ROLE OF NIGELLA SATIVA IN DECREASING MORTALITIES IN NILE TILAPIA CAUSED BY PSEUDOMONAS SEPTICEMIA

AYA GALAL SAAD EL-DEEN
Animal Health Research Institute, Assiut Laboratory
E-Mail: aya9galal@gmail.com

ABSTRACT

This work was carried out to investigate Pseudomonal septicemia in Nile tilapia, Oreochromis niloticus, at Assiut governorate and to evaluate the effects of oral administration of black cumin Nigella sativa on resistance against Pseudomonas aeruginosa infection. A total of 100 Nile tilapia were collected from River Nile and El-Ibrahemia canal, Assiut governorate, and were subjected to clinical and bacteriological examination. Pseudomonas like isolates were detected from 29 out of 100 collected and examined fish samples (The incidence of pseudomonas infection was 29%). Biochemically, the collected isolates were identified as P. auroginosa and P. putida and other unidentified pseudomonas species. The organisms were mainly isolated from liver, spleen and kidney. The pathogenicity of the isolated P. aureginosa from Nile tilapia was confirmed by an experimental pathogenicity challenge. A total of 90 Nile tilapia were divided into two equal groups with three replicates to investigate the effect of dietary supplementation of black cumin on diseased resistance throughout the experimental challenge. Fish of the first group were fed on basic diet, while fish of the second group fed on basic diet with 3% black cumin /kg diet (3 g. black cumin /100 g diet) for 30 successive days. Experimental challenge was done by immersion where the infected fish showed typical signs of pseudomonal septicemia; redness all over the body, ulceration, scales detachment, darkening of body and congestion of all internal organs. Cumulative mortalities of fish challenged were significantly less in groups fed on black cumin diet (13.33%) than those fed on the basic diets (53.33%). Moreover, lesions and symptoms were less or sporadically seen in group treated with black cumin. These results showed black cumin improve the fish resistance to disease.

Key words: Pseudomonal septicemia, Nile tilapia, black cumin

INTRODUCTION

Bacterial pathogens are the causative agents of most serious disease problems in both wild and cultured fish causing mortalities and severe economic losses (Roberts, 2001). Pseudomonas infection has been incriminated as an important bacterial infection among fish and appear to be stress related disease of freshwater fish especially under culture conditions (Kitao et al., 1993).

Pseudomonades are opportunistic Gram negative pathogens, naturally occur in aquatic environment and as a part of normal gut flora of healthy fish, it causes outbreak when the optimum environmental conditions change (Angelini and Seigneur 1988). The genus Pseudomonas contains five species which have been described as etiological agents of diseases in fish in Egypt. Pseudomonas fluorescens, P. angulliseptica, P. aeruginosa and P. putida were identified in various species of fish as causative agents of pseudomonas septicemia (Sakar and Azza 2008 and EL-Nagar 2010), which characterized by fin rot, petechial hemorrhage, darkness of the skin, detached scales, abdominal ascitis and exophthalmia (Khalil et al., 2010).

Using natural feed additive is becoming useful for fish feeding rather than classic chemical feed additives due to the cumulative effects of the chemical components induced deterrent effects on human health (El-Dakar et al., 2008). Black cumin, Nigella sativa, an annual herb that belongs to the botanical family of Ranunculaceae, showed antibacterial, fungicidal effects (Akgul, 1989). Black cumin have been used as enhancer for performance, growth and immune system of some fish species (Abdel-Ghaffar et al., 2003; Diab et al., 2008)

The aim of the present study was to investigate the incidence of pseudomonas species in Nile tilapia at Assiut governorate, as well as the pathogenicity of the
isolated bacteria to Nile tilapia. This study was also performed to evaluate the effect of dietary supplementation of black cumin on diseases resistance of Nile tilapia challenged with pseudomonas.

MATERIALS and METHODS

Clinical and Postmortem Examination of Naturally Infected Fish:
A total of 100 alive Nile tilapia, Oreoichromis niloticus, and weighing 100-350 g with total length of 14-26 cm were collected from El-Ibrahemia canal and River Nile from November 2012 to April 2013. Fish were transported immediately to the Aquatic Animals Wet Lab., Veterinary Hospital Clinic, Faculty of Veterinary Medicine, Assiut University. Fish were subjected to clinical and bacteriological examination (Plumb and Bowser, 1982), observed signs were recorded and detected lesions were reported.

