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Mass Kills Associated with *Pseudomonas fluorescens* and *Saprolegnia parasitica* Concurrent Infection in Earthen Pond-Farmed Nile tilapia (*Oreochromis niloticus*)

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Abstract

POOR WATER quality is considered the most critical predisposing factor for disease eruptions in aquaculture facilities. Abrupt decrease or fluctuation in water temperature is a detrimental factor in the occurrence of some low temperature dependent microbes. The ubiquitous pathogen *Pseudomonas fluorescens* and the saprotrophic water mold *Saprolegnia parasitica* are ideal examples of this disease pathway. In the current study, the two ubiquitous microbes were isolated from Nile tilapias at an acre-sized earthen pond that was overstocked with more than 25,000 fingerlings during an episode of mass mortalities at mid-February 2023. The peak of mass mortalities occurred after heavy rains and wind storms that hit the country during this time of the year. Most of the dead and moribund fishes were suffering from generalized skin ulcers, fin rot, gill congestion, and cottony wool-like hyphae intimately attached to skin ulcers and fins. Internally, almost all internal organs including the kidney, spleen, and liver were congested. Histopathological examination of affected fish showed hyalinization and necrosis of fin tissues together with inflammatory cell infiltration. Moreover, fungal hyphae were detected within the muscle bundles. The final identity of the retrieved *Pseudomonas fluorescens* was confirmed using 16S rRNA gene sequence analysis, while *Saprolegnia parasitica* isolates were confirmed using ITS rDNA gene sequence analysis. All supportive accession numbers for retrieved isolates were published through the Genbank official website.

Keywords: Winter kills, *Pseudomonas fluorescens*, *Saprolegnia parasitica*, Concurrent infection, Water quality, and Climatic stress.

Introduction

Aquaculture is a globally important industry that provides important animal protein to the swiftly growing world population. Even though the Egyptian aquaculture sector had emerged three decades ago, yet, it is steadily growing moving the country to the top list of global aquaculture producers. Aquaculture in Egypt has rapidly grown from 15.4% in 1982 to 78.7% of total fish production in 2021[1]. Egypt ranks the largest Nile tilapia producer among all African countries with a total production of 1.6 million tons [2].

Proper water-quality management is crucial for ensuring optimal fish health with consequent enhancement in the production cycle. Deteriorated water quality measures such as low dissolved oxygen, high ammonia, elevated pH, and fluctuation in water temperature are known to be the most critical/ direct triggers of the stress vicious cycle with consequent clinical disease and mortalities [3]. Water temperature is the main environmental factor responsible for the growth, activity, and safety of fish and other aquatic organisms. The optimal range of water temperature is 25 - 30°C for most tilapias to ensure optimal growth performance [4]. The optimum pH for freshwater aquaculture is ranging from 6.5 – 8.5. Very high (greater than 9.5) or very low (less than 4.5) pH values are unsuitable for aquatic organisms [3].

There is a proportional relationship between ammonia levels, water temperature, and pH where un-ionized ammonia levels rise as temperature and pH increase. Levels below 0.02 ppm are considered safe for aquaculture [5]. Levels of DO depend on water temperature, fish biomass and rate of water exchange. Optimal DO levels in freshwater aquaculture range from 4-6 ppm [3].

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Fish diseases are among the major challenging factors that limit aquaculture productivity and sustainability. Most infectious diseases of fish are opportunistic; this means that the single presence of the pathogen in the environment of the fish is insufficient to cause a disease outbreak [6]. Various environmental stressors such as high stocking densities, sharp decline/ elevation in water temperature, erratic management, underfeeding, and overfeeding are potential immunosuppressive factors that frequently result in the vast spread of pathogenic bacteria within aquatic environments [7-9]. Pseudomonas species is considered one of the most important fish pathogens affecting freshwater, brackish water, and marine fishes. Pseudomonas septicemia is a septicemic disease caused by several members of the family *Pseudomonadaceae* as *P. fluorescens, P. angulliseptic, P. diminuta, P. aeruginosa,* and *P. putida* [8, 10].

Pseudomonas fluorescens is a Gram-negative, rod-shaped organism, motile by polar flagella that might produce fluorescent pigment (fluorescein**)** on specialized bacteriological media [11]. *Pseudomonas fluorescens* is responsible for generalized septicemia in fishes as well as other aquatic animals [8, 12]. Mass mortalities linked to *P. fluorescens* infection were previously reported by [8]. The lesions associated with *P. fluorescens* include but are not limited to ulcerative syndrome, hemorrhagic septicemia, tail/fin rot, exophthalmia, and ascites [13].

