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COMPREHENSIVE STUDY OF *CONTRACAECUM SPP.* LARVAE INFECTING NILE TILAPIA IN ASSIUT GOVERNORATE: OCCURRENCE, MOLECULAR IDENTIFICATION AND EVALUATING THE *IN VITRO* NEMATICIDAL ACTIVITY OF SOFT CORAL EXTRACTS

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ABSTRACT

Contracaecum spp., a member of the Anisakidae family, presents significant challenges to both the marketability of fish and public health due to its zoonotic potential. This study explores the prevalence and seasonal variation of *Contracaecum* larvae in 100 randomly collected samples of Nile tilapia from Assiut Governorate, Egypt. Detailed microscopic and ultrastructural examinations were conducted, which were further supplemented by molecular sequencing and phylogenetic analysis. Our findings revealed an overall prevalence rate of 24%, with a notably high correlation between *Contracaecum* infection and the summer season, showing an infection rate of 50%. Molecular analysis identified *Contracaecum multipapillatum* as the dominant species in the region. Additionally, we explored the effectiveness of marine extracts-specifically Sarcophyton and Dendronephthya-as alternatives to conventional anthelmintics. In vitro assessments demonstrated that these extracts were highly effective in altering larval morphology and reducing viability in a time-dose dependent manner, with IC_{50} values of 12.52 mg/mL for Sarcophyton and 14.25 mg/mL for Dendronephthya. Treatment led to significant morphological changes observed through scanning electron microscopy, including severe cuticular damage and edema, as well as destruction at both the anterior and posterior ends. This study highlights the impact of *Contracaecum* larvae on fish in Assiut and presents promising marine-derived anthelmintic properties.

Keywords: Assiut, *Contracecum, Sarcophyton sp.* and *Dendronephthya sp*

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INTRODUCTION

Fish are a complementary part of the human diet and play a crucial role in the global diet security. However, consuming

fish also increases the risk of zoonotic parasites (Shamsi *et al*., 2019). Consuming live or undercooked fish, like smoked and marinated fish in some countries increases the risk of human infections with fish borne nematodes (Eiras *et al.*, 2016). One of the common zoonotic nematodes infecting are *Anisakis spp*. *Contracaecum* is the most common Anisakidae with worldwide distribution and mostly reported in human cases (Ziarati *et al.*, 2022)

Contracaecum species adult can infect the marine mammals and the piscivorous birds as their final hosts. Whereas third-stage larvae (which can be free or encapsulated) are able to infect the invertebrates and fish as intermediate or paratenic hosts. These third-stage larvae of *Contracaecum spp.* could also infect humans when consuming raw and undercooked invertebrates and fish (Pekmezci and Yardimci, 2019). Zoonotic infection in humans causes different symptom, such as allergic reactions, which can lead to anaphylactic shock, as well as nonspecific gastro-intestinal manifestations, including diarrhea or severe vomiting (Deng and Wang, 2022). *Contracecum sp*. larvae lack characteristic morphological features and weak organ development, so the traditional methods of diagnosis may not be valuable due to their low specificity, so it is better to use molecular identification (Aqeele *et al.,* 2024).

Various medications and drug candidates have been produced from marine natural compounds and are either in preclinical testing or clinical studies (Savić et al., 2022). The soft corals develop unique secondary metabolites to be protected from their hostile habitat. Marine natural compounds derived from soft coral have a wide variety of molecular structures, some of which have medicinal qualities (Santacruz *et al*., 2020).

One of the most distributed corals in the red sea is *Sarcophyton spp.,* Which have 14 identified species. *Sarcophyton* is rich in cembranoids 5 and steroids. terpenes and celebrants. Previous studies demonstrated that the extracted compounds from the soft coral *S. trocheliophorum* possessed a significant anticancer, as they are cytotoxic to human cancer cell lines. Moreover, it has an antimicrobial, and insecticidal potent activity (Bashar *et al.*, 2024).

The soft coral *Dendronephthya sp.* was first recorded in the Mediterranean Sea in 2023 (Nativ *et al.*, 2023). A previous study in South Korea investigated the potent anti-inflammatory effect of *Dendro-nephthya sp.* on stimulated inflammation zebrafish model. Moreover, it did not have any toxic impact on embryos (Lee, 2017). Another study done in Egypt demonstrated that *Dendronephthya sp.* ethanolic extract treatment effectively decreased the levels of nitric oxide (NO) and malondialdehyde (MDA) in induced breast cancer rats (Maued *et al.*, 2023).

In this study, we aimed to clearly identify the *Contracaecum* larvae which infect Nile tilapia in Egypt by using light and scanning electron microscopy, along with confirmation of the species of these larvae by molecular analysis, and evaluate the *in vitro* anthelmintic effect of some soft coral extracts (*Sarcophyton* and *Dendro-nephthya*) on these larvae.

MATERIALS AND METHODS

1. Ethical approval: this research has been approved by the ethical committee of the faculty of veterinary medicine under the no. 06\2025\0369

2. Study area: This research was conducted in Assiut Governorate, Egypt, which is located at approximately 27.1828° N latitude and 31.1837° E longitude

3. Sample collection:

Sampling was carried out between October 2024 to October 2025 to investigate a total number of 100 *Nile Tilapia* (*Oreochromis*

niloticus) 25 fish in each season were collected randomly from different local markets in Assiut. Fish were captured alive or freshly dead then transported to veterinary medicine parasitology lab in tightly closed plastic bags to perform parasitological examination.

4. Parasitological examination: Fish were dissected at the anal opening extended anteriorly along the fish's ventral region towards the gill chamber. In addition, another two lateral incisions were performed to reveal the body cavity for detection of *Contracecum spp.* larvae by naked eye in the body cavity (Abd-ELrahman *et al.*, 2023). The detected larvae were pulled out from the infected fish by using forceps, and any detected encapsulated larvae were released by a needle (Sameeh *et al.,* 2024).

5. Morphological identification of *Contracaecum spp.* larvae:

- **Light microscopy:**

The larvae isolated from each fish species were washed in saline solution, then killed and fixed in hot 70% ethanol. Subsequently, after they were preserved in 70% alcohol and 5% glycerin solution. They were clarified in lactophenol for 24 hours, then mounted in glycerin jelly preparation, covered with a cover glass, and examined by 10x objective lens (Elseify *et al*., 2015).

6. Molecular analysis:

- **DNA extraction:**

  From the middle portion of *Contracaecum* larvae using (QIAamp DNA Mini Kit, Catalogue no. 51304) according to manufacturer's protocol. Target gene used for DNA amplification 18S rDNA using specific *Contracecum* primers (*5*`CGCGAATRGCTCATTA-CAACAGC3′) and (5`GGGCGGTATCTG-ATCGCC3`) yielding about 900 bp (Floyd *et al*., 2005).

- PCR amplification: was done using Emerald Amp GT PCR master mix (Takara) Code No.RR310A. Each reaction mixture included (12.5*µl)* of (2x premix), (5.5 *µl)* PCR grade water, (1 *µl)* from each primer (20 pmol), (5 *µl)* from Template DNA, and the total volume was 25 *µl.* Cycling conditions started with denaturation for 5 min at 94 c, followed by 35 cycles for 30 s at 94 °C, Annealing 54 °C for 40 sec. and extension at 72 °C for 1 min, then a final extension at 72 °C for 10 min. The PCR products were analyzed to a 1.5% agarose gel in a horizontal cell (Compact M, Biometric, Germany) and then stained with ethidium bromide. DNA fragments were then observed using ultraviolet illumination.

