IMMUNE RESPONSE OF GENETICALLY DIFFERENT OREOCHROMIS FISH SPECIES TO *PSEUDOMONCE FLUORESCENCE* VACCINATION

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Abstract

This study have been designed to evaluate the effect of genetic variations on the degree of immune response of the three species (*O. niloticus & O. auraus* and their hybrid) to formalized whole culture of *Ps. Fluorescence* vaccine and variations in mortalities post challenge with virulent strains of *Ps . fluorescence.* Also to evaluate variations in the level of natural resistance among two Oreochromis fish species and their hybrid.

Ps. fluorescence have been isolated from clinically infected fishes and fully identified. The incidence of *Ps. fluorescence* infections in diseased fish were studied in examined naturally infected tilapia fish species to choice the most virulent strains of *Ps. fluorescence.* Vaccination of the three species under experiment was done using formalized whole culture of *Ps. fluorescence.* Detection of mean antibody titers were done 0,14,21 and 35 days post vaccination for all vaccinated fish groups. Challenge test were done 35 days post vaccination (peak antibody titers) using the most virulent strain of *Ps. fluorescence.* Mortalities of all groups were recorded 15 days post challenge.

The vaccine protection efficacy of *Ps. fluorescence* killed vaccine have been evaluated through detection of antibodies titers 7 days post challenge and mortality patterns 15 days post challenge in each Oreochromis species.

The Results obtained showed that:

Chromosomal analysis of the three Oreochromis species cleared that *O. niloticus; O. auraus* and their hybrid have the same chromosomal number.

* level of antibody titers at day zero (one day before vaccination) cleared a superiority for *O. niloticus* in the level of natural resistance followed by *O. auraus* then hybrid.
* The antibody titers after vaccination showed significant differences in the level of immune response to vaccine with a significant superiority to *O. niloticus* followed by hybrid while *O. auraus* peak antibody titers.
* Challenge test showed that *O. niloticus* recorded the highest survivability post challenge of vaccinated groups with virulent *Ps. fluorescence* followed by hybrid while *O. auraus* recorded the highest mortalities.
* Antibody titers and mortalities not significantly vary between vaccinated groups of the three Oreochromis species, but significant differences have been reported between the vaccinated and non vaccinated groups of each species, so the vaccine reported a variable degrees of protection efficacy with superiority to *O. niloticus* followed by hybrid tilapia then *O. auraus.*

INTRODUCTION

In Egypt, Oreochromis species have become very important and are cultured in fish farms throughout the country. Their economic importance is constantly increasing for their fast growth, disease resistance, different feeding habits and palatability (Dadzie, 1982). For these reasons the possibility of cross-breeding between them has accordingly been used to verify relationships within natural groups or species. Genetic resistance to disease is important method of disease prevention and control relative to other methods of disease control. Genetic resistance to disease is important not only in cases where no other effective means of control exists, but also for disease against which vaccines or medicament are available and even for those that have been successfully eradicated. Improvement of the ability to survive is also a crucial component of any practical breeding program, regardless of the primary production trait (s) under selection (Gavora and Spencer, 1978). It should be mentioned that various pathogens develop resistance against drugs and antibiotics. Under such circumstances, genetic resistance to disease would be of particular value (Gavora. and Spencer;1978).The objectives of this study was to throw light on the following :

Role of genetic variation in immune response of two breeds of Oreochromis spp. and their hybrid after vaccination with *Ps. fluorescence* as well as resistance of fish after challenge with virulent strains of *Ps. fluorescence*.

MATERIALS AND METHODS

(I) Morphology of *O. niloticus*, *O. auraus* and their hybrids

O. niloticus, *O. auraus* and their hybrids can be differentiated from their characteristic morphological differences through careful attention to the body fins especially the dorsal and the caudal ones, for their color, the presence of regular, vertical, black stripes or red margins on the caudal fins, the presence of grey or black margins of the dorsal one (*Trewavas, 1983*). *O. niloticus* characterized by that the caudal fin has regular vertical black stripes throughout its depth and the margin of the dorsal fin is grey or black (Fig.1), *O. auraus* characterized by that dorsal and caudal fins of the male have red margins (*Trewavas,1983*) (*Fig.2*). Hybrid tilapia characterized by the caudal fin has less regular or discontinuous vertical black stripes, there may be red margins in male dorsal and caudal fins (*Fig.3*).

(II) Fish hybridization:

O. niloticus and *O. auraus* having an average body weight of about 150 g/fish were collected according to their external features (*Rothbard* & *Prugnin, 1975*) from Abbassa fish farms then putted in a separate ponds for about two weeks to be acclimatized.

