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Experimental infection of atypical *Aeromonas salmonicida* in Nile tilapia *Oreochromis niloticus* and its treatment with carvacrol and cymene mixture

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The pathogenecity of atypical *Aeromonas salmonicida* was studied in healthy *Oreochromis niloticus*. Inoculum at concentration of 1.5×10^8 CFU/ml and 3×10^8 CFU/ml was injected into healthy fish through intramuscular and intraperitoneal injections. Experimentally infected *Oreochromis niloticus* showed ulceration at the dorsal musculature and trunk region in addition to black coloration, congested gills, exophthalmia, and ocular hemorrhage. Congested liver and kidney were recorded in post-mortem examination. Mortality of the experimentally infected *Oreochromis niloticus* reached 100% after intramuscular injection at concentration of 3×10^8 CFU/ml. Histopathological investigation of infected organs was also performed. There was a focal area of bundles of skeletal musculature showing hyalinization. In addition, hyperplasia, congestion, and fusion were noticed in the gill lamellae. There was also congestion in the blood vessels in the ocular chamber. Severe congestion was also noticed in the central vein of liver associated with focal aggregation of the melanin pigmented cells in the parenchyma. Degenerative changes were noticed in the epithelial cells lining of kidney tubules. Plant extracts carvacrol and its biological precursor cymene were found to be effective in treating experimentally infected *Oreochromis niloticus* at concentration of 100 or 200 ppm.

Key words: Atypical *Aeromonas salmonicida*, *Oreochromis niloticus*, Pathogenecity, Intramuscular, Intraperitoneal, Carvacrol, Cymene.

Nile tilapia (*Oreochromis niloticus*, *O. niloticus*) have become the third most important fish in the world aquaculture after carp and salmon and the first one in Egypt; worldwide production exceeded 1,500,000 metric tons in 2002 (Lim and Webster, 2006) and increases annually. Because of their high protein content, large size, rapid growth (6 to 7 months to grow to market size), and palatability, various species of tilapia are the focus of major aquaculture efforts (Parker and Parker, 2011). The expression 'atypical *Aeromonas salmonicida*, atypical *A. salmonicida'* was initially used for bacterial strains belonging to the species *Aeromonas salmonicida* showing different biochemical characteristics from those described for *Aeromonas salmonicida subsp. Salmonicida*, such as slow growth and non-pigmentation production (Wiklund and Bylund, 1993). In fact, the number of published reports of disease outbreaks associated with atypical strains has increased significantly, and these isolates have been reported from an increasing number of fish species and geographical areas (Wiklund and Dalsgaard, 1998).

Atypical *A. salmonicida* infection in non-salmonids often manifests itself predominantly as skin ulceration scattered at the dorsal musculature and over the whole

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trunk of the fish (Wiklund and Dalsgaard, 1998). The best described diseases showing these symptoms are carp erythrodermatitis (Bohm *et al*., 1986, Mirle *et al*., 1986), goldfish ulcer disease (Humphrey & Ashburner, 1993; Whittington *et al*., 1995), and ulcer disease of flounder (UDF) (Wiklund *et al*., 1994; Wiklund & Dalsgaard, 1995). Additionally, many other fish species have been reported to be affected by ulcerations associated with atypical *A. salmonicida.* (Wilson & Holliman, 1994; Larsen & Pedersen, 1996).

Regarding, there are few reports about the pathological picture of internal organs of non-salmonids infected with atypical *A. salmonicida.* Congestion of gills, degenerative changes of kidneys, enlargement of the hepatocytes and hyperplasia of spleen were detected in goldfish naturally infected with atypical *A. salmonicida* (Mawdesley-Thomas, 1969). Infection of atypical *A. salmonicida* caused high cumulative mortality (60%-65%) in farmed fish, in addition to high economical losses (Wiklund and Dalsgaard, 1998).

Less information about control of atypical *A. salmonicida* is available. It appears that potentiated sulphonamide, chloramphenicol, neomycin, nitrofurantoin and oxytetracycline are generally effective against these pathogens (Groman *et al.,* 1992; Pedersen *et al.,* 1994; Jeney & Jeney, 1995), but a development of resistance to these antibiotics among atypical strains of *A. salmonicida* was recorded (Hirvela-Koski *et al*., 1994).