- **Purification of PCR:**

  purification of the product of PCR by using QIAquick PCR Product extraction kit. (Qiagen Inc. Valencia CA).

- **DNA Sequencing reaction:**

  The purified product of PCR was sequenced in the forward and/or reverse directions on an Applied Biosystems 3130 automated DNA Sequencer *(*ABI, 3130, USA*).* Using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit. (Perkin-Elmer/Applied Biosystems, Foster City, CA). After sequencing A BLAST® analysis (Basic Local Alignment Search Tool) was initially performed to establish sequen-ce identity to GenBank accessions.

- **Phylogenetic analysis:**

  A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 12.1 of MegAlign module of Lasergene DNAStar software Pairwise (Madison, Wisconsin, USA), which was designed by Thompson *et al.* (1994). Phylogenetic analyses were done using maximum likelihood, neighbor joining and maximum parsimony in MEGA6 (Tamura et al., 2013).

7. Preparation of crude extracts from soft corals (*Sarcophyton sp.* and *Dendronephthya sp.*): steps of soft corals characterizations and preparations were performed according to (Mohammed *et al.*, 2024) and (Maued *et al.*, 2023).

8. In vitro study on the effect of soft coral extract on Contracecum:

- **Experiment design:**

For preparation of aqueous solution used for in vitro study 1g of raw powder of crude extract to 10 ml of DMSO. 100, in sterile 6-well plates, three concentrations were prepared from sarcophyton and Dendronephthya (15, 25 and 50 mg/mL) and three replicates were prepared for each concentration, with each well containing 10 alive *Contracaecum* larvae. The control group was placed in pure 0.9% saline then all worms were observed during the experiment period under the dissecting microscope. The worm was considered dead when it stopped moving for 5 minutes, even with light and needle stimulation.

- **Scanning Electron Microscopy (SEM):**

For SEM analysis, *Contracecum* larvae samples were fixed for 24h in 2.5% glutaraldehyde at room temperature. samples were then fixed in 1% osmium tetroxide (OsO4) in phosphate buffer solution for another 24 h, then subsequently, the specimens underwent dehydration using an ascending ethanol gradations (30%, 50%, 70%, 90% and 100%) for 30 min in each concentration .The larvae were then dried and were mounted with carbon tape on aluminum stubs, finally coated with layer of gold using a sputter coater , followed by examination of prepared specimens by scanning electron microscope (SEM) camera, a JEOL, JSM-5400 LV SEM (JEOL Ltd.) operated at 15 kV, at Electron microscopy unit in Assiut University (Buchter *et al.*, 2018).

9-Statistical analysis:

Data were verified, coded by the researcher and analyzed using IBM-SPSS 24.0, using the chi-square test to compare the difference groups. The survival curves using Kaplan–Meier method and compared using Log-rank (Mantel-Cox) test, a two-way ANOVA to compare viability between different concentrations. For *in vivo* study, One-way analysis of variance (ANOVA) and Post-hoc analysis using Tukey's HSD test. P-value <0.05 was considered significant.

RESULTS

1. *Contracaecum spp.* infection rate in relation to seasonal variation in Assiut Governorate:

Macroscopic examination of Nile tilapia revealed that the total prevalence rate of *Contracecum* larvae was 24% (24\100). The larvae were found encapsulated on the surface of various organs in the abdominal cavity or embedded in fish muscles. There was a highly positive significant relation-ship between the *Contracecum* infection and summer season (50%) (Table 1, Fig1).

Table 1: The effect of season on *Contracecum* infection in Nile tilapia (*Oreochromis niloticus*):

Parameter	Non-infected (n = 76)	Infected (n = 24)	P-value*
Season			
• **Winter (n=25)**	25(32.8%)	0(0%)	<0.001
• **Spring (n=25)**	16(21%)	9(37.5%)	
• **Summer (n=25)**	13(17.1%)	12(50%)	
• **Autumn(n=25)**	22(28.9%)	3(12.5%)	

Chi square test was used to compare the difference in frequencies among groups

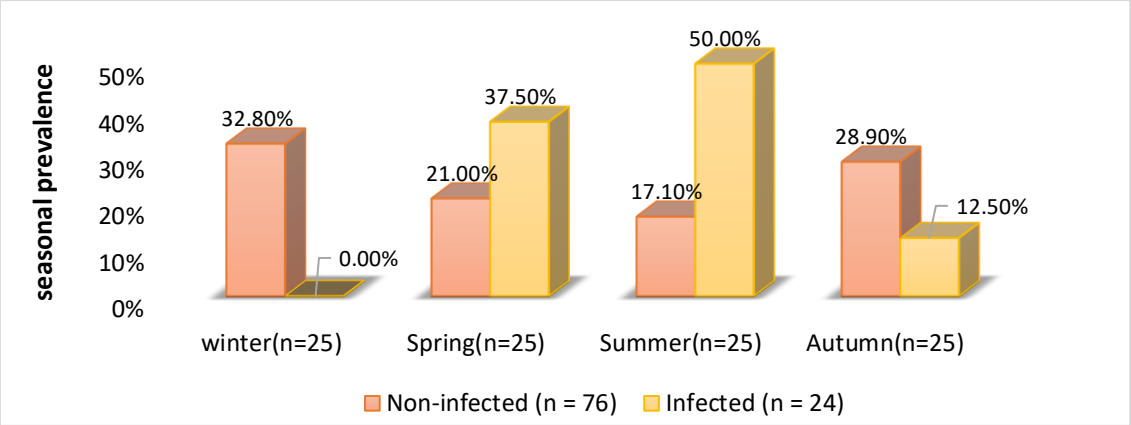

Fig. 1: Seasonal variation of *Contracecum* infection in Nile tilapia

2. Morphological identification of the collected *Contracaecum spp.* larvae:

- **Light microcopy:**
  Microscopic examination of *Contracecum* larvae revealed that the body was very long (from 2.7 to 3 cm) and opaque white when it is fresh. The body shape is cylindrical, with circular anterior end and pointed posterior end. The cuticle was homogenously finely striated, which were more distinguished in the anterior and posterior ends. The mouth was triangular with one small boring tooth anteriorly. Intestinal caecum is located anteriorly and longer than the ventricular appendix, which is located posteriorly, the anal opening is found near the posterior end. The tail was conical and ended with a spine **(Fig2).**

