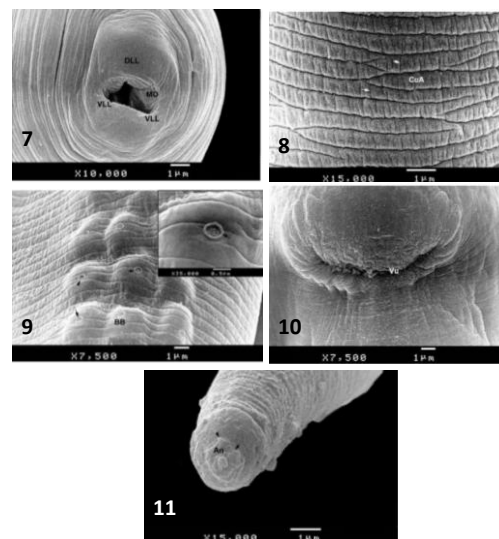
The present results agree with Arunyanart et al. (2009) who reported that the transmission electron micrographs of *Gnathostoma spinigerum* advanced third-stage larvae ABZ treated, revealed a damaged body wall, especially in the non-contractile part of the muscular layer. The body walls of the ABZ treated larvae demonstrated a significant decrease in the number of mitochondria in the non-contractile muscular part. Some mitochondria had large vacuoles, became degenerated and distorted. The nuclei also degenerated and developed irregular shapes.

The present findings are similar to those of Sant' Anna et al. (2011) of the ultrastructure of *Caenorhabditis elegans* treated in vitro with Oryzalin, a compound with similar action as the benzimidazole, where the cuticle detached from the hypoderm. Mitochondria appeared swollen and presented modified crista. The SEM observations made by Shalaby et al. (2012) showed that the changes in *H. contortus* adult worms after 24 h incubation with 50 ng/ml ivermectin affected the buccal capsule other than the cuticle.



Figs. (2-6): scanning electron micrographs of female *Capillaria sp*

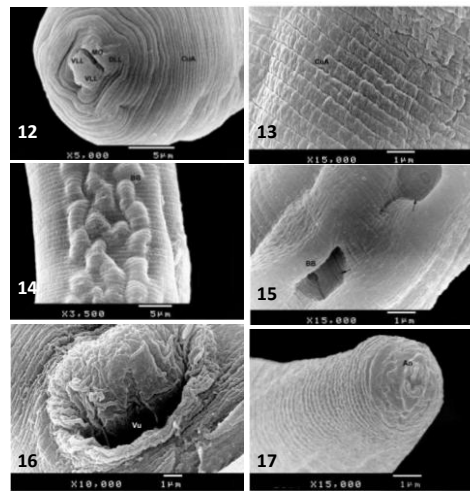
Fig.2:Enface view of cephalic end showing the mouth opening (MO), surrounded by three lips;two ventro lateral (VLL) and one dorso lateral lip (DLL). Note the characteristic cuticle annulations (cuA) at the anterior end,**fig.3:**High magnification showing cuticular annulations (arrow)(cuA),**fig.4:**High magnification of bacillary band opening (BBO) appear as round structures containing semi granula material (SGM), **fig.5:**Somatic region of the female showing the vulva (Vu) at the second third of the body,**fig.6:**Posterior end of female showing the anus (An) with small anal papillary structures (P).



Figs. (7-11): scanning electron micrographs of female *Capillaria sp.* Treated with LC90 of albendazole

