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Field studies on Ichthyophonasis (Ichthyosporidiosis) infecting Red Sea Cultured grouper, Taradi, *Plectropomus areolatus* in Jeddah, Saudi Arabia with a special trial for treatment using *Moringa oleifera.*

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ABSTRACT

Ichthyophonus is a fungus-like agent that causes a chronic, systemic, granulomatous disease, present study aimed to investigate the prevalence, clinical signs, postmortem lesions and trial for treatment of naturally infected Red Sea cultured grouper, Taradi, *Plectropomus areolatus* broadstock fish with Ichthyophonasis using *Moring oleifera* leaves water extract studying the infectivity and pathogencity of *ichthyophonus hoferi* when experimentally infected other marine fish species in Jeddah Saudi Arabia and studying the histopathological alteration caused by infection with Ichthyophonasis in naturally infected Red Sea cultured Taradi, *Plectropomus areolatus* broadstock**.** The disease causes bulging of eyes, loss of color, ulcers, respiratory distress, open mouth and some nervous manifestations, depression, sluggish movements swim in abnormal circular movements, distended abdomen. Internally macroscopical cysts in internal organs and inflammations especially in vasculrized organs liver, heart, spleen and kidney, distended swim bladder with formation of bloody fluids in the abdominal cavity, in advanced cases of icthyophonasis in Taradi fish almost all internal organs enveloped with sheath from spores and connective tissues. Squash preparation of small parts of tissues from internal organs of infected fish revealed the presence of various life stages of *Ichthyophonus hoferi* in heart, liver, kidneys, spleen and peritoneum membrane. The naturally infected and treated taradi fish with *M. oleifera* leaves water extract was 24 from 30 fish with percentage 80%, while treatment percentage in control non treated group was 0%. Experimental infection of icthyophonasis 30 fish in each group, taradi fish infected with percentage 100 % followed by *Oreochromus spirulus* 96.6 % followed by *Asian sea bass* 46.6 % and the lowest suspected species was *European Sea bream* 36.6 %, the route of experimental infection of ichthyophonasis through stomach tube (ST) infection was more efficient than intra peritoneal (IP). Histopathological alterations in naturally infected taradi fish was discussed in the study.

Key words: Ichthyophonus, *Plectropomus areolatus,* nervous manifestations, respiratory distress, vasculrized organs, Experimental infection, Histopathological alterations.

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INTRODUCTION

Ichthyophonus is a fungus-like agent that causes a chronic, systemic, granulomatous disease. It is endemic in many feral, cold water marine fish populations and has been reported in over 80 species of marine fish [1,2,3]. Its causative agent is *Ichthyophonus hoferi*, that has a very broad host spectrum and is known to infect marine and freshwater fishes, cultured and wild fish, causing granulomatous systemic disease in vascularized organs such as heart, spleen, liver and kidneys [4,5,6,7]. It is probably a significant cause of chronic mortality in some feral marine fish populations [2].

Epizootics of ichthyophonasis occasionally occur among commercial fish species [8,9,10,3]. I. hoferi attacks over 80 species of fish, including salmon, herring and induces systemic mycosis [11, 1, 2, 10, 3]. Epizootics among salmonids thought to be caused by I. hoferi have been reported from trout farms in Europe and North America [12]. In wild populations of salmonids a high prevalence of *Ichthyophonus hoferi* disease was reported for *Oncorhynchus tshawytscha* from the Yukon river [13].

Moringa oleifera is one of the most widely tropical plants and its leaves contain several chemical and biological active ingredients. *Moringa oleifera* is native to the western and sub- Himalayan region, India, Pakistan, Asia minor, Africa and Saudi Arabia [14]**.** Important medicinal properties of the plant include antipyretic, antiepileptic, anti-inflammatory, anti-ulcerative [15] antihypertensive [16]**,** cholesterol lowering [17]**,** antioxidant [18]**,** anti diabetic, hepatoprotective [19]**,** antibacterial and antifungal activities [18,20,21]**.**

