COMPARATIVE STUDY IN SALINITY TOLERANCE AND DISEASES RESISTANCE OF THREE NILE TILAPIA (*OREOCHROMIS NILOTICUS*) STRAINS CULTURED IN EGYPT

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Abstract

Salinity tolerance and resistance to *Aeromonas hydrophila* infection were investigated in three Nile tilapia strains cultured in Egypt: improved Abbassa, commercial Manzala and Kafr Elshiekh throughout two experimental studies. In the first experiment, 120 fish from each strain (15 g ± 0.25) were equally divided into four groups (30 fish each). Each group was transferred to three tanks (10 fish each). Fish of groups 1-4 were exposed to a direct transfer to four different concentrations of saline water 6, 12, 18, and 24 ppt for 96 hours. In the second experiment, 60 fish from each experimental strain (20 g ± 0.34) were divided into two equal groups including control group and tested group, three replicates each with 10 fish per replicate. Tested groups were challenged with pathogenic *A. hydrophila*. A 0.1 ml dose of 24-h broth from virulent bacterial pathogen of *A. hydrophila* ($5x10^5$cells/ml) was injected by intraperitoneal (IP) route. The challenge test lasted for 14 days. At the end of salinity tolerance experiment, Abbassa and Manzala strains showed significantly ($P<0.001$) higher survival rates (71.66 and 70 %, respectively) than Kafr Elshiekh (59.16%). Median lethal salinity (MLS_{96}) was significantly ($P<0.01$) higher in Abbassa (19.97) and Manzala (19.90) than Kafr Elshiekh (17.07). Lethal concentration 50 (LC_{50}) of both Abbassa and Manzala (19.13 and 19.4, respectively) were also significantly ($P<0.05$) higher than that of Kafr Elshiekh (16.26). In the second experiment, after challenging with *Aeromonas hydrophila* some of the tested experimental fish showed darkness in the color of the skin, detachment of the scales, and large irregular hemorrhages on the body surface. Survival rate% at tested groups was significantly ($P<0.001$) higher in Abbassa (93.3%) followed with Manzala (70 %) whilst Kafr Elshiekh showed the lowest survival rate (46.7%). It could be concluded from the current study that, Abbassa selection strain is a good choice for tilapia culture in brackish water and meantime have more resistance to *Aeromonas hydrophila* infection than the other two strains.

Keywords: Salinity tolerance, diseases resistance, *A. hydrophila*, improved strain.

INTRODUCTION

Tilapias are tolerable for low protein diets and wide ranges of adverse environmental conditions; comparatively free from serious disease and parasites infections in addition to its ease of handling and breeding in captivity (Eknath, 1994). Egypt is considered as the world second producer of tilapia after China (FAO, 2014). In order to develop the featured Egyptian tilapia production many challenges must be overcome such as open new prospects for production and controlling bacterial outbreaks in farmed fish.

The scarcity of freshwater in many countries complicated with the competition with agriculture and other urban activities has increased the pressure to develop aquaculture in brackish and sea water (El-Sayed, 2006). In some areas of Egypt, ground water salinity may exceed 10 ppt restricting the use of this water for agricultural activities. Therefore, efficient utilization of brackish water for aquaculture may constitute an important alternative. On the other hand, many Egyptian tilapia farmers and hatchery mangers complained of the existence of signs of bacterial infections and high mortality outbreaks during the last period. It has been known for decades that *Aeromonas,* plays as a common causative agent for fish diseases in aquaculture. Yet, many fish farms have been infected by epidemics of *Aeromonas hydrophila* which causes a great loss in cultured fish (Neilsen *et al*., 2001and He *et al.,* 2006). The existence and pathogenicity of *A. hydrophila* has likewise been reported in tilapia (Liu *et al.,* 1999 and Aly *et al.,* 2016).

The WorldFish center in Egypt developed the improved-growth tilapia strain “Abbassa” (Rezk *et al.,* 2009) which showed a superior growth rate in fresh water as compared with local commercial tilapia strain (Ibrahim *et al.,* 2013*).* Extensive dissemination of such superior tilapia strain should be accompanied with testing its tolerance for some environmental extremes like higher salinities as well as its resistance to infection with different pathogens.

