**Yucca plant as treatment for *Pseudomonas aeruginosa* infection in Nile tilapia farms with emphasis on its effect on growth performance**

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**Abstract**

*Pseudomonas aeruginosa* is a G-ve bacterium causing diseases threat the animals, poultry and fish resources. Recently medical plants are greatly used as replacements for antibiotics in diseases treatment of both avian and animals The present study was carried out to evaluate the effect of dietary supplementation of yucca extract on growth performance and diseases resistance of Nile tilapia challenged with *pseudomonas aeruginosa* which previously isolated. Total number of two**-**hundred and twenty O.*niloticus* fish were used in our work (70 diseased fish for isolation of *Pseudomonas aeruginosa* and 150 apparently healthy fish for experimental design). One**-**hundred and fifty fish in the experiment were divided into five groups, 30 fish each and fed on five experimental diets (first group received basal diet(control –ve), second group received basal diet and infected with *pseudomonas aeruginosa* in a dose of 3x107cfu/ml at the end of the experiment(control +ve). Third group received basal diet with 0.1% yucca extract and infected with *pseudomonas aeruginosa*, fourth group received basal diet with 0.14% yucca extract and infected with *pseudomonas aeruginosa*, fifth group received basal diet with 0.2% yucca extract and infected with *pseudomonas aeruginosa*). Results of this study showed a higher growth performance for 0.1% yucca group with significant differences with control, 0.14% and 0.2% groups. A total of 10 *pseudomonas aeruginosa* strains were isolated from 70 diseased fish samples with a percentage of 14.28%. The isolated strains from examined fish were identified by cultural, morphological, biochemical characters and PCR technique. The results of PCR examination revealed that all 10 isolated strains were positive for the specific gene of *pseudomonas aeruginosa* (*16S rDNA*) . Also the PCR results revealed that, *opr* Land *exo* S virulence genes were detected in all ten studied strains . Our results after experimental challenge showed that the re-isolation and mortality rate decreased in groups fed on yucca extract than the control group and the best survival was observed in group fed on 0.1% yucca extract. These results concluded that the optimum dietary Yucca extract inclusion level in the diet of Nile tilapia could be 0.1% as a feed additive to promote growth and increase disease resistance.

**Key words:** Yucca extract, growth performance, *Pseudomonas aeruginosa*, virulence genes, *Oreochromis niloticus*.

**Introduction**

In Egypt, fish production comprises 20% of the white animal protein production, 17% of which is derived from aquaculture **(Khalil *et al.,* 2010)** and the common cultured fish is *Tilapia nilotica* (*Oreochromis niloticus*) which have attained a great economic importance. Fish is an important source of cheap, high nutritive animal proteins. Fishing is an important economic activity in many countries. In this way, the Egyptian Government paid special interest to fish meat within its strategy of the food security **(Elsayed *et al.*, 2018).**

Bacterial pathogens are a great threat to fish production worldwide due to the high economic importance of diseases they cause **(Bondad- Reantaso *et al*. 2005)**.

The use of veterinary drugs is becoming more restricted since they present numerous side-effects for the environment and health safety. For example, massive use of antibiotics have resulted in the development of resistant bacteria strains **(Seyfried *et al*., 2010)** or the presence of residual antibiotics in the muscle of commercialized fish and thus has potential consequences on human health **(Romero Ormazábal *et al*., 2012)**. So some of the proposed solutions are the use of natural products (plant extracts) or probiotics (beneficial microbial strains) in the culture of fish and shrimp **(Citarasu, 2010; Lee *et al*., 2009 and Mohapatra *et al*., 2013).**

Pseudomonades are considered one of the most important fish pathogens which are responsible for ulcer type diseases including ulcerative syndrome, **(El-Nagar, 2010).** The disease is characterized by petechial hemorrhage, darkness of the skin, detached scales, abdominal ascites and exophthalmia Moreover, Pseudomonas can cause a problem for human consumers too, generally caused by only one species (most frequently *Ps. aeruginosa*), cause healthcare associated illnesses **(Bagshaw *et al*., 2006 and Zilberberg and Shorr, 2009)**. *Pseudomonas aeruginosa* is a gram-negative rod shape bacterium belonging to the family Pseudomonadaceae. This species is highly adaptable opportunistic pathogen, capable of surviving in a variety of environment, including aquaculture environment **(Abdullahi *et al*., 2013)**.

Several plant extracts are reported to stimulate appetite and promote weight gain when they are administered to cultured fish **(Harikrishnan *et al*., 2012a and Pavaraj *et al*., 2011)** and act as immunostimulants with antibacterial and antiparasitic properties in fish and shellfish **(Yeh *et al*, 2006 ; Zhang *et al*, 2009** and **Wang *et al*, 2015).** This attributed to their active molecules such as alkaloids, terpenoids, saponins and flavonoids **(Reverter *et al*. 2014).** *Yucca schidigera* extract (YE) contains two active ingredients: the steroidal saponin fraction, which has surface active properties, and the glycocomponent fraction, which binds to ammonia. Saponins also improve animal immunity and performance (**Ayasan *et al., 2005***). The ammonia may be used for microbial protein synthesis or may enter the blood stream.**(Yeo and Kim, 1997**).