The present study was aimed to investigate the prevalence, clinical signs, postmortem lesions and trial for treatment of naturally infected Red Sea cultured grouper, Taradi, *Plectropomus areolatus* broadstock fish with Ichthyophonasis using *Moring oleifera* leaf water extract studying the infectivity (experimental infection) and pathogencity of *ichthyophonus hoferi* when experimentally infected other marine fish species (Asian Sea bass, *Oreochromis spirulus* and European Sea bream) in Jeddah Saudi Arabia and study the histopathological alterations caused by infection with Ichthyophonasis in naturally infected Red Sea cultured Taradi, *Plectropomus areolatus* broadstock**.**

MATERIALS AND METHODS

Naturally infected Fish

A total number of 120 Red Sea cultured Taradi, *Plectropomus areolatus* broodstock were collected from cement ponds, Fisheries Research Center, Jeddah, Kingdom of Saudi Arabia. Fish suffered from emaciation, nervous manifestations and sudden death, fish transferred to the laboratory Fisheries Research Center, Jeddah, Saudi Arabia. Clinical signs and postmortem lesions were recorded [22]. Fish body weight range about 500-2500 g.

Clinical and postmortem signs

Infected fish clinically observed in the cement ponds for any external abnormalities, swimming behavior, respiration, feeding and escape reflex. eyes, ulcers, skin, fins, after dissecting all internal organs were examined, gills, swim bladder, intestine and gonads also vascularized organs heart, liver, spleen and kidney [23].

Fungal examination

Fresh smears from heart, liver, spleen, kidneys are and gills were examined by light microscope immediately or few hours after death with drops of Sea water searching for the presence of spores or any other stages of *Ichthyophonus*. The inoculum used consisted of fungal material removed aseptically from suspected organs or peritoneum to culture medium [24].

The inoculums was cultured aseptically in different broths, Eagles minimum essential medium (MEM, Sigma M5775), and Sabourauds dextrose broth medium (SDB, Difco) using sterile tubes with 5 ml medium/tubes [25]. The fungus was also inoculated into solid media of Sabourauds dextrose agar (SDA, Difco). All media were supplemented by different concentrations of fetal bovine serum (FBS, Sigma F4010), All media

were supplemented also by penicillin at 100IU/ml and with streptomycin at 100 µg/ml. The culture in tubes and plates were incubated at 15 ^{o}C for 15 days [5,6].

Identification of the fungal growth

Identification of the fungal growth was identified by microscopical examination of wet mount preparation and stained by lactophenol cotton blue as described by [26]. The isolates were sub cultured in MEM-10 pH 3.5 for experimental infection.

Experimental infection

A total 150 fish were divided into 8 groups each 15 from Red Sea cultured marine fish species Taradi, Asian sea bass , *Oreochromis spirulus* and European sea bream respectively . 1st 4 groups were given an inoculum 0.2 ml was prepared from homogenates from naturally infected tissues of Taradi with Hank's balanced salt solution (HBSS) supplemented with 100 IU mL^{-1} penicillin and 100 l g mL^{-1} streptomycin intra peritoneum (IP). Wet mounts of the inoculum were confirmed microscopically to contain Ichthyophonus, The 2nd 4 groups were given an inoculum 0.5 ml by intra gastric stomach tube at the same time to the same 4 fish species. The remainder of the inoculum was stored at -20 ^{o}C. Wet mounts of fresh tissue samples were prepared between glass cover slip and slide and examined microscopically for the presence of the fungus.

***Moringa oleifera* water extract**

Moringa oleifera leaves were collected from Fisheries Research Center, Jeddah, Saudi Arabia garden. Leaves washed dried and grinded, The powdered plant material (leaves) 50 g were soaked in 500 ml distilled water in one liter capacity conical flasks, stopper and kept for one week with intermittent shaking. The percolates were filtered with Whatman's No. 1 filter paper. The extracts were concentrated at 40°C under reduced using rotary evaporator (R110) The concentrated extracted were labeled (Moringa leaf aqueous extract) [27]**.**

Treatment trial for naturally infected Taradi fish

Ration

Ration was supplemented with 5 ppm of *Moring oleifera* water extract/kg ration. *M. oleifera* leaves water extract was added to dietary ingredients and thoroughly mixed in a mixer and extruded through a 5 mm diameter diet in a meat grinder. The pellets were air-dried at room temperature, broken into small pieces, sieved to obtain appropriate size and stored in a freezer at -4°C until used.