The objective of this study is therefore to investigate salinity tolerance and impact of experimental infection with *Aeromonas hydrophila* on the

improved strain Abbassa versus two local commercial strains, Kafr Elshiekh and Manzala. This is done to verify if Abbassa strain would be a good candidate for brackish water culture and to evaluate Abbassa relative resistance to bacterial infection as compared to local available strains.

MATERIALS AND METHODS

Experimental condition.

This study was carried out in the Aquatic Lab, Faculty of Fish Resources, Suez University, Suez, Egypt. The experiment was conducted in 40 plastic tanks (70 liter each) which were a part of a static water system. All tanks of the system were vigorously aerated by supplying air to each individual tank through air stones, which were connected to main air line. The water quality variables were measured during the experiment and they were appropriate for tilapia in the initial conditions: temperature 23 ± 2°C; pH between 7.2 and 7.5; dissolved Oxygen between 7.0 and 8.0 mg L^{-1}.

Fish for experiment.

Three strains of Egyptian Nile tilapia were used in this study: The genetically improved strain Abbassa which has been described by Rezk *et al.* (2009) and Khaw *et al.* (2009). Abbassa strain was introduced from WorldFish Regional Center, Abbassa, Abu-Hamad, Sharkia, Egypt. Kafr Elshiekh local commercial strain came from a civil tilapia hatchery, located at Al-Ryad, Kafr El Sheikh, Egypt. Manzala local commercial strain was obtained from a civil tilapia hatchery, located at Manzala, Dakahlia, Egypt. Experimental fish from each strain were hatched within mass spawning system and reared in separate replicated hapas for 12 weeks in a private tilapia hatchery located in San-El-Hagar, Sharkia, Egypt. Fish were transported to Suez in plastic bags filled with oxygen, each containing approximately 15 L of fresh water. Fish were acclimatized for a period of 3 weeks. During the acclimatization period, fish were fed to satiation twice a day with commercial pelleted feed.

Salinity tolerance experiment.

One hundred-twenty fish from each strain, with an average body weight of 15 g and standard error ± 0.25, were equally divided into four groups (30 fish each). Each group was split to three tanks (10 fish each). Fish of groups 1-4 were exposed to a direct transfer to four different concentrations of saline water 6, 12, 18 and 24 ppt for 96 hours. The behavior of fish was observed and the daily mortality was recorded. Dead fish were removed and approximately 20% of the water was replaced by clean aged water daily and then saline water concentrations were re-adjusted to maintain the targeted salinities. Three indices were estimated in order to compare salinity tolerance of the studied strains. Survival rate (%) in each tank was calculated after 96 h. Median Lethal Salinity (MLS_{96h}) is defined as the salinity at which survival of test species falls to 50% in 96 hours following direct transfer from freshwater to various test salinities (Watanabe *et al*., 1990). Linear regression equation (Y = a + bx) was used to predict the exact salinity (ppt) of the MLS_{96h}. Lethal concentration 50 (LC_{50}) was determined by Probit method according to Finny (1952). After 96 hours the relationship between the mortality% and the concentration of saline water was graphically represented for each studied strain. The concentration which resulted in 50% mortality after 96 hours (LC_{50}) was determined from the graph.

Bacterial challenge test.

Sixty fish from each strain (20 g ± 0.34) were divided into two equal groups including control group and tested group, three replicates each with 10 fish per replicate. A pathogenic *A. hydrophila* (ATCC 13037) was obtained as a reference strain from Microbiology Resources Centre in Faculty of Agriculture, Ain Shams University (MIRCEN). Fish of the tested groups were challenged with pathogenic *A. hydrophila.* A 0.1 ml dose of 24-h broth from virulent bacterial pathogen of *A. hydrophila* ($5x10^5$ cells / ml) was injected by intraperitoneal (IP) injection (Abdel-Tawwab *et al.,* 2008). While the control groups was IP injected by 0.1 ml of sterile saline solution. All groups were kept under observation for 14 days to record any abnormal clinical signs and the daily mortality rate.