Isolation and Identification of Pseudomonas spp. From Fish:
Samples from internal organs of the examined fish were streaked onto bile salt brilliant green agar (Lab M), Pseudomonas p. agar medium plates (Lab M) and brain heart infusion agar (Lab M), then incubated at 28ºC for 24hr. Bacterial colonies were identified according to colony morphology, bacterial staining character, and biochemical character (Palleroni, 1984 and Buller, 2008).

Experimental Fish:
Apparently healthy Nile tilapia with an average body weight of 100 ± 5 g were obtained from a private fish farm in waladya area at Assiut Governorate and transported to the Aquatic Animals Wet Lab., Veterinary Clinical Hospital, Faculty of Veterinary Medicine, Assiut University where they kept in well prepared aquaria. Random samples were used to check whether they are Pseudomonal septicemia free. Fish were acclimated for 2 weeks according to the protocol of maintaining bioassay fish as was previously described by Elsaeesser and Clem (1986) and received commercial food.

Experimental challenge:
Bacterial strain:
Bacterial strains were kept in BHI broth with 15% glycerol (El-Gomhurrhia, Cairo, Egypt) at -20ºC. Pseudomonas aeruginosa strain was passed three times in Nile tilapia through intraperitoneal injection before using for experimental challenge.

Bacterial challenge suspension and counts:
Colonies forming units (cfu) counts in bacterial suspensions were determined using spectrophotometry optical density values at wavelength of 600 nm and standard-plate-count method with ten-fold serial dilution (Elkamel and Thune, 2003).

Experimental challenge:
Acclimated Nile tilapia were divided into three groups with 15 fish each. The first group was infected through immersion in 1 X 10^7 cfu/ml suspension of P. aeruginosa for 30 minutes in 30L, while the second group was subjected to sterile BHI broth for the same duration and the other group remained unchallenged. The whole experiment was repeated three times. Resolation and identification of the inoculated organism from freshly dead and moribund fish were carried out as described above.

Diets and feed additives:
Two different diets with or without additives, representing two diet variants, were formulated to be used for feeding of fish. A basic diet (control) was formulated of grounded yellow corn (34.9%), soya bean meal (28.6%), fish meal (17.0%), wheat bran (9.3%), vegetable oils (6.5%), ground lime stone (0.7%), bone meal (0.30%), mineral mixture (1.7%) and vitamin mixture (1.0%). The other experimental diet was formulated as a Nigella diet (3 g. black cumin, N. sativa/100 g. of basic diets).

Experimental design:
Acclimated Nile tilapia were allotted into two replicates, one replicate received the nigella diet, while the other replicate received the basic diet. Each replicates were subdivided in to three groups (15 fish each). Each replicate was fed twice daily for 30 successive days.

The whole experiment was repeated three times. Re-isolation and identification of bacteria was done from freshly dead fish as mentioned above. The whole experiment was repeated three times.

RESULTS

Clinical and postmortem examination:
Clinical examination of naturally infected fish revealed the presence of septicemia signs on some fish represented by skin darkness and scales detachment in 6 examined fish. Fish exhibited congestion and petechiae on the body surface, especially on the ventral part of abdomen and fins in 22 fish. The postmortem examination revealed congestion of the spleen, kidney and liver in 18 fish. In 17 cases, showed enlarged gall bladder and distended with bile. The remaining fish appeared to be clinically healthy.

Bacterial isolation and identification:
Bacteriological examination resulted in isolation of 34 isolates suspected to be Pseudomonas aeruginosa
(n=18), *Pseudomonas putida* (n=6) and unidentified *Pseudomonas* spp. (10) according to Morpho-biochemical test. Bacterial colonies grown on BHI agar were circulated, convex, entire edge, glistening, creamy color and 1-2 mm in diameter. On pseudomonas p agar colonies were greenish white colonies, while on bile salt brilliant green agar, bacterial colonies were whitish, convex and 1-2 mm in diameter. Results of the biochemical characters and enzyme activities of suspected isolates are shown in Table (1).

Results also revealed that the organism could be mainly isolated from spleen, liver, kidney. *Pseudomonas* existence ratios in different fish organs was 12 in spleen, 10 from liver and 12 from kidney.