Experimental Design for treatment trial

One hundred and twenty Red Sea cultured Taradi, *Plectropomus areolatus* Broadstock were divided into two groups. First group (20 fish) was considered as a negative control. The second one (100 fish) was infected by using stomach tube containing *I. hoferi* MEM-10 pH 3.5 culture as 1 ml fish^{-1} for one time according to [28]**.** The mortality and clinical abnormalities were recorded during one month after experimental infection. At the end of 30 days of infection, 15 infected fish were scarified for mycological examination to verify the presence of infection and the survived fish (60) was subdivided into two equal sub-groups. 1st group was represented as infected positive control. 2nd group was fed on ration containing 5 ppm of *Moringa oleifera* leaves water extract/kg ration [20]. The treatment was continued for 60 days . At the end of experiment, all survivors were examined for infection. All groups were taken in 3 replicates.

Blood samples

Five blood samples were collected from each group from caudal vessels using hepranized syringe then serum was separated and kept at -20 °C until used for biochemical analysis.

Serum total protein, albumin and globulins were performed using 7150 Automatic blood chemistry analyzer (Ciba- Corn in g Diagnostic Crop). Red blood corpuscles, Total leucocytic count (TLC) was estimated according to [29] and differential leucocytic count was carried out according to [30].

Phagocytosis assay

Phagocytosis was determined as follow

Fifty µg of well identified Candida albicans strain culture (previously adjusted to be 100 mg/ml) were added to 1 ml of pooled whole blood samples collected from treated and control fish then shacked in water bath at 23-25^{o}C for 3-5 hours. Air dried blood smears fixed with methanol then stained with Giemsa stain, phagocytic index was calculated as follow:

Phagoytic index (PI) = Number of yeast cells phagocytized / Number of phagocytic cells according to [31].

Lysozyme activity assay

Serum lysozyme activity was modified as described by [32]. Briefly, 10 µl of individual serum was mixed with 200 µl of a Micrococcus luteus (Sigma) suspension at 0.2 mg ml^{-1} in 0.05 M sodium phosphate buffer (pH 6.2). The mixture was incubated at 27 °C, and its OD was detected after 1 and 6 min at 530 nm using an ELISA plate reader. One unit of lysozyme activity was defined as the amount of enzyme producing a decrease in absorbance of 0.001 min^{-1} ml^{-1} serum. Lysozyme concentrations were calculated from a standard curve of known lysozymes from chicken egg white (L4631-1VL, Sigma) concentrations.

Histopathological Examination

Tissue specimens from suspected examined organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with Haematoxylin and Eosin (H&E) according to [33].

Statistical analysis

The obtained data were analyzed statistically by analysis of variance (ANOVA) according to [34].

RESULTS

Clinical signs of naturally infected Taradi fish

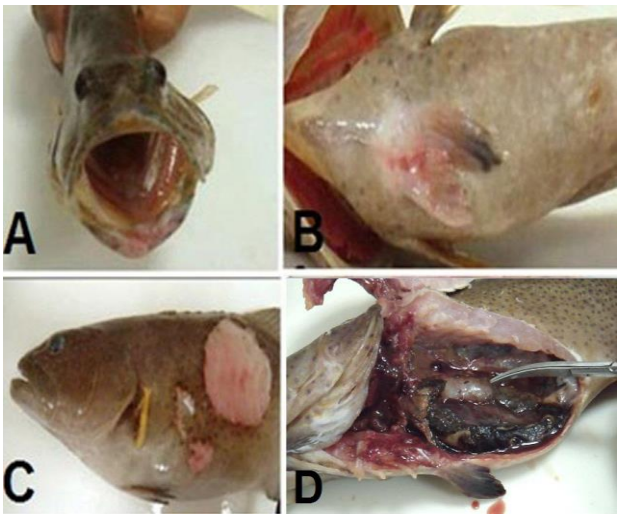

Figure 1: Naturally infected Taradi fish showing (A) exthophthalmia (plugged eyes) and respiratory stress (open mouth) (B) distended abdomen with ulcer in the flank region with open operculum, (C) ulcer in the lateral side (D) cysts in liver and heart with inflammation accompanied with bloody ascites in the abdominal cavity.

