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Efficiency of oral *Aeromonas hydrophila* vaccine and Tumeric powder mixture on immune response of Nile tilapia (*Oreochromis niloticus*)

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Abstract

The aim of the present study was to evaluate the dietary dosages of a mix from formalized killed vaccine and turmeric on enhancement the immune response and disease resistance against the pathogenic *Aeromonas hydrophila* that cause mass mortality in *Oreochromis niloticus*. Four different dosages of turmeric 0, 1, 3 and 5% kg^{-1} feed were fed to the fish for 15 days at rate of 3% body weight then a dose of a mix of *Aeromonas hydrophila* oral formalized vaccine wet-packed whole cell at level of 5 mg/g of diet and Tumeric mixed feed was giving as initial dose at the beginning of the experiment for one week followed by booster dose after 15 days. Blood was collected before and after vaccination; serum was separated for antibody titer assay, hematological parameters as (IGM, ALT and AST) were done. At the end of the experiment, fish was challenged with 0.2 ml *Aeromonas hydrophila* I/P per fish and the mortality (%) was recorded after ten days post challenge. The serum antibody titer were significantly ($P<0.05$) higher on 56 days of feeding of 1, 3 and 5 g of turmeric per kg of feed mixed with formalized killed vaccine wet-packed whole cell at level of 5 mg/g of diet. The survival rate was 0, 80, 95, 100 and 100% in challenge test groups T1, T2, T3, T4, and T5 respectively. IGM, ALT and AST were significantly ($P<0.05$) higher up to 56 days of feeding mainly T4. The result showed that turmeric at a dose of 3-5 mg / g^{-1} feed mixed with *Aeromonas hydrophila* oral formalized vaccine wet-packed whole cell at level of 5 mg/g of diet provided the greatest protection to pathogen challenge.

Introduction

Aquaculture has been growing rapidly for food production in the last few decades. Several commercial fish species have been cultured intensively in narrow or enclosed spaces such as earthen ponds, cages or tanks under high density conditions, thereby causing adverse effect on their health with a potentially stressful environment and infectious diseases susceptibility **(Jadhav *et al.*, 2006).** The outbreaks of infectious disease in cultured fish have emerged as constraints for the development of aquaculture and spread through the uncontrolled movement of live aquatic animals resulting in the transfer of pathogenic organisms among countries **(Lunden and Bylund 2000)**. Antibiotics and chemotherapeutics have been used to prevent or control bacterial infections in aquaculture for about 20 years **(Sakai 1999).** Unfortunately, antibiotics treatment is not completely successful and sustainable due to increase antibiotic-resistant bacteria and their negative effects on the indigenous micro flora of juveniles or adult fish **(Misra 2006).** In view of these undesirable effects caused by the antibiotics, there is an urgent need for echo friendly disease-preventive measures to promote sustainable aquaculture. During the last decade, attention has been increasingly focused on the use of herbal-based immunostimulants for fish disease control, particularly for those that elevate a non specific defenses mechanism activating early protection against infections. So, plant products are used effectively in aquaculture for various purposes as a toxicant **(Chiayvareesajji *et al.*, 1997; Thomas, *et al.*, 1997; Sharma, *et al.*, 1998),** anaesthetic **(Mgbenka and Ejiofor 1998),** growth promoter **(Jayaprakas and Euphrasia 1997),** bactericide **(Das *et al.*, 1999)** fungicide and other purposes.

Turmeric is a spice derived from the rhizomes of *Curcuma longa*, which is a member of the ginger family (*Zingiberaceae*). Rhizomes are horizontal underground stems that send out shoots as well as roots. The bright yellow color of turmeric comes mainly from fat-soluble, polyphenolic pigments known as curcuminoids. *Curcuma longa*, popularly known as 'Haldi', the active ingredients present are Turmeric oil and Curcumin. It also contains curcuminoids altatone, Bisdemethoxy curcumin, DimethoxyCurcumin, Diaryl heptanoids and Tumerone **(Phan, *et al.*, 2001),** possesses digestive and medicinal properties and is useful for humans **(Govindarajan, 1980; Ammon, *et al.*, 1992)** as anti-inflammatory effects **(Ammon and Wahl 1991),** antifungal **(Wuthi-Udomler *et al.*, 2000)** and antibacterial activities **(Mahady, *et al.*, 2002; Sahu 2004).** Polysaccharide naturally obtained from the rhizome of *C.*

longa L., has been reported to exhibit phagocytic, anticomplementary and mitogenic activities **(Gonda, *et al.*, 1992).**