At present, there is a growing concern about the use of plant extracts and their compounds for disease control in fish. Carvacrol is a biological compound naturally present in plants such as oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) and savory (*Satureja hortensis*). It has well-known antibacterial (Friedman *et al*., 2002), antifungal (Chami *et al*., 2005), insecticidal (Panella *et al*., 2005), and antiparasitic (Lindberg *et al*., 2000) properties as well as antitoxigenic effect (Ultee and Smid, 2001). It has a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria (Burt, 2004). It has been shown to inhibit many fish bacterial pathogens as *Edwardsiella tarda* ((Rattanachaikunsopon and Phumkhachorn, 2010; Hashiem and Abd El-Galil, 2012; Fatma, 2012), *Aeromonas hydrophila and pseudomonas fluorescens* (Desousa *et al.,* 2012).

The isolate used in this study was previously isolated from ulcerated sea bream *(Sparus aurata)* and identified using VITEK 2 system and polymerase chain reaction (PCR) technique. The present work describes the pathogenicity of atypical, oxidase-positive *A. salmonicida* in *O. niloticus* at different routes and concentrations, in addition to its treatment in the same fish by carvacrol and cymene mixture in the feed.

Materials and methods

Collection and maintenance of experimental *O. niloticus*

A total number of 150 *O. niloticus* with average body weight (40 ± 5 g) were collected alive from Abo-Saleh fish hatchery, Beni-suef governorate, Egypt, during May and July 2014. They were transferred alive to the wet lab. of Fish Department, Faculty of Veterinary Medicine, Beni-suef University, Egypt and fed commercial fish feed at a rate 3% of its body weight.

Aquaria

Three fiberglass tanks (800L for each) in addition to 15 glass aquaria of 70 × 25 × 40 cm, supplied with chlorine free tap water (water temperature 27±2°C) and air supply were used in this work.

Fish feed

A commercial fish feed with 25% protein (Zoo-Treatment Company, Egypt) was used.

Bacterial culture

Atypical *A. salmonicida* was isolated from ulcerated sea bream *(Sparus aurata)* and identified using VITEK 2 system and PCR.

Identification of atypical *A. salmonicida* by VITEK 2 system

Atypical *A. salmonicida* strain was identified by using VITEK 2 Gram-negative (GN) card which contains 64 biochemical tests used as confirmative aiding tools according to manufacture instructions. Inoculated cards were incubated in the VITEK 2 apparatus (BioMerieux, Marcy l Etoile, France) and a computer-assisted algorithm was optimized and used to generate kinetic identification results.

PCR technique

The primer for detection of 16S rDNA included a forward primer 5'-GGC CTT TCG CGA TTG GAT GA-3' and a reverse primer 5'-TCA CAG TTG ACA CGT ATT AGG CGC-3', with a length of 271 (bp). In addition, it was synthesized in Matrix company-Egypt and designed on the basis of published sequences (H0IE *et al*., 1996). The protocol of work was done according to H0IE *et al*., (1999).

Preparation of bacterial inoculum

The bacterial culture was grown overnight on nutrient agar for use in pathgenicity experiments. The pathogenicity of the bacterial isolate was tested by intraperitoneal (I/P) and intramuscular (I/M) injection. The bacterial count was adjusted to match McFarland standard no 0.5 (1.5×10^8 CFU/ml) and 1 (3×10^8 CFU/ml).

Pathogenicity of atypical *A. salmonicida* isolate in healthy *O. niloticus*

A total number of 60 *O. niloticus* were divided into six groups (10 fish each) in a separate aquarium, containing freshwater at 28°C ± 2. The bacterial isolate was inoculated into the first and second groups by intramuscular injection, near the dorsal fin at doses of 0.3 ml of 1.5×10^8 3×10^8 CFU/ml and 0.3 ml of 3×10^8 CFU/ml, respectively. The third control group received only sterile physiological saline (PBS). The fourth and fifth groups were injected intraperitoneal injection at doses of 0.3 ml of 1.5×10^8 CFU/ml and 0.3 ml of 3×10^8 CFU/ml respectively. The sixth control group received only sterile physiological saline (PBS) (Austin and Austin, 1993). All groups of fish were observed for two weeks.