The yucca also has been suggested suitable for water quality management in aquaculture systems **(Santacruzreyes and Chien, 2012).** The effect of feeding diets containing yucca extract or probiotic on growth performance, nitrogen utilization, digestibility, blood parameters, ceacal microbial activity were studied and the results showed that the yucca extract reduced blood and caecal urea and ammonia concentrations by using these additives. **(Amber *et al.;* 2004).**

The aim of the present study was to investigate the prevalence of *pseudomonas aeruginosa* in some Nile tilapia at Kafre EL-Sheikh governorate, and also evaluate the effect of dietary supplementation of yucca extract on growth performance and diseases resistance of Nile tilapia challenged with *pseudomonas aeruginosa*.

**Material and methods**

**A-Fish samples:**

1. **Diseased fish:** for isolationof *pseudomonas aeruginosa*

A total of 70 diseased *Oreochromis niloticus* fish were collected from different fish farms in Kafr El-Sheikh governorate. Fish were examined clinically for any abnormal lesions according to **(Noga, 1996)**. The fish samples were kept in tanks partially filled with the same water of the pond then transported immediately to the Lab.

**Clinical and post-mortem examination of naturally infected *Oreochromis niloticus*:**

Naturally infected fishes showed hemorrhages all over the fish body especially at the base of fins, tail and fins rot; detachment of scales, darkness in skin , skin ulceration, exophthalmia and abdominal distention. Post mortem, these fishes showed dark gall bladder , congested gills; enlarged and congested liver and spleen.

1. **Experimental Fish:**

A total of 150 Apparently healthy Nile tilapia with an average body weight of 20 ± 5 g were obtained from a private fish farm in Kafre EL-Sheikh Governorate and transported a live to Animal Health Research Institute laboratory at Kafr EL-sheikh and they kept in well prepared aquaria measuring (90x45x45 cm). These glass aquaria supplied with chlorine free tap water according to **(Innes, 1966)** and continuously aerated by electric pump and held at 27±20C and half of the water was daily changed. Fish were acclimated for 2 weeks during the acclimation period fish fed on the basal diet only. Random samples were used to check if they are Pseudomonal septicemia free.

**B- Isolation and identification of *Pseudomonas aeruginosa* from fish samples:**

Specimens from gills, liver, kidneys, brain and spleen of examined fish were taken under aseptic conditions and inoculated in trypticase soya broth then incubated at 370C for 24 hours aerobically. A loopful of the broth was streaked onto nutrient agar and Pseudomonas agar medium with supplement (cetrimide) then incubated at 37ºC for 24 - 48 hr aerobically. The growing colonies were purified in pure form and identification of all isolates was done by cultural, morphological and biochemical characters according to **(Austin and Austin, 2007).**

**C- Molecular identification of *Pseudomonas aeruginosa* and detection of some virulence genes:** It was performed in Animal Health Research Institute, in Dokki**.**

**DNA extraction.** DNA extraction from 10 isolates biochemically identified as *pseudomonas aeruginosa* was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer’s recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56OC for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer’s recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

**Oligonucleotide Primer.** Primers used were supplied from **Metabion (Germany)** are listed in Table (1).

**PCR amplification. For PCR,** primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix **(Takara, Japan)**, 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

**Analysis of the PCR Products.**

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the PCR products were loaded in each gel slot. Gelpilot 100 bp DNA ladder (qiagen, gmbh, Germany) and Generuler 100 bp ladder (Fermentas, Thermo Scientific, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

**Table (1):** Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Target gene** | **Primers sequences** | | **Amplified segment (bp)** | **Primary**  **denaturation** | **Amplification (35 cycles)** | | | **Final extension** | **Reference** |
| **Secondary denaturation** | **Annealing** | **Extension** |
| *opr*L | F  R | F ATG GAA ATG CTG AAA TTC GGC | 504 | 94˚C  5 min. | 94˚C  30 sec. | 55˚C  40 sec. | 72˚C  45 sec. | 72˚C  10 min. | **Xu *et al*., 2004** |
| CTT CTT CAG CTC GAC GCG ACG |
| *exo*S | F  R | GCGAGGTCAGCAGAGTATCG | 118 | 94˚C  5 min. | 94˚C  30 sec. | 55˚C  30 sec. | 72˚C  30 sec. | 72˚C  7 min. | **Winstanley *et al*., 2005** |
| TTCGGCGTCACTGTGGATGC |
| *P. aeruginosa 16S rDNA* | F  R | GGGGGATCTTCGGACCTCA | 956 | 94˚C  5 min. | 94˚C  30 sec. | 52˚C  40 sec. | 72˚C  50 sec. | 72˚C  10 min. | **Spilker *et******al*., 2004** |
| TCCTTAGAGTGCCCACCCG |

**D- Experimental design and feeding diets:**

150 fish were divided to five groups (30 fishes per group) were fed the prepared pelleted experimental diet for two months according to the experimental design Table (2). Fish diet were formulated according to **(NRC, 2011)** Table (3) and analyzed chemically for different nutrients acc. to **(AOAC, 1995)** Table (4)**.** Fish were fed to apparent visual satiation by hand twice a day at 9:00 and 14:00 according to **Blanquet and Aires (2010).** Extreme care was taken to assure that all supplied feed was consumed. All fish in each group were weighted at the beginning (Wi) and biweekly for a continuos 8 weeks. Feed intake was readjusted according to the average body weight each period. Weight gain (WG), gain percent (G%), relative growth rate (RGR), Feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER) and Metabolizable Energy were calculated.