The aim of the current study was to evaluate the dietary dosages of turmeric powder and formalized killed vaccine on enhancement of immune response and disease resistance against the opportunistic pathogens *Aeromonas hydrophila* in **(***Oreochromis niloticus***)**.

Materials and Methods

Fish

A total number of 380 apparently healthy *Tilapia nilotica* with an average body weight of 30 ± 5 g were collected randomly from Central laboratory For Aquaculture Research. They were kept for 2 weeks under observation for acclimatization in glass aquaria (40 × 60 × 100 cm). Third of the water column was removed daily; supplemented with aeration, pH maintained at 7± 1and temperature at 25 ± 1 °C. Groups and treatments showed in the **table (1).**

Table 1: Distribution of fish and groups

Test type	Treatment	Replicate			Total
		1	2	3	
Virulence test	Test	20	-	-	20
	Control	20	-	-	20
Safety test	Test	20	-	-	20
	Control	20	-	-	20
Vaccination test, challenge and blood sampling	Control food (No Vaccine): T1	20	20	20	60
	Control food + Vaccine: T2	20	20	20	60
	Control food + Curcumin 1% + Vaccine: T3	20	20	20	60
	Control food + Curcumin 3% + Vaccine: T4	20	20	20	60
	Control food + Curcumin 5% + Vaccine: T5	20	20	20	60
Total number		180	100	100	380

Feed preparation

Commercial fish feed components containing 25% protein was crushed and divided into five groups. Crude Curcumin was obtained from (Kimet Company) minced and were mixed by 0%, 1%, 3% and 5% with pelleted diet and let to air dry and stored in refrigerator for use**.**

Bacterial strain

Aeromonas hydrophila were isolated from liver of diseased (*Oreochromis niloticus*) fish obtained from El Abbassa fish farm on Brain heart infusion broth, nutrient agar, R. S. media, **Shotts and Rimler (1973)** and Tryptic Soy Agar with 5% sheep erythrocytes, **Chen and Levin (1975),** the biophysical and biochemical characters were carried out according to **Bergey's manual of systematic bacteriology, (2005).**

Virulence test

The level of virulence of the isolated *Aeromonas hydrophila* bacterial strain, was detected by using *Aeromonas hydrophila* strain solution, prepared from isolate incubated brain heart infusion agar 20 hs. culture and suspended in sterile physiological saline solution then estimated by McFarland 0.5**.** Forty Nile tilapia (30 ± 5) g body weight was divided into two equal groups each contains twenty fish. First group were injected intramuscularly with 0.2 ml of the bacterial suspension (adjusted spectrophotometrical at 625A^{o} and absorbance was 0.08 – 0.1 to be 1.5 × 10^{8} C.F.U/ml) / fish, **Wakabayashi, *et al.*, (1981) and Badran (1987),** the second group (Control group) was injected with 0.2 ml of sterile physiological saline solution. The tested fish were under observation for 2 weeks and results were recorded. Re-isolation of injected bacteria was done.

Preparation of formalized killed vaccine

Aeromonas hydrophila bacterin was prepared according to (**Yin *et al.*, 1996**). *Aeromonas hydrophila* strain was inoculated in Ten ml of brain heart infusion broth and incubated at 25°C for 12 hs. 2 ml of this broth culture inoculated in one liter broth culture and incubated at 25°C for 12 hs. The prepared one liter was used as inoculums for 15 liters of the broth medium and incubated for 12 hs at 25°C. Finally, 250 ml of 20% dextrose solution was added and the culture was incubated for an additional 12 hs. Formalin (40%) was added to the broth culture at a final concentration of 2% (V/V) and left overnight at room temperature. The inactivated cells were harvested by centrifugation at 4000 rpm for 10 min., then washed twice in 0.3% formalized PBS and re-suspended in normal saline to give concentration of 5 mg bacterial cell wet weight / ml equal to the density of (1.6 x 10^{9} cells / ml), then was tested for their sterility and safety.