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The disease causes bulging of eyes, loss of color, ulcers, respiratory distress, open mouth and some nervous manifestations, depression, sluggish movements swim in abnormal circular movements, distended abdomen. Internally macroscopical cysts in internal organs and inflammations especially in vasculrized organs (liver, heart, spleen and kidney), distended swim bladder with formation of bloody fluids in the abdominal cavity, in advanced cases of icthyophonasis in Tradi fish almost all internal organs was enveloped with sheath from spores and connective tissues (fig 1&2).

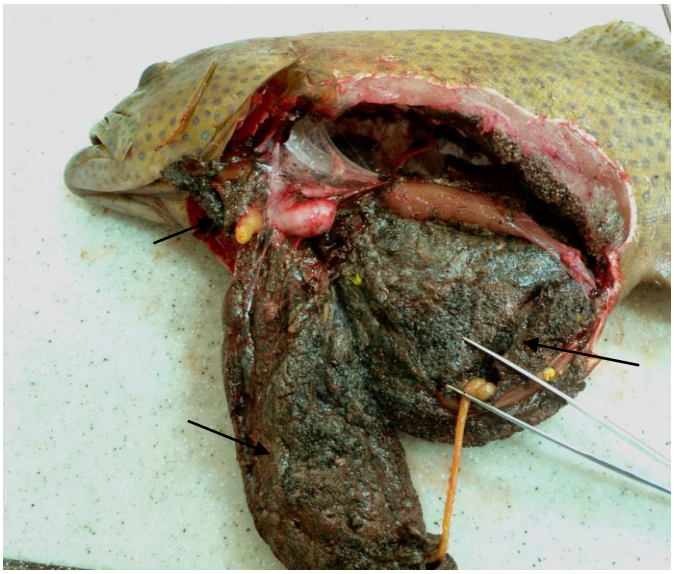

Figure 2 : Showing advanced ichthyophonosis in Taradi, *Plectropomus areolatus* with internal organs liver, gall bladder, heart, spleen, kidney and intestine of the fish enveloped with fibrous sheath of Ichthyophonus spores.

Mycological examination

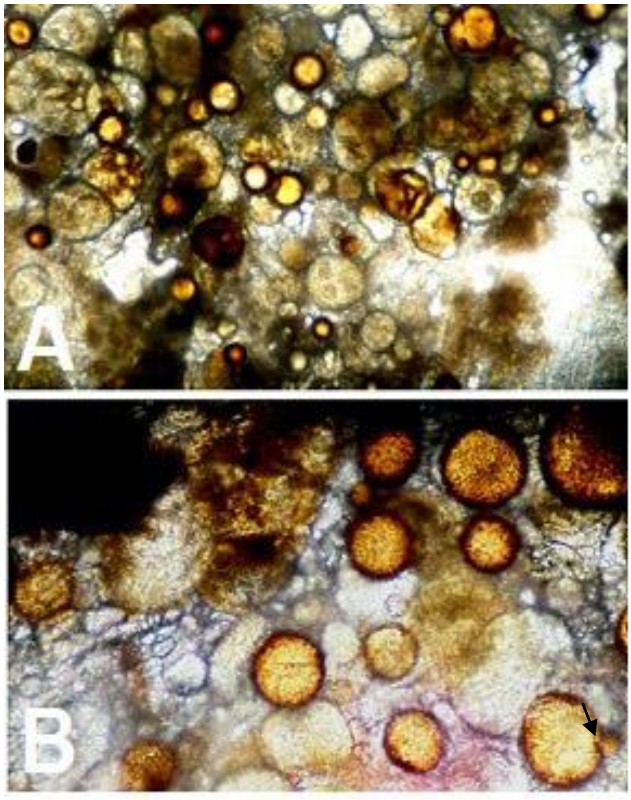

Figure 3: Showing wet mount preparation showing (A) low magnification of icthyophonus spores (B) high magnification brown and yellow germinating (arrow) and non germinating double walled spores isolated from peritoneal cavity membranes of infected Taradi *Plectropomus areolatus* fish.