The fish were classified as following:

(group I) *O. niloticus* ♀ X *O. auraus* o→

(group n) *O. auraus* ♀ X *O. niloticus* o→

Sexing of parent fishes were done by inspection of the genital papilla (*Maar et al., 1966*). The experimental ponds were supplied with drainage water. By using 4-5 mm mesh sized net the fry were seined completely (at 6-8 am) three times during the season (180 days) at the first day of June, August and October to allow the fry to grow enough (*Hepher & Prugnin, 1982*). At the end of November all spawning ponds were seined to check the presence of fry in this period.

Spawning and nursing operations:

All spawning operations were carried out according to the method of (Hepher and Prugnin, 1982).The parent brood stocks were stocked at the rate of 6kg/spawning pond (100m²) with sex ratio of 3♀ : 1o→ as following :

Group *I* *O. niloticus* 30 ♀ x *O. auraus* 10 o→ [pond I]

Group II *O. auraus* 30 ♀ x *O. niloticus* 10 o→ [pond II]

The spawners were fed with supplementary feed of 20% protein at the rate of 5% body weight daily through six days/week. The small fingerlings gained in the harvesting period were counted and restocked in the nursing ponds (400m^{2}each) at the rate of 10 fish/m^2 (Hepher & Prugnin, 1982).The nursed fingerlings were given supplementary feed of 20% protein at the rate of 3% body weight 6 days/week. The water of all nursing ponds was partially changed weekly. These ponds were covered by batches of water hyacinth (Enchoria Crassipes (mart) solmes) in addition to some aquatic plants around dicks to make a shelter for the fish in winter.

Cultivation of hybrid tilapia:

Fingerlings (43g/fish) were selected and restocked separately in concrete ponds (400m^2) at the rate of one fish/m^2 in June first till November fist (150 day rearing period) to grow out. They were given supplementary feed (15% protein) at the rate of 5% of body weight for six days/week

(III) Chromosomal analysis:

This work was done as described by (Bertolla *et al.*, 1978).

(IV)Isolation & identification of pure culture of Ps. fluorescence from naturally infected fish were performed and identified according to (*Schaperclaus et al., 1992*).

(V) Experimental infection with isolated bacteria (Pathogenecity test):

Six glass aquaria each containing 20 *O. niloticus* fish; Aquaria from 1 to 5 injected I/P with 0.2 ml/fish of 24 hour broth culture of *Ps. fluorescence* adjusted to McFarland 4. The 6th aquarium injected with 0.2 ml/fish of sterile broth. Mortality

patterns & clinical picture within two weeks after injection were recorded for detection of the most virulent strain of *Ps. fluorescence*. In all experimentally infected groups re-isolation of the inoculated bacteria on the previously mentioned media, were obtained from the internal organs and heart blood of infected fishes in a pure forms.

(VI) Preparation of *Ps. fluorescence* killed vaccines:

Formalin killed bacteria (FKB) was prepared according to (Sakai *et al.*, 1984 and Soliman, 1988). Formaldehyde 37% was added to 24 hour tryptic soy broth culture of Ps. fluorescence to a final concentration of 2% (v/v). After an overnight incubation at room temperature, the inactivated cells were harvested, centrifugation at 7000 xg for 5 minutes at 40C, washed twice in 3% formalized PBS (phosphate buffer saline) and re-suspended in sterile saline to the density of McFarland 4 standard (approximately 109 organism/ml), cultures were tested for sterility.

Tests performed to insure sterility of the vaccines:

1–Inoculation test was performed according to (Anderson *et al.*, 1970).

2–Sterility test was performed according to (*Ali, 1981*).

(VIII) Immunization schedule: A total number of 300 tilapia fish were classified as following:

I- Group A (a total number of 100 *O. niloticus*):

Consists of two glass aquaria each containing 50 fish of 60 ± 10g/fish average body weight and treated as following:

1–Glass aquarium number 1 immunized by I/P injection of 0.2 ml/fish of formalized whole broth culture of *Ps. fluorescence* emulsified in an equal volume of complete Freund's adjuvant.

2–Glass aquaria number 2 lifted as control injected I/P with sterile broth 0.2 ml / fish

II-Group B (a total number of 100 *O. auraus*):

Consists of two glass aquaria each containing 50 fish of 60 ± 10g/fish average body weight and treated as following:

1–Glass aquarium number 3 immunized by I/P injection of 0.2 ml/fish of formalized whole broth culture of *Ps. fluorescence* emulsified in an equal volume of complete Freund's adjuvant.

2–Glass aquaria number 4 lifted as control injected I/P with sterile broth 0.2 ml/fish

III-Group C (a total number of 100 hybrid tilapia):

Consists of two glass aquaria each containing 50 fish of 60 ± 10g/fish average body weight and treated as following:

1–Glass aquarium number 5 immunized by I/P injection of 0.2 ml/fish of formalized whole broth culture of *Ps. fluorescence* emulsified in an equal volume of complete Freund's adjuvant.