Sterility test

Sterility of the vaccine was performed according to **(Aly, 1981)**. An inoculum from the washed cells of vaccine was cultivated on Nutrient and Tryptic Soy Agar plates and incubated at 25°C for 24 hours then examined for positive bacterial growth. Negative growths indicate sterile vaccine.

Safety test

This test was performed by inoculating the susceptible fish (*O. niloticus*) with the prepared bacterin according to **Anderson *et al.*, (1970)**. Two groups of Nile tilapia each contained 20 fish with an average body weight (30 ± 5 g) were used. First group were injected intramuscularly through with 0.1 mg bacterin cells (1.6×10^9 cells / ml) / fish. The second group (Control) was injected with sterile formalized phosphate buffer solution (PBS). The fish of both groups were under observation during 15 days after injection. After that, fish were tested for re-isolation of injected bacterin on brain heart infusion broth that incubated at 25°C for 24 hs, to insure that there is no infection or disease will be occurred from living bacteria.

Preparation of vaccinating diet

Commercial diet containing 25% protein and 0% 1%, 3%, and 5% Curcumin was sprayed by bacterin solution contains wet-packed whole cell at level of 5 mg/g of diet, **Fryer, *et al.*, (1976),** then coated by soaking in melted gelatin flakes **(International Co. for gelatin Manufacture, Egypt)** and let to air dry.

Vaccine application

Three hundred *Oreochromis niloticus* were equally divided into five equal groups with three replicates in well prepared glass aquaria as shown in table (2). Fish groups to be vaccinated fed on vaccinating diet by 3% of body weight per day for 10 days then a booster was given after 15 days.

The potency of the vaccine was calculated as relative percent survival (RPS) using the formula **(Amend, 1981).**

$$RPS = 1 - \frac{\text{(\% mortality in vaccinated fish)}}{\text{(\% mortality in control fish)}} \times 100$$

Table 2: Feeding and vaccination program

Treatment	0-15th day	16-22th day	23-37th day	38-44th day	45 -60th day
T1	C.	C. + saline	C.	C.+ saline	C.
T2	C.	C. + Vac.	C.	C. + Vacc.	C
T3	C. + Cur.1%	C. + Cur.1% + Vac.	C. + Cur.1%	C. + Cur.1% + Vacc.	C. + Cur.1%
T4	C. + Cur.3%	C.+ Cur.3%+ Vac.	C. + Cur.3%	C. + Cur.3% + Vacc.	C. + Cur.3%
T5	C. + Cur.5%	C. + Cur.5% + Vac.	C. + Cur.5%	C. + Cur.5% + Vacc.	C. + Cur.5%

C.: Control feed containing 25% protein
C. + Cur.: Control feed containing 0, 1, 3 and 5 % of Curcumin
C. + Cur. + Vacc.: Control feed mixed with Curcumin and formalized killed *Aeromonas hydrophila* vaccine**.**

Challenge test

Vaccinated groups were challenged with *Aeromonas hydrophila* strain, the strain was activated by cultivation on TSA at 28 ^{o}C for 18 h for 3 successive cultivates then standardized at (1.5 x 10^{8} CFU/ml) and 0.2 ml / fish were injected intra-peritoneal. The mortality was recorded for 10 days and relative percent of survival were calculated.

Preparation of stained antigen used in antibody titration

Aeromonas hydrophila antigen for antibody titration was established. The formalin inactivated bacterial cells by wet-weight was diluted with equal volume of sterile physiological saline solution. One drop of Loffler's alkaline methylene blue was added to each 10 ml of the diluted antigen, **(Cruickshank, 1985).**

Blood collections

Blood sampling were obtained from different experimental groups and divided into 2 groups **(Lied *et al.*, 1975)** in two clean dry tubes, one containing dry dipotassium salt of EDTA as anticoagulant **(Hawk *et al.*, 1965)** and the other was used for preparation for sera separation.

