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In- vitro evaluation of antithelminthic activity of albendazole and three midicinal plant extracts on *Capillaria sp* and its characterization with RAPD-PCR.

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ABSTRACT

Capillaria species are nematodes of serious fish diseases which may be transferred to human. The present study aimed to evaluat the anthelminthic activity of different concentrations of albendazole and three medicinal plants (*Allium sativum, Lupinus termis,* and *Cucrbita pepo)*on *Capillaria sp.* Infested fresh water catfishes, *Bagrus docmac* and *B.bayad.*collected from Menoufia and Kalyobia Governorates, Egypt. Five different media; RPMI 1640(Formula 1), RPMI 1640 (Formula 11), RPMI 1640 (Formula 111), Hank`s saline and Natural calf serum in optimum conditions were used for in vitro maintaining of *Capillaria* sp. Natural calf serum was the best medium for maintenance of *Capillaria* sp. as observed by high activity and low mortality rate in optimum conditions for 24 hours (pH; 7&20^{o}C). Albendazole at a dose of 5.5x10^{6}ppm showed high mortality rate (86.6%) after 24 hours exposure time. Water extracts of the medicinal plants (*A.sativum ,l.termis,*and *C.pepo)* have higher effect on the mortality rate of adult *Capillaria* sp. in vitro than albendazole. *A.sativum* water extract was high effective than *L.termis* and *C. pepo* extracts where lower concentration (18x10^{3} ppm) gave maximum mortality rate (100%) 24 hours post exposure. DNA of *Capillaria* sp. was isolated and the RAPD-PCR was applied .using 6 arbitrary primers: [G-11^{b} (TGCCCGTGG), G-15^{b} (ACTGGGACTC), G-18^{b} (GGCTCATCTC), M-1(AGGTCACTGA), M-are(ATCTGGCAAC),G-7^{b} (GAACCTGCGG)].The obtained result indicated that RAPD-PCR assay can be used to differentiate *Capillaria* sp. from its geneticallay close species of the superfamily Trichuroidea in which genera *Trichinella, Trichuris* and *Capillaria* are included. RAPD-PCR has proved useful to detect genomic instability and DNA variation induced by treatments of sublethal concentrations (LC_{50}) of albendazole and *Allium sativum* water extract.

Keywords: *Capillaria,* In vitro, Albendazole, *Allium sativum,*anthelminthic plant extracts .

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INTRODUCTION

Capillaria is a parasitic nematode which parasitizes many classes of vertebrates, including humans. Small fresh water and brackish - water fish and probably fish- eating birds are the source of infection [1] .

Benzimidazoles success rates have been recorded to reach 100% in the treatment of most nematode [2].The benzimidazole anthelminthics represent the beginning of the modern chemical assault on helminth parasites [3]. Albendazole (ABZ) is a broad-spectrum anthelminthic drug used all over the world in human and veterinary medicine [4]. It had vermicidal, ovicidal, larvicidal activities by binding to intracellular tubulin, inhibiting essential absorptive functions in the organisms [5]. Boix ***et al.*** [6] studied the effect of triclabendazole sulfoxide on Embryolethality in Zebrafish and mouse in vitro.

Alternatives to the commonly used chemotherapeutics are needed for several reasons: Helminth parasites are becoming resistant to almost every chemical class of available anthelminthics, the presence of environmental pollution and human health concerns, the growing desire among the general population for more natural and environmentally friendly treatments[7]. The medicinal value and uses of lupine as anthelmintic described by [8] .It contain 0.3–3% of alkaloid and Lupinus alkaloids have pronounced effect on the nervous system of insects [9], lupin extract had suppressive effect on plant parasitic nematodes [10].

Cucurbitin is one of the major components in pumpkin seeds that has shown antiparasitic activity in vitro [11] they indicated that pumpkin was used against tapeworms and threadworms. Iqbal ***et al.*** [12] reported that the aqueous, ethereal and alcoholic extracts of *C. Mexicana* (Cucurbitaceae; Kuddu) seeds have exhibited good anthelminthic activity against *Moniezia expansa, Fasciola buski, Ascaris lumbricoides* and *Hymenolepis diminuta*.

Anthelmintic activities of crude aqueous and hydro-alcoholic extracts of the leaves of *Eucalyptus globulus* against the egg and larvae of nematodes naturally infected sheep was studied by [13] .*Capillaria* species are closely related to *Trichuris* and *Trichinella* species, and the eggs of *Trichuris trichura* and *C. philippinensis* are similar in appearance [14].The surveying of genomes of parasites is enhanced by the advantage of RAPD-PCR which is a very simple, fast, and inexpensive technique that does not require either prior knowledge of the DNA sequence or DNA hybridization [15].

RAPDs have been used to study genetic diversity in many parasites, including *Scistosoma mansoni* and *Trypanosoma cruzi* [16]. RAPDs have also been used to develop diagnostic assays by identifying regions of DNA that are polymorphic between strains or species of parasite e.g. nematodes [17]. RAPD-PCR assay has proved useful to detect genomic instability manifested such as point mutation, chromosomal rearrangements, deletion and insertions [18]. In genetic toxicology most RAPD-PCR studies describe changes such as differences in band intensity and/or gain or loss of RAPD-PCR bands [19].