Saprolegniasis is an infectious fungal disease that is widespread in all stages of the fish life cycle. It is caused by the genus Saprolegnia which belongs to Class Oomycetes, family saprolegniacae, and is commonly known as water molds [14]. *Saprolegnia parasitica* is ubiquitous constituent of the aquatic environment since they are saprotrophic and obtain their nourishment by decomposing organic matter. It is primarily considered a secondary invader associated with environmental stressors [15-16]. *S. parasitica* outbreaks aggravated with mass kills among different freshwater-farmed and ornamental fishes have frequently been reported [17,19]. Infected fish usually show cottony white to gray, brown, or greenish masses on the skin, eyes, and gills [20]. The disease occurs due to fluctuations in water temperature, it may occur in the winter, autumn, and spring seasons [21]. It acts as a co-factor for the presence of bacteria and parasites as it causes immunosuppression for different stages of fish species [19]. Death of infected fish is mainly due to osmoregulatory failure [19].

Therefore, the current study aims to assess the roles of poor water quality and environmental stressors in the development of *P. fluorescens* and *S. parasitica* concurrent infections in farmed Nile tilapia.

Material and Methods

Case history

At the mid-February 2023, a clinical *fluorescens* disease eruption together with mass kills among farmed Nile tilapia fingerings was reported in an acre-sized earthen pond-based private farm at Kafr El-Sheikh province, Egypt. The reported mass kills were 35% of the entire stock during the period from February 11 through February 13. Mortalities started on February 11 at a rate of 5 % of the entire stock with a peak of mortalities on February 12 (20 % of the entire stock) and 10 % on February 13. The pond was originally stocked with 25000 Nile tilapia fingerlings of average weight 50g ± 10. The water column was adjusted to 1 meter; the daily water temperature averaged 19 ºC ± 1 at noon and 13 ± 2 ºC during the late night; DO was 7 ppm at the mid-afternoon and pH was 7.6 ±0.2. On February 11, 2023, the province weather was generally stormy together with heavy rains driving the pond's water muddy and blackish brown in colour.

Moribund fish were seen surfacing as well as aggregated at the water inlet and pond sides. Dead fish were seen covered with whitish brown cottony wool-like masses at lateral sides and over gill covers and sometimes at the nuchal region of fingerlings.

Sample collection and processing

A total of 300 moribund and freshly dead fingerlings were collected and equally divided into 10 plastic bags (30 fingerling/bag). Collected samples were placed on ground ice in an isothermal box till transferred to the Aquatic Animal Medicine and Management Lab (AAMML) at the Faculty of Veterinary Medicine, Cairo University for fingerlings examination and sample processing.

Ethical approval

The current study is ethically approved by the Institutional Animal Care and Use Committee (Vet. CU. IACUC). The ethical approval number is "Vet CU 09092023748".

Clinical examination

Fingerlings were examined while in the pond's water for any possible abnormal behavioral changes. In the lab, fingerlings were visually inspected for any possible external lesions on skin surfaces, gill covers and gills then dissected using 3 line incisions for internal examination according to [18].

Mycological examination

Fish with cotton wool-like fungal mats were washed up with double distilled water to get rid of superficial bacterial contaminants then loopfuls from the deep cotton wool-like mats as well as deep skin lesions were spread onto sterile plates of Sabouraud dextrose agar with chloramphenicol (SDA, Difco Lab, USA) [19]. Culture plates were incubated at 20

ºC for 3 to 5 days with regular daily inspection for any expected fungal growths. Harvested fungal colonies were purified then the slide culture technique was adopted on retrieved colonies for initial morphological identification. Fungal spores were fixed with one drop of methyl alcohol and stained with lactophenol cotton blue as described by Willoughby.

Bacteriological examination

Loopfuls from skin ulcers kidney and spleen of moribund and freshly dead fingerlings were spread onto Tryptic soy agar (TSA) (Becton, Dickinson and Company (Becton, Dickinson, and Company -BD, NJ – USA) and then incubated at 25 ºC for 18–24 hr. Grown colonies were further purified and morphologically examined for their cultural characteristic and Gram staining criteria according to Eissa et al. [8]. The retrieved isolates were identified using API 20 NE semi-automated kit (bioMérieux Inc., NC, USA). Results were interpreted at 24–48 hr according to the manufacturer's instructions.

Histopathological examination

Skin, muscles and fins specimens were fixed in 10% formol saline for 24 h. Fixed tissues were washed in tape water and then dehydrated using serial dilutions of absolute alcohol. Specimens were cleared in xylene and embedded in paraffin at 56 °C in a hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 4 microns thickness (Leitz rotary microtonal for paraffin section). The obtained tissue sections were placed on glass slides, deparaffinized, and stained by hematoxylin and eosin (HandE) stain [22] for examination using low/high powers of light electric microscope (Olympus, USA).