Squash preparation of small parts of tissues from internal organs of infected fish revealed the presence of various life stages of *Ichthyophonus hoferi* in heart, liver, kidneys, spleen and peritoneum membrane. The recognized stages were distension of the spore wall was observed with the formation of hyphae or new budding yeast like spores. Development of uni or binucleated endospores and multinucleate spore (resting spore) which vary greatly in diameter and germinating flask shaped cyst or uninucleated endospores scattered between tissues of the organs (Fig, 3A & B).

The first isolation of *Ichthyophonus* from infected organs was achieved in all the media assayed, though optimal growths of the organism were observed in MEM-10 and SD broth-10. Results of cultivation of infected materials on SDA-1 revealed hyphae growth both on the surface and into the substance, the hyphae growth increased to full fill plate within 12-14 days post inoculation formation of hyphae and spores was observed and large elongated chlamydospores.

The examination of investigated fishes was classified as ; infected when the organism was isolated by in vitro culture, clinically infected or diseased when visible white lesions were observed on at least one organ and confirmed to be *Ichthyophonus* by culture, sub-clinically infected when visible lesions were not apparent but *Ichthyophonus* was detected microscopically or negative when *Ichthyophonus* couldn't be identified by any of the above techniques.

Treatment trials

The present study displayed that the naturally infected and treated taradi fish with *M. oleifera* leaves water extract was 24 fish from 30 with percentage 80%, while treatment percentage in control non treated group was 0%. Treatment trial exerted 6.66% mortality in *M. oleifera* water extract treated group, while mortality rate in control group was 56.6%. No fungus was re-isolated from organs of Taradi, *Plectropomus areolatus* after treatment with *M. oleifera* (Table, 1).

Table 1. The treatment percentage and mortality rate percentage among the naturally infected Taradi fish after treatment.

Fish groups	No of fish	No of treated fish	% of treated fish	No of dead fish	Mortality rate
Infected non treated group	30	0.0	0.0	17	56.6%
Moriga oleifera leaves water extract	30	24	80	2	6.66 %

Haematological and immunological studies

Table 2: Showing some physiological and immunological parameters post treatment with *M. oleifera* leaves water extract after 30 and 60 days

parameters	Before treatment	30 days post treatment	60 days post treatment
	Infected non treated	Treated with *M. oleifera*	Treated with *M. oleifera*
RBCs count	106±5.3B	104±5.2B	110.7±5.5B
WBCs count	4.13±0.21B	6.55±0.33b	5.50±0.28B
Total protein	3.92±0.37B	4.31±0.36B	4.05±0.2B
Albumin	1.36±0.39B	1.39±0.39B	0.97±0.01B
Globulin	2.56±0.32B	2.92±0.42B	3.08±0.47B
Phagocytic index	113.5±5.6 B	169.0±7.2b	152.3±6.2b
Lysozyme conc.	1.75 ± 0.26B	2.92 ± 0.31b	2.25±0.26b

Each value represents mean ± S.E.; N=5.

Small letters (a), (b), and (c) represent a significant change to capital letters A, B, and C Respectively at the same row (by LSD using ANOVA at $p < 0.05$).

The treated group with *M. oleifera* leaves water extract displayed non significant increase of RBSCs after 30 and 60 days post treatment. The treated group with *Moringa oleifera* leaves water extract showed that there is significant increase of total lecuocytic count at the period of 30 days post treatment while at 60 days showed non significant increase in comparison with the control group. There in non significant increase in total protein, albumin and globulin between the three groups.

The treated group with *Moring olifera* leaves water extract displayed significant increase of phagocytic index and serum lysozyme concentration along all the period of examination 30 and 60 days in comparison with the control group table 2.

Experimental infection

The present study displayed that a total number of 120 fish sp. included in the experiment of experimental infection of icthyophonasis 30 fish in each group, tradi fish infected with percentage 100 % followed by *Oreochromus spirulus* 96.6 % followed by *Asian sea bass* 46.6 % and the lowest suspected species was *European Sea bream* 36.6 %, from the present study it is clear that route of experimental infection of ichthyophonasis through stomach tube (ST) infection was more efficient than intra peritoneal (IP) table 3.