Babaei ***et al.*** [20] revealed that RAPD-PCR technique with multilocus pattern can be used as useful tool for evaluating genetic diversity and detecting mutantion. Magdalena and Nohynkova [21] described the effects of metronidazole treatment on DNA and cell cycle progression in vitro.

The present study aimed to evaluat the anthelminthic activity of different concentrations of albendazole and three medicinal plants (*Allium sativum, Lupinus termis,* and *Cucrbita pepo)*on*Capillaria sp.* Infested fresh water catfishes. DNA of *Capillaria* sp. was isolated and the RAPD-PCR was applied using 6 arbitrary primers.

MATERIALS AND METHODS

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. 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 powder of albendazole is white and insoluble in water [22] acetone and alcohol 70% make suspension with 98% DEMSO and soluble in 100% DEMSO.

Cucurbita pepo (pumpkin) and *Lupinus termis* (termis) seeds and *Allium sativum* (garlic) cloves were bought from Menofia Governorate markets and used in the present study.

Fish was examined immediately after collection. The stomach was removed from the viscera and opened with a pair of scissors, placed in saline solution (70%) and examined using a binocular dissecting microscope (10X). Nematodes were isolated in separate Petri-dishes containing 0.7% saline solution.

Maintenance of *Capillaria* in vitro:

No previous definite information was found about the in vitro maintenance of this fish parasite. Different laboratory conditions (e.g. pH and temperature) were tested to reveal the most suitable condition for in vitro maintenance of the parasite.

Five maintenance media (Table 1) were tested to choose the best for in vitro maintenance [23].To determine the most suitable maintenance medium, 10 ml of each maintenance medium 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) [24] .To each Petri-dish, 10 worms were added and incubated

Maintenance media:

Table (1): Components of different media in millimetre used for maintenance of *Capillaria* sp. in vitro:

Contents / Media	RPMI 1640	Hank's saline	Calf serum
Formula I	10	-	-
Formula II	5	5	-
Formula III	5	-	5
Hank's saline	-	10	-
Calf serum	-	-	10

Evaluation of anthelminthic activity of albendazole and three medicinal plants water extract against adult *Capillaria* sp. in vitro:

To detect the effective dose of albendazole against adult *Capillaria* sp; different concentrations of albendazole ($5x10^6$, $5.25x10^6$, $5.5x10^6$, $6.0x10^6$, $6.5\ x10^6$, $7.0\ x10^6$, $7.5\ x10^6$ ppm) were prepared in distilled water Dry seeds of *Cucurbita pepo* and *Lupinus termis* were blended to a fine powder while *Allium sativum* cloves were minced. To prepare 25000 ppm stock solution of plant extract, 25 gm of material (powder of seeds or minced garlic) was soaked in one litre of dechlorinated tap water for 24 hours. Then the solution was shaken for about half hour and boiled for five minutes [23].The aqueous extract was filtered through filter paper and different concentrations were prepared from the stock solution.

Ascending concentrations of *Cucurbita pepo* ($15.0\ x10^3$, $20.0\ x10^3$, $22.5\ x10^3$, $23.0\ x10^3$, $23.25\ x10^3$, $23.5\ x10^3$, $23.75\ x10^3$, $24.0\ x10^3$, $25.0\ x10^3$), *Lupinus termis* ($15.0\ x10^3$, $20.0\ x10^3$, $22.5\ x10^3$, $23.0\ x10^3$, $23.25\ x10^3$, $23.5x10^3$, $24.0\ x10^3$, $25.0\ x10^3$) and *Allium sativum* ($15.0x10^3$, $16.0\ x10^3$, $16.25\ x10^3$, $16.5\ x10^3$, $17.0\ x10^3$, $18.0\ x10^3$, $20.0\ x10^3$, $25.0\ x10^3$ ppm) were used .

Seven groups, each of 10 worms of almost the same length were transferred to sterile Petri-dishes, each containing 10 ml of maintenance medium, 2 ml of the antiseptic solution and 10 ml of the drug concentration. Triplicate experiments for each drug or plant extract concentration were performed at optimal temperature and pH, in addition to control which contained only the maintenance medium and the antiseptic solutions. Treated worms were examined and the mortality rate was calculated in all experiments after 24 hours.

$$\text{Mortality rate} = \frac{\text{Number of dead worms X 100}}{\text{Total number of worms}}$$

Treated worms were examined and the mortality rate was calculated after 24 hours. To construct the dose effect levels, the percentage of dead worms in each group was recorded. The drug which showed highest activity against *Capillaria* sp. (highest worm mortality rate) at the lowest sublethal dose was detected and used to study its effect on adult *Capillaria* sp. after 24 hours.

