# Studies on Anguillicoliasis in cultured *Anguilla anguilla* fish farms in Delta region, Egypt with special reference to hematological, biochemical changes and treatment

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**Abstract:** Nematode parasite, *Anguillicola crassus* (*A. crassus)* infests eels, *Anguilla anguilla*, causes pathological damage to the swim bladder, potentially compromising their ability to cope with hypoxic conditions and assist it for swimming. Present study aimed to investigate the clinical picture, hematological, biochemical changes in *A. crassus* infected eels in comparison with non infected eels and trials for treatment the *Anguillicola crassus* (*A. crassus)* infection for eels, *Anguilla Anguilla*. The study revealed that any change for vent color from normal to red or orange could be used as a diagnostic tool of disease. *A. crassus* infection more prevalent in small sized eels than larger ones, while larger eels harbored more parasites than smaller ones. The study recorded the clinical signs, postmortem lesions, prevalence, parasitic intensity of *A. crassus*, seasonal variation, hematological and biochemical changes in *A. crassus* infected eels, with a trial for treatment of cultured eels infected with *A. crassus*. using *Moringa Oleifera* leaf powder in the diet and Levamisol HcL as freshwater bath.

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## Introduction:

Eels fish are characterized by their excellent quality and flavor of the moist, boneless flesh which makes it of highly prized one as well as its adaptability to conditions of culturing; artificial food, tolerance the variation of temperature and salinity and high stocking densities which make it suitable species for culture **(Barker** and **Cone 2000)**. Eels are sensitive to environmental stresses (parasitic, fungal and bacterial diseases) which are responsible for lower survival, lower growth, and lower commercial value and for higher costs in preventive and curative **(Dosoky, 2007)**. *Anguillicola crassus* infestations may cause serious losses in eel production (**Pilecka and Sobecka 2004 Noor El-Deen *et al.*, 2012).**

Parasitic nematode is remarkable by the complexity of their cycle which often imply a migration inside the body of the host, and by their capacity to produce eggs in great quantity **(Justine, 2001)**. One will cite mainly the nematode *A. crassus*, which attacks the swim bladder and causes significant mortalities in the eel population in aquaculture or in the natural environment **(Molnar *et al.*, 1993)**. It was suggested that infection with this parasite may soon be widespread in natural basins in Europe and North Africa **(Maillo *et al.*, 2005 and Abdallah** and **Maamouri, 2006)**.

Levamisole Hc1 seemed to be the most effective, and bath treatment with 1 mg $l^{-1}$ per 24 h led to a complete cure **( Taraschewski *et al.*,1988).** The long-term effect of various dosages of Levamisole on the different developmental stages was conducted where adults and pre adults showed considerable loss of vitality for 3 week after therapy. Eggs and newly hatched larvae showed no reaction to the drug and a single treatment with levamisole under freshwater conditions seemed to be ineffective since some nematodes were able to regenerate after suffering sub-lethal damage **(Hartmann, 1989)**.

*Moringa oleifera* is known to treat over 300 diseases in human and animals, it is known as (The Miracle Tree of Life) blood Pressure, diabetes, fever, migraines, malnutrition, arthritis, tumors, ulcers, impotency, it has antioxidants, anti-aging and anti-inflammatory substances, it is also antibacterial, antiparasitic and antimycotic (**Ogbi and Affiku, (2011)** moringa grows quickly in many types of environments. it is edible by humans or by farm animals. The leaves contain all essential amino acids and are rich in protein, vitamin A, vitamin B, vitamin C and minerals **(Jules and Paull, 2008).**

Thus Present study was aimed to record the prevalence and intensity of *A. crassus* infection in eels in relation to seasons, body size, in addition to study the clinical signs and postmortem lesions of *A. crassus*

infected eels and investigate the efficacy of *Moringa oleifera* leaf as medicinal plant and levamisole Hcl as a chemical product as a trial for treatment of eels infected with *A. crassus* reared and cultured in Delta region, lower Egypt.

**Material and methods:**

**Fish for examination:**

A total of 200 eels fish were collected from private fish farm in Delta region (earthen ponds), 100 eel fish (50 in winter and 50 in spring) with average length of 23 cm and average body weight of 70 gm; and 100 eel fish (50 in summer and 50 in autumn) with average length of 35 cm and average body weight of 135 gm. The fish were examined for prevalence of worm infection according to season and size of eel fish.