Experimental challenge was done by immersion in 1 X 10^7 CFU/ml viable cells of *P. aeruginosa* in two groups of fish. The infected fish in two groups of fish display the same clinical signs and postmortem lesions but in different percentage (level). The challenged fish exhibited signs of ulceration on the body and fin rot (in four fish in group 1 and 2 fish in group two). Petechial haemorrhage on different part of the body surface especially on the ventral part of abdomen, fin and gill cover were recorded in six fish in group one, however 4 in group two. Dark pigmentation were observed in three fish in group one. Internally there were congestion of all internal organs in eight fish in group one and in two fish in group two. Gall bladder was enlarged and distended by bile. Intestine was filled with bloody serous fluid in two fish of group one (Fig 1).

Mortality rate after bacterial infection showed a significant decrease in *Nigella sativa* treatment (p<0.05) in a way that mortality rate in *Nigella sativa* treatment group, basic diets group and in control group were 13.33%, 53.33% and 0% respectively.

Table 1: Cultural and biochemical characters of the isolated bacteria (n=34).

<table>
<thead>
<tr>
<th>characters</th>
<th><em>P. aeruginosa</em></th>
<th><em>P. putida</em></th>
<th>Un-identify strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates</td>
<td>18</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Gram stains</td>
<td>G-ve</td>
<td>G-ve</td>
<td>G-ve</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O/F test</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H2S production</td>
<td>-</td>
<td>-</td>
<td>4/10</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>-</td>
<td>2/10</td>
</tr>
<tr>
<td>V.P.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M.R.</td>
<td>-</td>
<td>-</td>
<td>5/10</td>
</tr>
<tr>
<td>Gelatin liquification</td>
<td>+</td>
<td>-</td>
<td>4/10</td>
</tr>
<tr>
<td>Growth on 5%NaCl</td>
<td>+</td>
<td>+</td>
<td>7/10</td>
</tr>
<tr>
<td>manitol</td>
<td>+ acid only</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Nile tilapia, experimentaly infected with *Pseudomonase aeruginosa* showing congested liver, kidney and bloody fluids filling the intestine
DISCUSSION

Pseudomonas septicemia is one of the important pathogenic bacteria affecting fish farm in Egypt (Khalil et al., 2010). The present study was done to assess and characterize the pseudomonas infection in Nile tilapia, O. niloticus in Assiut governorate and to investigate the effect of dietary supplementation of black cumin, Nigella sativa on the diseases resistance of cultured Nile tilapia.

Results revealed that fish naturally infected with Pseudomonas spp. showed signs of infection were nearly similar to those reported by (Khalil et al., 2010, EL-hady and Samy 2011).

Bacteriological analysis of naturally infected fish resulted in the isolation of two different Pseudomonas spp. including P.aeruginosa and P.putida. The isolates showed phenotypic and biochemical characteristics of the isolated P. aeruginosa and P.putida were parallel to previous studies which identified the same organisms from other fish species (Austin and Austin, 2007 and Buller 2008).

In this study, the incidence of Pseudomonas spp. in the examined O. niloticus was 29%. The result supports previous studies of the examined O. niloticus 25.5% (Saleh et al., 2008) and 30.83% (Eissa et al., 2010). These results are not in agreement with those reported by EL-hady and Samy (2011) who isolated Pseudomonase spp. From O. niloticus with percentage of 55.3%.

Regarding the samples of bacterial isolates among various organs of fish, it was revealed that the isolation from liver, spleen and Kidney approximately had the same rates. This result agreed with (El-Refaey, 2013). This may be due to most of bacterial infections affect haemobiotic system mainly liver, kidney and spleen.

Experimental infection was successfully done by immersion. The result of the current study demonstrated that clinical picture of pseudomonas septicemia characterized by signs of dark pigmentation, petechial hemorrhage on different parts of the body surface, ulceration, especially at dorsal part and at the base of fins with eroded fin (fin and tail rot). It may be due to the toxic proteases produced by this organism, thus serving to destroy the body tissues. Hemorrhages at the base of fins could be primarily induced by release of powerful bacterial proteolytic enzymes which lead to electrolyte and protein loss together with disturbed blood circulation (Amlacker, 1970 and Mortia, 1975). Congested internal organs are a septicemic lesion, where the congestion and edema was seen to play a role in the enlargement of kidney, spleen and liver. The over distended gall bladder could be attributed to the enteritis or to encountered constriction of the common bile duct by peri-duct fibrosis, these results are conceited with those noticed by Eissa et al. (2010); Kalil et al. (2010).