Molecular Methods:

DNA extraction and RAPD PCR reaction

Nucleic acids extraction was carried out according to salting out extracting method **[24]** and modifi After isolation of parasites DNA, RNA was digested by adding DNAase-free RNAase (10µg/ml). The amplification reaction mixture was set in a volume of approximately 50µl consisting of 25 µl of master mix containing 2.5 mM $MgCl_2$, dNTPs (2.5 mM each), and 1 unit taq polymerase (from Fermentas, Germany). 1µl of (25 pmol) primer was added to each reaction, with cation introduced by **[25].** RAPD-PCR reaction was performed according to**[26].** the use of 6 random primers (from Metabion, Germany)M-1 (5`-AGGTCACTGA-3`), M-are (5`-ATCTGGCAAC-3`), G-7[b] (5`-GAACCTGCGG-3`) and G-11[b] (5`- TGCCCGTCGT-3`), G-15[b] (5`-ACTGGGACTC-3`), G-18[b] (5`- GGCTCATCTC-3`).
DNA was denaturated for 5 mins. at 94°C, amplification was programmed for 39 cycles of 1 min. at 94°C (denaturation), 1 min. at 38°C (annealing) , and 2 min. at 72°C (extension) then 7 mins at 72°C in Biometra thermal cycler.

DNA bands were measured by software Gel - Pro program as maximum optical density values using Gel- electrophoresis assay.

Table (2) showing the used random primers sequence and their C+G content.

Primer	Sequence	C+G content
M-1	AGGTCACTGA	50%
M-are	ATCTGGCAAC	50%
G-7[b]	GAACCTGCGG	70%
G-11[b]	TGCCCGTCGT	70%
G-15[b]	ACTGGGACTC	60%
G-18[b]	GGCTCATCTC	60%

RESULTS

Maintenance of *Capillaria sp*. in vitro

Incubation of adult *Capillaria* sp. in different media showed that natural calf serum is the most appropriate media as observed by the high activity and low mortality rate of the worms during the incubation period (24 hours) when compared with other media (Figs. 1,2). So, natural calf serum was selected for the in vitro screening of anthelminthic drug and medicinal plants on *Capillaria* sp. Different pH and temperatures showed that the optimal pH is 7 and the optimal temperature is 20°C, for 24 hours.

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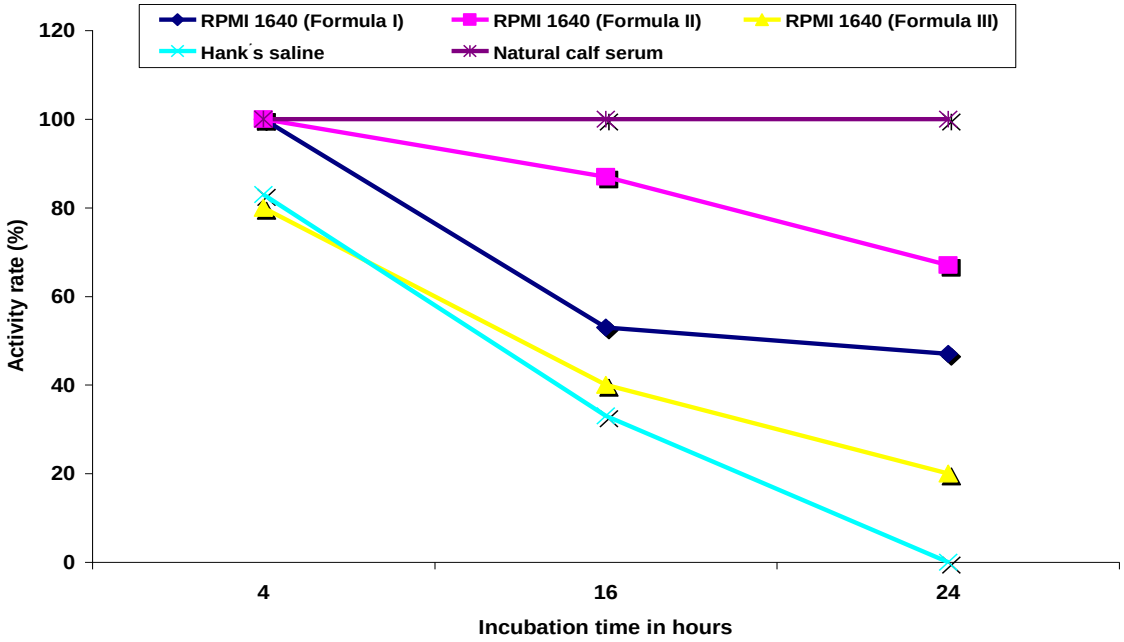

Fig. (1): The activity of *Capillaria* sp. in different incubated media.