**Experimental Fish:**

A total of 75 eel fish used for a trial of treatment of infested eels by *Moringa oleifera* leaf powder and Levamisole Hcl. The fish were divided into 3 groups, each 25 eels. 1st Group was control untreated while 2nd group treated with *Moringa oleifera* leaf 50g/kg diet for 2 weeks and group (3) treated with levamisole Hcl with a dose 5mg/L as a bath for 24h.. Eels at the end of experiment were counted to record the percentage of survival percentage, then dissected and record infestation, prevalence clinical and post mortem signs.

**Diet preparation:**

The diet was formulated from fish meal, meat meal, soybean meal, corn flour, wheat bran, beside vitamins and mineral mixtures. Diet contained 42% crude protein, all ingredients were finally ground to a size less than 1mm that could taken by fish easily, the ingredient were then weighted out according to the formulation, thoroughly mixed then water and oil were added to the dry ingredients mixture to form a paste, then the feed should be used quickly after preparation. The diet was given two times daily at a rate of 5% of body weight according to **(Dosoky, 2007)**.

**Drug used for trial of treatment:**

**a**- Levamisol Hcl 10% (Sigma,USA):

Levamisol, was applied as a freshwater bath 5 mg / L for 24 h. as described by **Hartmann, (1989).**

**b**- *Moringa oleifera* leaf powder: Green leaf were collected manually and obtained from nurseries, dried and finely chopped, grounded in blender then amount of 50g/kg ration was added to previous mentioned ration as described by **Ogbe *et al.* (2011),** the mixture was used immediately in the experiments of treatment or preserved at - 4 till used.

**Clinical examination of eels**:

The collected 120 fishes were examined externally and postmortem using the methods described by **(Lucky, 1977)** paying special attention to the, swim bladder and abdominal cavity.

**Parasitogloical examination & identification of nematodes:**

The nematode after being recovered, washed in saline solution and kept in refrigerator for killing and stretching. The worms were treated with 70% alcohol and 5% glycerol, after that for best clearing they were put in lactophenol for 24 hours and then mounted in polyvinyl alcohol as best clearing and mounting agent, then examined with dissecting microscope for morphological identification **(Lucky, 1977).**

**Diagnosis of Anguillicoliasis in eels:**

Diagnosis of Anguillicoliasis in eels is based mainly on characteristic clinical sings and post-mortem examination of infested eels.

**Blood sampling:**

Blood samples were taken from the caudal vessels with anticoagulant (EDTA) for hematological and without anticoagulant for separation of serum for biochemical examinations.

**Determination of hematological parameters:**

Blood samples were taken from the caudal vessels with anticoagulant, erythrocytes (RBCs) and leukocytes (WBCs) counts were made by standard clinical method (**Natt and Herrick, 1952 and Soliman, 1986**). Hematocrit (PCV) was measured according to **Jain (1986**), while hemoglobin concentration (Hb) was performed according to acid hematin method using Farstab haemometer as rapid correction (**Larson and Snieszko, 1961**) using Sahli's method. The blood indices were calculated according to **Jain (1986**). The obtained hemoglobin values were corrected according to equation of **Larsen (1964).**

**Biochemical analysis:**

Fresh blood samples (without anticoagulant) were collected from the caudal vein according to **Lied *et al.* (1975)** from 10 *Anguillicola crassus* infected eels and 10 *Anguillicola crassus* free eels. Serum was separated form each blood sample for the biochemical examination. Total protein level in serum was determined according to **Cannon *et al.* (1974).** Serum albumin concentration was measured as described by **Gustafsson (1976).** Blood serum globulin was calculated by subtracting the concentration of albumin from that of the total protein and albumin/globulin ratio (A/G ratio) was calculated by dividing albumin concentration over that of globulin **(Coles, 1986).** Urea concentration was measured according to **Pathson and Nauch (1977).** Creatinine level was determined after **Rock *et al.* (1987).** Uric acid concentration was determined according to **Schultz (1984).** Activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to **Reitman and Frankel (1957).**

**Statistical analysis:**

Data were presented as mean ± standard error (S.E.) and the significance of differences was estimated using ANOVA test as described by **Snedecor (1964).**