Table 3: Showing experimental infection of ichthyophonus inoculum once and examination 60 days post infection

fish	No of fish	Mode of Exp. infection				Infected fish	Percentage %
		I.P*	Inf.	S.T*	Inf.		
Taradi, *P. areolatus*	30	15	15	15	15	30	100.0
Asian Sea bass	30	15	6	15	8	14	46.6
Oreochromus spirulus	30	15	13	15	16	29	96.6
European Sea bream	30	15	4	15	7	11	36.6

*I.P. = Intra Peritoneal *S.T. = Stomach Tube

Histopathological studies

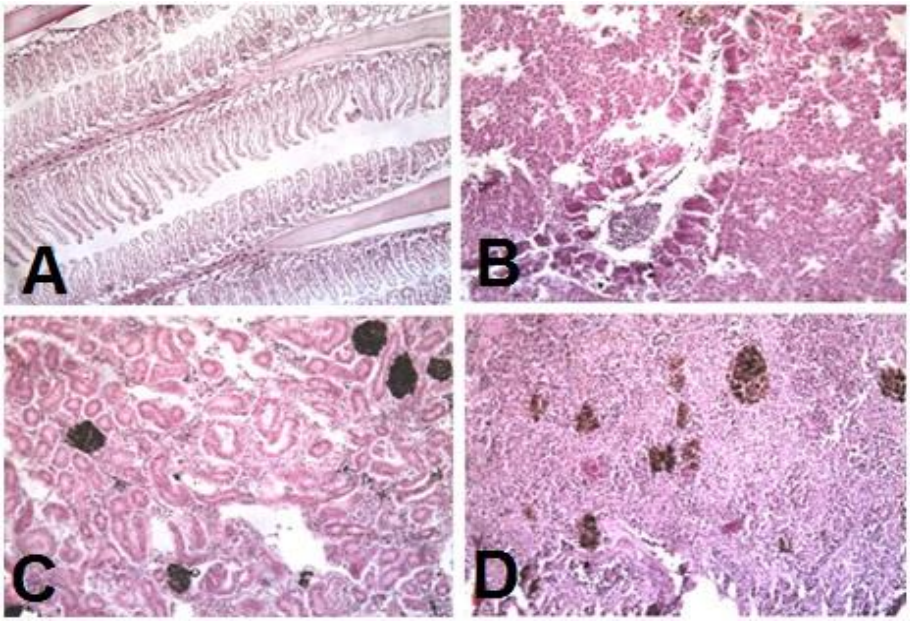

Figure 4 : Showing (A) Gills of infected Taradi, *Plectropomus areolatus* fish with icthyophonosis showed oedema with mononuclear leucocytic inflammatory cells infiltration as well as dilated and congested blood vessels with hyperplastic secondary gill lamellae, (B) liver, showed that the hepatocytes displayed different stages of necrobiotic changes with complete rupture of the cells associated with inflammatory cells infiltration with presence of bacterial cells, showed also thickening of all bill ducts (C) kidney showed swelling and degeneration of the epithelium cells lining the renal tubules with partial replacement of haemobiotic tissues with presence of focal prepcitation of haemociderin in between renal tissues and (D) spleen showed embedding of haemociderin with congestion of red and white pulps.

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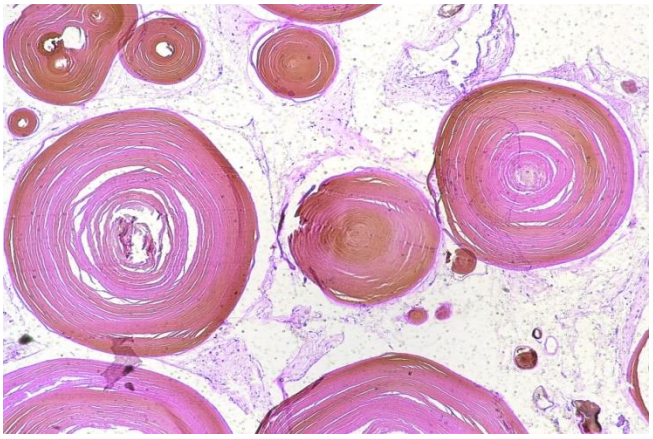

Figure 5 : Showing Taradi liver tissues replaced completely with different sized calcified icthyophonus spores surrounded with fibrous connective tissues H&E X100.