Determination of median lethal dose (LD50) of atypical *A. salmonicida* isolate in healthy *O. niloticus*

A total number of 60 *O. niloticus* fish were subdivided into 6 groups each of 10 fish. An overnight culture of the isolate was adjusted to densities 1.5×10^7, 1.5×10^6, 1.5×10^5 and 1.5×10^4. Each dilution was injected I/P into a fish group at a dose of 0.3 ml/fish and the fish of the 6^{th} group were used as control and injected with 0.3 ml of physiological saline. All fish groups were closely observed for 2 weeks. Mortalities were recorded daily and the organs were aseptically streaked on TSA agar for re-isolation and re-identification.

Histopathological examination

For histopathological investigations, organs such as gills, eyes, underlying musculature, liver and kidney were dissected out from injected and control fish. The organs were fixed in 10% formol saline for twenty four hours for histopathological examination (Banchroft *et al*., 1996).

Treatment of *O. niloticus* experimentally infected with atypical *A. salmonicida* by using a plant extract carvacrol & its biological precursor cymene mixture in feed.

1. Plant extracts

Carvacrol and Cymene (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

2. Preparation of fish carvacrol & cymene supplemented diets

Fish diets supplemented with the combinations of carvacrol and cymene were prepared at concentrations of 100 ppm (diet-1) and 200 ppm (diet-2) according

to Rattanachaikunsopon and Phumkhachorn, (2010). The control fish diets (diet 3) were prepared using the same process as the other fish diets except adding no additives.

3. Design of treatment experiment

Three equal groups of *O. niloticus* (10 fish each) were challenged with atypical *A. salmonicida* isolate by I/P injection of 0.3 ml of LD_{50} and left without feeding for 24 hrs and then were fed at feeding rate 5% of their body weight twice a day for 7 days. The 1st group was fed on diet-1, the 2nd group was fed on diet-2 while the 3rd group was fed on diet-3. Mortality, clinical abnormalities, feeding response, and behavioral alterations of the fish were observed daily for 2 weeks after feeding stop (Rattanachaikunsopon and Phumkhachorn, 2010).

Results

Identification of atypical *A. salmonicida* by VITEK 2 system

The tested atypical *A. salmonicida* strain showed excellent confidence with a probability of 99% (Table 1).

PCR technique

PCR technique was done for detection of 16S rDNA gene which is found in atypical *A. salmonicida* at 271 bp. By using 16S rDNA primer, the isolate was PCR positive (Fig. 1).

Experimental pathogenicity

The bacterial inoculum when injected into healthy *O. niloticus* produced characteristic clinical signs. The major signs were ulceration at the dorsal musculature (Fig. 2, a, b) and trunk (Fig. 2, c) which appeared in both I/M and I/P injections and in both concentrations (1.5×10^8 and 3×10^8 CFU/ml), skin black coloration and congested gills (Fig. 2, d), in addition to exophthalmia and ocular hemorrhage (Fig. 2, e). Additionally, congested liver and kidney were noticed in post-mortem examination. Other clinical signs were

Table 1. Biochemical identification of atypical *A. salmonicida* by VITEK 2 system.

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-		
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-		
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BALaP	-		
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-		
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-		
40	ILATK	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-		
46	GLyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-		
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-					

APPA. Ala-Phe-Ppro-ARYLAMIDASE; ADO. ADONITOL; PyrA. L-Pyrrolydonyl-ARYLAMIDASE; lARL. L-ARABITOL; dCEL. D-CELLOBIOSE; BGAL. BETA-GALACTOSIDASE; H2S. H2S PRODUCTION; BNAG. BETA-N-ACETYL-GLUCOSAMINIDASE; AGLTp. Glutamyl Arylamidase pNA; dGLU. D-GLUCOSE; GGT. GAMMA-GLUTAMYL-TRANSFERASE; OFF. FERMENATATION/GLUCOSE; BGLU. BETA-GLUCOSIDASE; dMAL. D-MALTOSE; dMAN. D-MANNITOL; dMNE. D-MANNOSE; BXYL. BETA-XYLOSIDASE; BAlap. BETA-Alanine arylamidase pNA; ProA. L-Proline ARYLAMIDASE; LIP. LIPASE; PLE. PALATINOSE; TyrA. Tyrosine ARYLAMIDASE; URE. UREASE; dSOR. D-SORBITOL; SAC. SACCHAROSE/SUCROSE; dTAG. D-TAGATOSE; dTRE. D-TREHALOSE; CIT. CITRATE (SODIUM); MNT. MALONATE; 5KG. 5-KETO-D-GLUCONATE; ILATk. L-LACTATE alkalinisation; AGLU. ALPHA-GLUCOCSIDASE; SUCT. SUCCINATE alkalinisation; NAGA. Beta-N-ACYTYL-GALACTOSAMINIDASE; AGAL. ALPHA-GALACTOSIDASE; PHOS. PHOSPHATASE; GlyA. Glycine ARYLAMIDASE; ODC. ORNITHINE DECARBOXYLASE; LDC. LYSINE DECARBOXYLASE; ODEC. DECARBOXYLASE BASE; IHISa. L-HISTIINE assimilation; CMT. COUMARATE; BGUR. Beta- GLUCORONIDASE; O129R. O/129 RESISTANCE (comp.vibrio); GGAA. GLU-Gly-Arg- ARYLAMIDASE; IMLTa. L-MALATE assimilation; ELLM. ELLMAN; ILATa. L-LACTATE assimilation.