Calculation:

Weight gain=Final body weight- Initial body weight

Gain %= (Total gain/Initial weight) x100

Relative growth rate= (Wf-Wi)/ 0.5x (Wf+Wi) x100 **(Brody,1968).**

Feed conversion ratio (FCR) was calculated by dividing total feed intake per aquarium by the total body weight gain per the same aquarium **(Lambert *et al*., 1936).**

Protein intake= Feed intake ×Protein% of the feed used/ 100

Protein efficiency ratio (PER) = Weight gain/Protein intake was calculated according to **Mcdonald *et al*., 1987**

Feed efficiency (FE) was calculated by dividing the total body weight gain per aquarium by total feed intake per the same aquarium.

Metabolizable Energy was calculated according to **(Shiau and Hang, 1990).**

**Table (2):** Experimental design outline

|  |  |
| --- | --- |
| **Groups** | **Treatments** |
| **1** | Basal diet for 8 weeks and without challenge\*. |
| **2** | Basal diet for 8 weeks and challenged with *pseudomonas aeruginosa* after 8 weeks. |
| **3** | Basal diet and addition of 0.1% yucca extract\*\* for 8 weeks and challenged with *pseudomonas aeruginosa* after 8 weeks. |
| **4** | Basal diet and addition of 0.14% yucca extract for 8 weeks and challenged with *pseudomonas aeruginosa* after 8 weeks. |
| **5** | Basal diet and addition of 0.2% yucca extract for 8 weeks and challenged with *pseudomonas aeruginosa* after 8 weeks. |

\*.The challenge was performed intraperitoneal with 0.2 ml of PBS containing 3x107cfu/ml *pseudomonas aeruginosa* at the end of the experiment after 8 weeks according to **(Younes *et al*., 2015)**  and this dose adjusted by using McFarland standard tube according to **(Ezzat *et al*., 2018)**

\*\*Yucca extract (Yucca 100-AP **®**) contain Yucca schidigro extract, polyhydroxyl stilbene, saponin, Batch No.031800892.

**Table (3):** Ingredients proximate analysis of the experimental different dietary treatments

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ingredients** | **Basal diet** | | **Group 3** | **Group 4** | **Group 5** |
| **1** | **2** |
| **Yucca**  **Yellow corn(6.83%)**  **Soybean(42.88%)**  **Fish meal (59%)**  **Corn gluten (58.92%)**  **Bran (14.44%)**  **Oil**  **Dical ph**  **Salt**  **Vit and mineral mixture**  **Dl meth** | -----------  34.336  37.40  2.934  17.17  2.00  4.0  1.5  0.200  0.300  0.160 | | 0.100  34.236  37.40  2.934  17.17  2.00  4.0  1.5  0.200  0.300  0.160 | 0.140  34.196  37.40  2.934  17.17  2.00  4.0  1.5  0.200  0.300  0.160 | 0.200  34.136  37.40  2.934  17.17  2.00  4.0  1,5  0.200  0.300  0.160 |

**Table (4):** Chemical analyses of the experimental diet used in the present study:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Items** | **% Composition** | | | |
| **Group 1 and 2** | **Group 3** | **Group 4** | **Group 51** |
| **Moisture%**  **DM %**  **CP%**  **EE%**  **Ash%**  **CHO%**  **ME Kcal/Kg**  **P/E ratio**  **Ca%**  **Ph%**  **Methionine%**  **Lysine%** | 9.27  90.73  30.59  3.25  5.89  51.00  3424.54  8.9  0.68  0.82  0.7  1.49 | 10.41  89.59  31.2  4.85  6.08  47.59  3469.26  8.99  0.68  0.82  0.7  1.49 | 9.80  90.20  31.09  3.87  5.94  49.3  3440.53  9.04  0.68  0.82  0.7  1.49 | 9.33  90.67  30.72  3.45  6.12  50.38  3425.8  8.97  0.68  0.82  0.7  1.49 |

**Sample collection and analysis of kidney and liver functions:**

At the end of the feeding trial before the challenge, all the fish in each aquarium were counted and weighed to calculate weight gain (WG), gain% (G%), Feed conversion ratio (FCR) , feed efficiency (FE), protein efficiency ratio (PER), and relative growth rate (RGR). For the blood collection, five fish per aquarium were randomly selected and euthanized with ethylene glycol phenyl ether (200 mg/L). Blood samples were obtained from the caudal vein of the fish with 1 ml syringes. Serum samples were obtained from the blood by clotting in room temperature then centrifugation at 3000 rpm for10 min and stored at – 70o C for biochemical determination(Urea, Creatinine, AST and ALT).

