# Experimental studies on cultured mullet fingerlings mortalities at Kafr Elsheikh Governorate

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**Abstract**

The study was designed to study causes of mortalities among cultured mullet fingerlings at Kafer El-Sheikh governorate. Cultured mullet fingerlings suffered from ascities, hemorrhagic spots over the skin, easily detached scales with skin ulceration and fin & tail rot. Water analysis registered that pH was 7.4 and salinity was 1‰. Parasitic and Bacteriological investigation revealed heavily *Trichodina* infestation and presence of *Pseudomonas fluorescencs*. In addition, biochemical examinations registered highly significant increase of K in gills, LDH, CPK in musculaturess, liver enzymes (ALT and AST) and ammonia and creatinin among diseased cultured mullet fingerlings. Isolated *Pseudomonas fluorescencs* had sensitive to Danofloxacin, nalixidic acid, ciprocin and oxytetracycline. Experimentally infected fish with *Pseudomonas fluorescencs* were studied.

***Key words:*** *Pseudomonas fluorescencs , ascities, musculaturess.*

## Introduction

Aquaculture is a fast growing industry, which represented one third of the world fisheries production in 2003 **(Lowther, 2005)**. In Egypt, the production of fish coming from aquaculture represents about 65% of the total fish production sources **(GAFRD, 2009).** The intensification of fish culture has caused the emergence of new pathogens, and the need for sustainable treatments and prophylactic measures **(Gatesoupe, 2008).** Prevention or control of fish diseases are essential to the success of the large-scale, intensive production of fish in culture **(Popoff, 1984)**. Mullet fish live within very specific salinity levels (levels of salt in the water). Their bodies work hard to maintain the osmotic gradient between themselves and their environment. If their environmental salinity is not specific to their needs and is not held at a steady level, they have to work harder to maintain their osmotic gradient, which generates chronic stress **(Tullock, 2001).** Bacterial fish diseases constitute one of the major challenges facing sustainable aquaculture production. Most bacterial pathogens of fish are aerobic Gram- negative rods **(Woo and Bruno, 1999).** Ps. fluorescens is a dominant component of freshwater ecosystem (**Allen *et al*., 1983).** Ps. fluorescens has been associated with septicemic and ulcerative condition in a wide range of fish species. Pseudomonas septicemia is a hemorrhagic condition of fish usually associated with stress or improper health management **(Moustafa *et al*., 2010)**. In Egypt pseudomonas sp. were isolated from tilapia, cat fish, mugle and carp from different localities **(Eissa *et al*. 1996; Ahmed and Shoreit 2001; Dardiri *et al*. 2002; Atwa 2007; Abou El-Atta and El- Tantawy 2008).**

The diagnosis of bacterial infections is traditionally based on examination of external signs of infection and isolation of the pathogen by culture **(Suomalainen *et al*., 2005).** Also **Teal and Saggers, (1997)** mentioned that, septicemic diseases leads to clinical and pathological situations in term of problems associated either with specific enzymes, or with the factors that control and integrate enzymatic activity. Enzymes are biochemical macromolecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism **(Roy, 2002 and Humtsoe**

***et al*., 2007).** LDH, CPK, ALT and AST are cellular metabolic key enzymes with no evident function in vertebrate plasma. They were found in small concentrations in plasma, including fish plasma, derived probably from the regular physiological shedding of cells **(Schmidt & Schmidt, 1974).** Therefore any detectable increase of their activity in plasma can be used as a reliable indicator of changed metabolic functions or structural damage at the tissue level **(Krajnovic-Ozretic and Ozretic, 1987).**

Measurement of blood and tissue biochemistry parameters is commonly used diagnostic tool in biomonitoring and according to **Coz-Rakovac *et al*. (2008)** it presents good indicator of the artificial feed effects. In order to document and better understand variability and dynamics of blood and tissue chemistry parameters in general **(Ferri *et al*., 2011).**

## Materials and Methods

The present work was applied in the Fish Diseases Department, Animal Health Research Institute Dokki Giza and in Kafer Elsheikh governorate in the year 2012 in order to know causes of mortality among mullet in Kafr Elsheikh.