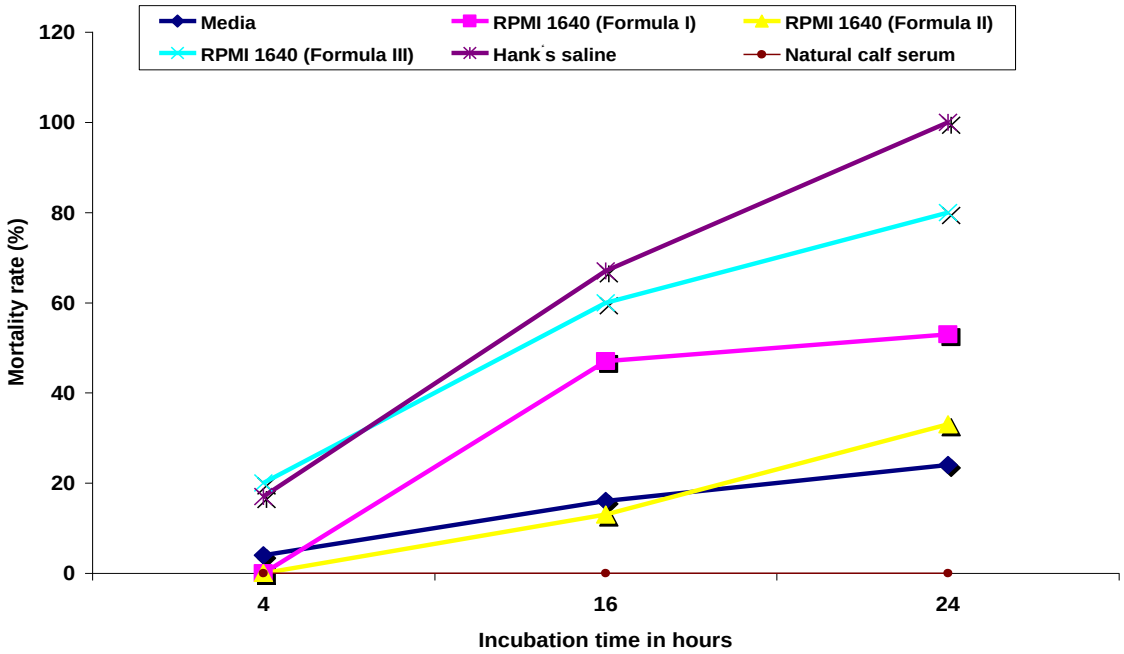

Fig. (2): The mortality rate of *Capillaria* sp. in different incubated media.

Effect of anthelminthics on *Capillaria sp*. in vitro:

Chemical drug:

The effect of albendazole was tested against adult *Capillaria* sp. at different concentrations for 24 hours exposure time . High concentrations of albendazole (6.0 x10^6 ppm, 6.5 x10^6, 7.0 x10^6, and 7.5 x10^6 ppm, respectively) showed maximum mortality rate (100%). While lower concentrations, 5.25 x10^6, and 5.5 x10^6 ppm, allowed worms to survive longer, where the mortality rates reached 53% and 86.6%, respectively after 24 hours exposure time (Fig. 3).

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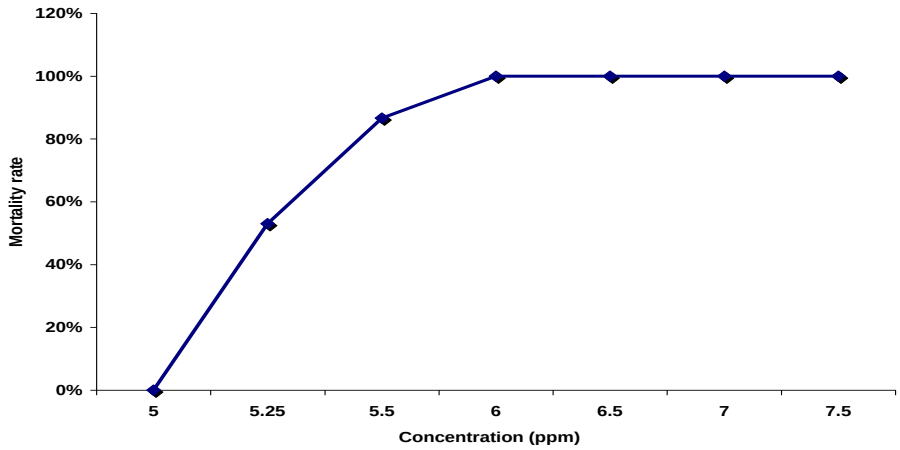


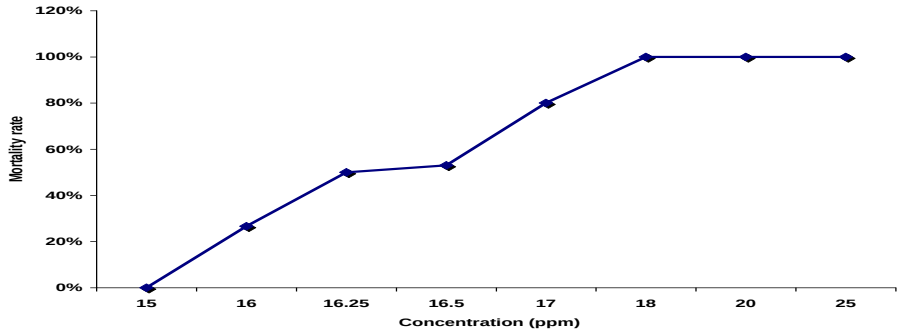
Fig. (3): Mortality rate of *Capillaria* sp. at different concentrations of albendazole after 24 hours exposure time.