**Preparation of the bacterial strain for experimental challenge:**

The selected virulent bacterial isolate was sub-cultured in tryptic soya broth at 370C for 24 hrs. Bacterial pellets were then taken after centrifugation of the broth solution. Bacterial pellets were then suspended in sterile physiological buffer saline (PBS) solution according to **(Hardi *et al*., 2014b)** and adjusted to (3x107cfu/ml) by using McFarland standard tube according to **(Ezzat *et al*., 2018)**

**Experimental challenge:**

After the end of the experiment (after 8 weeks), fish in all groups were challenged intraperitoneal (IP) with one of the virulent *pseudomonas aeruginosa* strain that previously isolated from *Oreochromis niloticus*. Each fish injected with 0.2 ml containing 3x107cfu/ml according to **(Ezzat *et al*., 2018).** Fish were observed for seven days after challenge in which clinical signs and mortality were noticed. Dead and survivor fish were subjected to clinical and bacteriological examination.

**Bacteriological investigation:**

For bacterial re-isolation samples from gills, liver, kidneys, brain and spleen of fishes were collected from 10 fishes (dead and survivor) in each group in the 5th day after challenge under aseptic conditions and inoculated in trypticase soya broth and incubated at 370C for 24 hours.Then a loopful of the broth was streaked onto nutrient agar and Pseudomonas agar medium with supplement (cetrimide) then incubated at 37ºC for 24 - 48 hr .

**Statistical analysis:**

Statistical analysis was made using Analysis of Variance (ANOVA) one-way analysis of variance for study the effect of different treatment groups on the different studied variables studied that includes (growth performance parameters, hematological and biochemical) variables using **(SAS, 2004).**

**Results and Discussion**

Increased research on the use of plant-derived products for replacement of chemotherapies in fish farms noted in the recent past **(Bulfon *et al*., 2015).** Extracts of plants or their by-products contain some exclusive compounds that can be effective as chemotherapists and vaccines. They promote the growth performance, improve survival, enhance the immune function, modulate the innate (non-specific) immune responses and modify physiological function of animals **(Jeney *et al*., 2015).**

Our results showed that fishes infected fishes with *Pseudomonas aeroginosa* showed hemorrhages all over the fish body especially at the base of fins, tail and fins rot; detachment of scales, darkness in skin , skin ulceration, exophthalmia and abdominal distention. Post mortem, these fishes showed abdominal dropsy with reddish ascetic exudates; enlarged and congested liver and spleen. Nearly similar findings were recorded by **Abd El-Ghany *et al*., 2009;** **Salama and Gharib, 2009;** **Khalil *et al.* 2010** and **EL-hady and Samy, 2011.**

**Clinical and post-mortem examination of naturally infected *Oreochromis niloticus*:**

|  |  |
| --- | --- |
| *Oreochromis niloticus* showing petechial haemorrhages all over the body | لا يتوفر وصف.  *Oreochromis niloticus* showing Skin ulcer |
| E:\صور تجربة السمك فى بحث الايروموناس\IMG-20190716-WA0012.jpg  *Oreochromis niloticus* showing exophthalmia and tail rot | لا يتوفر وصف.  *Oreochromis niloticus* showing dark gall bladder enlarged and congested liver and congested gills |

**Prevalence of *Pseudomonas aeruginosa* isolated from diseased *Oreochromis niloticus*:**

In this study, *Pseudomonas aeruginosa* was isolated from diseased *Oreochromis niloticus* in which10 positive samples out of 70 tested ones with a percent of 14.28. Our results were nearly similar to that obtained by **El-Kader and Mousa-Balabe (2017)** who isolated*P. aeruginosa* from *Oreochromis niloticus* witha percentage of 15.6%. And higher than that obtained by **Younes *et al.* (2015)** who isolated*P. aeruginosa* from naturally infected *Oreochromis niloticus* with a percentage of 10% . And lower than that obtained by **EL-DEEN (2014)** and **Atwa (2017)** and who isolated *P. aeruginosa* from *Oreochromis niloticus* with a percentage of 29% and 21.3% % respectively.

**The cultural and biochemical characterizations of the isolated *Pseudomonas aeruginosa*:**

Also our study revealed that all the isolates of *Pseudomonas aeruginosa* produced blue green colonies on nutrient agar due to production of pyoverdin and pyocynin pigments. Also they produced green colonies on Pseudomonas agar supplemented with Cetrimide. These results were in agreement with **Rasheed *et al*. (2016); Aya *et al.* (2018)** and **Noha *et al*. (2018).**

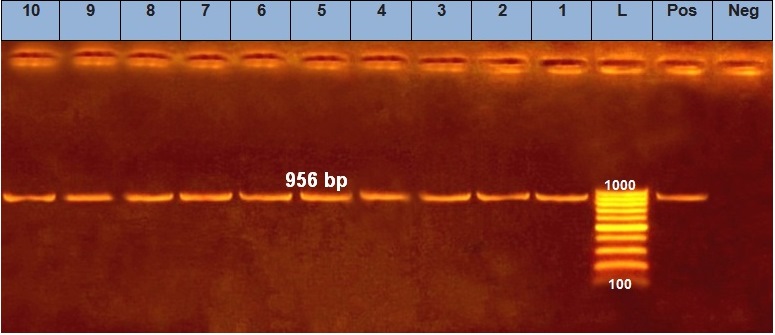
Also as shown in table (5), the biochemical tests indicated that *Pseudomonas aeruginosa* positive to citrate utilization test, positive to catalase test, oxidase and citrate ters, positive to motility test while negative to indole test, methyl red, vogaus proskauer, indole production, urease and H2S production on triple sugar iron (TSI). Also, these results were in agreement with those of **Hossain *et al.* (2006)**; **Musa *et al*. (2009); Aya *et al.* (2018)** and **Noha *et al*. (2018).**