Statistical analysis.

In salinity tolerance experiment survival data were statistically analyzed by two-way analysis of variance (ANOVA) with interaction according to the following model: $Y_{ijk} = \mu + S_i + T_j + S_i X T_j + e_{ijk}$, where, μ is the overall mean, S_i is the fixed effect of i^{th} strain (i = 1 …3), T_j is the fixed effect of j^{th} concentration of saline water (j = 1 …4), $S_i X T_j$ is interaction between strain and concentration of saline water, and e_{ijk} is random error. LC_{50} and Median lethal salinity (MLS_{96h}) were analyzed by one-way analysis of variance (ANOVA). In the bacterial challenge test survival data were analyzed by two-way analysis of variance (ANOVA) with interaction according to the following model: $Y_{ijk} = \mu + S_i + I_j + S_i X I_j + e_{ijk}$, where, μ is the overall mean, S_i is the fixed effect of i^{th} strain (i = 1 …3), I_j is the fixed effect of j^{th} injection treatment (j = 1 or 2), $S_j X I_j$ is the interaction between strain and injection treatment, and e_{ijk} is random error. Survival data were treated as a binary trait, for each tank the counted alive fish at the end of the experiment were coded as "1", whereas the counted dead ones coded as "0". Bonferroni's Test (Bonferroni, 1936) mean separation test was used to distinct statistical differences among the means of main effects. Significant differences are stated at $P < 0.05$. All statistical analyses were performed using statistical software SPSS 22 (IBM SPSS, 2013).

RESULTS AND DISCUSSION

Salinity tolerance.

The results showed that the mortality rate% was highly positively correlated with the saline water concentration in all studied strains. Consistent coefficients of correlation between mortality% and saline water concentration were observed for Abbassa, Manzala, and Kafr Elshiekh strains (0.94, 0.95, and 0.93, respectively). The results (Table 1) revealed that at the end of the salinity tolerance test, Abbassa strain showed the higher survival rate (71.66%) which didn't differ significantly from that of Manzala local commercial strain (70%). Kafr Elshiekh strain showed significantly lowest ($P<0.001$) survival rate (59.16%). As for the effect of the saline water concentration, results (Table 1) indicated significantly ($P<0.001$) progressive decrease in the survival rates% with the developing increase in concentration of saline water. The highest

survival rate% was recorded in the concentration 6 ppt (being 100%), while the lowest was noticed in the concentration of 24 ppt. Figure 1 shows the survival rate% for the experimental strains during the 96 hours under concentrations of saline water 12, 18, and 24 while no mortalities were recorded in the level of 6 ppt. Most of mortalities were recognized at 24 hours of expose and ended at 72 hours from the direct transfer to saline water. The results (Table 2) indicated that the salinity tolerance index Median Lethal Salinity (MLS_{96}) was significantly ($P<0.01$) higher in the growth improved strain Abbassa (19.97) and the local commercial strain Manzala (19.90) than that of Kafr Elshiekh strain (17.07). As for the other salinity tolerance index Lethal Concentration 50 (LC_{50}), the results revealed that outputs were compatible to the MLS_{96} results where both Abbassa and Manzala strains showed significantly ($P<0.05$) higher LC_{50} (19.13 and 19.4, respectively) than that of Kafr Elshiekh strain (16.26).

Table 1. Least squares means for survival rate% of three Nile tilapia strains subjected to different concentrations of saline water for 96 hours.