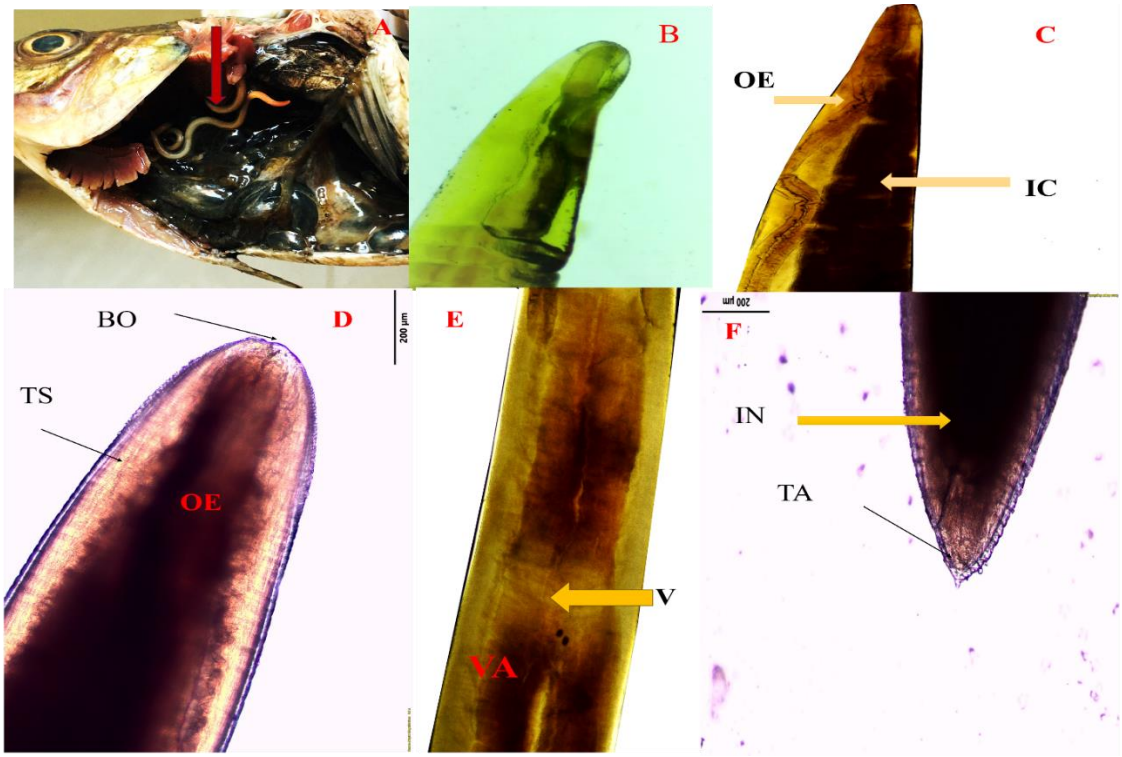

Fig. 2: A- third stage larvae of ***Contracaecum*** sp .in the body cavity of Nile tilapia (B-D) anterior region of ***contracecum*X100 (E) -body of larva (F**) posterior region of ***contracecum* lava. bo:**boring teeth **OE:** oesophagus **Ic**: intestinal caecum **Ts**: transverse striation V: ventriculus **VA:** Ventricular appendix Ta:tail IN: Intestine

3. Molecular identification of *Contracaecum sp.* larvae using conventional PCR:

PCR products of this region consisted of 1000 bp, the nucleotide sequences of *Contracaecum* larvae ITS2 region (had the typical band of *Contraceum* gene (900 bp) under the UV light on (1.5%) agarose gel) **(Fig.٣).** The sequences data were settled in the GenBank with accession number (PQ660931). Phylogenetic analysis of the nucleotide sequence revealed the presence of an identical clade with 100% similarity to the resulting gene. This clade was then subdivided into two main subclades, one of them including family *Anisakis simplix* and the other one including genus *Contracaecum* with the studied species in our study. The resulted strain 18s rDNA alignment showed that the ITS2 region sequences of the studied strain were identical to the established genes of previously studied *Contracaecum multipapillatum* with accession number U94370 (Nadler and Hudspeth, 1998), another species found in the same subclade with 99% similarity were *Contracaecum ovale* and *Contracaecum rudolphii* **(Fig.4).**

4. Impact of *Dendronephthya* and *Sarcophyton* extracts on the survival of *Contracecum* spp:

The survival curves demonstrate a marked inverse relationship between concentrations of *Dendronephthya* and *Sarcophyton* extracts and survival time of *Contracaecum* spp. *in vitro*. In *Dendronephthya* extract, the high concentration (50 mg/mL) exhibited dropped in survival to 80% within 10 hours and reached complete mortality by 18 hours. At 25 mg/mL, survival sharply declined to 50% within 18 hours and complete mortality by 22 hours of exposure. Whereas 15 mg/mL reduced survival gradually after 22 h of exposure and reached approximately 50% survival probability within 30 h, with complete mortality at 40 h after exposure. The Log-rank (Mantel-Cox) test showed highly significant differences in the survival of *Contracecum* spp. against *Dendronephthya* extract (Chi-square= 35.87, df= 1, *P* <0.0001) across different concentrations **(Fig 5).**

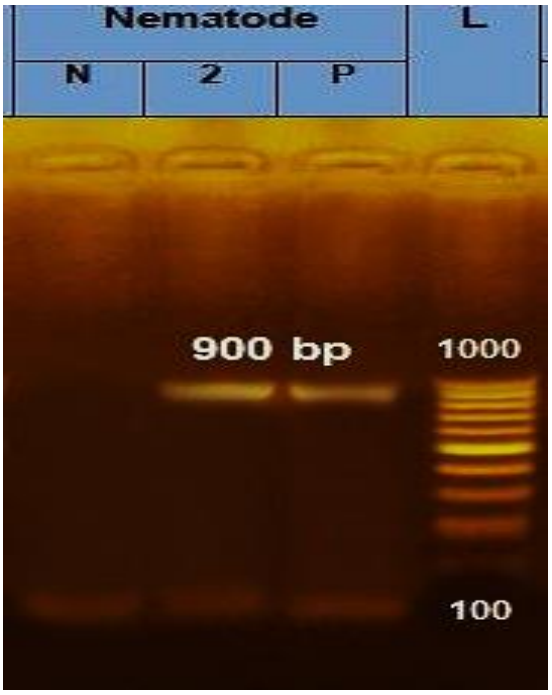

Fig. 3: Agarose (1.5%) gel showing PCR products (900 bp) of *Contracaecum multipapillatum*. (P) is the positive control, lane (N) is the negative control, and lane (L) DNA size marker.

Exposure of *Contracecum* spp. to *Sarcophyton* extract exhibited faster and high mortality in the high concentration (50 mg/mL) with a complete mortality within 20h, whereas the moderate concentration (25 mg/mL) showed similar pattern with slight delay, compared to the high concentration. However, the low concentration (15 mg/mL) exhibited the least mortality and survival extended to approximately 44 h, indicating weaker lethality. The Log-rank (Mantel-Cox) test showed a highly significant difference in the survival of *Contracecum* spp. against Sarcophyton extract (Chi-square= 45.59, df= 1, *P* <0.0001) across different concentrations **(Fig 5).**