**Results:**

**Clinical signs and postmortem findings:**

The infested eels showed loss of vitality, inverted swimming, retard in growth rate, absence of escape reflex, increase mortality rate, distended abdomen in addition, the anal opening appear pink or red coloration it was characteristic sign. Congestion and inflammation of swim bladder and viscera. The swim bladder of *A. carssus* infested eels showed numerous different sized worms filled the swim bladder giving a picture of a case engorged with worms (sausage like) worms appeared from outside the intact swim bladder, others ruptured due to more engorgement with adult worms and larvae Fig. (2)**.**

**Results of parasitogloical examination and Identification of nematode:**

The nematode after being recovered were washed in saline solution and kept in refrigerator for killing and stretching. The worms were treated with 70% alcohol and 5% glycerol, after that for best clearing they were put in lactophenol for 24 hours and then mounted in polyvinyl alcohol as best clearing and mounting agent, then examined with dissecting microscope for morphological identification. The nematode was identified after examination as *Anguillicola crassus* due to morphological characters and measurements according to **Mo & Steien (1994).**

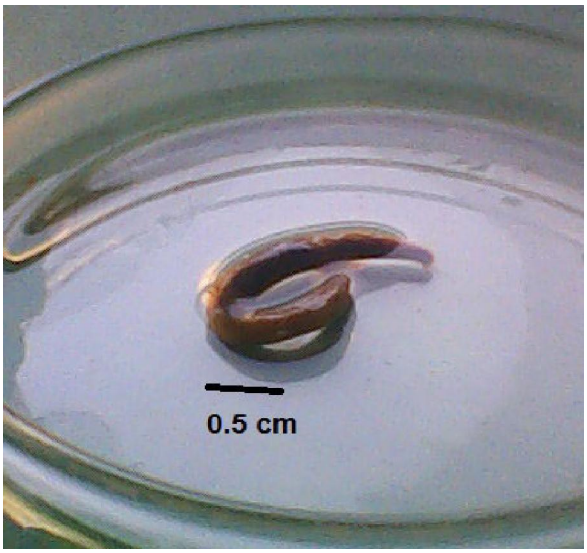


Figure 1: Adult *A. crassus* worm in Petri dish (arrow).

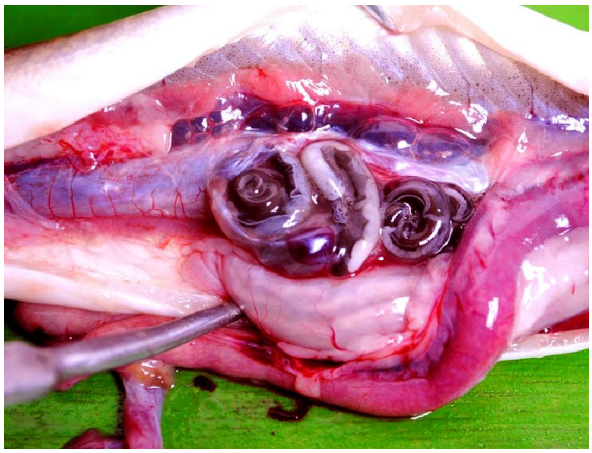

Figure 2: Showing adult worms of *A. crassus* appeared after ruptured of swim bladder (arrows).there was also congestion and inflammation of swim bladder and viscera

**Prevalence and intensity in relation to Seasonal variation:**

The total prevalence of *A. crassus* infection in cultured eels was 55% and total parasitic abundance was 1-5.5 parasites. Also, it was detected that the summer season showed highest prevalence rate (83.3%), followed by autumn (66.6%) then spring (50%) while the lowest prevalence was in winter (16.6%)**.** The total parasitic intensity was 4.2, the highest parasitic intensity was in spring, 8 followed by winter 3.5 then autumn, 3 while the lowest intensity was in summer figure3.