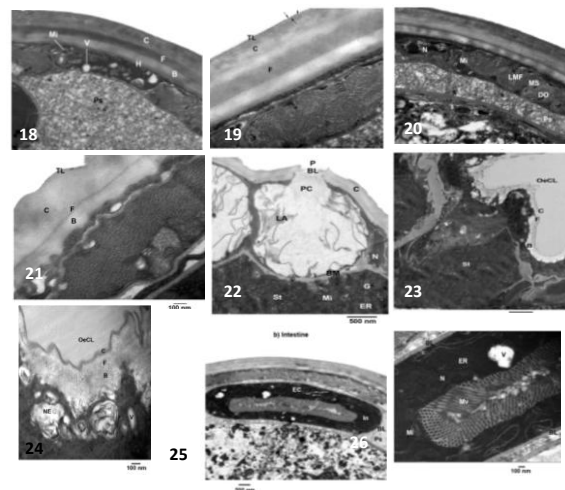
Fig.7:Enface view of cephalic end showing the mouth opening (MO), become swollen and flaccid with loss of the normal structure of the lips; dorso-lateral (DLL) and ventro-lateral (VLL), **fig.8:**Somatic region show corrugated cuticle annulations (cuA) (arrow),**fig.9:**Bacillary bands (BB) transformed into swollen structures. The cuticular rim of bacillary pores is swollen and inflated (arrow),**fig.10:**Vulva (Vu) is deformed with swollen cuticle, **fig.11:**Posterior end of female

showing deformation of anus (An) and swallowing of the cuticle around it (arrow heads) and disappearance of anal papillary structures .



Figs. (12-17): scanning electron micrographs of female *Capillaria sp.* treated with LC90 of *Allium sativum* (garlic)

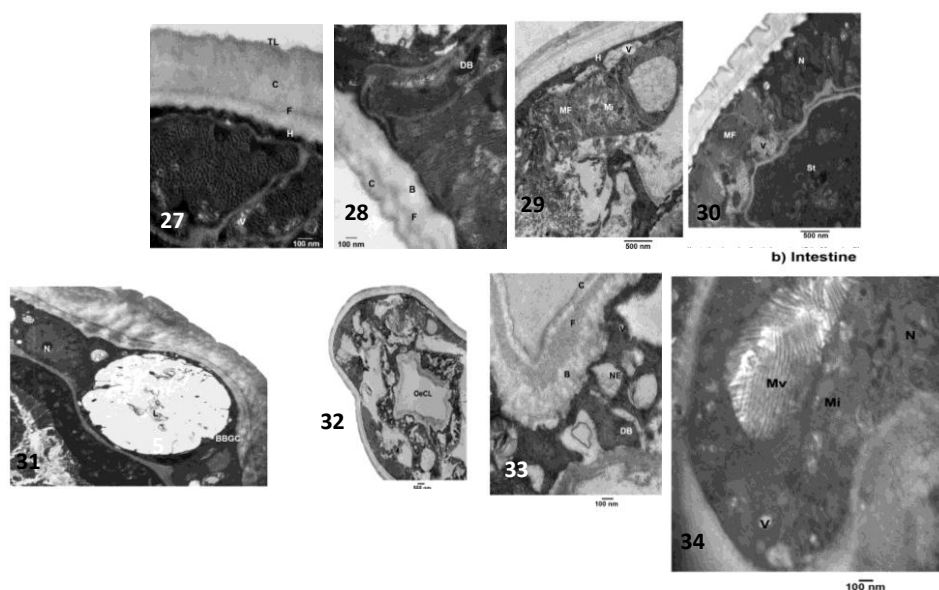
Fig.12:Enface view of cephalic end showing the mouth opening (MO) is deformed with contraction of cuticular annulation (cuA),**fig.13:**Somatic region showing wrinkled and corrugated cuticular annulations (cuA),**fig.14:**Bacillary bands (BB) are extensively swollen ,**fig.15:** Bacillary bands (BB) gland opening deeply embedded in cuticular grooves (arrow),**fig.16:**Vulva (Vu) is deformed with notable damage of cuticular edge,**fig.17:**The cuticle around the anus (An) become swollen and corrugated .