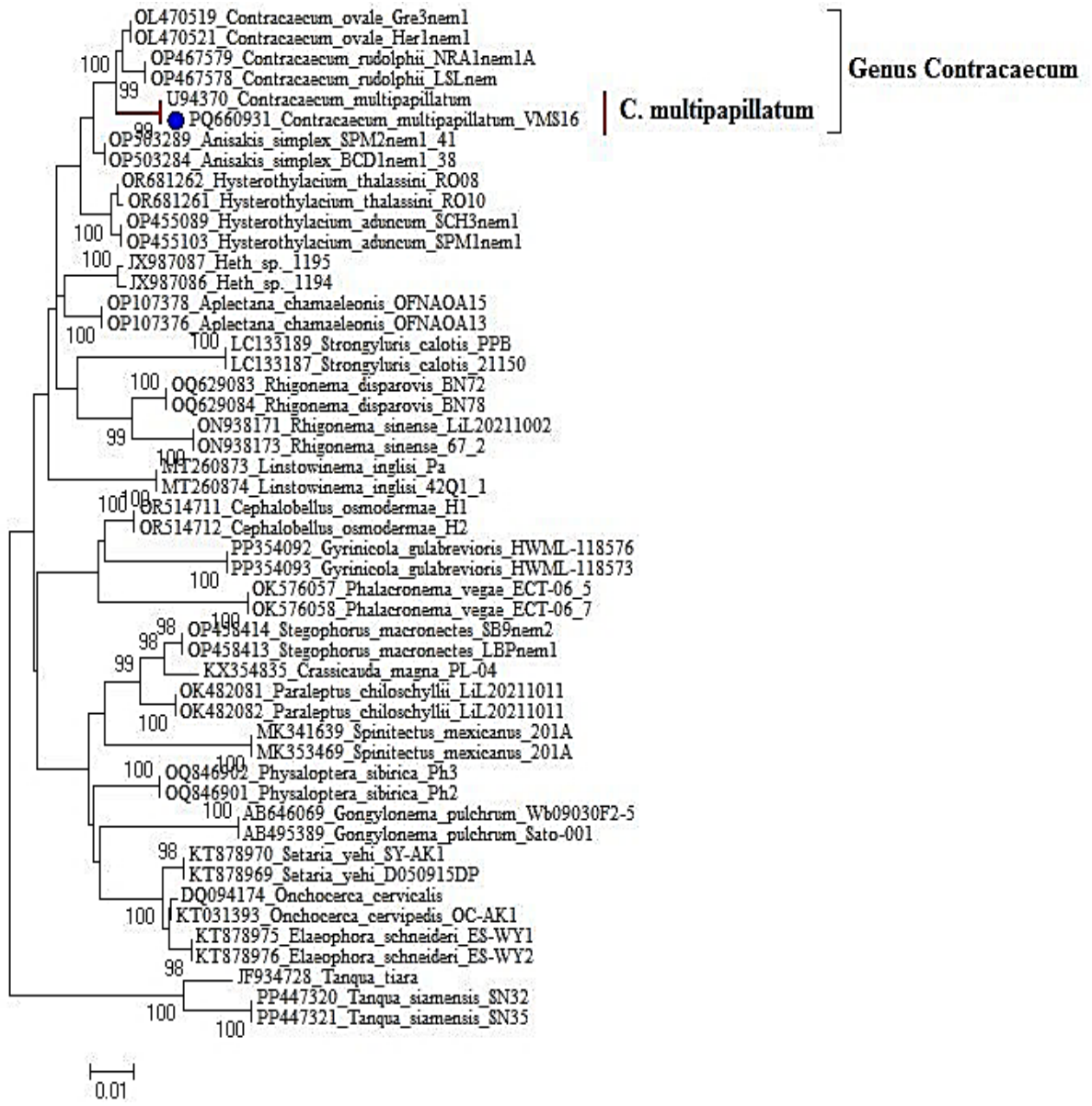

Fig. 4: Phylogenetic tree of *Contracaecum multipapillatum* (blue circle) recovered from Nile tilapia.

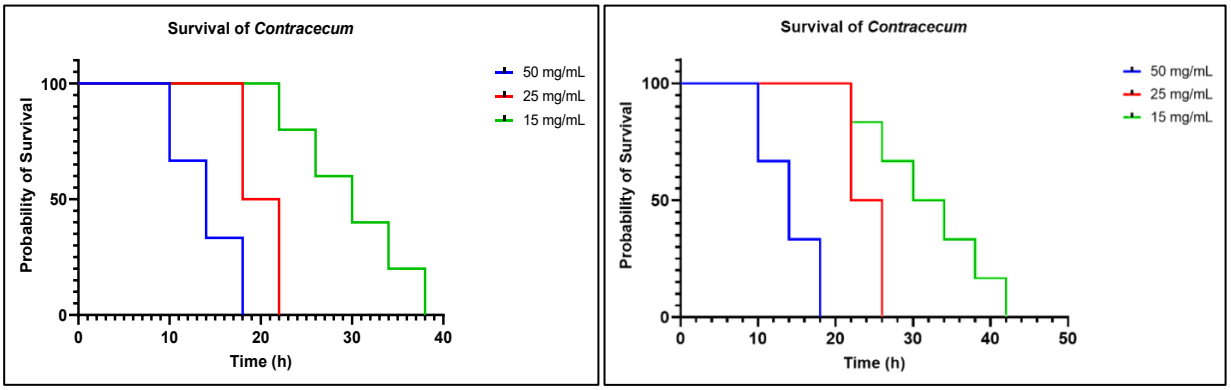

Fig. 5: Survival probability of Contracecum spp. against *Dendronephthya* extract (a) and *Sarcophyton* extract (b) over time (42h) at different concentrations. Each concentration was tested on 10 *Contracecum* worms. The control group maintained 100% survival.

5. The viability rate (%) and inhibition concentration (IC50) of *Dendronephthya* and *Sarcophyton* extracts against *Contracecum* spp.:

The inhibition concentration (IC50) was estimated at 34h, based on the extract effect on different concentrations after prolonged exposure. The *Dendronephthya* and Sarcophyton extract exhibited a dose-dependent inhibitory effect on *Contracecum* spp., with IC50 of 14.25 mg/mL (R^2 = 0.9448) and 12.52 mg/mL (R^2 = 0.9500) for *Dendronephthya* and Sarcophyton extract, respectively. These results indicate that both extract have moderate anti- parasitic effect, which is time and dose- dependent **(Fig 6).**

Viability rate showed a dose-dependent response, with 100% viability rate as a baseline. There was significant differences in the viability rate between all groups at 14h, 18h, 22h, 26h, 30h, and 34h. The high concentration of *Dendronephthya* **(Fig. 7A)** and Sarcophyton extracts **(Fig. 7B)** showed significantly decrease in the viability rate of *Contracecum* spp. (30% and 26.67%, respectively) at 34 h. However, the moderate concentration of *Dendronephthya* extract (25mg/mL) exhibited significant decrease in the viability of *Contracecum spp.* compared to Sarcophyton extract after 18h (53.33% and 100%, respectively) and 22h (0% and 66.67%). However, gradual decrease in the viability rate of *Contracecum spp.* after exposure to low concentration of *Dendronephthya* and *Sarcophyton* extracts with complete inhibition at 38h and 42h, respectively **(Fig 7).**

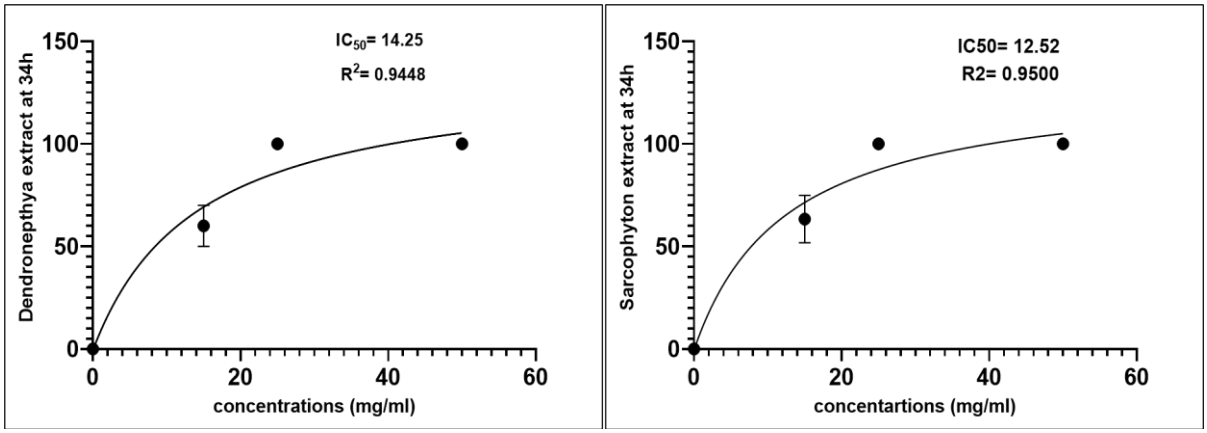

Fig. 6: The lethal inhibition concentration (IC_{50}) of *Dendronephthya* and *Sarcophyton* extracts against *Contracecum spp.* at 34h