2–Glass aquaria number 6 lifted as control injected I/P with sterile broth 0.2 ml/fish.

Injections were done as described by (*Schaperclaus et al.,* 1992). Fish were anaesthetized before handling using Quanalidine (Jolly *et al.*, 1972). Blood samples were taken just before immunization and then weekly for 6 weeks post immunization from caudal blood vessels after anaesthetization of fish using Quanalidine (Jolly *et al.*, 1972). Blood serum was separated, inactivated at 56^{0}C for 30 minutes then kept at -20^{0}C till being used.

Analytical procedures:

1- Measurement of antibody titers:

Antibody titers were measured by direct hem-agglutination test from pooled blood sera of each group of test fish before and after immunization with *Ps. fluorescence.*

2- Estimation of serum total proteins:

Colorimetric determination of serum total protein level in the serum of fish was carried out using the method of (Doumas,1975).

3- Estimation of serum albumin:

Colorimetric determination of serum albumin was carried out according to (Baure, 1982).

4- Estimation of serum globulin:

Globulin value was determined by subtracting the albumin value from the total protein value of the same sample (Coles, 1974).

(IX) Challenge test:

The immunized as well as the control fish groups were challenged by I/P injection of o.2 ml/fish of 24 hour broth culture of virulent *Ps. fluorescence* at the end of the 5th week post immunization. Fishes were observed for two weeks post-challenge for mortalities patterns and clinical abnormalities. Blood samples were collected at the end of the 5th week post-immunization (1week post-challenge), serum separated, inactivated at 56^{0}C for 30 minutes then kept at -20^{0}C till being used. The level of protection was calculated according to the equation of *(Newman and Majnarichs 1982)*

$$\text{Relative level of Protection} = \left(1 - \frac{\text{Percent immunized mortality}}{\text{Percent control mortality}} \times 100\right)$$

(X) Statistical analysis:

Data were analyzed using SAS Statistical analysis system package (Littel *et al.*,1991)

RESULTS AND DISCUSSION

In Egypt, Oreochromis species have become very important and are cultured in fish farms throughout the country, their economic importance is constantly increasing. For their fast growth, disease resistance, different feeding habits and palatability (*Dadzie, 1982*). For these reasons chromosomal investigation of these fishes and the possibility of cross-breeding between them have accordingly been used to verify relationships within natural groups or species. Surveys of chromosome numbers are less complete for fishes than for other groups of animals in spite of the fact that fishes constitute a greater number and diversity of species than all other vertebrates. The karyological differences among different Oreochromis species (*O. niloticus, O. auraus* and hybrid) will be reflected on immune status level and the degree of resistance to different bacterial agents. Karyotyping is one of the species characteristic and can help in studying the characteristic of chromosomal complement. Little information is available on selection procedures for increasing disease resistance. An artificial challenge is the most convenient way to establish genetic differences in disease resistance between fish families (*Sarder et al.,2001*). In this study, a relationship between the genetic build-up and immunological capacity of different tilapia species have been estimated. It was found that the diploid chromosome number of each of the three species (*O. auraus*, hybrid and *O. niloticus*) is 44 chromosomes (2n =44) (*FIG.4*). These results agree with the findings of *El-Feky et al.; (1993)*. and disagree with *Badr and El-Dib; (1977)* who stated that chromosomes number is 40 in case of *O. niloticus* (2n=40). The chromosomes of all the three species forms homologous pairs. The morphological distribution of the karyotypes of the species is almost identical, small differences were found in average size of certain chromosome pairs. From the karyological analysis described above, it is evident that the three species of Oreochromis fish have a very close genetic relationship so *O. niloticus, O. auraus* can produce hybrids with favorable characteristics. The results support the close similarity in feeding, breeding, and biological characteristics. However, the homogenecity in chromosome number and structure may increase the possibility of cross breeding between *O. niloticus, O. auraus*. The apparent morphological variation between the three Oreochromis species resulted from minor chromosomal variations.

Genetic variations of immune response have been evaluated through measurement of mean antibodies titers and mortalities patterns of different Oreochromis species under experiment. The results showed that vaccinated *O. niloticus* have the highest immune response through high mean antibodies titers (3.287) compared to that of hybrid (2.866) and *O. auraus* (2.866) 35 days post

vaccination (*Table 3*). These variations may be due to variations in genetic control of immune response. This agree with the findings of Balfry *et al.* (1997a) who identified a significant differences between red and wild type *O. niloticus* in lysosyme activity following *Vibrio parahemolyticus* challenge with no differences between *O. auraus* and *O. Mossambicus*. Sarder *et al.*,(2001) reported that there was variations in the level of immune response of *O. niloticus* breeds against *A. hydrophila.*