Bacterial agglutination activity

Agglutination test was conducted in U shaped microtitre plate. Two-fold serial dilution of 25 µL fish serum was made with an equal volume of PBS in each well, to which formalin- killed *A. hydrophila* (1.6×10^{9}

CFU/ml) suspension was added. The plates were incubated overnight at room temperature. The titer was calculated as the reciprocal of the highest dilution of serum showing complete agglutination of the bacterial cells, **(Silva *et al*., 2009).**

Hematological assay

Immunoglobulin assay was performed by fish immunoglobulin M (Igm) ELISA Kits (Cat. No: MBS042385) compared with the typical standard curve. Alanine aminotransferase (ALT), Asparate aminotransferase (AST) tests were applied using Blood parameters reagents (SPINREACT, Ctra. Santa Cloma, 7E-17176 SANT ESTEVE DE BAS (Gl) SPAIN), **Murray R. (1984) [a, b].**

Results

Characters of the isolated *A. hydrophila* from naturally infected fish was circular, smooth and raised colonies 1 to 2 mm diameter on Nutrient agar media, after inoculation on Shotts and Rimler (R-S) agar incubated at 37°C for 24 to 48 h, produced yellow colonies. On TSA with 5% sheep erythrocytes hemolysis produced. Upon microscopic examination, hanging drop showed motile bacteria and gram stained slide showed short (0.5 X 1.0 ~m) gram-negative bacilli. The biochemical characters were shown in table (3).

Table 3: Bacteriological characters of isolated organism

Item		Finding	Item		Finding
AGS		KK	Growth at 40°C		+
Oxidase		+	Growth at 42°C		+
Arginine dihydrolase		+	Growth at 45°C		-
Gelatinase		+	Sucrose		+
Urease		-	Glucose (gas)		+
Catalase		+	D-Cellobiose		+
Voges-Proskauer		+	Lactose		V
Indol		+	Arabinose		V
Methyl red		-	D-Mannitol		+
Growth on	0% NaCl	+	Maltose		+
media with	3% NaCl	+	Sensitivity	O/129 (150 µg)	R
NaCl	6% NaCl	+	to	Ampicillin	R
	8% NaCl	-		Novobiocin	R

KK: Slant alkaline (purple) / Butt alkaline (purple), R: resist. V; variable

The present result indicated that the prepared vaccine was sterile and safe (Table 4)

Table 4: Characters of the prepared vaccine

Characters	Result
Bacterial strain	*Aeromonas hydrophila* virulent strain isolated from diseased fish of local area
Type of vaccine	Bactrin (formalized killed vaccine) mixed food coated with gelatin
Safety test	Safe
Sterility test	Sterile
Concentration	5mg/g food mixed
Route of administration	Oral
Duration of administration	For one week
Booster dose and duration	After 2 week for on week
Daily feeding rate	3 % from body weight

The relative percent of survivability of groups T4 and T5 were 100% protection than T3 and T2 as shown in the tables (5).

Table 5: Cumulative deaths after challenge

Treatment	No of Fish	Cumulative death's										Deaths	Survival	RPS.
		1	2	3	4	5	6	7	8	9	10			
T1	40	0	0	0	0	4	16	34	40	40	40	40	0	0
T2	40	0	0	0	0	1	3	8	8	8	8	8	32	80
T3	40	0	0	0	0	0	1	2	2	2	2	2	38	95
T4	40	0	0	0	0	0	0	0	0	0	0	0	40	100
T5	40	0	0	0	0	0	0	0	0	0	0	0	40	100

As shown in table (6) the level of antibodies titer was higher in group T4 than other groups and continuity higher after 56 days post vaccination.

Table 6: Antibody titer of *Oreochromis niloticus* before and after feeding on tumeric and formalized killed vaccine supplemented diet

Group	Type	14 day	28 day	56 day
T1	Control feed (No Vaccine)	5.33 ±0.002	8.56 ±0.003	6.39 ±0.003
T2	Control feed + Vaccine	85.33 ±0.002	97.24 ±0.004	66.67 ±0.003
T3	Control feed + Curcumin 1% + Vaccine	111.23 ±0.004	118.59 ±0.004	82.67 ±0.004
T4	Control feed + Curcumin 3% + Vaccine	172.61 ±0.003	175.35 ±0.003	113.33 ±0.012
T5	Control feed + Curcumin 5% + Vaccine	123.94 ±0.004	126.81 ±0.002	94.66 ±0.010
Two Way ANOVA		*P* Value		
Treatment			0.0001	
Day			0.0001	
Treatment X day			0.0001	

Data are expressed as mean ± SEM

The immunoglobulin M was higher in T4 than other groups and continuity higher after 56 days post vaccination, (table 7).