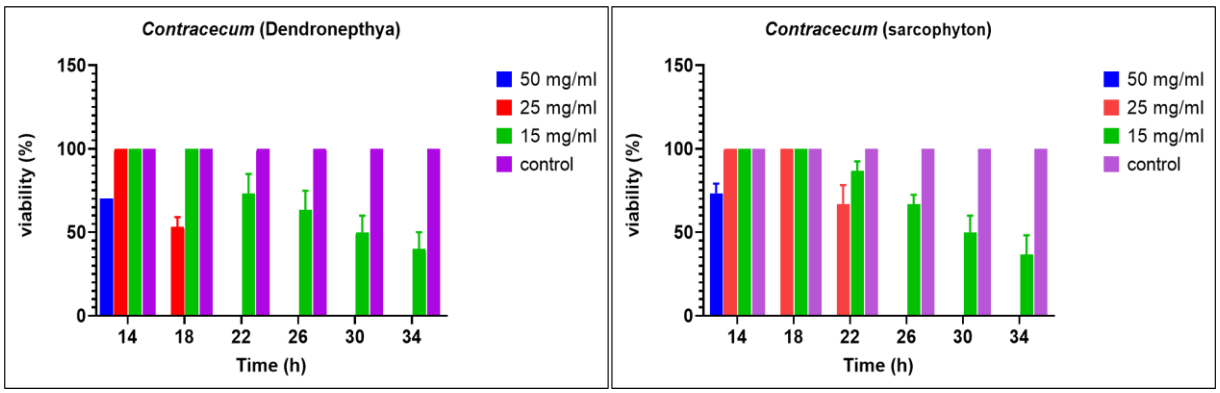

Fig. 7: The viability rate of *Contracecum* spp. after exposure to *Dendronephthya* and *Sarcophyton* extracts at 14h, 18h, 22h, 26h, 30, and 34h. Comparing viability between different concentrations with a two-way ANOVA. Each concentration was tested in three replicates. The viability rate of the control was 100% as a baseline.

6. Evaluation of the marine extract effect using SEM:

Scanning electron microscope of *Contracecum* larvae control group showed normal smooth cuticles with transverse striation. The anterior extremity contains triangular mouth and 3 lips (one dorsal and two ventral) with uniform transverse annulations. The posterior end contains anus, and the tail was short-ended with a distinct mucron **(Fig 8).**

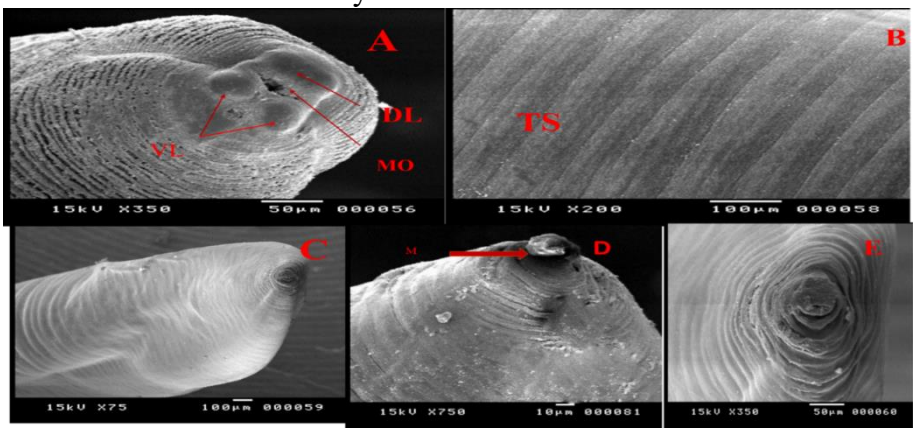

Fig 8: **A)** anterior end of the body showing MO: mouth opening surrounding with 3 lips one DL: Dorsal lip and two VL: ventrolateral lips B) Cuticle with smooth transverse striations **C-E:** the posterior end of the body contains TA: tail with M:mucron AN: anus

SEM conducted to observe ultrastructural changes in Contracaecum larvae by using different concentrations (15\25\50 mg/mL) from *Dendronephthya* and *Sarcophyton* extracts after the end of the experiment. These substances caused notable changes in different parts of larvae. *Sarcophyton* with concentration 15 mg/mL is the least effect on *Contracaecum* larvae showed damage and sloughing in the cuticle with separated striation in some regions and some changes in the posterior end, and with closure to anal opening. Higher concentrations of *Sarcophyton* leads to deeper rupture in the cuticle, the edema in the posterior region worsens with separation of striation and damage of the anterior and posterior regions **(Fig 9)**.

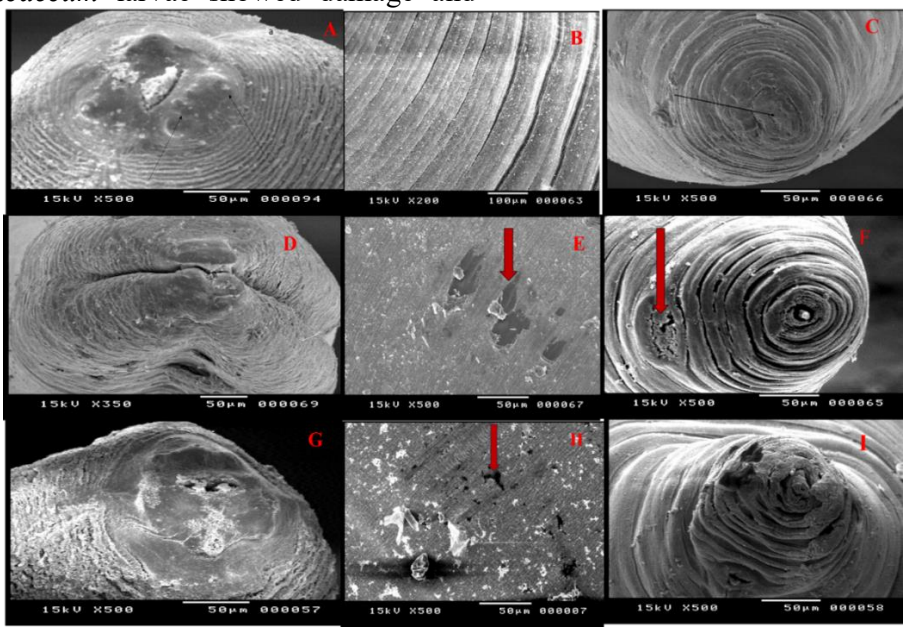

Fig 9: (A-C) **15 mg/mL of sarcophyton: A)** closed mouth opening with multiple blebs on lips b) separated striation c) posterior end showed edema and closure to anal opening (D-F) 25 mg/mL concentration D) oedema of lips with closure to mouth E) Sloughing of the cuticle F) degeneration of some area of the posterior region (G-I) 50 mg/mL G: complete destruction of lips H) pores in the cuticle i) Destruction of anal region

Dendronepthya spp. in 15 mg/mL leads to several cracks and sloughing in the cuticle, as well as oedema on the anterior end, dryness and disfiguration in the posterior end. At concentration 25 mg/ml, there was more edematous closed mouth, separation of striation, pores in cuticle and sloughed posterior end. The 50 mg/mL showed complete separation of the striation from the hypodermis and complete destruction of the posterior end, with pores or holes observed within the cuticle **(Fig 10)**.