Anthelminthic medicinal plants:

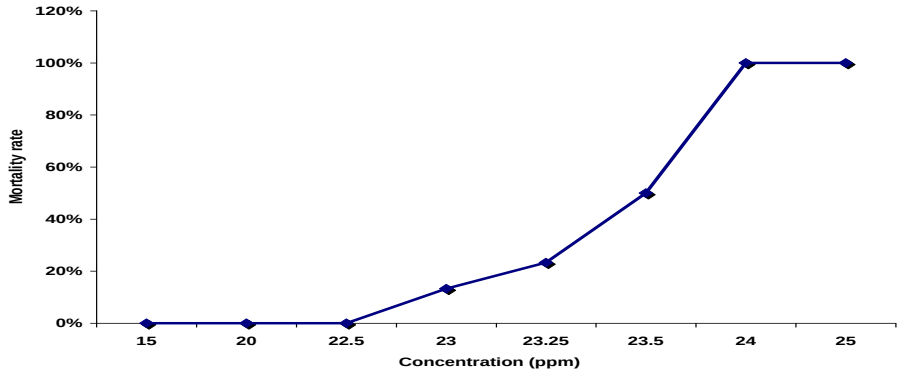
Water extracts of Allium sativum cloves**,** Lupinus termis seeds, and Cucurbita pepo seeds were tested in vitro against adult Capillaria sp. Results showed that 17.0 $x10^3$ ppm of Allium sativum water extract gave high mortality rate 80% after 24 hours exposure time. While higher concentrations of Lupinus termis and Cucurbita pepo 23.5 $x10^3$ ppm and 23.75 $x10^3$ ppm respectively, gave relatively lower mortality rate (50%) up till 24 hours post exposure time.

Water extract of Allium sativum was more effective than that of Lupinus termis and Cucurbita pepo. The highest mortality rate (100%) was recorded at the concentrations of 18 $x10^3$ ppm for Allium sativum, while the same ratio was achieved at relatively higher concentration (24 $x10^3$ ppm) for both Lupinus termis and Cucurbita pepo (Figs. 4, A.B&C).

A, ***Allium sativum***,



B , ***Lupinus termis)***



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C, ***Cucurbita pepo)***

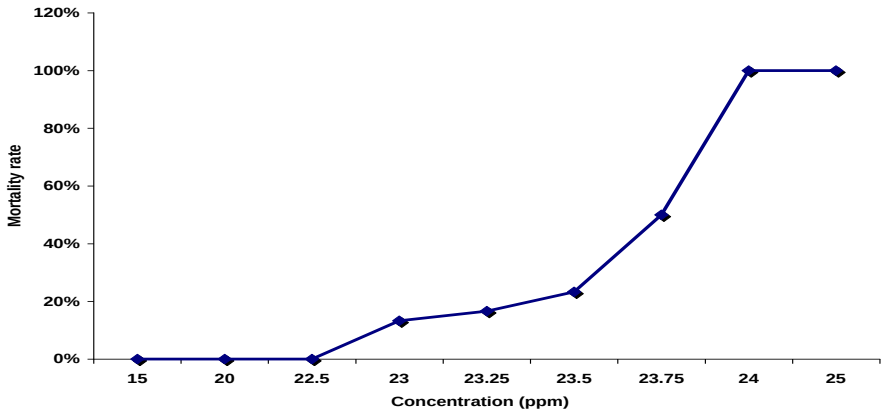

Fig. (4): Mortality rate of *Capillaria* sp. at different concentrations of midicnal plants water extract after 24 hours exposure time(A *Allium sativum*, B *Lupinus termis*, C *Cucurbita pepo*)

Molecular Studies

Characterization of *Capillaria sp*. by RAPD-PCR -PCR assay*:*

The present study was attempted to use RAPD-PCR technique, using 6 arbitrary primers to determine the specific bands of *Capillaria* sp. given by these primers. DNA was isolated from *Capillaria* sp. collected from *Bagrus bayad* and *B. docmac* fishes.The PCR products were analyzed by electrophoresis in 1.3% agrose gel and visualized with ethidium promide staining. Sequences of used primers and their GC content were shown in table 2.

Figure (5) illustrates the electrophoretic results of RAPD-PCR amplification of the fish nematode, *Capillaria* sp., DNA combination with primer M-1, M-are, G-7 ^{b} (GC 70%), G-11^{b} (GC 70%), G-15 ^{b}, G-18 ^{b} , respectively. Where M is 1kb plus DNA ladder.

Table 3 shows that primers M-1 (5`- AGGTCACTGA -3`), M-are (5`- ATCTGGCAAC -3`), G-7^{b} (5`- GAACCTGCGG -3`), G-15^{b} (5`- ACTGGGACTC -3`), G-18^{b} (5`- GGCTCATCTC -3`) gave no bands with *Capillaria* sp. DNA. While RAPD-PCR amplification product of *Capillaria* sp. DNA and primer G-11^{b} oligonucleotides, 5`- TGCCCGTCGT -3, gave 4 distinct sharp bands ranging in molecular weight from 2807.3 to 7080.5 base pairs.

Table (3): RAPD-PCR data report analysis for molecular weight by base pair for *Capillaria* sp. using 6 arbitrary primers:

M-1	M-are	G-7^{b}	G-11 ^{b}	G-15 ^{b}	G-18 ^{b}
-	-	-	7080.573	-	-
-	-	-	5902.484	-	-
-	-	-	3901.806	-	-
-	-	-	2807.321	-	-

RAPD- PCR application for in vitro studies*:*

DNA instability of *Capillaria* sp. in vitro treated by LC_{50} concentration of albendazole and *Allium sativum* water extract was evaluated by RAPD-PCR using the previously mentioned 6 arbitrary primers. The present study illustrated the disappearing of three bands in DNA material of specimens treated with albendazole with the primer G-11^{b} and the presence of one band at 5901.974 base pair (Fig. 6, Table 4).