Effect				**Survival rate%**							
Strain											
Abbassa				**Manzala**				**Kafr Elshiekh**			
71.66^{a} ±2.2				70^{a} ±2.2				59.16^{b} ±2.2			
Salinity											
6ppt				**12ppt**				**18ppt**			**24ppt**
100^{a} ± 2.54				83.3^{b} ± 2.54				57.77^{c} ± 2.54			26.66^{d} ± 2.54
Strain * Salinity (ppt)											
Abbassa				**Manzala**				**Kafr Elshiekh**			
6	**12**	**18**	**24**	**6**	**12**	**18**	**24**	**6**	**12**	**18**	**24**
100^{a}± 4.4	100^{a} ±4.4	56.66^{d} ±4.4	30^{f} ±4.4	100^{a} ±4.4	83.33^{b} ±4.4	70^{c} ± 4.4	26.66^{f} ±4.4	100^{a} ±4.4	66.66^{c} ±4.4	46.66^{e} ±4.4	23.33^{f} ±4.4

$^{ab...}$Within classification any two means having the same script are not significantly different using Duncan test p ≤

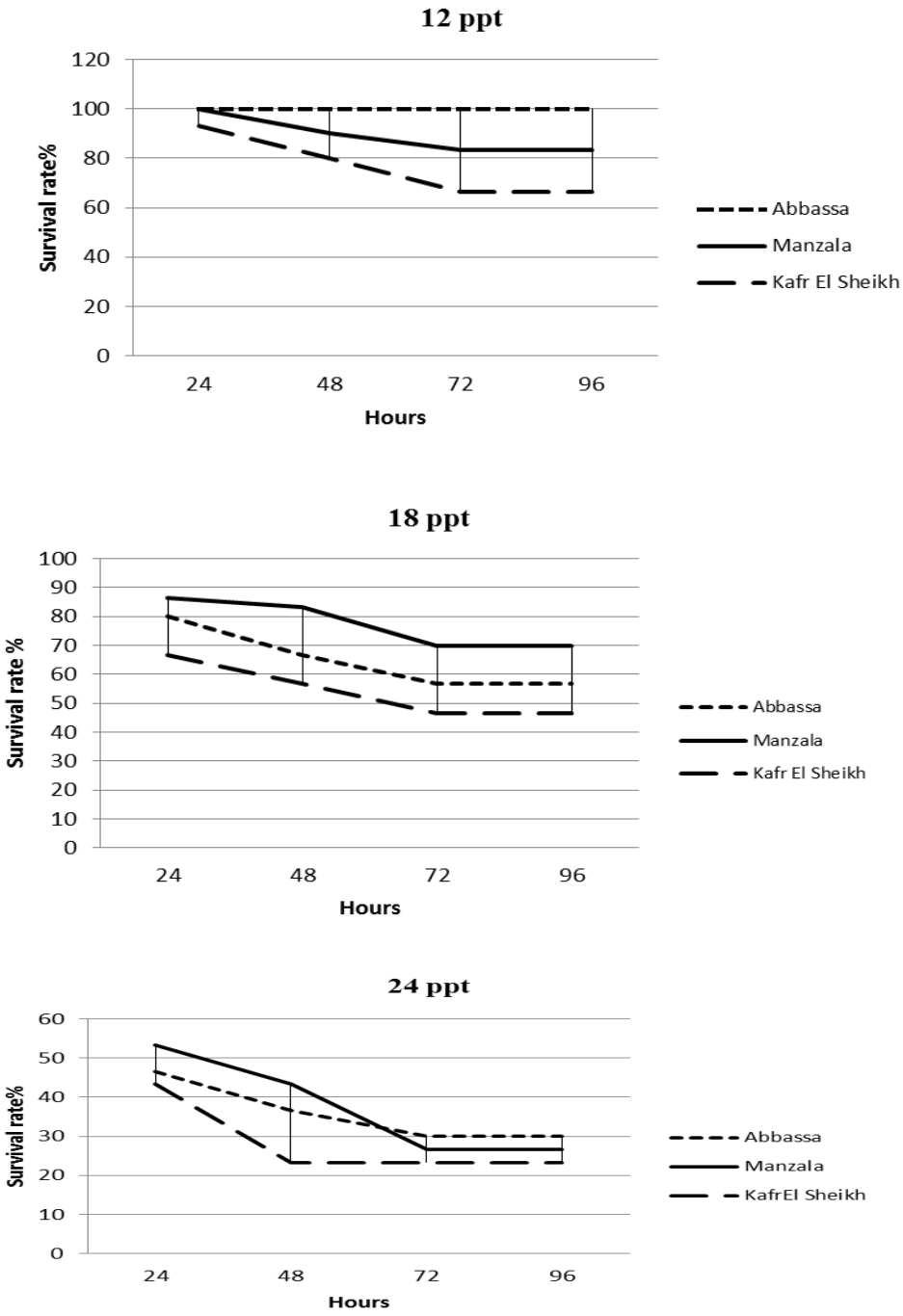

Fig. 1. Survival rate% of three Nile tilapia strains subjected to different concentrations of saline water for 96 hours.