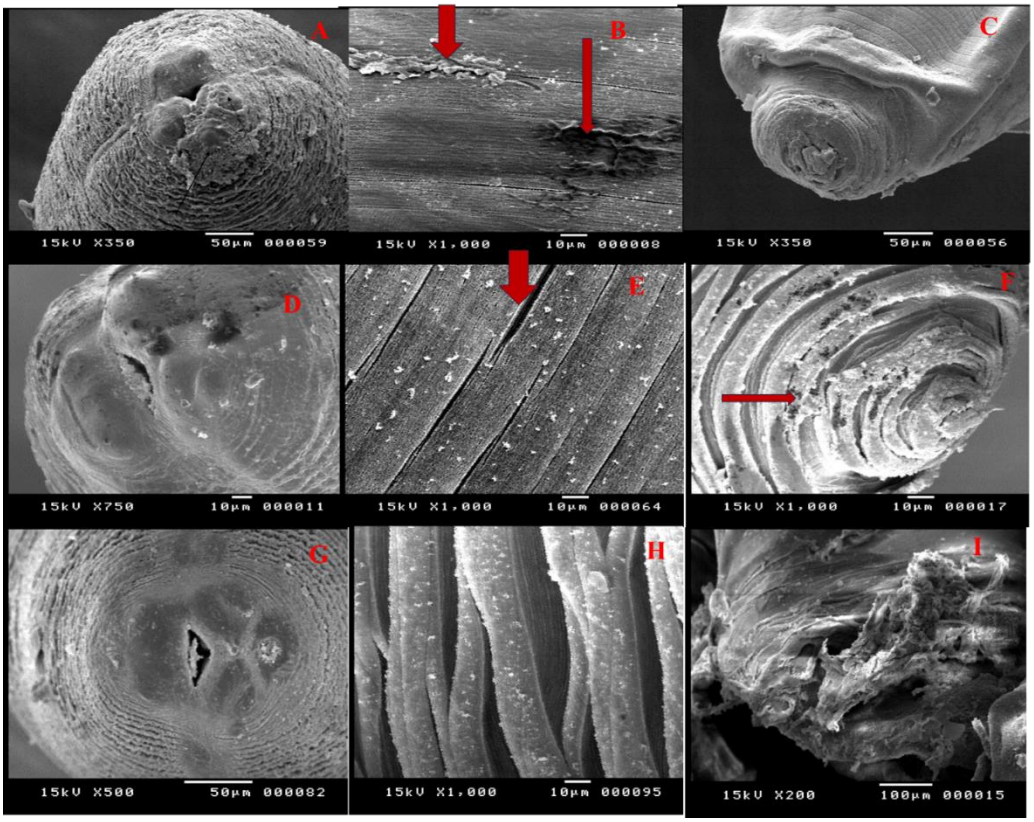

Fig 10: (A-C) 15 mg/mL of Dendronepthya **A)** Sloughing of some area of anterior region **B)** Sloughing of some areas on the cuticle **C)** entrance of posterior end inside and closure of anal opening (D-F)25 mg/mL concentration D)oedema f lips with cloure to mouh **E)** Cracks in the cuiticle and changes in normal striations **F)** complete changes in morphology with degenerations in some area of posterior region (G-I) 50 mg/mL **G**: complete destruction of lips **H)** opening in cuticle i) complete destruction of posterior region.

DISCUSSION

The distribution and occurrence of *Contracaecum* larvae in the abdominal cavity of fish, especially those which are more common for consumption by humans, is unfortunately poorly known in Egypt (Taha, 2020). *Contracaecum* third stage larvae can infect human accidentally by consumption of the raw and undercooked fish (Vuić *et al*., 2022). Contracaecosis is the resulted disease in human and recognized by stomach aches, fever, intestinal colic with vomiting and diarrhea (mattiucci *et al*., 2018).

Prevalence rate of *Contracaecum* spp. was 24%, agreeing with previous studies, where 22% of *M. surmuletus* were infected with *C. multipapillatum* in Libya (Almashay 2021), 18.5% in Ethiopia (Gebreegziabher *et al*., 2020) 25.1% were infected with *Contracecum multi-papillatum* in Mexico (Choc *et al.,* 202٠) and 21.50% in Iraq (Aqeele *et al.,* 2024). In Egypt, the results were nearly compatible with Hamouda & Abd Alkareem (2021) in Lake Nasser (20%).

The obtained result was higher than that recorded before (2.08%) in Ethiopia (Areda *et al*., 2019) and (2.3%) in Qena

(Elseify *et al*., 2015). However, the results were lower than Thabit & Abdallah (2022), who reported that 66.96% of examined Nile perch Lates niloticus were infected in Assiut, (50.0%) in El Minia (Hefnawy *et al*., 2019) and 35.60% in *O. niloticus* from lake Nasser (Younis et al., 2017).

The differences in the infection rates mainly depend on climatic conditions, as *Contracaecum* larvae flourish in autumn and spring and decreased significantly in summer and winter (Abd-ELrahman *et al.*, 2023). Another factors including pollution and presence of paratenic hosts may also affect the infection rates.

Regarding SEM study of control Contracecum larvae, it was shown that the cuticle with transverse striation, anterior end showed three lips surrounding the mouth opening, one dorsal and two lateral ventricles with a small papilla in each lip, and the posterior end contains anal opening. These findings are compatible with those obtained by Younis *et al*. (2017) who described *Contracecum multi-papillatum* morphology using SEM from Lake Nasser in Egypt.

Molecular identification confirmed the obtained species in this study is *Contracaecum multipapillatum* and this species was previously identified molecularly by Shamsi, & Aghazadeh (2011), who identified the larvae of *Contracaecum multipapillatum* in Iran. Also, Motamedi *et al*. (2019) have reported the infection of *Contracaecum multi-papillatum* isolated from small killifish in Iran, while the identified species in Assiut by using the nuclear ribosomal internal transcribed spacer (ITS1) and the mitochondrial cytochromeoxidase c subunit I (COI) gene is *Contracaecum quadripapillatum* (Thabit, & Abdallah, 2022).

Latterly, the need for using therapeutic plants or their bioactive extracts in order to control zoonotic diseases, as an alternative for using synthetic drugs which produce drug resistance in humans and animals (Tavares-Dias, 2018). Nowadays, *in vitro* studies have explained that *Dendro-nephthya spp.* extracts have anti-inflammatory power (Lee, 2017). *Sarco-phyton* has antileishmanial potency (El-Ayouty *et al.*, 2021), and it has insecticidal activity (Bashar *et al.*, 2024).

This study revealed the *in vitro* anthelmintic effect of soft coral extracts of (*Dendronephthya* and *Sarcophyton*) on the *Contracaecum multipapillatum* larvae. The larvae showed severe cuticular damage and edema. There was a previous *in vitro* study conducted on *Contracaecum* larvae and other nematodes, including the use of citronella oil that causes death of larvae after one hour exposure, which by microscopic examination, there were ruptured areas of cuticle (Justino and Barros, 2008), which resembles the cuticular damage in our study.

The IC50 of 14.25 mg/mL (R^2 = 0.9448) and 12.52 mg/mL (R^2 = 0.9500) for *Dendronephthya* and *Sarcophyton* extract, respectively. This result indicates a good nematicidal activity, up till now there are a few invitro studies targeting parasites clearing the anti-parasitic activity of soft coral extracts. However, the *Sarcophyton glaucum* extracts have a promising cytotoxic antileishmanial activities (El-Ayouty *et al.*, 2021). Also, the fractions extracted from *Nephthea mollis* have a potential anti Trypanosomal activity with LC50 of 6.4 and 3.7 µg/ml.