Molecular identification of Pseudomonas fluorescens

The bacterial Genomic DNA was extracted from purified bacterial isolates using QIAamp DNA Mini Kit, (Qiagen, Germany) following the manufacturer's instructions. The eluted DNA was used as a template for PCR detection of the universal 16S rRNA gene. The 16S rRNA from the strains was amplified using the primer set 16SPSEfluF and 16SPSER (16SPSEfluF 5´-TGCATTCAAAACTGACTG-3´); (16SPSER5´-AATCACACCGTGGTAACCG-3´) [23]. PCR amplification was carried out in a total volume of 50 µl comprising of 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan) (2x premix), 1 µl of each primer (20 pmol), 5 µl Template DNA and 18 µl PCR grade water. The amplification was performed using a thermal cycler (Biometra, Germany) with the following conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of amplification (denaturation at 94°C for 35s, primer annealing at 55°C for 50s, extension at 72°C for 1 min) and a final extension step of 72°C for 10 min. The PCR amplicons from five representative isolates was purified with a QIAquick PCR purification kit and directly sequenced in both directions with a 3500/3500xL Genetic Analyzer (Applied Biosystems, at Faculty of Agriculture Research Park, Faculty of Agriculture, Cairo University).

Molecular identification of Saprolegnia parasitica

Genomic DNA was extracted from the pure mycelia using DNeasy kits, (Qiagen, Germany) following the manufacturer's instructions. The eluted DNA was used as a template for PCR detection of ITS region of ribosomal DNA. The (ITS) rDNA was amplified using the universal primers 5′-TCCGTAGGTGAACCTGCGG-3′ (ITS1) and 5′-TCCTCCGCTTATTGATATGC-3′ (ITS4) [24]. PCR amplification was carried out in a total volume of 25 µl comprising of 12.5 µL of Dream Taq Green PCR Master Mix (Thermo Scientific, USA), 2 µL of forward and reverse primers (100 pmol), 100 ng/µL of extracted DNA and sterile water up to the final volume. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and directly sequenced in both directions with a 3500/3500xL Genetic Analyzer (Applied Biosystems, at Faculty of Agriculture Research Park, Faculty of Agriculture, Cairo University).

Phylogenetic analysis

The raw sequences of the *P. fluorescens* and *S. parasitica* were checked and assembled using Bio-Edit version 7.0 [25]. The assembled sequences were submitted to the database of GenBank and compared to the related sequences by BLASTN search. Phylogenetic trees were constructed using MEGA X 11 using the Neighbor-Joining method, with 1000 bootstrap values [26].

Results

Clinical examination

On farm visits, fingerlings were seen restless, jumping from the water, aggregated at water inlets, surfacing, and gasping in some cases. Flashing behavior was also noticed among fingerlings with cotton wool-like growths attached to their skins. On clinical examination, whitish to brownish cottony wool-like masses were seen over eyes, gills, skin, fins, and sometimes on the mouth of moribund and freshly dead fingerlings. Different degrees of skin erosions, ulcerations, and fin rot/ hemorrhages were commonly detected. Upon dissection, congested gills, liver, spleen, and kidneys were also detected.

Bacteriological examination

The results of colonial characteristics on enriched media and biochemical identification using a miniaturized API 20 NE system have revealed that the identity of retrieved bacterial isolates is *P. fluorescens*. Isolates retrieved from the internal organs of moribund fingerlings were associated with

a clinical form of septicemia which is typical for such pathogen.

Mycological examination

Visual inspection of the cultured SDA plates has exposed the eminent growth of mold colonies. The colonies can be morphologically depicted as cysts of whitish cottony long hairs that quickly shifted to grey and then black after 96 hrs (Fig.1A and B). Microscopically, fungal colonies were characterized by an extensive and dense mycelium. By examination, isolates showed the characteristic appearance of branched non-septated hyphae (Fig. 2 A) together with masses of mature and immature sporangia, which is indicative of asexual reproduction (Fig. 2B). Such sporangia were filled with a large number of spherical sporangiospores, which were separated from the basal somatic hyphae by a small septum.

Histopathological examination revealed complete hyalinization and necrosis of fin tissues associated with inflammatory cell infiltration and melanophore activation together with the appearance of mold spores as well as hyphae embedded within the fin tissues (Fig. 3).

Molecular identification of Pseudomonas fluorescens

The assembled 16S rRNA gene sequences were submitted to GenBank. Depending on sequence analysis, the current sequences were confirmed to be *P. fluorescens*. The nucleotide sequences of five bacterial strains were deposited in GenBank under accession numbers from (PP464130) to (PP464134). The alignment of these sequences showed 100–99.77% identity to the accession numbers of *P. fluorescens* (ON430515.1, ON430510.1, and AF094731.1). The neighbor-joining phylogenetic tree constructed based on the sequenced 16S rRNA genes of *P. fluorescens* was grouped with their relevant *P. fluorescens* sequences which were genetically apart from other related species *P. aeruginosa* (99%) and *P. putida* (97%) depending on their degree of similarities (Fig. 4).