There were significant differences in the antibody titers between the three species under experiment with superiority to O. niloticus (2.385) than hybrid (1.722) and *O. auraus* (1.903) at day zero before vaccination. Also, although there were no significant variations in the Chi-square analysis of mortalities patterns post challenge with Ps. fluorescence virulent strains, O. niloticus have the highest survivability (33.3%) than *O. auraus* (20%) and hybrid (16.67%). Also O. niloticus recorded the highest survivability post challenge of vaccinated groups (83.33%) followed by hybrid (70%) while *O. auraus* recorded the lowest survivability (60%) (Tables 4 & 5). These variations in the level of natural resistance may be due to hereditary factors. These agree with many studies. Sarder *et al.*,(2001) reported a significant differences in natural resistance of *O. niloticus* breeds to *A. hydrophila* infection. *Abd El-Rahman et al.,(2002)* cited that *T. zilli* and *S. galileous* had higher resistance to bacterial diseases relatively than other tilapias, as *T. zilli* can live at lower temperature and a higher water salinity than other tilapias. Hybrid tilapia has an intermediate resistance between that of *O. niloticus* and *O. auraus*. From the results of the study it was evident that vaccination of Oreochromis species through I/P injection of formalin killed whole culture of *Ps. fluorescence* can give protection against natural or experimental infection with variable degrees according to fish species. These findings agree with Schaperclaus, *(1972)*; where they reported an increase in the concentration of naturally acquired antibody through oral or parental immunization of carp against *A. punctata*; also Ruangpan *et al.*, (1986) noted that 100% protection was obtained 2 weeks after I/P injection of Nile tilapia formalin-killed *A. hydrophila* vaccines. El-Ashram, (2002) cited that the immunization of *O. niloticus* with formalized whole culture vaccine of virulent strains of *A. hydrophila* was effective in protecting fish against infection and controlling MAS out break among cultured tilapia with economic benefit. With respect to serum total proteins, albumin and globulin (Tables 6,7&8). There was no significant value except for globulin at 35 day post vaccination with superiority to *O. niloticus*. However, the globulin level values are higher in all other periods in *O. niloticus* than *O. auraus* and hybrid to the vaccine. Significant variations *in vaccine protective efficacy were* recorded in different Oreochromis species in response to killed vaccination through I/P route. Vaccine protective efficacy was

74.96, 62.5 and 51.98 in *O. niloticus*, hybrid and *O. auraus* respectively (Table 9). These results agree with more antibody titers and more resistance in O. *niloticus* than other two species of fish. From the previous results, *O. niloticus* species have the highest level of antibody titers against the vaccine used at many different periods. Moreover, it more survive to natural infection with the virulent strain of *Ps. fluorescence*. On the other hand the *O. auraus* showing the lowest level of antibody and of low resistance as the number of dead fishes is high. However, crossing of *O. niloticus* and *O. auraus* give promising results in improving the immune response and resistance to both diseases.

Table 1. Morphological, cultural and biochemical characters of *Ps. fluorescence*

Character	**Response**
Gram Stain	Negative
Shape	Rods
Motility	Motile
Growth on R-S media	Dark green colonies
Cytochrome oxidase test	Positive
Oxidase test	Positive
Growth at $4^{O}C$	Positive
Growth at $42^{O}C$	Negative
H2S production	Negative
Indol	Negative
Vogues Prauskeur	Negative
Citrate utilization	Positive
Gelatin hydrolysis	Positive
Methyl red	Negative
O/ F (Oxidation Fermentation)	O (Oxidative)
Fluorescent pigment (fluorescen)	Produced
Nitrate reduction	Positive
Acid produced from glucose	Positive
Sucrose	Positive
Manitol	Positive
Maltose	Positive
Lactose	Negative
Dulcitol	Negative

Table 2. Mortality patterns 15 days post I/P injection of *O. niloticus* with virulent *Ps.*

Fish group	Fish number	No. of dead fishes 15 days post-injection															Dead fish	Survived fish
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	No.	No.
Isolate1	20	7	1	2	1	-	-	1	1	-	-	-	-	-	-	-	13	7
Isolate2	20	4	3	2	2	-	-	1	1	1	-	-	-	-	-	-	14	6
Isolate3	20	5	4	2	1	-	-	1	1	-	1	-	-	-	-	-	15	5
Isolate4	20	3	2	2	-	2	-	-	2	1	-	-	-	-	-	-	12	8
Isolate5	20	5	6	3	1	-	-	2	-	-	-	-	-	-	-	-	17	3
Isoate6	20	4	2	3	1	-	-	1	-	-	-	-	-	-	-	-	11	9

fluorescence strains (0.2 ml of 24 hours whole broth culture

Table 3. Mean antibody titers in different Oreochromis species before vaccination 0 day) and after vaccination (14, 21, 35 days) and 7 days post-challenge with *Ps. fluorescence* (42 day).