This is the first study that highlighted the potential nematicidal activity of soft coral extracts against *Contracaecum multi-papillatum* larvae but in the future, *in vivo* research is necessary to confirm the tested extract's safety and therapeutic potential.

CONCLUSION

In conclusion, this study found a 24% prevalence of *Contracaecum spp*. larvae in Nile tilapia sourced from Assiut, with the highest prevalence occurring in the summer at 50%. We identified *Contracaecum multipapillatum* as the dominant species. Marine extracts from Sarcophyton and Dendronephthya, which effectively reduce larval viability and induce significant morphological damage. These findings highlight the importance of addressing the issue of *Contracaecum* larvae in fish and the potential of marine extracts to serve as effective anthelmintics.

REFERANCES

Abd-ELrahman, S.M., Gareh, A., Mohamed, H.I., Alrashdi, B.M., Dyab, A.K., El-Khadragy, M.F., Khairy Elbarbary, N., Fouad, A.M., El-Gohary, F.A., Elmahallawy, E.K. and Mohamed, S.A.-A. (2023): Prevalence and Morphological Investigation of Parasitic Infection in Freshwater Fish (Nile Tilapia) from Upper Egypt. *Animals*, *13*(6), 1088. https://doi.org/10.3390/ani13061088

Almashay, A. (2021): Study on some types of parasitic nematodes, Anisakissimplex, Contracaecum multipapllatium and Hysterothylacium adun-cum affecting fish, Mullus surmuletus in the Beach City of Sirte-Libya. Egyptian Academic Journal of Biological Sciences, E. MedicalEntomology & Parasitology, 13(1), 17–21 DOI: 10.21608/EAJBSE.2021.175749

Areda, T.A., Mitiku, M.A., Woldearegay, Y.H., Teklu, A. and Getachew, S. (2019): Prevalence of major parasites of Nile tilapia (Oreochromis niloticus) in south west Showa zone selected fish farms, Oromia region, Ethiopia. International Journal of Fisheries and Aquatic Studies, 7(3), 165-170.

Aqeele, G.J., Kadhim, D.K., Alali, F., Alhesnawi, A.S. and Taher, T.M.J. (2024): Morphology and molecular confirmation of Contracaecum spp. in Fish. *East J Agric Biol Sci*, *4*(1), 43-50. https://qabasjournals.com/index.php/ejabs

Bashar, M.A.E. et al. (2024): 'Anticancer, antimicrobial, insecticidal and molecular docking of sarcotrocheliol and cholesterol from the marine soft coral Sarcophyton Trocheliophorum', *Scientific Reports*, 14(1), pp. 1–16. Available at: https://doi.org/10.1038/s41598-024-75446-6.

Buchter, V. et al. (2018): 'Assessment of tegumental damage to Schistosoma mansoni and S. haematobium after in vitro exposure to ferrocenyl, ruthenocenyl and benzyl derivatives of oxamniquine using scanning electron microscopy', *Parasites and Vectors*, 11(1), pp. 1–12. Available at: https://doi.org/10.1186/s13071-018-3132-x.

Choc-MM, S., Jiménez-R Ph, A., Arguedas-C Ph, D. and Dolz Ph, G. (2020): Contracaecum multipapillatum (Nematoda: Anisakidae) en peces de aguas continentales de Guanacaste, Costa Rica e Izabal, Guatemala. *Revista colombiana de ciencia animal recia*, *12*(2), 40-50. https://doi.org/10.24188/recia.v12.n2.2020.767

Deng, Y. and Wang, H. (2022): 'Characterization of the Complete Mitochondrial Genome Sequence of Contracaecum sp. (Nematoda : Ascarididae) from China' journal of Nematology, 54**,** e2022-1. https://doi.org/10.21203/rs.3.rs-1513649/v1

Eiras, J.C. (2016): 'Potential risk of fish-borne nematode infections in humans

in Brazil – Current status based on a literature review', *Food and Waterborne Parasitology*, 5, pp. 1–6. Available at: https://doi.org/10.1016/j.fawpar.2016.08.002.

El-Ayouty, M. et al. (2021): 'Cytotoxic and Antileishmanial Activities of the Red Sea Soft Coral Sarcophyton glaucum Extract and Some of Its Isolates', *Records of Pharmaceutical and Biomedical Sciences*, 5(Pharmacognosy-Microbiology), pp. 115–120. Available at: https://doi.org/10.21608/rpbs.2021.90767.1111.

El-Seify, M.A., El Shihawy, I.S., Metwally, A.M. and Fawaz, M. (2015): Studies On Nematode Parasites Infecting Freshwater Fish In Qena Governorate. Kafrelsheikh Veterinary Medical Journal, 13(1), 19-34. doi:10.21608/kvmj.2015.109533

Floyd, R.M., Rogers, A.D., Lambshead, P.J.D. and Smith, C.R. (2005): Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Molecular Ecology Notes*, *5*(3), 611-612.

Gebreegziabher, H., Degefu, H. and Tsegay, A.K. (2020): Prevalence of internal helminth parasites of fish in gilgel-gibe river and three selected ponds in and around jimma town, south west ethiopia. Turkish Journal of Fisheries and Aquatic Sciences,20(9),693–699. https://doi.org/10.4194/1303-2712-v20_9_04

Hamouda, A.H. and Younis, A.E. (2022): Molecular characterization of zoonotic anisakid Contracaecum spp. larvae in some fish species from Lake Nasser, Egypt. *Aquaculture Research*, *53*(6), 2548-2561. https://doi.org/10.1111/are.15774

Hefnawy, Y.A., Ahmed, H.A., Dyab, A.K., Abdel-Aziz, A.R. and Boules, M.S. (2019): Fish as a Potential Source of Parasites of Public Health Importance in El-Minia Governorate, Egypt. *PSM Microbiol*, *4*(2), 44-52. https://psmjournals.org/index.php/microbiol/article/view/317

Justino, C.H. da S. and Barros, L.A. (2008): 'In vitro evaluation of the resistence of the Contracaecum sp. larvae (Railliet & Henry, 1912) (Nematoda: Anisakidae), to the essential oil of citronella (Cymbopogon sp.) (Poaceae)', *Revista Brasileira de Ciência Veterinária*, 15(3), pp. 122–125. Available at: https://doi.org/10.4322/rbcv.2014.212.

Lee, S.H. (2017): 'Developmental toxicity and anti-inflammatory effect of the soft coral Dendronephthya gigantea collected from Jeju Island in zebrafish model', *Fisheries and Aquatic Sciences*, 20(1), pp. 1–7. Available at: https://doi.org/10.1186/s41240-017-0077-5.

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M. and Nascetti, G. (2018): Molecular epidemiology of Anisakis and anisakiasis: an ecological and evolutionary road map. *Advances in parasitology*, *99*, 93-263. DOI: 10.1016/bs.apar.2017.12.001

Maued, A.Z.F. et al. (2023): 'Anti-Tumor Effects of Dendronephthya putteri Ethanolic Extract in DMBA-Induced Breast Cancer in Adult Female Rats', *The Egyptian Journal of Hospital Medicine*, 93(1), pp. 7153–7158. Available at: https://doi.org/10.21608/ejhm.2023.321798.