In LC_{50} of *Allium sativum* treated specimens, three bands, (4902.820, 2089.482 and 1480.364 base pairs) of which are different rather than those in the control one (2806.223 base pair) matches with the same primer (G-11^{b}) in comparison with control. In addition, four bands with primer G-7^{b} were detected (Fig. 7, Table 5). RAPD-PCR assay was employed to detect genomic alterations in the treated worms and it could

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clearly show that some DNA changes had occurred. Differences were apparent in the form of the appearance of new amplified bands or the disappearance of some bands (Figs, 6 and 7).

Table (4): RAPD-PCR data report analysis for molecular weight by base pair for *Capillaria* sp. treated with LC_{50} concentration of albendazole using 6 arbitrary primers:

M-1	M-are	G-7[b]	G-11[b]	G-15[b]	G-18[b]
-	-	-	5901.974	-	-

Table (5): RAPD-PCR data report analysis for molecular weight by base pair for *Capillaria* sp. treated with LC_{50} concentration of *Allium sativum* water extract using 6 arbitrary primers:

M-1	M-are	G-7[b]	G-11[b]	G-15[b]	G-18[b]
-	-	6098.543	4902.820	-	-
-	-	4708.421	2806.223	-	-
-	-	1870.862	2089.482	-	-
-	-	980.321	1480.364	-	-

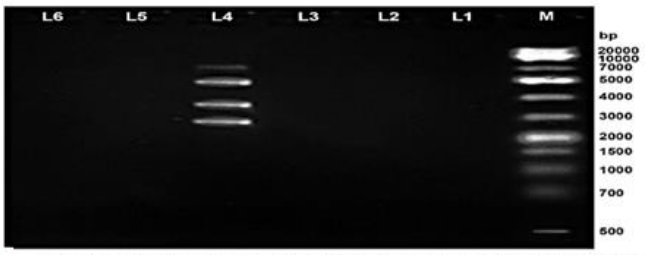

Fig. (5) : RAPD-PCR results for *Capillaria* sp. using 6 arbitrary primers, Lane 1 = M-1 (5`- AGGTCACTGA -3`), Lane 2 = M-are (5`- ATCTGGCAAC -3`), Lane 3 = G-7[b] (5`- GAACCTGCGG -3`), Lane 4 = G-11[b], (5`- TGCCCGTCGT -3), Lane 5= G-15[b] (5`- ACTGGGACTC -3`), Lane 6 = G-18[b] (5`- GGCTCATCTC -3`), M = 1 kb plus ladder.

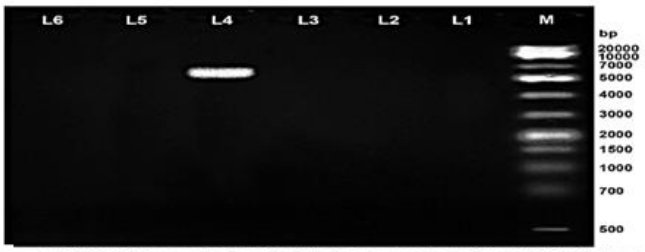

Fig. (6) : RAPD-PCR results for *Capillaria* sp. treated with sublethal dose (LC_{50}) of albendazole in vitro using 6 arbitrary primers, Lane 1 = M-1 (5`- AGGTCACTGA -3`), Lane 2 = M-are (5`- ATCTGGCAAC -3`), Lane 3 = G-7[b] (5`- GAACCTGCGG -3`), Lane 4 = G-11[b], (5`- TGCCCGTCGT -3), Lane 5= G-15[b] (5`- ACTGGGACTC -3`), Lane 6 = G-18[b] (5`- GGCTCATCTC -3`), M = 1 kb plus ladder.

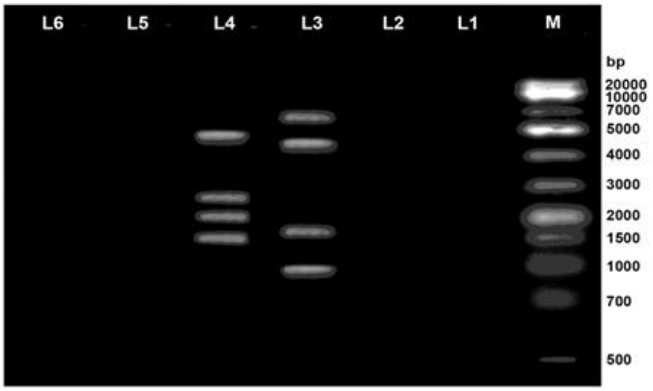

Fig. (7) : RAPD-PCR results for *Capillaria* sp. treated with sublethal dose (LC_{50}) of *Allium sativum* in vitro using 6 arbitrary primers, Lane 1 = M-1 (5`- AGGTCACTGA -3`), Lane 2 = M-are (5`- ATCTGGCAAC -3`), Lane 3 = G-7[b] (5`- GAACCTGCGG -3`), Lane 4 = G-11[b], (5`- TGCCCGTCGT -3), Lane 5= G-15[b] (5`- ACTGGGACTC -3`), Lane 6 = G-18[b] (5`- GGCTCATCTC -3`), M = 1 kb plus ladder.