**Table (5):** Biochemical characters of *Pseudomonas aeruginosa* isolated from cultured fishes.

|  |  |
| --- | --- |
| **Item** | ***Pseudomonas aeruginosa*** |
| **Gram stain** | **-ve** |
| **Pigment** | **+** |
| **Motility** | **+** |
| **Oxidase** | **+** |
| **Catalase** | **+** |
| **H2S production** | **-** |
| **Urease** | **-** |
| **Citrate** | **+** |
| **TSI** | **k/k** |
| **Indol production** | **-** |
| **Voges Proskour** | **-** |
| **Methyl red** | **-** |

PCR used in diagnosis of bacterial fish diseases, isolated from cultured fish,it is a very rapid and accurate method **(Oliveira *et al*., 2012)**. In the present study as shown in Fig (1),(2) and (3) polymerase chain reaction (PCR) was used for detection of 16s rDNA gene of *Pseudomonas aeruginosa* giving bands at 956bp. and used for detection of two virulence genes which were *opr*L(Lipoprotein L) and *exo*S giving bands at 504 bp and 118 bp respectively in ten isolates of *Pseudomonas aeruginosa*. These results were agreement with **Abd El Tawab *et al*. (2016)** who detected PCR could be used for detection of *Pseudomonas aeruginosa* virulence genes as *opr* L*, tox*A*, phz* Mand *exo* Sgiving product at 504 bp., 396 bp, 875 bp and 118 bp respectively and **Eman *et al.* (2016)** who reported polymerase chain reaction (PCR) can be used for detection of virulence genes of *Pseudomonas aeruginosa* giving positive results for outer membrane lipoprotein gene (*opr*L) at 504 bp.

**Detection and identification of *Pseudomonas aeruginosa* by using polymerase chain reaction (PCR):**

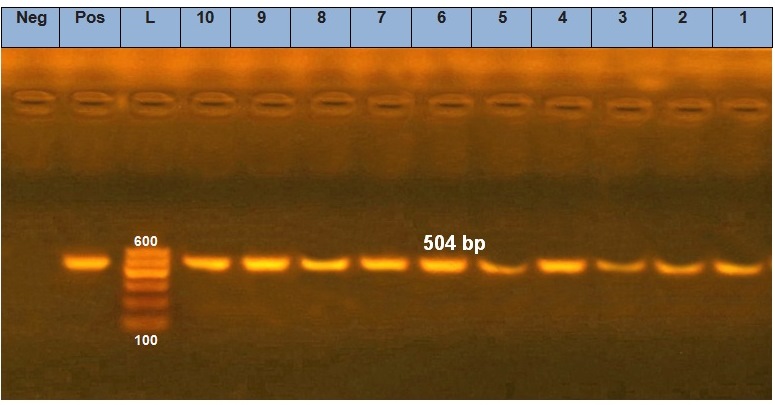


**Fig (1): Agarose gel electrophoresis of PCR amplification products of *16S rDNA* gene for identification of *Pseudomonas aeroginosa.***

**Lane L:** Molecular size marker (100-1000 bp).

**Lane Pos. and Neg.:** Positive and negative controls.

**Lane1-10:** Positive for *Pseudomonas aeroginosa* strains at amplicon of 956 bp.

**Detection of *oprL* genes of *Pseudomonas aeruginosa* by using polymerase chain reaction (PCR):** 

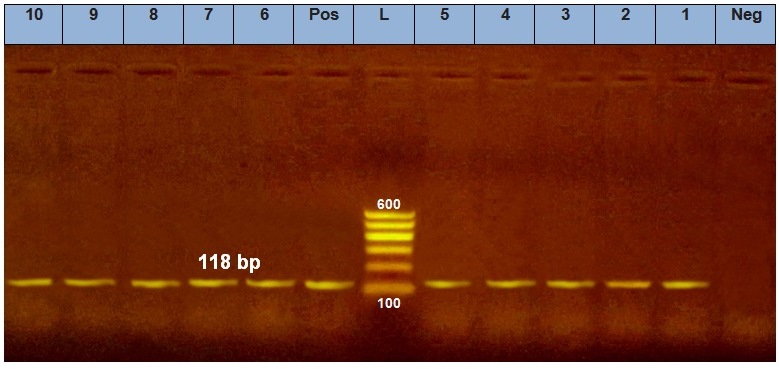
**Fig (2): Agarose gel electrophoresis of PCR amplification products of *opr*L gene for characterization of *Pseudomonas aeroginosa.***

**Lane L:** Molecular size marker (100-600 bp).

**Lane Pos. and Neg.:** Positive and negative controls.

**Lane1-10:** Positive *Pseudomonas aeruginosa* strains for *opr*L gene at504bp.

**Detection of *exoS* genes of *Pseudomonas aeruginosa* by using polymerase chain reaction (PCR):**



**Fig (3): Agarose gel electrophoresis of PCR amplification products of *exo*S gene for characterization of *Pseudomonas aeruginosa.***