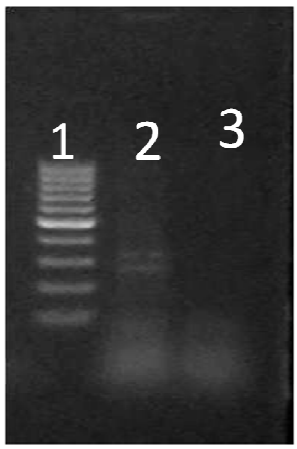

Fig. 1. Lane 1, 100bp ladder DNA marker. Lane 2, atypical *Aeromonas salmonicida* isolate (at 271 bp). Lane 3, negative control.

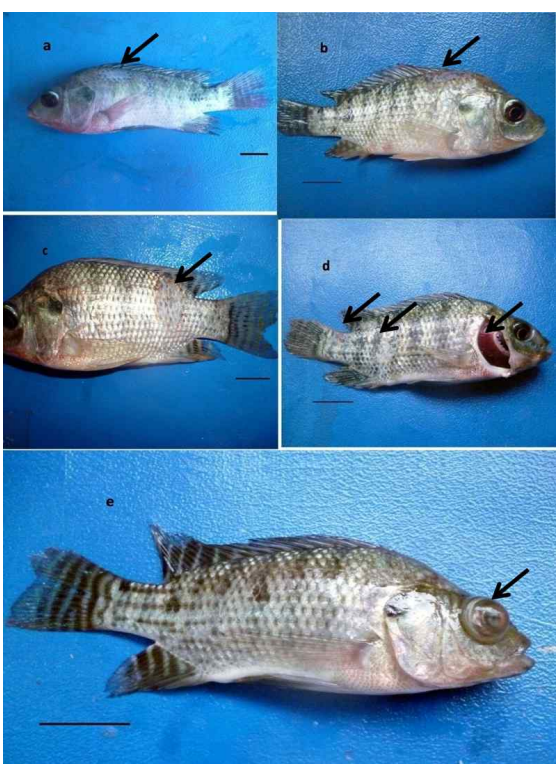

Fig. 2. Clinical signs of experimentally infected *O. niloticus*. a&b skin ulceration at the dorsal musculature and ocular hemorrhage(Scale bar 1cm). c. skin ulceration at trunk region(Scale bar 0.5cm). d. congested gills, loss of scales at trunk region and ulceration at peduncle(Scale bar 1cm). e. exophthalmia (Scale bar 0.5cm)

recorded, as lethargy, off food in addition to respiratory distress as gasping and accumulation at the air source. By the end of observation time (14 days) the mortality of the experimentally infected fish reached to 80%, 70%,100%, 90% in 1.5×10^8 CFU/ml I/M, 1.5×10^8 CFU/ml I/P, 3×10^8 CFU/ml I/M and 3×10^8 CFU/ml I/P respectively (Table 2), comparing to zero % mortality in the control groups. Atypical *A. salmonicida* reisolated and reidentified from the experimentally infected fish.

Median lethal dose (LD50)

The mortality of the experimentally infected *O. niloticus* was reported for 2 weeks after I/P injection with different concentrations of the isolate. The fish death occurred during the 1st week of the experiment. The LD50 of atypical *A. salmonicida* for *O. niloticus* was 1.5×10^6 CFU/mL. (Table 3).