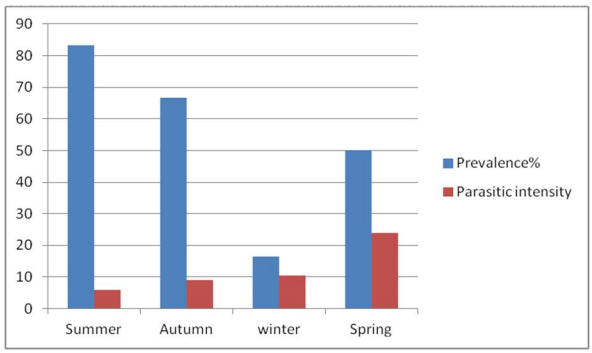


Figure 3: Seasonal variation of *Anguuillicola crassus* infestation in cultured eel fish

**Prevalence rate and intensity of infection in relation to size of cultured eel:**

As shown in table (2) observed that the high prevalence of *Anguillicola crassus* infection was seen in eels of small size 83.2 % in eels of less than 20 cm in length while the prevalence decreased as the eel size increased and reached the lower prevalence in the larger sized eels 16.6 % in eels of more than 60 cm length. In contrast the mean intensity of infection

increase as the eel body size increased which was the lowest 2 worms per infected eels in the smallest eels (less than 20 cm in length) while it was highest 8 worms in the biggest sized eels (more than 60 cm in length).

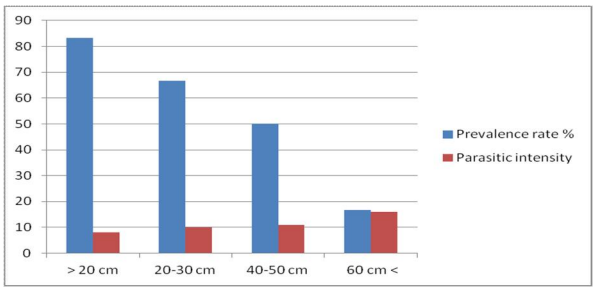


Figure 4: Length, prevalence rate and intensity of *Anguillicola crassus* infestation in cultured eel fish.

Table (1): Survival percentage and curative percentage of *M. oleifera* leaf powder & levamisole Hcl treated groups in relation to untreated.

| groups | No. of infected eels | No. at the end | Surviv. % | Treated fish | Curative % |
|---|---|---|---|---|---|
| **Control (untreated)** | 25 | 15 | 60 | --- | 0 |
| **Treated with leaf of *Moringa Oleifera*** | 25 | 24 | 96 | 23 | 92 |
| **Treated with levamisole Hcl** | 25 | 21 | 84 | 24 | 96 |

Table (2) Some hematological changes in healthy control and infected cultured eels with *Anguillocola carssus.*

| parameter / fish | RBCs $10^6$/µL | Hb gm/dl | PCV % | MCV Fl | MCH Pg | MCHC % |
|---|---|---|---|---|---|---|
| ***A. crassus* free fish (control)** | 3.18 ± 0.10 | 11.18 ± 0.41 | 24.85 ± 0.94 | 113.40 ± 4.72 | 42.11 ± 1.89 | 38.49 ± 1.54 |
| ***A. crassus* infected fish untreated** | * 1.41 ± 0.07 | * 7.94 ± 0.40 | * 17.86 ± 0.98 | * 126.67 ± 5.81 | * 56.31 ± 3.10 | * 44.46 ± 2.18 |
| ***A. crassus* infected fish treated with levamisole** | 3.65 ±0.18 | 9.34 ±0.46 | 19.34 ±0.96 | 122.12 ± 6.10 | 49.15* ±2.45 | 41.85 ±2.09 |
| ***A. crassus* infected fish treated with *M. oleifera*** | 4.17* ±0.20 | 15.19* ±0.75 | 28.73* ±1.43 | 108.32 ±5.41 | 39.18* ±1.95 | 40.47 ±2.02 |

*: Significant at $P < 0.05$. n=10
Each value represents mean ± S.E;

Table (3): Biochemical alteration in blood serum in *A. crassus* infected eels treated with levamisole and *M. oleifera* leaf powder