The histopathologicl studies revealed that infected organs with *Ichthyophonus hoferi* displayed that gills showed oedema with mononuclear leucocytic inflammatory cells infiltration as well as dilated and congested blood vessels with hyperplastic secondary gill lamellae Fig (4,A).While infected liver there was severe dilatation and congestion of the central veins. The hepatocytes showed different stages of necrobiotic changes with complete rupture of the cells associated with inflammatory cells infiltration .Thickening of all bill ducts Fig (4,B). kidney showed swelling, necrosis and degeneration of the epithelium cells lining the renal tubules with partial replacement of haemobiotic tissues with presence of focal prepcitation of haemociderin in between renal tissues Fig (4,C). The infected spleen showed embedding of haemociderin with congestion of red and white pulps Fig (4,D). In advanced cases of icthyophoniasis vital organs like liver cells was replaced with calcified spores Fig.5.

DISCUSSION

Ichthyophoniasis is the fungal disease of both freshwater and marine fish, affecting any species of both types. Disease caused by *Ichthyophonus hoferi* characterized by rough or granulomatus lesions of the skin and white to gray-white lesions in the internal organs and different parts of the body. *Ichthyophonus* is a fungus-like agent that causes a chronic, systemic, granulomatous disease. It is endemic in many feral, cold water marine fish populations and has been reported in over 80 species of marine fish [1,2,3]. Epidemics have occurred in Atlantic herring and yellowtail flounder in the northwest Atlantic Ocean, haddock and plaice in the northeast Atlantic, Pacific herring and rockfish in the eastern Pacific, and cod in the Baltic Sea [35,36]. It is probably a significant cause of chronic mortality in some feral marine fish populations [2, 23].

The present study was aimed to determine pathogencity, the prevalence, clinical signs, postmortem lesions with a trial for treatment of naturally infected Red Sea cultured Taradi, *Plectropomus areolatus* broodstock fish with marine Ichthyophonosis and study the infectivity (experimental infection) of *ichthyophonus hoferi* when experimentally infected other marine fish species (Asian Sea bass, *Oreochromis spirulus* and European sea bream) in Jeddah Saudi Arabia with studying the histopathological alteration due to infection with Ichthyophonosis in naturally infected Red Sea cultured Taradi, *Plectropomus areolatus* broodstock fish.

Regarding the clinical signs and postmortem lesions The ability of observers to detect *Ichthyophonus* from gross signs is linked closely to the level of infection and pathogenicity in the host and the tissue sampled. As this differs substantially between different species, obvious signs of disease may vary considerably between different fish [37]**.** These include behavioral abnormalities and changes associated with organ failure, such as lethargy, emaciation, color anomalies, fluid accumulation, nervous disorders and an increase in mortality.

Regarding the treatment of the present study revealed that the naturally infected and treated taradi fish with *M. oleifera* leaves water extract was 24 fish with percentage 80%, while treatment percentage in control non treated group was 0%. Treatment trial exerted 6.66% mortality in *M. oleifera* water extract treated group, while mortality rate in control group was 56.6% . No fungus was re-isolated from organs of Taradi,

Plectropomus areolatus after treatment with *M. oleifera*. Most literatures reported that there is no reported treatment for this disease. Infections in culture areas have been commonly associated with the use of contaminated marine fish as feed; therefore caution should be exercised when using raw trash fish feed as these could be infected with the pathogen [38].