Mohammed El-Sawi, N., Mohamed Hamed, M., Hefny Gad, M. and Ali Ahmed Hefny, Y. (2024): Comparative Assessment of the Antibacterial Potential of Four Soft Corals Collected from the Red Sea (Egypt) Against Selected Pathogenic

Bacteria. Sohag Journal of Sciences, 9(2), 121-126. DOI: 10.21608/sjsci.2023.240439.1134

Motamedi, M., Iranmanesh, A., Teimori, A., Sadjjadi, S.M. and Nasibi, S. (2019): Detection of Contracaecum multipapillatum (Nematoda: Anisakidae) in the indigenous killifish Aphanius hormuzensis (Teleostei; Aphaniidae) and its histopathological effects: A review of Iranian Aphanius species parasites. *Journal of Applied Ichthyology*, *35*(2), 558-569. https://doi.org/10.1111/jai.13848

Nadler, S.A. and Hudspeth, D.S.S. (1998): 'Ribosomal DNA and Phylogeny of the Ascaridoidea (Nemata: Secernentea): Implications for Morphological Evolution and Classification', *Molecular Phylogenetics and Evolution*, 10(2), pp. 221–236. Available at: https://doi.org/10.1006/mpev.1998.0514.

Nativ, H., Galili, O., Almuly, R., Einbinder, S., Tchernov, D. and Mass, T. (2023): New Record of Dendronephthya sp.(Family: Nephtheidae) from Mediterranean Israel: Evidence for Tropicalization?. *Biology*, *12*(9), 1220. https://doi.org/10.3390/biology12091220

Pekmezci, G.Z. and Yardimci, B. (2019): 'On the occurrence and molecular identification of Contracaecum larvae (Nematoda: Anisakidae) in Mugil cephalus from Turkish waters', *Parasitology Research*, 118(5), pp. 1393–1402. Available at: https://doi.org/10.1007/s00436-019-06278-x.

Sameeh, S.E.; Mahmoud, A., Huseein, E., Monib, M. and Abd Elhamid, M. (2024): Morphological and molecular characterization of Anisakis simplex (Nematoda: Anisakidae) third-stage larvae isolated from retail-marketed smoked herring fish in Assiut, Egypt. *Parasitologists United Journal*, *17*(1), 46-53. DOI: 10.21608/PUJ.2024.279456.1242

Santacruz, L., Hurtado, D.X., Doohan, R., Thomas, O.P., Puyana, M. and Tello, E. (2020): Metabolomic study of soft corals from the Colombian Caribbean: PSYCHE and 1H-NMR comparative analysis. *Scientific Reports*, *10*(1), 5417. https://doi.org/10.1038/s41598-020-62413-0

Shamsi, S. (2019): Seafood-borne parasitic diseases: A "one-health" approach is needed. *Fishes*, *4*(1), 9. https://doi.org/10.3390/fishes4010009

Shamsi, S. and Aghazadeh Meshgi, M. (2011): Morphological and genetic characterisation of selected contracaecum (nematoda: anisakidae) larvae in iran. iranian journal of fisheries sciences, 10(2), 356-362. sid. https://sid.ir/paper/585047/en

Savić, M.P., Sakač, M.N., Kuzminac, I.Z. and Ajduković, J.J. (2022): Structural diversity of bioactive steroid compounds isolated from soft corals in the period 2015–2020. *The Journal of Steroid Biochemistry and Molecular Biology*, *218*, 106061. https://doi.org/10.1016/j.jsbmb.2022.106061

Taha, R.G. (2020): Role of sea bream fish P. pagrus (Family: Sparidae) in harboring some larval ascaridoids (Family: Anisakidae) in Cairo governorate, Egypt. J. Egypt. Soc. Parasitol. 50(3): 504–512. DOI: 10.21608/jesp.2020.131075

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013): MEGA6: molecular evolutionary genetics analysis version 6.0. Mol.

Biol. Evol. 30, 2725–2729. doi: 10.1093/molbev/mst197

Tavares-Dias, M. (2018): 'Current knowledge on use of essential oils as alternative treatment against fish parasites', *Aquatic Living Resources*, 31. Available at: https://doi.org/10.1051/alr/2018001.

Thabit, H. and Abdallah, E.S.H. (2022): Morphological and molecular identification of third-stage Contracaecum larvae (Nematoda: Anisakidae) parasitizing Nile perch Lates niloticus in Egypt. *Aquaculture Research*, *53*(13), 4869-4881https://doi.org/10.1111/are.15980

Thompson, J.D.; Higgins, D.G. and Gibson, T.J. (1994): CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research,22(22):4673-4680. DOI: 10.1093/nar/22.22.4673

Vuić, N., Turković Čakalić, I., Vlaičević, B., Stojković Piperac, M. and Čerba, D. (2022): The Influence of *Contracaecum* larvae (Nematoda, Anisakidae) Parasitism on the Population of Prussian carp (*Carassius gibelio*) in Lake Sakadaš, Croatia. *Pathogens*, *11*(5), 600. https://doi.org/10.3390/pathogens11050600

Younis, A.E., Saad, A.I. and Rabei, J.M. (2017): "The occurrence of Contracaecum sp. larvae (Nematoda: Anisakidae) in four teleostean species from Lake Nasser, Egypt: morphological and molecular studies," *The Journal of Basic and Applied Zoology*, 78(1). Available at: https://doi.org/10.1186/s41936-017-0012-4.

Ziarati, M. et al. (2022): 'Zoonotic diseases of fish and their prevention and control', *Veterinary Quarterly*, 42(1), pp. 95–118. Available at: https://doi.org/10.1080/01652176.2022.2080298.

دراسة شاملة ليرقات الكونتراسيكوم التي تصيب أسماك البلطي النيلي في محافظة أسيوط: التواجد، والتعريف الجزيئي، ودراسة النشاط القاتل للنيماتودا في المختبر لمستخلصات المرجان الناعم

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تنتمي يرقات دودة الكونتراسيكوم إلى فصيلة الأنيساكيدا. تُشكل يرقات الكونتراسيكوم التي تُصيب الأسماك خطرًا على الإنسان، كما تؤثر على قابلية تسويق الأسماك. كما أن لهذه اليرقات أهمية وبائية مشتركة بين الإنسان والحيوان، إذ يُمكن أن تُصيب الإنسان عند تناول لحوم الأسماك النيئة أو غير المطهوة جيدًا، مُسببةً داء الكونتراسيكوم. لذلك الهدف من هذه الدراسة إلى تحديد يرقات دودة الكونتراسيكوم مجهريًا وجزيئيًا نظرًا لضئالة المعلومات المتوفرة عنها في محافظة أسيوط. كما أُجريت دراسة معملية باستخدام بعض المستخلصات البحرية لاجراء التجربة العلاجية عليها. جُمعت ١٠٠ عينة من أسماك البلطي النيلي عشوائيًا من محافظة أسيوط للتحقق في وجود دودة الكونتراسيكوم، وخضعت اليرقات المُجمعة للفحص المجهري والبنيوي الدقيق، وقد دُعمت هذه النتائج بتفاعل البوليميراز المتسلسل (PCR). علاوةً على ذلك، استُخدمت اليرقات الحية في الدراسة المخبرية باستخدام بعض المستخلصات العشبية البحرية مثل السرقوفيتون وديندرونيفثيا. أظهرت نتائج هذه الدراسة معدل إصابة (٢٤٪)، خاصةً في فصل الصيف بنسبة (٥٠٪)، وأكد التحليل الجزيئي تشابهًا بنسبة ٩٨,٦٪ مع Contracaecum mulipapillatum. كشفت الدراسة المخبرية باستخدام المستخلصات البحرية عن تلف شديد في الطبقة الكيوتكلية لليرقات، والذي يُمثل بديلاً واعدًا لمضادات الديدان، بتركيز القاتل الوسيط (IC50) يبلغ ١٤,٢٥ ملغم/مل.