Table 2. Least squares means for median lethal salinity (MLS_{96}) and lethal concentration50 (LC_{50}) of three Nile tilapia strains subjected to different concentrations of saline water for 96 hours.

Strain	MLS_{96}	LC_{50}
Abbassa	19.97[a]	19.13[a]
Manzala	19.90[a]	19.40[a]
Kafr Elshiekh	17.07[b]	16.25[b]
S.E.	0.55	0.71
Sig.	**	*

[ab…]Within classification any two means having the same script are not significantly different using Duncan test $p \leq 0.05$.* Significant differences at $P < 0.05$** Significant differences at $P < 0.01$.

Tilapia species are generally tolerant to a wide range of environmental comparatively radical conditions such as salinity. Tilapia, which, euryhaline, grows well in brackish and fresh water, while its growth rate decreases considerably in sea water (Assem, 1995). Extensive work had been investigated tilapia salinity tolerance and factors affecting it. Several factors and interaction between some of these factors were reported to affect salinity tolerance in tilapia, like size and age (Suresh & Lin 1992 and Ridah, 2008), temperature (Linkongwe *et al.,* 1996), and genetic effects (Lutz, 2006). Differences in salinity tolerance were also noted between tilapia species, hybrids, and strains (Watanabe *et al.,* 1985 and Villegas, 1990 a & b). Varied effects of salinity on growth performance were reported in different tilapia species and strains (Suresh and Lin, 1992 and Garcia-Ulloa *et al.*, 2001). There is a demand for tilapia genotypes which are capable to and competent with elevated salinities (Kamal and Mair, 2005; Lawson and Anetekhai, 2011). There is increasing attention in tilapias that can tolerate salinity and still display agreeable growth (Armas-Rosales, 2006).

The results of the current study indicated significant differences in salinity tolerance between different Nile tilapia strains. Abbassa and Manzala strains were significantly higher than Kafr Elshiekh concerning survival rate%, MLS_{96}, and LC_{50} after exposing to direct transfer to different salinities ranged from 6 to 24 ppt. The higher salinity tolerance of Abbassa strain may have been due to some advantages in the founding population concerning salinity tolerance. In freshwater, growth rates of the genetically improved Abbassa strain were superior to those of Egyptian local strains of the Nile tilapia (Ibrahim *et al.*, 2013; Said and Mekkawy, 2016). According to the reviewed higher growth rate and the current superior salinity tolerance Abbassa selection strain may constitute a good choice for tilapia culture in brackish water areas in Egypt.

Bacterial challenge test.

Diseases have become a primary constraint to the culture of many aquatic species. These diseases cause large-scale mass mortalities of cultured species, inducing devastating losses to regional aquaculture production, impending both economic and social developments and a significant constraint on aquaculture production and trade (Nagasawa and Cruz-Lacierda, 2004). *Aeromonas hydrophila* are Gram negative, motile rods that are oxidase and catalase positive and are fermentative in nature (Sabur, 2006). Septicemia, ascitis, erosion, ulceration, detachment of scale, exophthalmia and muscular necrosis were reported to be the most predominant clinical signs of Motile Aeromonas Septicemia (MAS) in Nile tilapia (Okpkowassili & Okpkowassili, 1994 and Ali, 1996). In the present study, some of the tested experimental fish showed one or more of the following signs; darkness in the color of the skin, detachment of the scales, large irregular hemorrhages on the body surface, ulcers on the skin varied from shallow to deep necrotizing ulcers, fin erosions, inflamed vent, exophthalmia, and abdominal distension with sero-hemorrhagic fluids exuded from the vent.