Recently, the application of medicinal plants in disease management is gaining momentum because herbal treatment is cost – effective, minimal side effect and environment friend. It is therefore very necessary that the search for newer antibiotic sources be a continuous process. Plants are safer alternative sources of antimicrobials [39, 40, 41]. *Moringa oleifera* Lam. is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [42] which is widely used for treating bacterial infection, fungal infection, anti-inflammation so that it is suitable to be effective in treatment of icthyophonosis in grouper tradi fish, in addition that [18, 20, 43] and [21] who reported that *Moringa oleifera* extracts act as antibacterial and antifungal activities and added that the use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

The disease is of economic significance, in both cultured fish and wild fisheries, marine and fresh water fishes and has a wide host and geographical distribution. It has been the subject of several comprehensive reviews [44, 45]. Fish predominate as hosts, and [46] who reported infections in 35 marine fish species and 48 freshwater species. More recently, [4] noted more than 80 fish species were susceptible to infection. The literature therefore indicates low parasite–host specificity in fish [1]. Consequently, new fish host records and a host list are probably of little scientific significance and they may largely reflect whether a particular species of fish has been examined sufficiently and appropriately [47, 48].

Regarding the transmission of the disease, the widespread natural reservoir of infection in marine and estuarine waters and the relative ease of horizontal experimental transmission of the disease between most species of fish could suggest that wild and farmed fish stocks in these areas would be at risk from the infection. However, in practice, there have been no records of farmed stocks becoming infected because of the proximity of infected wild stocks, as in European fish [49].

Concerning the effect of treatment on the physiological and immunological state, the present study displayed that, the treated group with *M. oleifera* leaves water extract displayed non significant increase of RBSCs after 30 and 60 days post treatment. The treated group with *Moringa oleifera* leaves water extract showed that there is significant increase of total lecuocytic count at the period of 30 days post treatment while at 60 days showed non significant increase in comparison with the control group. There in non significant increase in total protein, albumin and globulin between the three groups. The treated group with *Moring olifera* leaves water extract displayed significant increase of phagocytic index along all the period of examination 30 and 60 days in comparison with the control group. The result nearly agree with that obtained by [50] who reported that Moringa oleferia enhance the hematological, biochemical and immune parameters of treated fish.

Lysozyme is a bacteriolytic enzyme that is widely distributed throughout the body and is part of the nonspecific defense mechanisms in most animals. In salmonids, lysozyme has been detected in serum, secretions, mucous membranes and tissues rich in leucocytes, mainly the kidney and intestine [51]. Apparently, the main sources of lysozyme are monocytes/ macrophages and neutrophils. However, recent studies have detected this enzyme in the granules of the eosinophilic granular cells of the intestine [52]. The bactericidal action of this enzyme involves the hydrolyzation of the peptidoglycan of bacterial cell walls resulting in cell lysis. Lysozyme was initially associated with the defense against Gram-positive bacteria, but has been found to lyse Gram-negative bacteria as well, furthermore, this enzyme is known to trigger an opsonin of the complement system and phagocytic cells [53].

Regarding the histopathological studies of the naturally infected tradi fish with icthyophonus, the present study revealed that infected organs with *Ichthyophonus hoferi* displayed that gills showed oedema with mononuclear leucocytic inflammatory cells infiltration as well as dilated and congested blood vessels with hyperplastic secondary gill lamellae. While infected liver there was severe dilatation and congestion of the central veins. The hepatocytes showed different stages of necrobiotic changes with complete rupture of the cells associated with inflammatory cells infiltration, thickening of all bill ducts . kidney showed swelling,

degeneration and necrosis of the epithelium cells lining the renal tubules with partial replacement of haemobiotic tissues with presence of focal prepcitation of haemociderin in between renal tissues . The infected spleen showed embedding of haemociderin with congestion of red and white pulps, the present result nearly confirmed by [6, 33,7].

From the present study it was concluded that ichthyophoniasis in taradi broadstock fish (Red Sea cultured marine) is chronic disease affect fish in all ages not easily treated but treatment using *Moringa oleifera* leaf water extract was efficient, *Moringa oleifera* is good medicinal plant used as feed additive in ration of fish also, the present study indicate that leaf water extract of *M. oleifera* has significant effect on hematological, immunological and biochemical parameters of Taradi fish. This study helps to establish the safe limits of aqueous extracts of *M. oleifera* on treatment ichthyophoniasis in taradi fish it could be used as alternative of antibiotic in treatment of pathogenic organisms in aquaculture.

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