| Group / Parameter | *A. crassus* free fish (control) | *A. crassus* infected fish without treatment | *A. crassus* infected eels treated with levamisole | *A. crassus* infected eels treated with *M. oleifera* |
|---|---|---|---|---|
| **Total protein (g/dl)** | 4.42±0.22 | 1.85±0.12* | 4.99±0.24* | 5.12±0.24* |
| **Albumin (g/dl)** | 1.30±0.08 | 0.50±0.03* | 1.23±0.06* | 1.79±0.09* |
| **Globulin (g/dl)** | 3.12±0.20 | 1.35±0.10* | 3.76±0.18* | 3.33±0.17* |
| **A/G ratio (g/dl)** | 0.49±0.02 | 0.35±0.02* | 0.32±0.02 | 0.53±0.04* |
| **Urea (g/dl)** | 27.54±1.85 | 28.23±1.73 | 28.06±1.40* | 27.66±1.38 |
| **Creatinine (g/dl)** | 0.81±0.02 | 0.85±0.02 | 0.83. ±0.05 | 0.75±0.07* |
| **Uric acid (g/dl)** | 3.10±0.17 | 3.35±0.20 | 3.23±0.17 | 3.13±0.15* |
| **AST (U/dl)** | 53.70±2.16 | 98.41±5.67* | 59.34±2.97 | 52.40±2.52* |
| **ALT (U/dl)** | 36.65±1.80 | 83.58±3.70* | 36.62±1.83 | 36.0 ±1.65* |

Each value represents mean ± S.E; n=10
* Significant difference by student t-test at $p \leq 0.001$

**Results of biochemical findings in blood serum:**

As shown in table (3), serum total protein, albumin, globulin levels, and albumin/globulin (A/G) ratio were significantly declined in *Anguillicola crassus* infected eels comparing with non infected. Aspartate aminotransferase and alanine aminotransferase enzymes activities in the serum of infected eels were significantly increased in comparison with the non infected fish. However the serum urea, creatinine, and uric acid levels in infected fish were not significantly changed against the *Anguillicola crassus* free eels values. It was noticed that biochemical parameters of *A. crassus* infected eels treated with *M. oleifera.*

**Results of treatment of infected eels:**

As shown in table (2 ) the survival percentage of untreated (control) group, *M. oleifera* leaf powder and levamisole treated groups all over the breeding period was 60, 96 and 84 % respectively. While the curative percentage was 0, 92, and 96 respectively.

**Discussion:**

*Anguilla anguilla* is one of high commercial value in the cultured fish farms in lower Egypt. *Anguillicola crassus* accidentally introduced from Asia has been progressively expanding its range in the eel-inhabited inland waters of Europe and Africa and dissemination has been linked to the extensive commercial transport of live eels and restocking of eel fisheries with infected fish, as well as natural movements of infected eels (**Kirk,2003 Noor El-Deen *et al*., 2012**).

Present study showed that, the infected eel displayed loss of appetite, hanging near the surface, collected near water inlet with clear retard of growth with increasing mortality and the anal redness in eel as a characteristic diagnostic feature and an indicator of infection by *A. crassus*. These results were in agreement with **(Dosoky, 2007** and **Noor El-Deen *et al*., 2012)** who described the clinical signs of *Anguillicola crassus* infected that eel showed loss of appetite and vitality, reduced swimming ability, reduction growth rate, abnormal behavior by hanging near the surface of water, increase mortality rate, distended abdomen, the anal opening showed yellow-orange, pink or red coloration. The postmortem lesion of *A. carssus* infected eels**,** was represented as narrowing or collapsing the lumen of the swim bladder of *A. carssus* infected eels and the worms appeared from outside the intact swim bladder. The swim bladder wall in some infected eels showed thickening and extreme inflammation, it had become markedly enlarged and hyperemic, pneumatic duct showing inflammation **(Liewes and Hanen, 1995)**.

In severe cases, the bladder was dilated and its wall became thickened, opaque and showed signs of inflammation. These in agreement with **(Mohamed and Nouh 2004 and Noor El-Deen *et al*., 2012)**.

Prevalence rate of infestation in cultured eels during winter was 16.6 % while in summer was 83.2%, it may be attributed to that the increase of temperature could be lethal for larvae of the parasite, the results agree with that observed by **(Charleroy *et al*., 1989)**.

Concerning the prevalence of *A. crassus* infection in relation to body size in cultured eel, the study reported that the high prevalence of A. crassus infection was seen in eels of small size 80 % in eels of 23 in length while the prevalence decreased as the eel size increased and reached the lower prevalence in the larger sized eels 50 % in eels of more than 40 cm length, these may attributed to the crustaceans as intermediate host serve as a source of feeding and infection for smaller eels than larger, which agree with that observed by **Kirk (2003), Schabuss *et al*. (2005) & Abdallah and Maamouri (2006**).