The results (Table 3) indicated highly significant (P<0.001) differences between survival rates% of the studied strains after challenged with *A. hydrophila*. Abbassa showed the highest survival rate (96.7%) followed with Manzala (85%) whilst Kafr Elshiekh strain showed significantly lowest survival rate (73.3%). The effect of the bacterial infection was highly significant (P< 0.001). The survival rate in the control groups (100%) was significantly higher than that of the tested groups (70%). Survival rate at tested groups was significantly (P<0.001) higher in Abbassa (93.3%) followed with Manzala (70%) which in turns significantly higher than Kafr Elshiekh (46.7%). Figure 2 shows that mortalities happened earlier in the Kafr Elshiekh strain and also indicated that most mortalities occurred between the 2^{nd} and the 8^{th} day post-infection. The noted significant differences between the experimental strains in resistance to infection with *A. hydrophila,* indicated a superiority of Abbassa strain that may partly be a result of selection for nine generations concerning growth (Rezk *et al.,* 2009 and Khaw *et al.,* 2009). This explanation may be aligned with that of Gjedrem, (2000) who postulated that by increasing growth rate, a correlated positive genetic response can be obtained for disease resistance. Additionally, other studies do suggest a positive genetic correlation between survival and growth (Imsland *et al.,* 2002; Bilodeau-Bourgeois *et al.,* 2008). On the other hand, a genetic correlation between growth and disease resistance has not been detected in other investigations (Barroso *et al.,* 2008; Silverstein *et al.,* 2008). Another prober explanation of Abbassa strain advantage in resistance to *A. hydrophila* is the genetic material of the base population concerning resistance to diseases. Recent studies showed that single nucleotide polymorphism SNPs in some immune-related genes were associated with resistance against bacterial and viral pathogens in some fish species. Fu *et al.* (2014) concluded that two SNPs were associated with tilapia resistance to *A. hydrophila* and may be useful in the selection of tilapia resistant to *A. hydrophila*. The current results led to suggest superior resistance for *A. hydrophila* of Abbassa selection strain compared to local commercial strains.

Table 3. Least squares means for survival rate% of three Nile tilapia strains challenged with pathogenic *Aeromonas hydrophila* for 14 days.

Effect			**Survival rate%**		
Strain					
Abbassa		**Manzala**		**Kafr Elshiekh**	
$96.7^{a}\pm3.9$		$85^{ab}\pm3.9$		$73.3^{b}\pm3.9$	
Tretment					
Control				**Tested**	
$100^{a}\pm3.2$				$70^{b}\pm3.2$	
Strain * Treatment					
Ab*Control	**Ab*Tested**	**Ma*Control**	**Ma*Tested**	**Kf*Control**	**Kf*Tested**
$100^{a}\pm5.5$	$93.3^{a}\pm5.5$	$100^{a}\pm5.5$	$70^{b}\pm5.5$	$100^{a}\pm5.5$	$46.7^{c}\pm5.5$

Ab: Abbassa; Ma: Manzala and Kf: Kafr Elshiekh

$^{ab...}$Within classification any two means having the same script are not significantly different using Duncan test $p \leq 0.05$.