Table 7: Immunoglobulin M. Concentration in mg before and after vaccination

Group	**Type**	**14 th day**	**28 th day**	**56 th day**
T1	Control food (No Vaccine)	65.01 ±0.002	66.11 ±0.005	66.82 ±0.012
T2	Control food + Vaccine	69.54 ±0.012	96.83 ±0.008	78.65 ±0.123
T3	Control food + Curcumin 1% + Vaccine	111.04 ±0.009	122.07 ±0.004	119.12 ±0.006
T4	Control food + Curcumin 3% + Vaccine	118.95 ±0.004	132.78 ±0.10	122.68 ±0.021
T5	Control food + Curcumin 5% + Vaccine	89.29 ±0.003	103.34 ±0.014	90.08 ±0.000
Two Way ANOVA		*P* Value		
Treatment			0.0001	
Day			0.0001	
Treatment X day			0.0001	

Liver enzymes were shown in groups T3, T4, and T5 in comparison with T1 and T2 control group indicate that Tumeric has no destructive effects on the liver and improve liver functions, (Table 8).

Table 8: Alanine Aminotranferase (ALT) and Asparate Aminotranferase (AST) before and after vaccination.

T.	ALT *u/l*			AST *u/l*		
	14 th day	**28 th day**	**56 th day**	**14 th day**	**28 th day**	**56 th day**
T1	18.74 ±0.14	45.27 ±0.08	90.37 ±0.32	22.54 ±0.10	57.63 ±0.32	62.36 ±0.02
T2	65.84 ±0.29	68.68 ±0.29	65.60 ±0.09	40.45 ±0.29	50.09 ±0.06	43.42 ±0.32
T3	47.79 ±0.17	34.09 ±0.04	22.02 ±0.04	45.24 ±0.44	22.20 ±0.08	27.51 ±0.09
T4	36.91 ±0.06	46.12 ±0.13	52.62 ±12.27	42.23 ±0.07	33.00 ±0.04	66.49 ±0.09
T5	39.52 ±0.22	28.79 ±0.10	32.12 ±8.84	17.42 ±0.08	16.36 ±0.17	26.83 ±0.12
Two Way ANOVA	*P* Value					
Treatment	0.0001					
Day	0.0001					
Treatment X day	0.0001					

Discussion

Vaccinations were identified as active immunization that results in the increase of the concentration of acquired antibodies **(Schaperclaus 1972).** The efficiency of vaccination is largely dependant on the immune status of the fish and the conditions under which the fish were kept **(Robohm and Koch, 1995).**

Immunization against *Aeromonas hydrophila* is difficult because of its heterogeneity as well as *its sereo-typing. To cope with this problem, several researchers planned to produce different types* of vaccine such as the formalized whole culture vaccine **(Gado 1995)**, the hyper- osmotic infiltration vaccine **(AQUIGRUP 1980)**, the toxoids **(Baba *et al.*, 1988)** and the genetically engineered live bacteria with removal of one of the aerolysin genes (virulence gene), **(Soliman *et al.*, 1989).** Successful vaccination programs were ones targeted against the *Aeomonas*

hydrophila isolates endemic to particular areas**.** The oral route offers the most attractive approach of immunization of fish for a number of reasons: the ease of administration of antigens, it is less stressful than parenteral delivery and in principle, it is applicable to small and large sized fish; it also provides a procedure for oral boosting during grow-out periods in cages or ponds. There are, however, not many commercial vaccines available at the moment due to lack of efficacy and challenges associated with production of large quantities of antigens. These are required to stimulate an effective immune response locally and systemically, and need to be protected against degradation before they reach the sites where immune induction occurs, **Mutoloki *et al*., 2015**. So in our study we made protection of the antigen by adhesive material of plant origin as Gelatin for encapsulation of food mixed vaccine particles.