Period (days)	*O. niloticus*	hybrid	*O. auraus*
0	2.385 ± 0.074 ^{de}	1.722 ± 0.074 ^{fg}	1.903 ± 0.074 ^{f}
14	2.626 ± 0.074 ^{cd}	2.264 ± 0.112 ^{e}	2.264 ± 0.112 ^{e}
21	3.047 ± 0.06 ^{ab}	2.385 ± 0.22 ^{de}	2.686 ± 0.074 ^{c}
35	3.287 ± 0.074 ^{a}	2.866 ± 0.112 ^{bc}	2.866 ± 0.06 ^{bc}
42	1.722 ± 0.074 ^{fg}	1.963 ± 0.112 ^{f}	1.722 ± 0.095 ^{fg}

*Mean having different small letters are significantly different at ($P < 0.05$)

Table 4. Number of dead and survived Oreochromis fish 15 days post- challenge of vaccinated *O. niloticus*, *O. auraus* and hybrid with virulent *Ps. fluorescence*

Fish type	Number of fish	Dead fishes		Survived fishes	
		No.	%	No.	%
O. niloticus	30	5	16.67	25	83.33
hybrid	30	9	30	21	70
O. auraus	30	12	40	18	60

$\chi^2 = 4.002$ (Non significant at level $P \leq 0.05$)

Table 5. Number of dead and survived Oreochromis fish 15 days post- challenge of non vaccinated *O. niloticus*, *O. auraus* and hybrid with virulent *Ps. fluorescence*

Fish type	Number of fish	Dead fishes		Survived fishes	
		No.	%	No.	%
O. niloticus	30	20	66.67	10	33.33
hybrid	30	24	80	6	20
O. auraus	30	25	83.33	5	16.67

$\chi^2 = 2.609$ (Non significant at level $P \leq 0.05$)

Table 6. Mean total serum proteins in different Oreochromis species before vaccination (0 day) and after vaccination (14,21,35 days) and 7 days post-challenge with *Ps. fluorescence* (42 day)

Period (days)	*O. niloticus*	hybrid	*O. auraus*
0	2.396± 0.04 ^{bcde}	2.352± 0.04 ^{bcde}	2.364± 0.04 ^{bcde}
14	2.556± 0.06 ^{bcd}	2.458± 0.06 ^{bcd}	2.458± 0.06 ^{bcd}
21	2.730± 0.059 ^{bcd}	2.676 ± 0.058^{bcd}	2.612± 0.062 ^{bcd}
35	2.912± 0.06 ^{ab}	2.844± 0.066 ^{bc}	2.816± 0.072 ^{bcd}
42	2.550± 0.038 ^{bcd}	2.298± 0.057^{cdef}	2.278± 0.064 ^{cdef}

*Mean having different small letters are significantly different at ($P < 0.05$)

Table 7. Mean albumin levels in different Oreochromis species before vaccination (0 day) and after vaccination (14,21,35 days) and 7 days post-challenge with *Ps. fluorescence* (42 day)

Period (days)	*O. niloticus*	hybrid	*O. auraus*
0	0.962 ± 0.039 ^{cdefg}	0.970 ± 0.04 ^{cdefg}	0.962 ± 0.04 ^{cdefg}
14	0.916 ± 0.033 ^{efgh}	0.870 ± 0.049 ^{ghi}	0.950 ± 0.034 ^{cdefg}
21	0.952 ± 0.017 ^{cdefg}	1.050 ± 0.038 ^{abc}	0.910 ± 0.022^{fghi}
35	1.006 ± 0.046 ^{b}	1.080 ± 0.046 ^{a}	0.984 ± 0.028 ^{c}
42	0.920 ± 0.026 ^{ab}	0.886 ± 0.023 ^{b}	0.940 ± 0.023 ^{ab}

*Mean having different small letters are significantly different at ($P < 0.05$)

Table 8. Mean globulin levels in different Oreochromis species before vaccination (0 day) and after vaccination (14,21,35 days) and 7 days post-challenge with *Ps. fluorescence* (42 day)

Period (days)	*O. niloticus*	hybrid	*O. auraus*
0	1.442 ± 0.043 ^{efgh}	1.392 ± 0.036 ^{ghi}	1.418 ± 0.047 ^{fgh}
14	1.644 ± 0.035 ^{bcd}	1.570 ± 0.026 ^{cde}	1.544 ± 0.033 ^{def}
21	1.768 ± 0.043 ^{ab}	1.632 ± 0.026 ^{bcd}	1.706 ± 0.028 ^{abc}
35	1.906 ± 0.019 ^{a}	1.750 ± 0.044 ^{b}	1.694 ± 0.033 ^{bc}
42	1.628 ± 0.032 ^{a}	1.406 ± 0.04 ^{b}	1.338 ± 0.033 ^{bc}