Histopathological alterations

1. Intramuscular injection

1.1. At concentration of 1.5×10^8 CFU/ml.

1.1.1. Skeletal musculature

Focal area of the bundles showed hyalinization (Fig. 3, a).

1.1.2.Gills

Hyperplasia, congestion and fusion were noticed in the lamellae at the tips of the filaments (Fig. 3, b).

1.1.3.Eyes

There was congestion in the blood vessels in the chamber (Fig. 3, c).

1.1.4. Liver

Severe congestion was noticed in the central vein associated with focal aggregation of the melanin pigmented cells in the parenchyma (Fig. 3, d).

1.2. At concentration of $3x10^8$ CFU/ml.

1.2.1.Skeletal musculature

Focal inflammatory cells infiltration was detected in between the hyalinized bundles (Fig. 4, a).

1.2.2.Gills

The lamellae showed hyperplasia and fusion (Fig.

Table 2. Pathogenicity design of atypical *A. salmonicida* isolate in *O. niloticus*

Fish groups	Route of injection	Dose / fish (0.3ml of concentration)	No. of I/P inculatedFish	Number of dead fish / day									Total number of dead fish	Mortality %
				1	2	3	4	5	6	7 to 10	11	12 to 14days		
1	I/M	1.5×10^8	10	2	2	2	1	1	0	0	0	0	8	80
2		3×10^8	10	3	3	2	1	1	0	0	0	0	10	100
3 (control)		Saline	10	0	0	0	0	0	0	0	0	0	0	0
4	I/P	1.5×10^8	10	2	2	1	1	0	1	0	0	0	7	70
5		3×10^8	10	3	2	2	1	1	0	0	0	0	6	90
6 (control)		Saline	10	0	0	0	0	0	0	0	0	0	0	0

Table 3. Determination of LD_{50} of atypical *A. salmonicida* in *O. niloticus*

Fish groups (10 fish/each)	Dose / fish (0.3ml of concentration)	No. of dead fish / day												Total number of dead Fish	Mortality rate %
		1	2	3	4	5	6	7	8	9	10	11	12 to 14		
1	1.5×10^7	0	2	1	0	1	0	0	0	0	1	1	0	6	60
2	1.5×10^6	0	1	1	1	0	0	0	0	1	1	0	0	5	50
3	1.5×10^5	0	0	0	1	0	1	0	0	1	0	0	0	3	30
4	1.5×10^4	0	0	1	0	1	0	0	0	0	0	0	0	2	20
5	1.5×10^3	0	0	0	1	0	0	0	0	0	0	0	0	1	10
Control	Saline	0	0	0	0	0	0	0	0	0	0	0	0	0	0

4, b), while the arch showed focal haemorrhage (Fig. 4, c,).

1.2.3.Eyes

The anterior chamber showed focal haemorrhage (Fig. 4, d)

1.2.4.Liver

Focal melanin pigmented cells aggregation was detected in the hepatic parenchyma associated with congestion in the portal vein ((Fig. 4, e)). The hepatic parenchyma showed also focal necrosis, in association with severe congestion in the central vein (Fig. 4, f).

2. intraperitoneal injection

2.1. At concentration of 1.5×10^8 CFU/ml.

2.1.1. Skeletal musculature

Focal area of the bundles showed hyalinization (Fig. 5, a).

2.1.2. Gills

Hyperplasia of the lamellae was noticed.

2.1.3 Eyes

Multiple numbers of congested blood capillaries were detected in the chamber (Fig. 5, b).

2.1.4. Liver

There was severe congestion in the central vein (Fig. 5, c & d).

2.2. At concentration of 3×10^8 CFU/ml.

2.2.1. Skeletal musculature

Hyalinization with homogenous eosinophilic appearance were detected in the bundles (Fig. 6, a).

2.2.2. Gills

Congestion was observed in the hyperplastic lamellae in the filaments (Fig. 6, b).

2.2.3. Eyes

There was focal haemorrhage in the anterior chamber (Fig. 6, c).