The result of **table (5 and 6)** showed that their pronounced increase in antibody titer and imunoglobuline M in treatment 2 (vaccinated group and no Curcumin additive) than in treatment 1(non vaccinated and no Curcumin additive**).** This attributed to the effect of production of antibodies (active immunization), similar results obtained by **Noor El Deen *et al.*, (2010)**. In comparison with other treatments T3, 4, and 5 (vaccinated groups with Curcumin additive) the titer of immunoglobuline M and antibody titer showed marked increase under the effect of Curcumine products that continuous production of antibodies that give more longer protection, and this showed high survivability in challenged fish as shown in **table (7)** . Also it was found that T4 was the best treatment in which Curcumin added by 3% than 1% or 5%. This may attributed to 1% is not enough concentration while 5% had adverse effect, **Ayoub and El Tantawy, (2015).**

On the other hand the level of liver enzymes was indicator for the liver function, any increase or decrease in them can indicate the level of a function in the fish body system or organ. Alanine aminotranserase (ALT) formerly called Glutamate pyruvate transaminase (GPT) is a cellular enzyme found in highest concentration in liver and kidney, it catalyses the reversible transfer of amino group from alanine to α-ketogultarate forming glutamate and piruvate, the piruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH into lactate and NAD^+, the rate of decrease in concentration of NADH, measured photometrically is proportional to the catalytic concentration of ALT in the sample, so, high level is observed in liver diseases like hepatitis, disease of muscles like traumatism, its better application is in the diagnosis of the disease of liver, **Murray, 1984[a],** but Asparate

aminotransferase, AST, formerly called glutamate oxaloacetate (GOT), it's a cellular enzyme found in highest concentration in heart muscle, liver cells and skeletal muscle cells, it catalyse the reversible transfer of an amino group from aspirate to α-ketoglutarate forming glutamate and oxalacetate, the oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH. The rate of decrease in concentration of NADH, measured photometrically is proportional to the catalytic concentration of AST in the sample, although an elevated level in the serum is not specific in hepatic disease, is used mainly to diagnose and verify the course of this disease with other enzymes like ALT and ALP, **(Murray, 1984[b]).** There is obvious decrease in the level of ALT and AST in treatment 3 in comparison with other treatments as shown in **table (8),** indicating improvement in health stat of the liver, muscles and heart and this will improve the health stat of the fish.

Phagocytosis is the first step of macrophage to kill invading microorganisms, Phagocytic and Neutrophil activities can act as indicators of the non-specific immune response, **Weeks and Warinner (1986),** their roles in defense mechanisms have been reviewed by **Secmbes (1996)**, these cells can engulf bacteria and kill them principally by production of reactive oxygen species (ROS) during the so-called respiratory burst. These products include the superoxide anion (O2 "), H2O2 and the hydroxyl free radical (OHv) which has potent bactericidal activity and measured by NBT activities. Neutrophil and Macrophages also contain lysozyme and other hydrolytic enzymes in their lysosomes. Fish macrophages can also produce nitric oxide (NO), which form potent bactericidal agents like peroxynitrites and the hydroxyl free radical **(Secmbes, 1996).** This explain the effect of turmeric in increasing the level of Neutrophil and Macrophages and role in enhancement the immune cells.

El-Bahr and Saad, (2008) attributed the desired effect of turmeric to its potent antioxidant and hepatoprotective properties. On other focus **Mahmoud *et al.*, (2014**) described the improvement of immunity and performances attributed to hyperplasia of lymphoid follicles and melanomacrophage centers of spleen. Polysaccharide naturally obtained from the rhizome of *C. longa L.*, has been reported to exhibit phagocytic, anti-complementary and mitogenic activities **(Gonda, *et al.*, 1992).**