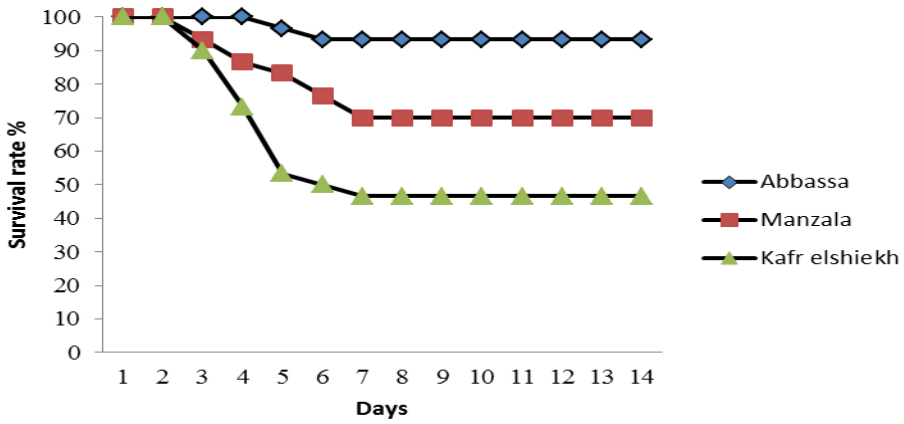

Fig 2. Survival rate% of three Nile tilapia strains during 14 days of bacterial challenge test with pathogenic *Aeromonas hydrophila.*

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دراسه مقارنه عن تحمل الملوحه ومقاومة الأمراض لثلاث سلالات من البلطى النيلى مستزرعه فى مصر

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الملخص العربى

تم دراسه تحمل الملوحه و مقاومه العدوى البكتيريه بميكروب الايروموناس هيدروفيلا فى ثلاث سلالات مصريه من البلطى النيلى: عباسه المحسنه ومنزله وكفر الشيخ التجاريتين ذلك من خلال تجربتين معمليتين. فى التجربه الأولى تم تقسيم ١٢٠ سمكه (١٥ جرام± ٠.٢٥) من كل سلاله الى أربعه مجموعات، كل مجموعة ٣٠ سمكه. كل مجموعه تم نقلها ثلاثه تانكات (١٠ سمكات فى كل تانك). الأسماك فى المجموعات ١-٤ تم نقلها مباشرة لأربعه تركيزات ملوحه مختلفه ٦ و ١٢ و ١٨ و ٢٤ جزء فى الالف لمده ٩٦ ساعه. فى التجربة الثانية تم تقسيم ٦٠ سمكه من كل سلاله (٢٠جرام ± ٠.٣٤) الى مجموعتين متساويتين: مجموعه مقارنه و مجموعه مختبرة، ثلاثه مكررات لكل مجموعه و١٠ سمكات فى كل مكررة. المجموعات المختبره تم حقنها بالمسبب المرضى البكتيرى الايروموناس هيدروفيلا. جرعة ٠.١ مللى من المسبب المرضى ايروموناس هيدروفيلا تم أعطاؤها بالحقن داخل التجويف البريتونى . تم وضع الأسماك تحت الملاحظه لمده ١٤ يوما بعد الحقن. فى نهايه تجربة تحمل الملوحه أظهرت سلالتى عباسه ومنزله معدلات إعاشة (٧١.٦٦ و٧٠% على الترتيب) أعلى معنويا($P<0.001$) عن كفر الشيخ (٥٩.٦١%). متوسط الملوحه القاتله (MLS_{96}) كان أعلى معنويا ($P<0.01$) فى عباسه (١٩.٩٧) ومنزله (١٩.٩٠) عنه فى كفر الشيخ (١٧.٠٧).التركيز القاتل النصف مميت (LC_{50}) فى كلا من عباسه ومنزله (١٩.١٣ و ١٩.٤ على الترتيت) كان أعلى معنويا ($P<0.05$)عنه فى كفر الشيخ (١٦.٢٦). فى التجربه الثانيه ظهرت على بعض الأسماك التى تم حقنها بميكروب ايروموناس هيدرو فيلا بعض العلامات المرضيه مثل دكانه لون الجلد و انفصال القشور و بقع دمويه نزفيه كبيرة مختلفه الاحجام على السطح الخارجى للسمكه. معدل الإعاشة فى المجموعات المختبره كان أعلى معنويا ($P<0.001$) فى سلالة عباسه (٩٣.٣%) يليها منزله (٧٠%) فى حين ان كفر الشيخ أظهرت أقل معدل إعاشة (٤٦.٧%). يمكن ان نستنتج من الدراسه الحاليه ان السلاله المنتخبه عباسه ستكون اختيار جيد لاستزراع البلطى فى المياه الشروب كما ان لها مقاومه مميزه لميكروب الايروموناس هيدرو فيلا.