Figs. (18-26): Transmission electron micrographs of female *Capillaria sp.*

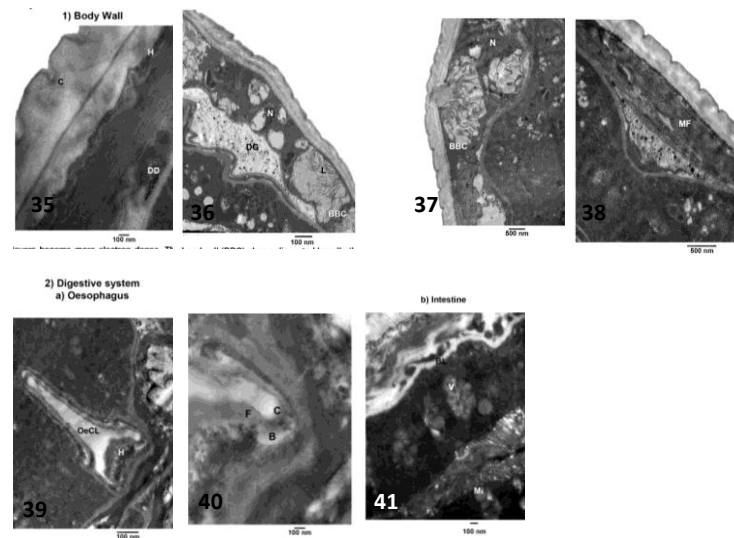
Fig.18:Body wall showing cortical (C) , fibrillar (F), and basal (B) layers of the cuticle. Hypodermis (H) is lying under the cuticular layer and contains numerous mitochondria (Mi) and round electron lucent vacuoles (V). pseudocolom (Ps) appears below the body wall,**fig.19:**The outer most triple-layered epicuticle (TL) consists of two electron dense layers enclosing an electron opaque one (arrow). The corticale layer (C) is electron dense layer with inner electron lucent region while the fibrillar layer (F) is electron opaque,**fig.20:**The contractile part of longitudinal muscle fibers (LMF) encloses mitochondria (Mi) and dense ovoid structures (DO). Bundles of arranged muscle fibers are separated by muscle septa (MS). The nucleus (N) of muscle cells are large and flattend. Pseudocoleom (Ps) appear below the body wall,**fig.21:**Cuticle in posterior region showing outer tripled-layered epicuticle (TL). Fibrillar layer (F) is composed of

circularly arranged myofibrils, **fig.22:** Bacillary band gland cell have plug (P) penetrating the cuticle (C) and possess an extensive lamellar apparatus (LA). Across the pore at the level of the base of the cuticle is the boundary layer (BL). The region between the boundary layer and the lamellar apparatus is the pore chamber (PC). Basement membrane (BM) separate the bacillary band cell from the stichocyte (St). the nucleus (N) of the bacillary band cell is located near the basal part of the cell. The stichocyte cytoplasm contains a high concentration of rough endoplasmic reticulum (ER), Golgi system (G) and mitochondria (Mi), **fig. 23:** Transverse section of the part of the oesophagus showing trifurcated cuticle - lined lumen (OeCL) and its cortical (C), fibillar (F) and basal (B) layers and stichocyte (St), **fig. 24:** Oesophagal wall showing oesophagal cuticle lining lumen (OeCL) , and formed of corticle (C), fibrillar (F) and basal (B) layers. Vacuoles (V) and nerve elements (NE) are present, **fig. 25:** Intestine (In) lies free in the pseudocoelom (Ps). The intestinal wall is formed of single layered epithelial cells (EC) lying on basal lamina (BL), **fig. 26:** Intestinal epithelial cells showing basal lamina (BL), numerous mitochondria (Mi), large nucleus (N), endoplasmic reticulum (ER), vesicle (V) and long microvilli (Mv).



Figs. (27-34): Transmission electron micrographs of female *Capillaria sp.* treated with LC90 albendazole .