### 1.0 Samples

### 1.1 Naturally infected diseased fish:

One hundred diseased cultured mullet fingerling were collected from farms at Damro, in Kafer Elsheikh governorate were transported alive in large plastic bags to the Fish Diseases Department, Animal Health Research Institute Dokki Giza and in Kafer Elsheikh governorate.

### 1.2 Experimentally infected fish sample:

A total number of 45 apparently healthy cultured mullet fingerling weighing 10.0 $\pm$5g were collected alive from a private fish farm were stocked in glass aquaria with aid of oxygen generators for three weeks to be acclimatized prior to the experimental infection. Five fish were randomly selected for bacteriological and parasitological examinations to insure that they free from natural infection. 40 fish were divided into 2 groups 20 of each (Groups 1, 2). All fishes were fed twice daily with commercial pellets as 1% of body weight and maintained in well aerated water in glass aquaria at 25ºC and supplied with decolorinated tap water according to **Abdelhamid *et al*., (2002).**

### 1.3 Water sample:

Water samples from different fish farms were obtained at depth of 15-20 cm below the water surface of each pond in sterile colorless glass determined pH and salinity by methods as described by **A. P. H. A. (1998)**.

### 2.0 Examination of the samples:

### 2.1 Full clinical and post mortem examination:

The naturally infected fish were examined for any abnormalities according to **(Schaperclaüse, 1992).**

### 2.2 Bacteriological examination:

Swabs taken under aseptic condition from skin lesion and samples from liver, kidney and spleen were inoculated on trypticas soya broth (Oxoid) at 25$^{o}$ C for 24 hrs then streaked into MacConkey and T.S.A. agar (Oxoid) Selective colonies were restreaked onto Aeromonas base media (Oxoid) and an TCBS media and Ordal S media. Pure colonies were streaked onto soft agar to be used for further studies. Bacterial isolates were identified by colonial morphology, growth characters on specific media and microscopically appearance as well as phenotypic characteristics according to **(Balows *et al*., 1992, Cowan and Steal, 1993 and Buller 2004) Table(1).** Identification by API 20 NE**:** according to the instruction of the **bioMerieux company**.

### 2.3 Parasitological examination

Naturally infected diseased fish were examined according to **(Paperna, 1996)** for the presence of external protozoa and the prevalence of each parasite was recorded.

### 2.4 Antibacterial sensitivity test:

Different types of antibacterial sensitivity discs (Oxoid) with variable concentrations (in µg/disc) were used in the present work to detect the susceptibility of the isolates according to **Stokes and Ridgeway (1980)** and **Finegold and Martin (1982).**

**2.5 Determination of some biochemical parameters:**

Tissue samples collected from diseased and apparently healthy cultured mullet fingerling (1gm from each organ) and kept at -80 until used where takes filaments of gills for detection of Na and K , musculatures for detection of LDH and CPK , liver for detection of ALT and AST and kidney for detection of ammonia and creatinin. Where all tissues homogenized according to **(Trinder, 1969**)where all parameters takes from homogenized tissue 1 ml and measure but in Na and K homogenized tissue centrifuged at 3000 rpm for 15 min. then takes from supernatant and measure. Na and K were measured by flame photometer according to **Fuhrman and Crismon, (1951)**, LDH, CPK, ammonia and creatinin were measured according to **(young, 1995),** ALT and AST were measured according to **(Schumann and Klauke, 2003**).