The pathogenicity of Pseudomonas spp. For experimentally infected Nile tilapia may be attributed to the production of extracellular enzymes and total toxins (as protease, haemolysins, enterotoxins, enterotoxins cytotoxins and others) (El-Attar and Mostaf 1996) Abou El-Geit et al., 2013).

The challenge infection revealed cumulative mortalities of Nile tilapia were significantly less in fish fed on nigella diets (13.33 %) than those of fish fed on the basic diets (53.33%). Moreover, lesions and symptoms were minimized or sporadically seen in group treated with black cumin. In this study, stimulation of the immune system of Nile tilapia as a result of feeding of black cumin diets have positively impacted the resistance of fish to P. aeruginosa infection as was indicated by the significantly lower mortality rates of fish challenged with virulent P. aeruginosa. It was reported that Ocimum sanctum enhanced the disease resistance in Oreochromis mossambicus against A. hydrophila infections (Logambal and Michael 2000). Furthermore, Nile tilapia fed with probiotics and challenged with A. hydrophila showed significant decrease in mortalities (Ali et al., 2010). Moreover Yilma et al. (2012) reported that cumulative mortality was 60% in fish fed the 0% control diet and challenged with Streptococcus iniae. However, in fish fed the 2.0% supplemented diets with black cumin, mortality was only 37.50%. These results are in fair agreement with the administration of herbal supplemented diets showing resistance against streptococcal disease in tilapia fed Rosmarinus officinalis (Abutbul et al., 2004; Zilberg et al., 2010).

This result agreed and explained by (Elkamel and Mosaad 2012) who reported that dietary supplementation of black cumin enhanced the overall immune response of Nile tilapia as was indicated by the significant increase of the WBC numbers, (White blood cells (WBCs) of fish play a crucial role in the cellular immunity and resistance to infectious diseases (Whyte, 2007), globulin proteins and the phagocytic activities of fish phagocytes. This modulation of the fish immunity has greatly enhanced the resistance of challenged fish to A. hydrophila as was indicated by the significant increase in mortalities in fish received the nigella diets. Al-Dubakel et al. (2012) reported that black cumin enhance T cell immunity and production of cytokines (Haq et al. 1995), natural killer cell and compliment (Mahdi, 1993). It also inhibit some microbe and has anti-helminthic activity against nematodes and cestodes (Agarwal et al. 1997). Black cumin extract has positive effect on leukocytes (Mona et al., 2002). Diab et al. (2008) argued that black cumin could increase the survival rate and the resistance of fish to some infectious diseases. Black cumin seed could be recommended to be used for farmed fish to decrease...
mortalities caused by pathogenic microorganisms (Dorucul et al., 2009).

On conclusion, P. aeruginosa can be considered as accountable fish infection under culture condition. The use of 3% black seeds for 30 days could increase the survival rate and the resistance of fish to some infectious diseases.

REFERENCES


**Title:**

Doctoral Thesis: Water Quality in the Nile Tilapia Farm in Assiut, Upper Egypt

**Author:**

Ayman Elnaggar

**Email:** aya@galal@gmail.com

A new study was conducted on the effects of water quality on the health of Nile Tilapia (Oreochromis mossambicus) cultivated in Assiut, Upper Egypt. The study aimed to investigate the role of water quality on the health of Nile Tilapia. The study was conducted on a Nile Tilapia farm located in Assiut Governorate, Upper Egypt. The study included 100 Nile Tilapia fish, which were divided into two groups: a control group and an experimental group. The control group was fed a normal diet, while the experimental group was fed a diet supplemented with dried leaves of Rosmarinus officinialis. The results showed that the water quality significantly affected the health of Nile Tilapia. The water quality parameters, including pH, temperature, dissolved oxygen, and turbidity, were significantly lower in the experimental group compared to the control group. The study also found that the Nile Tilapia in the experimental group had a higher growth rate and weight gain compared to the control group. The study concluded that the supplementation of dried leaves of Rosmarinus officinialis in the diet of Nile Tilapia significantly improved its health and growth rate.