**Lane L:** Molecular size marker (100-600 bp).

**Lane Pos. and Neg.:** Positive and negative controls.

**Lane1-10:** Positive *Pseudomonas aeruginosa* strains for *exo*S gene at 118 bp.

**Growth performance**

Results of this study showed a higher growth performance for group fed 0.1% yucca schidigera with significant differences with control, yucca 0.14% and yucca 0.2% groups as shown in table(6) . The yucca schidigera 0.1% group significantly increased final body weight by about 13.56% more than control groups (groups 1 and 2) moreover, increased significantly the total gain, gain percent and relative growth rate by about 39.66%, 38.92% and 29.36% respectively. Also supplementation of the Yucca schidigera extract in the Nile tilapia diet by about 0.1% improved FCR, FE and PER by about 16.95%, 21.65% and 18.5% respectively throughout whole experimental period while increasing Yucca schidigera extract in tilapia fish diet by level 0.14 and 0.2% had no significant effect on the previous mentioned parameters when compared with control and 0.1% yucca groups.

**Table (6):** Effect of different treatments on growth performance of *Oreochromis niloticus* (0-8weeks):

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **G1**  **(Mean+SE)** | | **G2**  **(Mean+SE)** | | **G3**  **(Mean+SE)** | | **G4**  **(Mean+SE)** | **G5**  **(Mean+SE)** |
| **Intial weight(g/fish)** | 24.07+0.26 | | 24.07+0.26 | | 24.29+0.22 | | 24.31+0.08 | 24.29+0.30 |
| **Final weight(g/fish)** | 35.49+0.26b | | 35.92+0.13b | | 40.54+0.43a | | 36.22+0.35b | 35.93+0.48b |
| **Total weight gain(g/fish)** | 11.42+0.07b | | 11.85+0.15b | | 16.25+0.22a | | 11.691+0.28b | 11.64+0.24b |
| **Gain%** | 47.45+0.62b | | 49.25+1.16b | | 67.17+0.45a | | 48.98+1.03b | 47.92+0.80b |
| **TFI**\*\*\*\* | 23.4+0.00 | | 24.09+0.00 | | 27.52+0.00 | | 22.69+0.00 | 22.14+0.00 |
| **FCR**\* | 2.04+0.012b | | 2.03+0.026b | | 1.69+0.025a | | 1.90+0.043b | 1.90+0.041b |
| **Feed Efficiency(FE)** | 0.48+0.003b | | 0.49+0.005b | | 0.59+0.010a | | 0.52+0.013b | 0.52+0.012b |
| **PER**\*\* | 1.59+0.008b | | 1.60+0.018b | | 1.89+0.025a | | 1.69+0.040b | 1.71+0.036b |
| **RGR**\*\*\* | 37.98+0.32b | | 39.51+0.75b | | 50.12+0.21a | | 39.34+0.66b | 38.65+0.52b |
| **Survival rate%** | | 93.33 | | 96.66 | 100 | 100 | | 100 |

Means within the same column of different letters are significantly different at (P≤0.05)

\*FCR (Feed Conversion Ratio). \*\*PER (Protein Efficiency Ratio) \*\*\*RGR (Relative Growth Rate)

\*\*\*\*TFI (Total feed intake).

The results of current study indicated an improved growth performance (final weight, total gain, gain% and relative growth rate) for Nile Tilapia when fed diet supplemented with 0.1% Yucca schidigera extract. **Njagi** ***et al.,* 2017** stated that in this level of 0.1 % yucca, diet contains 23.9mg Kg saponin. In addition, there was an increase in the feed intake and feed utilization (Feed efficiency, protein efficiency ratio and feed conversion ratio).

Growth enhancement of several fish species by yucca schidigera extract additives has been reported previously **(Kelly** **and Kohler, 2003,** **Gaber, 2006**, **Guroy *et al*., 2016)**. In addition, **Njagi** ***et al*., (2017)** carried out their experiment on juvenile Nile tilapia, *Oreochromis niloticus* and the best results (higher growth performance) in their study were obtained at yucca meal inclusion levels of 0.1% in diet (YMS0.1) with signiﬁcant differences with YMS0.5, YMS1.0 and YMS2.0 groups.

However, this is not in agreement with results presented by **Amoah *et al*., 2017** when they added Yucca in the diet in level of 0.05%. Their results revealed more positive response (WG, SGR, FE, PER, lysozyme, SOD and resistance against bacteria) of juvenile Amur catﬁsh to diets containing Song-gang stone (SG) as well as combination of SG and b-glucan (SG + BG) rather than diets containing either yucca meal (YM), or b-glucan (BG) separately.

On the other hand, our results also demonstrated that increasing dietary levels of yucca up to (0.14 and 0.2%) did not have significant effect on the same, parameters when compared with control and 0.1% yucca groups due to increase of intensification of saponin in the diet.

**Chen *et al*. (2011)** hypothetised that increasing growth in fish fed the diets with lower saponin rates could be due to intensification in the nutrient absorption from the intestine, induced by a slight permeabilization of the intestinal walls with saponins. This would explain why low doses (<1000ppm) of saponin-rich plant extracts can exert a beneficial impact on fish growth, whilst too high doses participate in the development of enteritis.