**2.6 Experimental Design**

**2.6.1 Preparation of bacterial suspension of pseudomonas fluorescens:**

The bacterium was cultured on Trypticase soya agar at 27ºC for 24 hrs. The pure cultures from isolate were suspended into a sterile saline to give final concentration of 3 x$10^7$ CFU/ ml by count after one hour **Abo El atta *et al*. (2008).**

**2.6.2 Experimental infection**

Fish in group 1 were exposed to pseudomonas fluorescens by using intraperitoneal (I/ P) routes injection**.** Experimental infection (for group 1) was carried out to determine the pathoginicity of pseudomonas isolate. The fish in group 2 were kept as a control and exposed to the same amount of TSB without pseudomonas fluorescens. Fishes were kept under observation for 14 day to record the mortality rate according to **Amos (1985)**. The tissue samples were taken from second group for biochemical analysis.

Mortality rate % =

$$\frac{\text{No. of death in specific period} \times 100}{\text{Total population during that period}}$$

**3.0 Statistical analysis:** It was performed by run on the computer using the SAS program **(SAS, 1998).**

**Results and Discussion**

Examination of naturally infected mullet fingerling revealed the presence of excessive mucus secretion covering the skin and dark grey coloration or even blackness of the skin. Hemorrhagic spots over the skin were occasionally seen. In addition to that there were easily detached scales with skin ulceration and fin & tail rot (figure 1). Postmortem examination revealed slightly amount of reddish exudates observed in body cavity in most cases. Liver was pale, enlarged in some fishes and congested with necrotic patches in other fishes. Spleens was congested and enlarged in most examined fishes. Kidneys were congested in some fishes and apparently normal in the others. Gallbladder was enlarged and engorged with bile. Intestine were hemorrhagic and inflamed in some fishes and apparently normal in the others. Stomach was enlarged and hemorrhagic in most fishes. This agree with those reported by **Austin and Austin (1999), salama (1999), Buller (2004), Atwa (2007)** and **Abo El Atta *et al*. (2008)**.

Bacteriological examination of internal organs (liver, spleen and kidney), musculatures and ascetic fluid of examined fish revealed isolation of motile gram negative, cytochome oxidase, catlase, citrate, gelatin liquefaction and glucose fermentation are positive and negative for urease, H2S production, Vogas Proskour, Methyl-Red was identified as *P. fluorescencs*, this agree with reported by **Abd EL-Rahman *et al*. (2002).**

The colonial characters are shown in Table (1). Pseudomonas fluorescence is likely to be spread through water, which will serve as the primary reservoir of infection **(Austin and Austin, 2007).** This agree with that reported by **Abd EL-Rahman *et al*. (2002)** who isolated Ps. Fluorescencs from O. niloticus reared in concrete ponds. Also, this agree with **Roberts, (1987) and (Woo and Bruno, 1999)** who re-

ported that, bacterial diseases, particularly those caused by gram negative organisms are the major causes of mortality in both wild and cultured fish**.** A wide variety of bacteria, normally living in harmony with their fish host (e.g. pseudomonas spp.) in water or inhabitant of the gastrointestinal tract are harmless to the host. Under certain circumstances as the effect of crowdness, pollution and parasitic infestation, exerting much stress on the fish, thus upsetting the balance between bacteria and These commensal bacteria become secondary opportunistic pathogens and invade fish. This agree with **Inglis *et al*., 1993)** who reported that Pseudomonas species are known to be more common associated with disease in freshwater fish.

Parasitological examination of fishes showed that, fishes highly infested with Trichodina The morphological characteristic of Trichodina spp. were more or less nearly similar to description given by **Hanaa (2001), El-Mowafy (2004)**. This may be due to high feeding where fish before transportation from production farm to growing farm were fed bran and this result agree with **Durborow, (2003)** who reported that, Trichodina infestations are typically caused by high stocking densities and generous feeding rates, both done to maximize production and profits. Such aggressive management can be profitable up to a point, but when the culture system's limits are exceeded, adverse conditions such as poor water quality can lead to lower production levels, higher mortalities from disease, off-flavor problems and, ultimately, lower profits. High feeding rates can lead to high ammonia concentrations, creating an ideal environment for the reproduction of Trichodina and a greater chance of a full-blown infection. If the fish are also crowded (as in cage-raised fish) the infestation can spread very rapidly among the fish. This agree with those reported by **Osman (2001)** and **Eissa (2002).**