*Mean having different small letters are significantly different at ($P < 0.05$)

Table 9. Level of vaccine efficacy

Type of fish	Type of vaccine	Type of bacteria	No. of dead fishes	Vaccine efficacy%
O. niloticus	immunized	*Ps. fluorescence*	5	74.96
O. niloticus	Non-immunized	*Ps. fluorescence*	20	
Hybrid	immunized	*Ps. fluorescence*	9	62.5
Hybrid	Non-immunized	*Ps. fluorescence*	24	
O. auraus	immunized	*Ps. fluorescence*	12	51.98
O. auraus	Non-immunized	*Ps. fluorescence*	25	

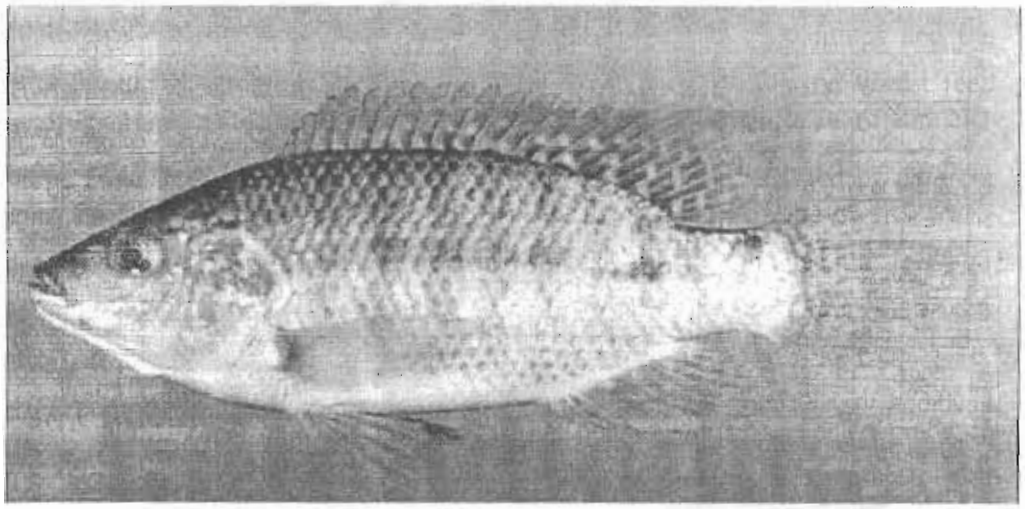

Fig. 1. *O. niloticus* with caudal fin has regular vertical black stripes throughout its depth and the margin of the dorsal fin is grey or black

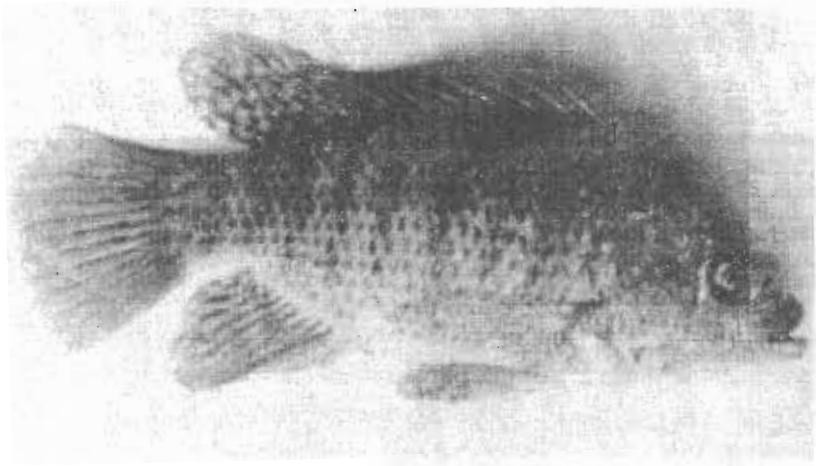

Fig. 2. *O. auraus* with dorsal and caudal fins of the male have red margins

Fig. 3. Hybrid tilapia with caudal fin has less regular or discontinuous vertical black stripes, there may be red margins in male dorsal and caudal fins.