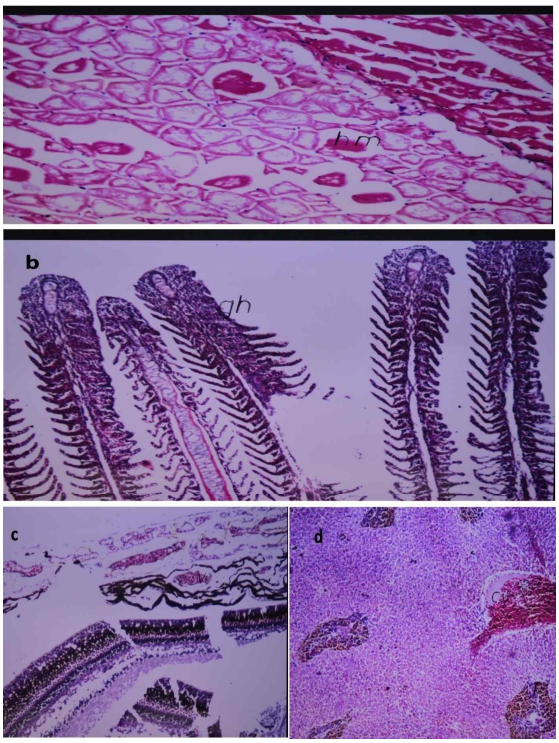

Fig. 3. Histopathological alterations of *O. niloticus* organs experimentally infected with *Aeromonas salmonicida* isolate (I/M, at concentration of 1.5×10^8 CFU/ml). (a) Skeletal musculature, focal area of the bundles showed hyalinization. (b) Gills, hyperplasia, congestion and fusion were noticed in the lamellae at the tips of the filaments. (c) Eyes, there was congestion in the blood vessels in the chamber. (d) Liver, severe congestion was noticed in the central vein associated with focal aggregation of the melanin pigmented cells in the parenchyma.

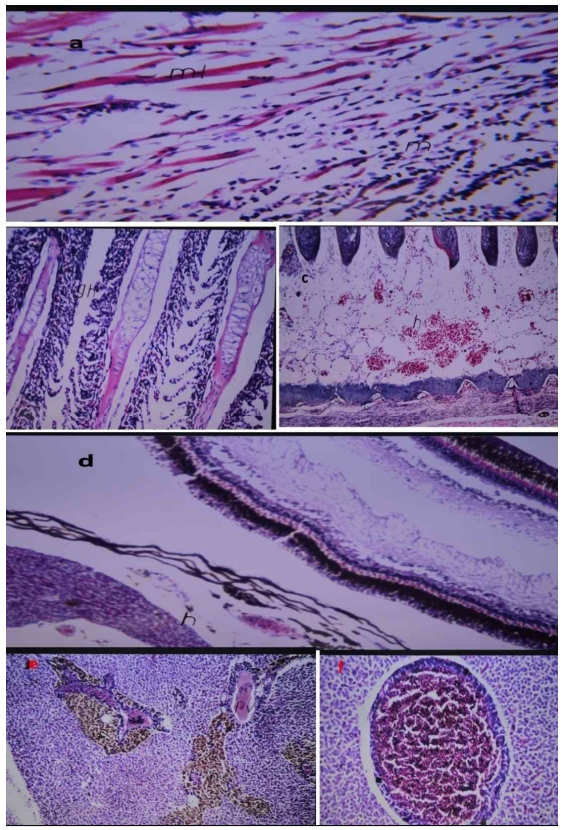

Fig. 4. Histopathological alterations of *O. niloticus* organs experimentally infected with *Aeromonas salmonicida* isolate (I/M, at concentration of 3×10^8 CFU/ml). (a) Skeletal musculature, focal inflammatory cells infiltration was detected in between the hyalinized bundles. (b) Gills, the lamellae showed hyperplasia and fusion, while the arch showed focal haemorrhage (c). (d) Eyes, there was focal hemorrhage in the anterior chamber. (e) Liver, focal melanin pigmented cells aggregation was detected in the hepatic parenchyma associated with congestion in the portal vein. (f) Liver, severe congestion in the central vein.

2.2.4. Liver

The central veins were congested (Fig. 6, d), associated with focal necrosis with melanin pigmented cells infiltration in the hepatic parenchyma (Fig. 6, e).

2.2.5. Kidneys

Degenerative changes were noticed in the epithelial cells lining the tubules (Fig. 6, f).