The sensitivity of the isolated *P. fluorescencs* to different antibiotic discs it was found that the organism was sensitive to Danofloxacin, nalixidic acid, ciprocin and oxytetracycline and resistant to Ampicillin, Spiramycin, Lincomycin, Nitrofurantaion and Oxalinic acid. These results agree with **Abd El-Rahman (1996)** and **Salama (1999).**

Results of pathogencity of Pseudomonas fluorescence in mullet fingerling group (1) revealed different mortalities as recorded in Table (2) with similar clinical signs Fig. (2) as hemorrhagic signs, fin and tail rot and superficial ulcer. Also, revealed congestion of internal organs especially liver, spleen and kidneys and distention of gall bladder. Pseudomonas fluorescence was isolated from liver, spleen and kidney of all artificially infected fish. This result agree with **Gharib and salama (2009)**. Fish of control group remained clinically healthy without any pathological lesions.

Water sample investigation showed that PH was 7.4 and salinity was 1‰. All fish survived from accumulating salinity after acute transfer to 20, 10, 5, and 3 ‰. But all fish died while transferred to 0 ‰ **(Cheng *et al*., 2012).**

Biochemical parameters in tissue of diseased and healthy cultured mullet fingerling:

**1-gills:** concerning the effect of naturally infected mullet fingerling with pseudomonas fluorescence on Na and K in gill tissue showed that, there were a highly significant increase in the mean value of gill tissue K in diseased group compared to control group. Meanwhile there were no significant differences in the mean value of gill tissue Na between both groups (table 3). The present study revealed that, the mean value of gill tissue Na in diseased group exhibited no significant change compared to control group. This finding explained by **Yusuf *et al*., (2005**) who stated that, the major cation of the extracellular fluid is sodium and its concentration depends on PH and salinity of the water and in this result PH was 7.4 and salinity 1‰. Potassium is the major cation found intracellularly and its concentration depends on salinity of the water, where up to 10‰ present negatively relationship **(Yusuf *et al*., 2005).** This finding agree with this work where salinity 1‰ and K highly significant in diseased group compared to control

group.

**2-musculatures tissue:** there were a highly significant increase in the mean values of musculatures tissue LDH and CPK in diseased group compared to control group (table 4). Concerning the effect of disease on musculatures tissue LDH and CPK , the present work showed that, presence of a highly significant increased in the mean values of LDH and CPK in diseased group compared to control one and this may be due to musculatures damaged as a result of pseudomonas fluorescence infection. These result was agree with **Wootton (1982)** who reported that, the skeletal musculatures is particularly containing large amounts of the enzyme LDH and CPK and the damage of these tissue is accompanied by arise in the tissue and serum LDH and CPK levels. Also **Burness *et al*., (1999)** stated that, The CPK enzyme is found in high concentration in heart and skeletal musculatures; low concentration is brain tissue.

**3-liver tissue:** there were a highly significant increase in the mean values of liver tissue ALT and AST in diseased group exhibited a highly significant increased compared to control group (table5). These result may be due to liver damaged as a result of pseudomonas fluorescence infection. This finding agree with **Schaperclaus *et al*., (1992)and Huang *et al*., (2006)** who stated that, ALT and AST are enzymes found mainly in the liver, but also found in red blood cells, heart cells, musculatures tissue and other organs, such as the pancreas and kidneys. ALT or AST levels are a valuable aid primarily in the diagnosis of liver disease. After severe damage, in the liver tissue ALT levels rise 10 to 20 times and greater than normal, whereas AST can reach higher levels (up to 50 times greater than normal