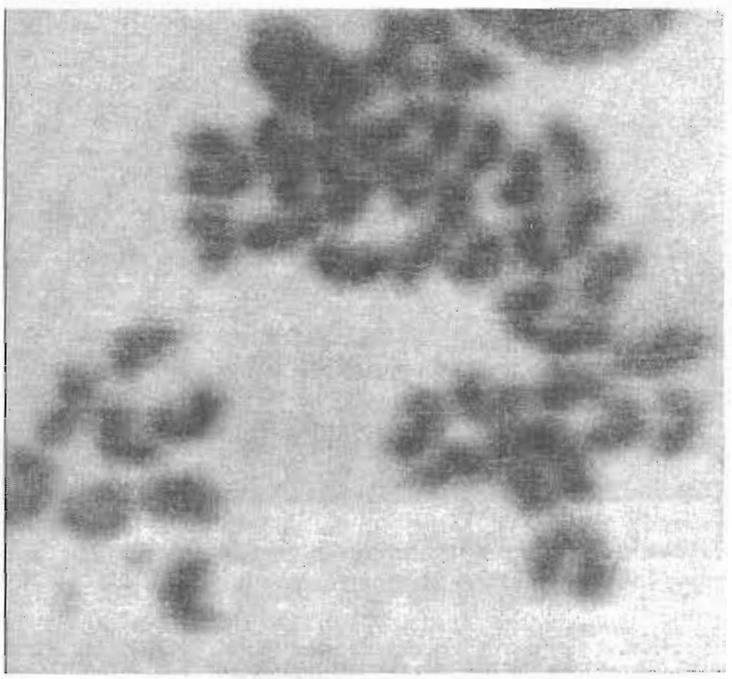

Fig. 4. Chromosomes of Oreochromis fish.

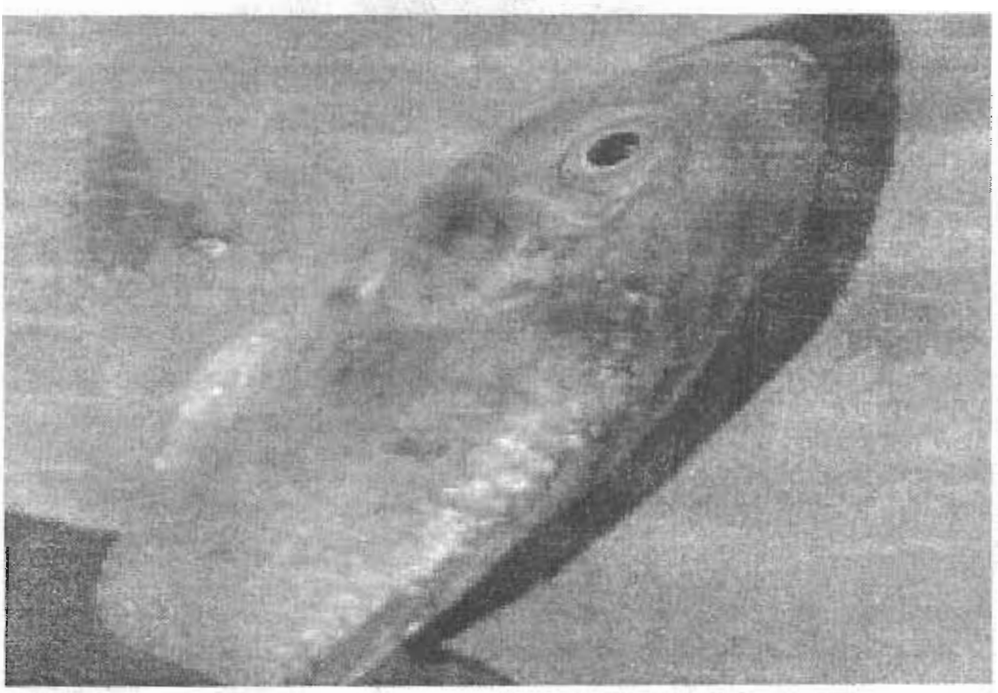

Fig. 5. Experimentally infected *O. auraus* showing detached scales, skin erosions and ulcers.

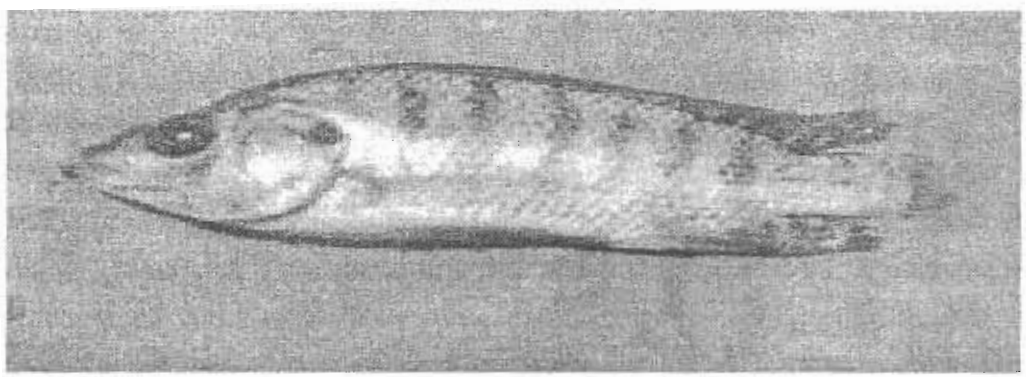

Fig. 6. Naturally infected *O. niloticus* showing tail and fin ro

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الإستجابة المناعية للأنواع المختلفة وراثيا من أسماك البلطي للتحصين ضد السودوموناس فلورسنس