According to **Antony *et al.*, (1999),** Curcuminoids have dose dependent inhibitory effects on reactive oxygen species production and myeloperoxidase release by activated Neutrophils, however, our results

showed that, at higher doses, the activities of Curcumin remained unaltered. **Hilda *et al.*, (2006),** turmeric with the active Curcuminoids and water soluble Turmerin, has antioxidant properties and hence effectively inhibits the free radical damage to biomolecules. **Manju *et al.*, (2012)** applied two doses of Curcumin 0.5 and 1% were supplemented in the 40% protein feed and fed to Anabas testudineus (Bloch) for the periods, 2 and 8 weeks and the lipid peroxidation product, thiobarbituric acid reactive substances content either decreased or unaffected. The glutathione content increased while the antioxidant enzyme activity pattern varied with time and dose. The histological analysis also confirmed the safety of Curcumin retaining the normal arrangement of hepatocytes, hepatopancreas, and macrophage-melanocyte centers. The immunostimulant status of fish fed Curcumin attributed to enhancement of the immune system through activation of secretion of the digestive enzymes and growth rate. **Rojtinnakorn *et al.*, (2012)** showed that all turmeric extract fed fish had significant higher specific activities of digestive enzymes and indicated that growth rate enhanced in follow up.

Conclusion

From the above results we can concluded that Tumeric at a dose of 3-5 g kg^{-1} feed mixed with *Aeromonas hydrophila* oral formalized vaccine wet-packed whole cell at level of 5 mg/g of diet was one of the solution to mass mortality in tilapia fish farms by increasing survivability and providing the greatest protection against pathogenic *Aeromonas hydrophila* in Tilapia fish farms.

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تقييم كفاءة تحصين الايروموناس هيدروفيلا و الكركم على الاستجابة المناعية لاسماك البلطى النيلى ضد مرض التسمم الدموى الايرومونياسى

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الملخص العربى

الهدف من هذا البحث هو تقييم الجرعات الغذائيه لمخلوط لقاح الايروموناس هيدروفيلا الميت الفورمالينى و الكركم على تحفيز الاستجابه المناعيه و نسبة الوفيات ضد الايروموناس هيدروفيلا الممرض الذى يحدث موت جماعى فى البلطى النيلى. تم إجراء التجربه على عدد ٣٨٠ من اسماك البلطى النيلى حيث تم تقسيمها الى ٥ مجموعات، مجموعه ضابطه و ٤ مجموعات للاختبار و تم تغذيه المجموعات المختبره على جرعات مختلفه من الكركم بواقع صفر، ١، ٣، ٥ % لكل كجم عليقه لمده ١٥ يوم بمعدل ٣ % من وزن الجسم ثم جرعات من مخلوط اللقاح الميت الفورمالينى بالفم ٥ مجم خلايا كامله طريه مضغوطه لكل جم وجبه غذائيه مخلوطه مع الكركم اعطيت كجرعه ابتدائيه من بدايه التجربه لمده اسبوع تلاها جرعه منشطه بعد ١٥ يوم. تم تجميع عينات الدم قبل و بعد التحصين حيث تم فصل السيرم لمعايره الاجسام المضاده واجريت بعض القياسات الهيماتولوجيه. فى نهايه التجربه تم الاستعداء بحقن ٠.٢ مل من الايروموناس هيدروفيلا بالحقن بالبريتون لكل سمكه وسجلت الوفيات لمده ١٠ ايام. اشارت النتائج ان مستوى الاجسام المضاده كانت مفيده و كانت ($P<0.05$) عاليه لليوم ٥٦ بعد التغذيه على ١ او ٣ او ٥ % من الكركم مخلوطه مع للقاح الميت الفورمالينى بالفم ٥ مجم خلايا كامله طريه و كانت نسبه الاعاشه صفر، ٨٠، ٩٥، ١٠٠، ١٠٠% فى المجموعات من المعاملات ١، ٢، ٣، ٤، ٥ على التوالى. و لذا فإن النتائج اوضحت ان خلط الكركم بنسبه ٣-٥ مجم لكل جم عليقه ممزوجا باللقاح الميت الفورمالينى بمعدل ٥ مجم من الخلايا الكامله الطريه المضغوطه من الايروموناس هيدروفيلا لكل جم عليقه يعطى حمايه كبيره ضد الايروموناس هيدروفيلا الممرض فى اسماك البلطى.