4-**kidney tissue:** there were a highly significant increase in the mean values of kidney tissue ammonia and creatinin in diseased group compared to control group (table 6). In fresh water fishes, ammonia is excreted across the branchial epithelium via passive NH3 diffusion (**Michael, 2002**). **Homer and Smith, (2012**) said that, Approximately 6 to 10 times as much nitrogen is excreted by the gills as by the kidneys. The branchial excretion consists largely if not entirely of the readily diffusible substances, ammonia, urea, and amine or amine oxide derivatives. The less diffusible substances (creatine, creatinine, and uric acid) are excreted by the kidneys. Also **Kouri and Honkanen, (2001)** said that, the tissue and plasma creatinin level depends on the creatinin production rate and the rate of elimination in the glomular filtrate. In the body creatinin is formed by a spontaneous and irreversible conversion from creatine and creatine phosphate, which is the source of high-energy phosphate bonds for the immediate reformation of ATP during muscular contraction. Creatinin production is not affected by illness, such as sepsis and trauma, or dehydration, but may increase with increased dietary protein intake. So we can concluded that increased ammonia may be due to gills tissue damaged or due to kidney tissue damaged. Also creatinin may be due kidney damaged or as a result of high dietary protein intake where in this work case history said that, fish before transportation from production farm to growing farm were feed bran only.

Biochemical analysis: the present work revealed that, there was a highly significant increase in K, LDH, CPK, ALT, AST, ammonia and creatinin in diseased group compared to control group. These finding coincide with the work of **Krajnovic-Ozretic and Ozretic, (1987**) who stated that, after functional damage to tissues and organs of fish due to infection or toxins , some specific cellular enzymes increased in tissue and leak into blood plasma where they have been detected.

Finally, we conclude that Pseudomonas fluorescence was the cause of death of the Mullet and recommended that must avoid any environmental stress on fish aquaculture.

Figure (1): showed clinical examination of naturally infected diseased mullet fingerlings

Figure (2): Showed clinical examination of experimentally infected mullet fingerlings.

**Table (1).** Biochemical characters of Pseudomonas fluorescens isolated from infected mullet fingerlings by using API20E.

| Biochemical test | Result | Biochemical test | Result |
|---|---|---|---|
| ONPG | - | GLU | + |
| ADH | + | MAN | - |
| LDH | - | INO | - |
| ODH | - | SOR | - |
| CIT | + | RHA | - |
| H2S | - | SAC | - |
| URE | - | MEL | - |
| TDA | - | AMY | - |
| IND | - | ARA | + |
| VP | - | OX | + |
| GEL | + | Nitrate reduction | - |
| Code no | 2206006 | | |

ONPG Mannitol = MAN hydrolase Arginine=ADH = β-galactosidase =GLU Glucose
Lysine drcarboxylase= LDH Sorbitol =SOR Ornithie drcarboxylase=ODH INO = Inositol
Sucrose=SAC H2S =H2S production Melobinose=MEL Citraste=CIT RHA = Rhaminose
Amyldain =AMY Urease= URE Arabinose= ARA Tryptophane deaminase =TDA
Cytochrome oxidase =OX Indole= IND Vogus prescour =VP Gelatinase= GEL

**Table (2),** Mortality rate of mullet fingerlings experimentally infected by *P. fluroscencs*.

| Group No. | No. of fish | Type of infection | Morbidity rate | Beginning of fish death | Mortality rate |
|---|---|---|---|---|---|
| **1** | **20** | **IP injection With bacteria*** | **14/20 70%** | **4 days post infection** | **6/20 30%** |
| **2** | **20** | **IP injection With sterile saline** | **------** | **-----** | **------** |

**Table (3).** shows the mean values of Sodium and Potassium mEq/gm in gills tissue of control and diseased fish.

| parameter | Control | Diseased |
|---|---|---|
| Na | 142.2 ± 1.4 | 145.8 ±1.3 |
| K | 4.35 ± 0.2 | K5.17 ± 0.21 * |

**Table (4).** Shows the mean values Levels of LDH(u/gm) and CPK(u/gm) enzymes in musculatures tissues of control and diseased fish

| parameter | Control | Diseased |
|---|---|---|
| LDH | 288.6 ±3 | 594.2 ± 3.9 * |
| CPK | 19569.6 ±1379.9 | 76026.1 ± 4376.7 * |