Supplementation of basal diet with a yucca schidigera extract 0.1% significantly increased feed intake, relative growth rate (RGR), protein efficiency ratio PER) and improved feed conversion ratio (FCR) compared with *O. niloticus* fed the basal diet. This means a decrease in the amount of feed necessary for animal growth which could result in a reduction in the production cost, **Table (6).**

**Engler *et al.,* (2018)** found that supplementation with low doses of the yucca as feed additive (100 or 500ppm) induced beneficial effects on total ammonia-nitrogen excretion (TAN) management.

**Effect of dietary supplementation of yucca extract on kidney and liver functions:** As shown in Table (6). There were no signiﬁcantly effect of Yucca schidigera on urea levels for YMS0.1, YMS0.14 and YMS0.2% than YMS0 groups (P < 0.05). Also, there were no signiﬁcant differences in creatinine levels among YMS0.1, YMS0.14, YMS0.2 and YMS 0 groups (P > 0.05).However, there were no signiﬁcant differences in AST levels for the basal diet (YMS0) , YMS 0.1%, YMS0.1, YMS0.14 and YMS0.2% than YMS0 groups There were not any signiﬁcant effect of Yucca on ALT among control and other Yucca schidigera groups.

**Table (7):** Effect of different treatments on kidney and liver functions of *Oreochromis niloticus* during experimental period:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **G1**  **(Mean+SE)** | **G2**  **(Mean+SE)** | **G3**  **(Mean+SE)** | **G4**  **(Mean+SE)** | **G5**  **(Mean+SE)** |
| **Urea(mg/dl)** | 24.85+0.31a,b | 24.15+0.17b | 25.20+0.11a | 25.20+0.10a | 25.30+0.15a |
| **Creatinine(mg/dl)** | 0.34+0.005a | 0.33+0.006a | 0.32+0.006a | 0.33+0.006a | 0.34+0.006a |
| **AST(u/ml)** | 33.90+0.32b | 34.5+0.26a,b | 35.12+0.20a,b | 35.40+0.26a | 35.50+0.25a |
| **ALT(u/ml)** | 34.50+0.28a,b | 33.89+0.20b | 34.98+0.26a,b | 35.55+0.29a | 35.50+0.25a |

Means within the same column of different letters are significantly different at (P≤0.05)

Addition of YMS0.1% has not any adverse effect on kidney and liver health. Also groups which received yucca diets YMS0.14 and YMS0.2% there was no significant increase in levels of AST so it is advisable that inclusion be carried out with good adherence to dosage to avoid inducing clinically negative effects (**Njagi** ***et al*., 2017;** **Lee *et al*., 2015a)**.

**Fayed *et al.* (2019)** added yucca extract in water and studied its effect on AST and ALT activities and they found that a considerable decrease in liver enzymes in groups treated with yucca extract in water compared with the standard group. Which could attributed to the eﬀectiveness of yucca extract in the improvement of liver health of European seabass (Dicentrarchus labrax) juveniles.This result may be due to that Yucca effect on water differ from its effect in diet.

The results of the serum parameters which studied by **Amoah *et al*. (2017)** suggested that the dietary additives of yucca had no adverse effects on health status of ﬁsh. These results are consistent with those of **(De Oliveira *et al*., 2001**; **Haridas *et al*.,** **2001)**, who reported that the active component of yucca extract reduced the stress conditions for the ﬁsh, triggering serum enzyme activity for improvement of liver health.

Most of the herbal substances are poorly absorbed, and they produce some effects in animals such as diarrhea. If used in higher doses, they are able to harm intestine and even destruction of red blood cells resulting from haemolysis could occur **(Sollmann 1957; George 1965).** Thus, high levels of yucca meal can be detrimental to fish health.

**The re-isolation and survival rate after experimental challenge:**

It was reported that some mode of action of medicinal plants in fish diseases includes stimulation of the cellular and humoral immune response through elevation in immune parameters **(Awad and Awaad, 2017).**

In our study as shown in Table(8), the re-isolation rate of *pseudomonas aeruginosa* from 10 survival fishes after challenge decreased in groups fed on yucca extract than the control group and this agreed with **Chakraborty and Hancz (2011)** who reported that herbal preparations play an important role in disease control due to their active ingredients as phenolics, alkaloids, steroids, flavonoids, and essential oils which act as antimicrobial, antioxidant, growth promotion, anti-stress, tonic, appetite stimulation, and immune stimulation. Also in our study, after challenge with *Pseudomonas aeruginosa* all treated groups showed a reduced mortality and morbidity rate compared to the control group and the best survival was observed in group fed on 0.1% yucca extract. These results were similar to that obtained by **( El Araby and El-Arabey, 2016)** who observed that Nile tilapia fish challenging against *Ps. aeruginosa* were shown no mortalities in all diets containing different levels of *Origanum vulgare* extractand the highest overall fish mortality rates were observed in the control group**.**

**Table (8):** The re-isolation, mortality rate, morbidity rate and survival rate after experimental challenge:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Groups** | **No.of fish before challenge** | **No.of fish after challenge** | **Mortality**  **rate%\*** | **Morbidity**  **rate %\*\*** | **Survival rate% after challenge\*\*\*** | **Re-isolation rate** | |
| **No** | **%\*\*\*\*** |
| **1** | 28 | 28 | 0 | 0 | 100 | 0/10 | 0 |
| **2** | 29 | 11 | 62.06 | 37.93 | 37.93 | 9/10 | 90 |
| **3** | 30 | 25 | 16.66 | 6.66 | 83.33 | 3/10 | 30 |
| **4** | 30 | 23 | 23.33 | 10 | 76.66 | 4/10 | 40 |
| **5** | 30 | 23 | 23.33 | 6.66 | 76.66 | 4/10 | 40 |

\*% calculated according to No. of dead fish after challenge.