Treatment trial

The synergistic effect of the plant extracts carvacrol and its biological precursor cymene at concentrations of 100 ppm and 200 ppm could be able to control atypical *A. salmonicida* in *O. niloticus*. No clinical signs or mortality occurred in fish injected firstly with 0.3 ml of median lethal dose (1.5×10^6 CFU/ml) then fed on carvacrol and cymene supplemented diet for one week. The fish groups which fed carvacrol and cymene supplemented diets were silvery bright, active, alert and had good appetite.

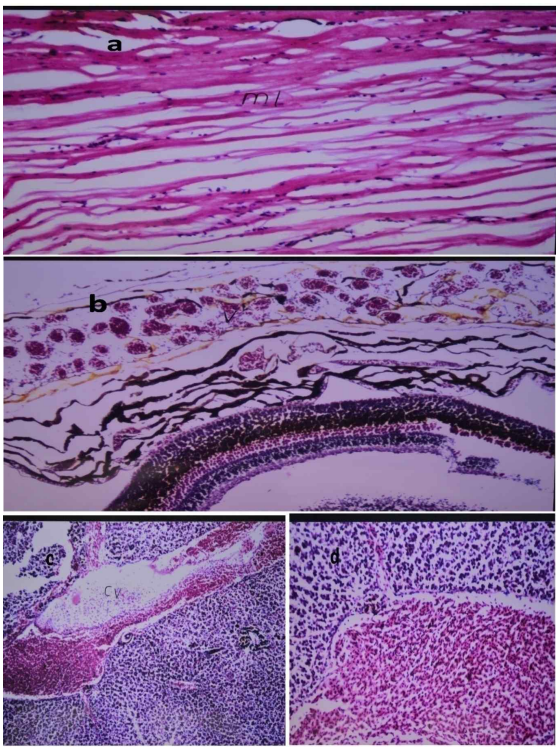

Fig. 5. Histopathological alterations of *O. niloticus* organs experimentally infected with *Aeromonas salmonicida* isolate (I/P, at concentration of 1.5×10^8 CFU/ml). (a) Skeletal musculature, focal area of the bundles showed hyalinization. (b) Eyes, multiple numbers of congested blood capillaries were detected in the chamber. (c & d) Liver, there was severe congestion in the central vein.

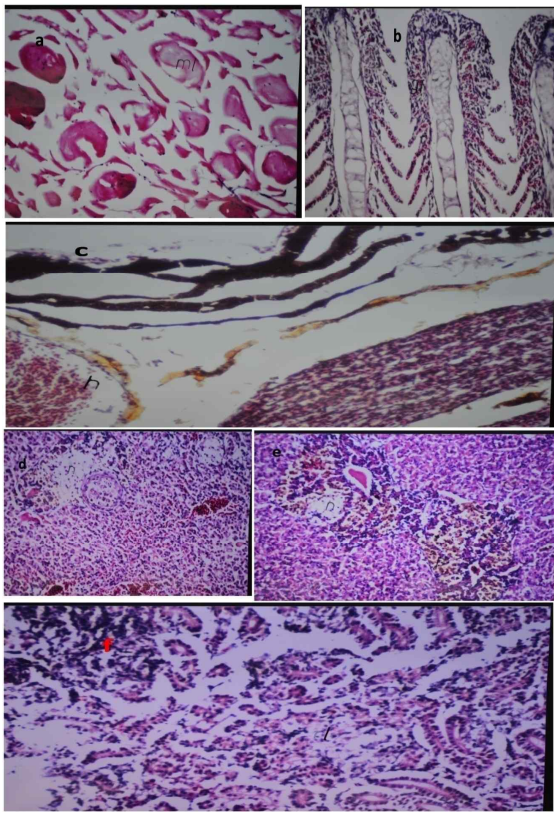

Fig. 6. Histopathological alterations of *O. niloticus* organs experimentally infected with *Aeromonas salmonicida* isolate (I/P, at concentration of 3×10^8 CFU/ml). (a) Skeletal musculature showed hyalinization with homogenous eosinophilic appearance in the bundles. (b) Gills showed congestion in the hyperplastic lamellae in the filaments. (c) Eyes, there was focal haemorrhage in the anterior chamber. (d) Liver, the central veins were congested, associated with focal necrosis with melanin pigmented cells infiltration in the hepatic parenchyma (e). (f) Kidney, degenerative change was noticed in the epithelial cells lining the tubules.