**Table (5).** Shows the mean values of ALT (u/gm) and AST(u/gm) enzymes in liver tissues of control and diseased fish.

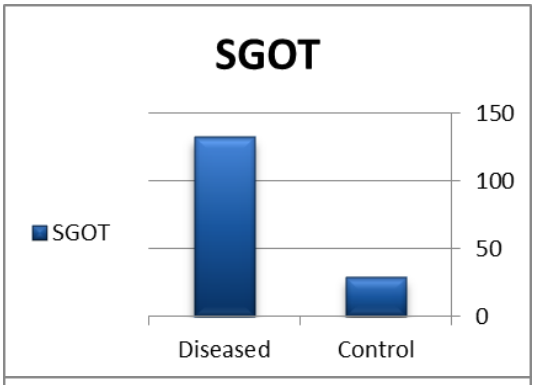
| parameter | Control | Diseased |
|---|---|---|
| ALT | 28.81 ± 0.4 | 132.52 ± 4.5 * |
| AST | 184.3 ± 3.2 | 475.7 ± 13.3 * |

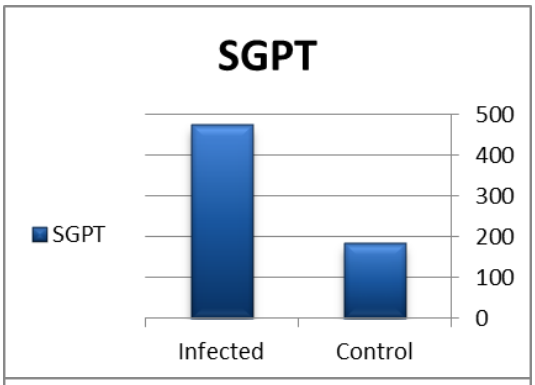
* Significant deference at ($P<0.05$).

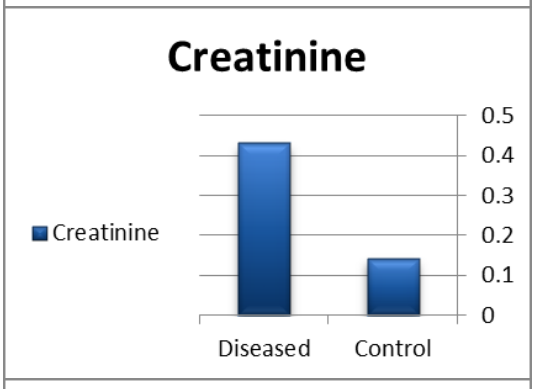
**Table (6).** Shows the mean values Levels of Creatinin (u/gm) and Ammonia (u/gm) in kidney tissues of control and diseased fish

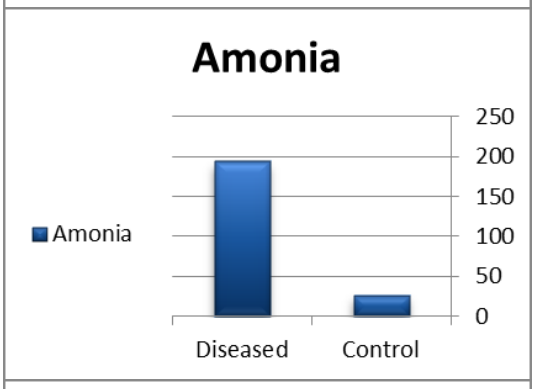
| parameter | Control | Diseased |
|---|---|---|
| creatinin | 0.1433 ± 0.12 | 0.4322 ± 0.014 * |
| ammonia | 26.37 ±1.13 | 194 ± 1.9 * |

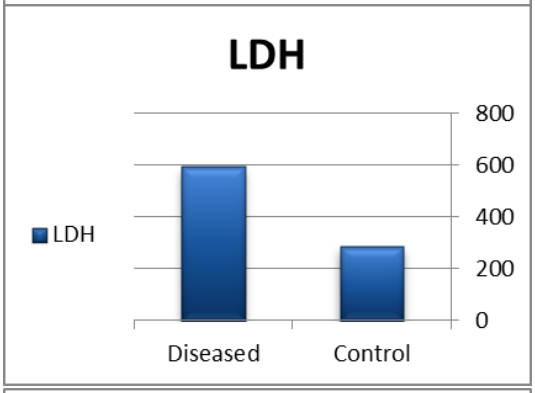
* Significant deference at (P<0.05).