Fig. 27: Cuticle showing the outer triple-layered epicuticle (TL) became dense and granular. Fibrillar layer (F) decreased in size and became less electron dense. Hypodermis (H) appears more electron dense, **fig.28:** Cuticle showing that no change was detected in the basal layer (B). electron dense bodies (DB) detected between the muscle bundles, **fig.29:** Hypodermis (H) contains a lot of vacuoles (V) and appears detached from the cuticle in many places. Muscular layer shows disruption of the shape and orientation of muscle fibers (MF). Mitochondria (Mi) appear swollen and decrease in number, **fig. 30:** Body wall at the level of stichocyte (St) . Muscle fibers (MF) enclose electron lucent vacuoles (V) containing electron opaque material . The cytoplasmic part of muscle cells degenerated nucleus (N) with irregular shape, **fig. 31:** Bacillary band gland cell (BBGC) lost its characteristic lamella (L). Notice : fragmented chromatin of nucleus (N), **fig. 32:** Oesophagal region showing the cuticle-lined lumen (OeCL), **fig. 33:** Oesophagal cuticular lining appears thicker with heterogeneous appearance in its layers, cortical (C), fibrillar (F) and basal (B) layers. The oesophagal wall encloses distorted nerve elements (NE), dense bodies (DB) and large vacuoles (V), **fig. 34:** Intestinal wall showing enlargement of mitochondria (Mi), formation of electron lucent vacuoles (V) and degeneration of nucleus (N) enclosing heterogeneous chromatin. Intestinal microvilli (Mv) appear disrupted.



Figs. (35-41): Transmission electron micrographs of female *Capillaria sp.* treated with *Allium sativum* (garlic)

Fig. 35: Cuticular layers become more dense. the corticle layer (C) become thicker. Hypodermis (H) become more electron dense and encloses electron dense deposites. Cytoplasmic part of muscle cells encloses electron dense deposites (DD), **fig. 36:** Bacillary band cell (BBC) shows disrupted lamella (L) and nucleus (N) with fragmented heterogenous chromatin. Body wall is separated from the internal organ by wide space filled with electron dense granules (DG), **fig. 37:** Bacillary band cell (BBC) shows nucleus (N) with dispersed fragmented heterogenous chromatin, **fig. 38:** The muscle contractile part reveals the disorientation of muscle fibers (MF) with hardly distinguishable muscle septa, **fig. 39:** Oesophagus shows electron dense, trifurcated cuticle-lined lumen (OeCL). Hypodermis (H) in the body wall encloses electron lucent areas, **fig. 40:** The three characteristic layers, cortical (C), fibrillar (F), basal (B) of the cuticle become hardly differentiated from each other, **fig. 41:** Intestinal wall shows dilation of basal lamina (BL), swallowing of mitochondria (Mi) and accumulation of vacuoles (V) enclosing electron opaque material.

The buccal capsule which presented a smooth surface in control worm lost its normal aspect and showed distortion with severe blebbing of the lips.

The present study revealed that *Allium sativum* water extract proved to be an effective in vitro anthelmintic against *Capillaria sp.* However, Pena et al. (1988) reported that minced garlic used in *Cyprinus carpio* infested with *Capillaria spp.* showed the greatest activity (75%) by the hexane extract, while the aqueous extract showed no anthelmintic effect. Our study is in accordance with Sutton and Haik (1999) who proved the efficacy of boiled garlic water extract as anthelmintic against Strongylidae in horses and donkeys in Israel.

The present study revealed that LC90 of *Allium sativum* water extract and albendazole (17.161×10^3 ppm, 5.54×10^6 ppm, respectively) had a high lethal (90%) effect on *Capillaria sp.* in vitro after 24 hours exposure time.

Damage of the cuticular surface was apparent in both treatments, but higher with *Allium sativum* than in case of albendazole. The mouth opening was swollen and lost the natural shape of the lips. Both treatments had severe effects on the parasite bacillary bands and caused pronounced effect on the muscles as revealed by changes in the form and integrity of shape after 24 hours incubation time.

5 References

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