**\*\***% calculated according to No. of affected fish after challenge.

**\*\*\***Survival rate in group 2 calculated according to No. of diseased fish after challenge and groups 3,4 and 5 calculated according to No. of healthy fish after challenge.

\*\***\*\***% calculated according to No. of re-isolated *pseudomonas aeruginosa* from 10 survival fishes after challenge.

**Conclusion**

Based on the results obtained, it can concluded that yucca extract in the diet can be used as a cost-effective, safe and biocompatible feed additive for supplementation in Nile tilapia diets to improve growth performance and enhance disease resistance in cultured ﬁsh. The optimum inclusion level of yucca extract on juvenile Nile tilapia diets was found to be 0.1% in the diet in which this concentration give the best growth performance and disease resistance than yucca 0.14% and yucca 0.2%. Further studies on the utilization of yucca meal as a feed additive for commercial ﬁsh species in aquaculture are required.

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**نبات اليوكا كعلاج عدوى السيدوموناس ايروجينوزا فى مزارع البلطى النيلى مع التركيز على تأثيرها على معدلات النمو**

**عبير محمد شحاته الكريدى1, نيهال على عبدالله نعينع2**

وحدة الكيمياء وأمراض النقص الغذائى1- وحدة البكتيريولوجى2

**الملخص العربى**

السيدوموناس ايروجينوزا هى بكتيريا سالبة الجرام تسبب أمراض تهدد الحيوانات والطيور والموارد السمكية.مؤخرا تستخدم النباتات الطبية بشكل كبير كبديل للمضادات الحيوية فى علاج الطيور والحيوانات. أجريت هذه الدراسة لتقييم تأثير المكملات الغذائية لمستخلص اليوكا على أداء النموومقاومة الامراض فى البلطى النيلى المعدى بالسيدوموناس ايروجينوزا التى سبق عزلها. تم استخدام إجمالي عدد مائتي وعشرين من أسماك الزنابق في عملنا (70 من السمك المريض لعزل السيدوموناس ايروجينوزا و 150 سمكة سليمة ظاهريا للتصميم التجريبي). تم تقسيم مائة وخمسين سمكة في التجربة إلى خمس مجموعات ، 30 سمكة لكل منها وتغذيتها على خمس وجبات غذائية تجريبية (المجموعة الأولى تلقت نظامًا غذائيًا أساسيا ، المجموعة الثانية تلقت نظامًا غذائيًا أساسيا وتم عدوتها بالسيدوموناس ايروجينوزا بجرعة 3x 710/cfu مل في نهاية التجربة، المجموعة الثالثة تلقى النظام الغذائي الاساسى مع 0.1 ٪ مستخلص اليوكا وحقنت بالسيدوموناس ايروجينوزا، المجموعة الرابعة تلقت النظام الغذائي الاساسى مع 0.14 ٪ مستخلص اليوكا وحقنت بالسيدوموناس ايروجينوزا ، المجموعة الخامسة تلقت النظام الغذائي الاساسى مع 0.2 ٪ مستخلص اليوكا وحقنت بالسيدوموناس ايروجينوزا). أظهرت نتائج هذه الدراسة أعلى أداء نمو لمجموعة اليوكا 0.1 ٪ مع وجود اختلافات كبيرمع مجموعات 0.14 ٪ و 0.2 ٪ ومجموعات الكنترول. تم عزل 10معزولات سيدوموناس ايروجينوزا من 70 عينة سمك مريض بنسبة 14.28 ٪. تم التعرف على السلالات المعزولة من الأسماك التي تم فحصها من خلال صفات الزرع على الميديا والصفات المورفولوجية والكيميائية و PCR. كشفت نتائج ال PCR أن 10 معزولات كانت إيجابية للجين الخاص بالسيدوموناس ايروجينوزا(*16S rDNA*) . كما كشفت نتائج PCR أنه تم اكتشاف جينات opr L و exo S vululence في كل العشرة معزولات المدروسة. أظهرت نتائجنا بعد العدوى التجريبية أن معدل العزل والنفوق انخفض في المجموعات التى تتغذى على مستخلص اليوكا عن المجموعة الكنترول ولوحظ أن أفضل معدل بقاء في المجموعة التى تتغذى على 0.1 ٪ مستخلص يوكا ولخصت هذه النتائج إلى أن المستوى الأمثل لاستخدام مستخلص اليوكا في نظام غذائي للبلطي النيلي يمكن أن يكون 0.1٪ كمضاف غذائي لتحسين النمو وزيادة مقاومة الأمراض.