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DISCUSSION

Three media, RPMI 1640 (in 3 formula), Hank's saline and natural calf serum at different concentrations were used for in vitro maintenance of *Capillaria* sp.

The present study revealed that the medium containing RPMI 1640 only did not allow *Capillaria* sp. to survive for the needed time of the experiment. Maintenance of the parasite in all RPMI 1640 formulas showed decrease in the viability and an increase in the mortality rate of the worms during the time of the maintenance. Maintenance of the parasite in Hank's saline decreased the activity and increased the mortality rates of the maintained worms.

The current study reported a successful maintenance of *Capillaria* sp. in natural calf serum with minimum mortality rate during 24 hours maintenance period. This finding shows that calf serum has the optimal nutritional requirements necessary for worm survival. For this reason natural calf serum was used to incubate *Capillaria* sp. for in vitro evaluation of the anthelminthic activity of selected drug. The present findings are in agreement with **[27]** who reported that this maintenance medium is typically used for screening potential anthelminthics or for generating excretory/ secretory products of the parasites.

Effect of anthelminthics:

Generally, anthelminthic drugs treat parasitic infestations either by: destroying the worm on contact or by paralyzing them or by altering the permeability of their plasma membrane[28].Suitable targets in anthelminthics 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 [29].

Benzimidazoles are thought to disrupt enzymes of the respiratory pathways, therefore disrupting energy synthesis, resulting in insufficient ATP [30]. Others reported mode of action includes inhibition of glucose, amino acid and fatty acid uptake and inhibition of various metabolic enzymes. The effect of benzimidazoles on tubulin polymerization and on respiratory enzymes has also been reported [31]**.** The primary action of these drugs at the biochemical and cellular levels involve their interaction with the eukaryote cytoskeletal protein, tubulin [28]**.**

Canete *et al.* [32] illustrated that tubulin is the first molecular target of benzimidazoles. These compounds act on the inhibition of the polymerization of tubulins and disrupt the function of microtubules in parasite cells. The second target is to block glucose uptake in adult helminthes inducing depletion of the parasite's glycogen stores.

In the present study, ABZ was in vitro effective drug against *Capillaria* sp.*,* where 5.5 x 10^6 ppm caused 86.6% mortality rate at 24 hours incubation time. **Arias-Diaz et al.[33]** reported that ABZ has shown activity against *Anisakis* in vitro with maximum activity at 50 µg/ml, it was able to kill almost all larvae at 48h. They added that ABZ efficacy was dependent on the pH value of medium, being less officious in acidic pH than in pH 7.4 or 8.

Effect of medicinal plants against Capillaria sp.:

Medicinal plants from ancient time were considered as the God's gift to human beings as they are having profound use in the treatment of various diseases and disorders **[34]** .For these reasons there is an urgent need to develop newer, selective and eco-friendly agents to control helminth infections. Plant based anthelmintics can be both sustainable and environmentally acceptable. The present study evaluates the efficacy of three medicinal plants namely: *Allium sativum* (garlic), *Lupinus termis* (termis), and *Cucurbita pepo* (pumpkin) water extracts against *Capillaria* sp. in vitro for 24 hours exposure time.

The most well known and widely studied garlic compound is allicin. Allicin and other thiosulfinates are unstable. Dilution and dissolving in water can greatly improve their stability **[35].**

Mansson **[36]** 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 activity of *Allium sativum* extract or many of its purified components has been tested *Haemonchus contortus* **[37]**. The present study revealed that *Allium sativum* water extract proved as an effective in vitro anthelminthic against *Capillaria* sp. (LC50) of *Allium sativum*, 16.25 $x10^3$ ppm caused 50% mortality rate of *Capillaria* sp. after 24 hours incubation time in vitro it is in accordance with **[38]** who proved the efficacy of boiled garlic water extract as anthelminthic against Strongylidae in horses and donkeys in Israel.

In the current study, *Lupinus termis* was in vitro effective against *Capillaria* sp*.,* where 23. 5 $x10^3$ ppm caused 50 % mortality rate after 24 hours incubation time. **Tahara *et al.* [39]** noted that *Lupinus termis* has been traditionally used as anthelminthic against most common nematodes and that lupins were known to contain various flavonoids with antimicrobial activity. In the present study, *Cucurbita pepo* was in vitro effective against *Capillaria* sp*.,* where 23. 75 $x10^3$ ppm caused 50 % mortality rate at 24 hours incubation time. The present results are in agreement with **[40]** who revealed that the aqueous extract of *C. mexicana* possesses the most significant efficacy against *Fasciola buski, Ascaris lumbricoides* and *Hymenolipis diminuta* in vitro as compared to alcoholic and etheral extracts. Bruneton **[41]** reported that the chemical structure of the cucurbitin compound is close to that of nematicidal compound named kainik which has a neurodegenerative action on nematodes by substituting for glumate, for this reason cucurbitin could have the same mode of action on *Haemonchus contortus*. The non-proteic amino acid cucurbitin is well known for its potential anthelminthic properties against *Taenia* in humans and against the growth of the immature trematode *Schistosoma japonicum* **[42]** and **[43]** reported that cucurbitin is an amino acid (3- aminocarboxy pyrrolidine) that has shown antiparasitic activity in vitro.