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صممت هذه التجربة لتقييم مدى الاختلاف في المقاومة الطبيعية لأسماك البلطي النيلي والأوريا و الخليط بينهما للإصابة بمكروب السودوموناس فلورسنس وتحديد أفضل الأنواع في الاستجابة المناعية للتحصين و تقليل نسب النفوق بعد الإصابة الصناعية لانتقاء أفضل الأنواع مقاومة لاستخدامها في التربية. بدأت التجربة بالعزل و التوصيف الكامل لمكروب السودوموناس فلورسنس من حالات مصابة إكلينيكيا. تم تحديد أكثر العترات ضراوة باستخدام اختبار الحقن في اسماك البلطي النيلي تم عمل التحصين من ميكروبي السودوموناس فلورسنس باستخدام الفورمالين ٣٧ %. تم تقسيم الأسماك في أحواض زجاجية إلى ثلاث مجموعات (مجموعة البلطي النيلي ١٠٠سمكة و مجموعة البلطي الأوريا ١٠٠ سمكة و مجموعة الهجين ١٠٠ سمكة) كل مجموعة منها مقسمة علىحوضين زجاجين بمعدل ٥٠سمكة لكل حوض . بعد انتهاء فترة الأقلمة تم تحديد معدل الأجسام المناعية الطبيعية في كل نوع .تم حقن الأسماك باستخدام التحصين من مكروب السودوموناس فلورسنس بمعدل ٠,٢ مل لكل سمكة داخل الغشاء البريوتوني. تم تجميع عينات الدم و قياس معدلات الأجسام المناعية بعد ١٤ ، ٢١ ، ٣٥ يوم من التحصين. تم عمل عدوى صناعية عن طريق حقن كل سمكة ٠,٢ مل من اشد العترات ضراوة من مكروب السودوموناس فلورسنس داخل الغشاء البريوتوني تم تحديد نسب النفوق لمدة ١٥ يوم بعد الحقن و تحديد نسب الأجسام المناعية في اليوم السابع بعد الحقن تم تحديد مدى كفاءة التحصين في كل نوع من الأسماك عن طريق حساب أعداد الأسماك الميتة و الحية لكل نوع. تم إجراء الدراسات الو راثية على السلالات الثلاثة و تجهيز الكر وموسومات الخاصة بكل نوع.

أوضحت النتائج ما يلي: وجود نفس العدد من الكر وموسومات في الأنواع الثلاثة من البلطي تحت الدراسة (٤٢ زوج من الكر وموسومات).

بقياس معدل الأجسام المناعية الطبيعية تبين وجود تفوق واضح لسمك البلطي النيلي حيث سجل٢,٤٤٥ يليها البلطي الهجين حيث سجل ١,٧٢٢ بينما كانت اقل معدلات الأجسام المناعية الطبيعية في البلطي الأوريا حيث سجل ١,٨٢٤. أثبت البلطي النيلي تفوق بالاستجابة المناعية للتحصين حيث سجل أعلى معدل أجسام مناعية ٣,٣٢٧ بعد ٣٥ يوم من التحصين يليه البلطي الهجين حيث سجل ٢,٨٦٦ و أخيرا البلطي الأوريا و الذي سجل ٢,٨٦٦.

بعد الحقن بالميكروب شديد الضراوة من السودوموناس فلورسنس سجل البلطي النيلي اقل عدد من الوفيات ٥ يليه الهجين ٩ وأخيرا الأوريا ١٢. كانت كفاءة التحصين في حالة البلطي النيلي هي الأعلى و سجلت ٨٩,٤١% يليه الهجين حيث سجل ٦٢,٥% ثم الأوريا حيث سجل ٥١,٩٨%.

خلاصة نتائج التجربة :

١- أن أسماك البلطي النيلي هي أكثر الأنواع تحت الدراسة من حيث المقاومة الطبيعية للإصابة لميكروب السودوموناس فلورسنس.

٢- سجلت البلطي النيلي أعلى معدلات الأجسام المناعية ضد ميكروب السودوموناس فلورسنس بعد التحصين باستخدام العترة المعاملة بالفورمالين.

٣- بعد الحقن باستخدام العترة شديدة الضراوة من ميكروب السودوموناس فلورسنس سجلت البلطي النيلي اقل معدل من الوفيات يليه الهجين ثم البلطي الأوريا وبذلك يمكن تكثير استخدامه في التربية نظرا لمقاومته الأفضل لميكروب السودوموناس فلورسنس.

٤- يمكن عن طريق التهجين بين البلطي النيلي و البلطي الأوريا إنتاج خليط يحمل صفات وراثية مقاومة أفضل من البلطي الأوريا.