In times of low water temperature when eels are in quiescent period and off food, the number of larvae could decrease as no new infections should take place and increased during summer (**Wurtz *et al*., 1998** and **Dosoky, 2007)**.

The infestation affects especially the fish of small size because of the feeding behavior of the later which is based on a primary food made up of annelids and small shell fish especially cyclops which is the intermediate host of this parasite, whereas the fish of large size feed on small fish and crabs. In addition, significant mortality of fish infested by this parasite decreasing the number of parasitized fish that arrive to the adult stage (**Molner *et al*., 1991**; **Noor El-Deen *et al*., 2012)**

Regarding to the hematological and biochemical changes of eel infected with *Anguillicola crassus*, it was noticed that there was a picture of microcytic hypochromic type of anemia in eels infected with *Anguillocola carssus*, represented by reduction in erythrocytic count, hemoglobin concentration, packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) associated with an elevation in mean corpuscular volume (MCV) of *Anguillocola carssus* infected eels compared with non infected eels. These results may be due to that the damage to eels caused by the parasite is considered to be related mainly to the blood-sucking activity of the (pre) adult stages of the nematode in the swim bladder. These because the gut of the adults is completely filled with eel erythrocytes, so the direct damage done by A. crassus a rose from the blood-sucking of mature worms as well as,changes produced in the swim bladder wall by the migration of larvae and the

pathological effects become progressively more pronounced as repeated infections occur (**Haenen *et al*., (1996**). Also off food of infected eels may cause anemia. The results nearly agree with the results previously recorded by **Boon *et al.* (1990)**. **Benajiba *et al.* (1994)** and **Dosoky (2007).**

Concerning the trial for treatment, present study investigate two different types of therapies, have been tested for treatment of eels infested with *Anguillicola crassus* (*A. crassus)*, chemical drug represented as levamisole hydrochloride and medicinal plant represented as dried leaves of the plant Moranja olifera (The Miracle Tree of Life ) the plant used for first time in treatment of fish diseases specially parasitic. It is difficult to identify if the treatment occurred or not. It was proved through previous studies and literatures (**Dosoky, 2007; Noor El-Deen *et al*., 2012** ) that any change in color of the anus of eels to red or pink, it was characteristic sign of worms infestation so present study use these clinical sign as a good tool for identification of performance of treatment.

Regarding the trial for treatment, group treated with levamisol Hcl.showed high curative ability. (**Choi *et al*.1998)** reported that levamisol enhance the non - specific in eels *Anguilla japonica* also **Mesalhy *et al*.(2010)** reported that, levamisole help to enhance the immune response of catfish to some vaccines and against bacterial infection (*aeromonas hydrophila*), it was noticed that levamisole efficiently enhance the non- specific immune response against infection with bacteria, virus and parasites in fish but not affect the nutritional and physiological status of treated fish comparatively, the treatment of *Anguillicola crassus* infected eels with *M. oleifera* leaf showed survivability of 93.3% all over the period while that of control group was 84.0%. the prevalence rate and mean intensity of infection of *M. oleifera* leaf powder treated group were 18.6% and 8.5 worms per infected eel, respectively. While those of control untreated group were 93.7 % and 2.2 worm per infected eel, respectively. These results indicated that *M. oleifera* leaf has much more curative ability for treatment of *Anguillicola crassus* infected eels without any side effects contrary treated fish become physiologically better than control and infested fish. The result nearly in agreement with **Soetan and Oyewole (2009) & Ogbi and Affiku ( 2011)** who reported that the presence of essential nutrients and minerals in Moringa leaves imply they could be utilized to improve growth performance and health status of animal. Certain bioactive chemical compounds (like saponins, tannins and other phytochemicals), which are known as secondary metabolites of plants are said to have pharmacologically active agents**.** They have antibacterial and anti-parasites properties.

From the present investigation we can concluded that the divergence from normal to coloration could be used as a diagnostic tool of *Anguillicola crassus* infection in eels, higher prevalence of infection was in summer, young or small sized eels was more susceptible than larger ones, while larger eels harbored more parasites than smaller ones. Anguillicolosis can be safely treated in eels using *M. oleifera* leaf powder in diet without any side effects improving physiological, immunological, nutritional, hematological and biochemical status of eels while levamisole Hcl has higher curative efficiency but affect lesser on physiological condition of infected eels.

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