It could be very well concluded that Cucurbita pepo has anthelminthic effects against different parasites **[44]** & [13] .They also noted that*, Cucurbita* seeds are known to contain several secondary metabolites of medicinal interests such as amino acids and terpenoid compounds which could be suspected for anthelminthic activity.

In the present study, six primers were used according to their ability to amplify, by RAPD-PCR, species of the superfamily Trichuroidea**[45]** in which genera *Trichinella*, *Trichuris* and *Capillaria* are included. No amplification occurred for the primers M-1 and M-are, $G\text{-}7^b$, $G\text{-}18^b$, disagreeing with the results of Humbert and Cabaret**[46]** who were able to amplify DNA from *Trichuris* with the primers $G\text{-}7^b$ and $G\text{-}18^b$ and Rodriguez**[48]** who applied M-1 and M-are with DNA from *Trichinella* and Martinez **[45]** who applied M-1, M-are, $G\text{-}7^b$, $G\text{-}11^b$, $G\text{-}15^b$ and $G\text{-}18^b$ with DNA from *Thichuris trichiura* eggs.

Comparing the present results with those of other trichuroides revealed that primer $G\text{-}11^b$ differentiate *Capillaria* sp. from *Trichinella* and *Trichuris* species. In the present study DNA extraction of adult worms containing eggs was used. Since, RAPD-PCR is a method based on the detection of genetic markers using arbitrary primers; it is very sensitive to DNA quality. Its molecular approaches have contributed significantly to specific identification of individual species and are of great importance for epidemiological and phylogenetic studies**[48]**. The identification of single egg to species can aid in determining the source of infection and the clinical source in infected host (human, animal, fish,.. etc) **[49].**Until now, *Capillaria* and *Trichuris* infections can interfere because of the great similarity of their egg appearance. Hence, the treatment and control of such diseases would not be put on the wright line, because every parasite has its treatment manner, especially with relapses. The present study revealed a powerful, easy, simple, and rapid technique (RAPD-PCR) to differentiate between these nematodes that have similar eggs.

In comparison with the control, RAPD-PCR assay showed changes in number and absence or presence of new amplified bands in the ABZ and *Allium sativum* treated worms. These findings may be related to such events as DNA damage, point mutations and complex chromosomal rearrangements induced by treatment. This in accordance with **Zhoua *et al.* [50]** who reported that one or both priming sites can be lost due to mutations that reduce or eliminate priming.Thus the present results demonstrated the potential of the RAPD-PCR assay for application as a powerful tool for detecting genotoxicity and possibly apoptosis induced by albendazole and *Allium sativum* in vitro.

Benzimedazoles exert their effect by binding selectively and with high affinity to the β-subunit of helminth microtubule protein, tubulin, leading to subsequent disruption of the tubulin-microtubule dynamic equilibrium **[51].** By binding to β-tubulin, benzimedazoles inhibit the polymerization of α- and β-tubulin molecules and the microtubule dependent uptake of glucose, resulting in paralysis and death.
Cytoskeleton containing microtubules plays an important role in cell functioning. Microtubules exhibit highly dynamicity due to assembly and disassembly at their ends. The involvement of microtubules in critical process like mitosis helps to design suitable chemotherapeutic agents that show antitubulin activity and thus promoting programmed cell death through different apoptotic pathways **[52].**

Molecular mechanism of benzimidazoles as an anthelmintic relies on the impairment of microtubular organization by disrupting its polymerization when bind to the β-tubulin **[53].**
Disruption of normal cytoskeletal texture through inhibition in microtubule polymerization by ABZ is thought to be a key cause of apoptosis induction by this drug. BZs are mainly orally ingested by nematodes, which cause starvation to nematodes by intestinal disruption due to ABZ-induced apoptosis in these cells. The fact that antitubulin agents can induce the initiation of several apoptotic pathways through impairment of the cytoskeletal structure is well known **[54].**

In the present study, RAPD-PCR profiles of *A. sativum* treated parasite DNA showed the appearance of new bands referring to DNA damage. The possibility of existence of the apoptotic process as an immune defense mechanism. The present results are similar to those of **Hammami *et al.*** **[55]** on plant germ cells.
In conclusion, albendazole and the three medicinal plants were efficient against *Capillaria* sp. in vitro but with dosage differences. Albendazole and *Allium sativum* showed higher damage to parasite, however, garlic is considered as higher effective by using a very lower concentration than that in albendazole.

RAPD-PCR assay proved a useful mean for differentiation between *Capillaria* and the genetically close similar species and in discovering genomic alterations induced by drug and medicinal plant treatments in vitro.

Albendazole and *Allium sativum* have anthelminthic effect on *Capillaria* sp., where treatment with LC_{50} doses of albendazole and *Allium sativum* water extract in vitro, induced DNA instability which increased the mortality rate in worms. Variations in the loci and number of bands were recorded in both treatments. We recommend mixing garlic with fishes before cooking or eating garlic after a fish meal, especially fresh water fish. Garlic serves as natural, safe, and available alternative treatment for such parasitic infections.

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