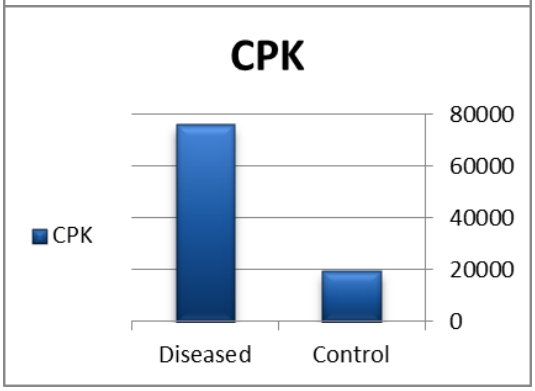


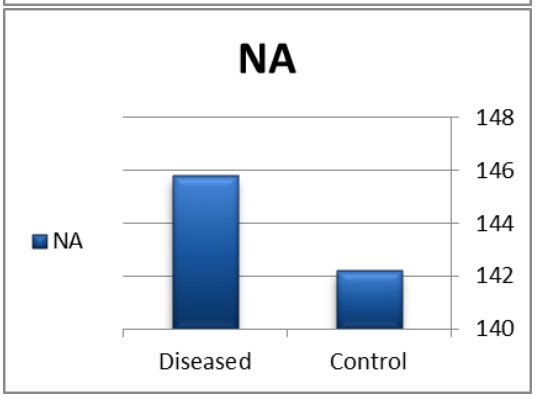



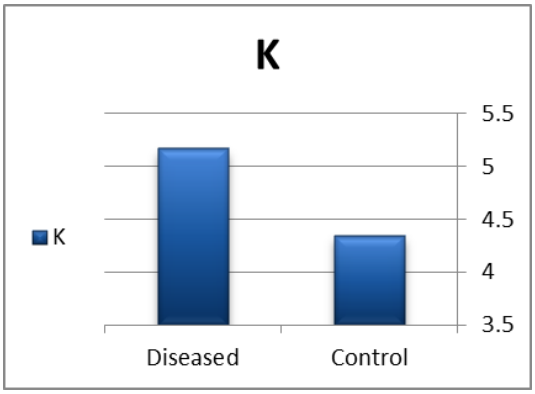



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## دراسات على نفوق اصبعيات البورى المستزرع فى محافظة كفر الشيخ

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**الملخص العربى**

تم تصميم هذه الدراسة لدراسة اسباب نفوق بين اصبعيات البورى المستزرع فى محافظة كفر الشيخ. اصبعيات البورى المستزرع تعانى من استسقاء وبقع نزفية على الجلد وتساقط قشورمع تقرحات جلدية تتاكل فى الذيل والزعانف. تحليل المياة اظهرت الاس الهيدروجينى 7.4 والملوحة 1 بالالف.الفحصى الطفيلى والبكتيرى اظهر اصابة شديدة بالتريكوديناوعزل ميكروب السيدوموناس فلورسنس.والفحص الكيميائى اظهر زيادة معنوية عالية فى Kفى الخياشيم و و LDH, CPKفى العضلات وانزيمات الكبد /AST ALT والامونيا والكريتنين بين اسماك البورى المستزرع. اوضح اختبار الحساسية لميكروب السيدوموناس فلورسنس انه حساس لدانوفلوكساسين وسبروسين والاوكسى تتراسيكلين. وتم دراسة العدوى التجريبية لميكروب السيدوموناس فلورسنس.