Contrarily, the control group showed dark coloration ulcerations on the dorsal musculature, off food, lethargy and 50% mortality.

Discussion

The incidence of the disease outbreaks caused by atypical *A. salmonicida* is world-wide, appears to be increasing and could potentially affect both aquaculture and natural fisheries.

Here, in this study, we used strain of atypical *A. salmonicida* isolated from ulcerated wild sea bream and identified by using VITEK2 system and PCR technique which was positive for detection of 16S rDNA, these results were supported by (H0IE *et al*., 1999) who mentioned that the 16S rDNA was 100 times more sensitive than other primer-sets used in detection of 205 atypical *A. salmonicida* strains.

This study evaluated the pathogenicity of atypical *A. salmonicida* in *O. niloticus* by two routes, namely, I/P and I/M injections and by using two different con-

centrations (1.5×10^8 and 3×10^8 CFU/ml). The results indicated that the mortality rate was higher in I/M route than I/P one. Contrarily, the pathological alterations of kidney appeared only in I/P route. In addition, the pathological picture of fish groups injected with concentration of 3×10^8 CFU/ml was more severe than those of 1.5×10^8 CFU/ml.

The most characteristic clinical signs of *O. niloticus* experimentally injected with atypical *A. salmonicida* were skin ulceration at the dorsal musculature (site of injection) and trunk region, the similar findings were recorded by Bohm *et al*. (1986); Mirle *et al*. (1986); Humphrey & Ashburner (1993); Wiklund *et al*. (1994); Whittington *et al*. (1995); Wiklund & Dalsgaard (1995); Wiklund & Dalsgaard (1998). Similarly, the skin ulceration appeared also in I/P injection and this means that atypical *A. salmonicida* has the ability to cause septicemic pathological changes which supported by the findings of histopathology in the kidney, liver, gills and eyes and the macroscopical lesions in gills, eyes, liver and kidney. These results were supported by Dalsgaard & Paulsen (1986); Hellberg *et al*. (1996); Anwar *et al*. (2012) who isolated atypical *A. salmonicida* from kidney and intestine of *Oreochromis niloticus* but disagreed with Wiklund (1995) who mentioned that the flounders inoculated I/P with atypical *A. salmonicida* did not develop skin ulcerations.

The mortality rate reached to 100% in I/M experimentally injected *O. niloticus*. This means that atypical *A. salmonicida* strain has the ability to cause mortality in non-salmonid species as recorded by Boomker *et al*. (1984); Groman *et al*. (1992); Gudmundsdottir *et al*.(1995).

The synergistic effect of the plant extracts carvacrol and its biological precursor cymene at concentrations of 100 ppm and 200 ppm could be able to control atypical *A. salmonicida* experimentally infection in *O. niloticus*, as there was no mortality in compared to 50% mortality of control group. Our results agreed with the findings of Rattanachaikunsopon and Phumkhachorn (2010); Fatma (2012); Hashiem and Abd El-Galil (2012) who used the two compounds in the control of Edwardsiellosis and recorded no mortality at concentration of 200 ppm. Also, our results were supported by Zheng *et al*. (2011) who evaluated the effect of carvacrol against *Aeromonas hydrophila* in channel catfish.

Carvacrol acts on microorganism itself as it causes cell death by damaging cytoplasmic membrane which leads to the collapse of the proton motive force and depletion of the ATP pool (Ultee *et al*., 2002). Cymene found to be able to enhance the bactericidal activity of carvacrol when used together (Ultee *et al*., 2000; Kisko and Roller, 2005). Based on characteristics and mechanism of action of carvacrol and cymene, it is possible that the synergistic effect between both compounds may be due to the fact that cymene accumulating in and causing the expansion of the plasma membrane enables carvacrol to be more easily transported into the cell (Rattanachaikunsopon and Phumkhachorn, 2010).

Conclusion

It is the first research describing the pathogenicity of atypical *A. salmonicida* isolate in *O. niloticus*, in addition to its treatment in the same fish. Atypical *A. salmonicida* infection in *O. niloticus* was highly pathogenic as there were severe pathological changes in most organs, in addition to 100% mortality. The synergism between plant extracts carvacrol & its biological precursor cymene succeeded in treatment of atypical *A. salmonicida* infection in *O. niloticus* at concentrations of 100 & 200 ppm.

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