Molecular identification of Saprolegnia parasitica

The assembled ITS rDNA gene sequence was submitted to GenBank. Depending on sequence analysis, the current sequences were confirmed to be *S. parasitica*. The nucleotide sequence of one representative isolate was submitted to Gene Bank under accession number (PP467684). BLAST alignment of this sequence showed 100–99.86% identity to the accession numbers of *S. parasitica* (KX494868.1, KT807577.1, OP642442.1, OP629498.1, ON818276.1, AM228808.1 AM228807.1, MW819631.1, MW819629.1, OP629505.1 and OP629503.1). The phylogenetic analysis using the neighbor-joining method showed that the amplified sequence of *S. parasitica* was grouped with the known *S. parasitica* from other studies. Furthermore, the obtained sequence from this study was separated from other groups belonging to *S. salmonis* and *S. hypogyna* (Fig. 5).

Discussion

Heavy rains, storms, and windy weather have very deleterious effects on the pond's water dynamics where sludge from the pond's bottom gets disturbed with consequent dispersion through all water layers resulting in high turbidity with a high possibility of impeding sunlight passage through water [27]. Such alterations in water dynamics usually disperse all settled chemicals, pathogens, and suspended particles throughout the water column with consequent lodging of these deleterious pollutants into the gills and skin [28]. Gill/skin respiratory, immunological, and osmoregulatory functions are either partially or fully damaged with possible pathogenic invasions including saprophytes (oomycetes) and ubiquitous pathogens such as obligate aerobes (*P. fluorescens*) [29]. Ultimately, all these bad environmental conditions will trigger fish to stop food and face generalized emaciation with consequent immunosuppression and eminent death due to osmoregulatory failure [8, 30].

The remarkable increase in mortalities among fingerlings on the 2nd and 3rd day of this episode could possibly be because of the gradual increase in water turbulence, suspended particles dispersion, and pollutant movement from the bottom to disperse throughout the water column post-storm and heavy rains that happened in 1st day [31]. These dynamic alterations in the pond's bottom including nasty pollutants and pathogens could have tremendously damaged the gill lamellar epithelium as well as dermal epithelium including those cells responsible for osmoregulation and respiration [8, 32]. This acute damage to respiratory and osmoregulatory cellular machines will swiftly result in mass kills which concords with the magnitude of dynamic alterations within the water column and amounts of released pollutants from the bottom's altered sludge [8].

Low water temperatures as those reported in the current episode together with luxurious DO levels generated by both low temperature and high-water turbulences during the rainy stormy weather that hit Kafr El-Sheikh province on February 11th could have triggered a drastic increase in load and virulence of low temperature depended on obligate pathogens such as *P. fluorescens* [8]. This potential increase in *P. fluorescens* load and virulence could have initiated acute invasion into the fins, skin, and gills of overwhelmed Nile tilapia fingerlings. Once *P. fluorescens* reaches the minute blood capillaries at these highly energetic/ vascularized tissues, acute septicaemia starts with the massive secretion of exotoxins such as hemolysins and proteases that

infuse through the epidermal, dermal, and hypodermal layers of skin and fins leading to various degrees of dermal erosions, ulcers, and fin rot [8, 33, 34].

In the current study, the histopathological picture of HandE-stained slides made from skin, fins, gills, kidney, and spleen of moribund and mortal tilapia fingerlings, concords with the *P. fluorescens* histopathological alterations of acute septicemia reported by [35, 36].

Pseudomonas fluorescens was isolated from internal organs (liver, kidney) from moribund or mortal *O. niloticus* fingerlings. These *P. fluorescens* isolates were presumptively identified by conventional morpho-chemical tests as well as API20NE miniaturized tests comparable to those reported by [7, 8].

The presumptively identified isolates of *P. fluorescens* were molecularly confirmed using standard PCR utilizing 16S rRNA sequences which is a useful method for the identification of different bacterial strains [35].

The assembled 16S rRNA gene sequences were submitted to GenBank. Depending on sequence analysis, the current sequences were confirmed to be *P. fluorescens*. The nucleotide sequences of five bacterial strains were deposited in GenBank under accession numbers from (PP464130) to (PP464134). The alignment of these sequences showed 100–99.77% identity to the accession numbers of *P. fluorescens* (ON430515.1, ON430510.1, and AF094731.1). The neighbor- joining phylogenetic tree constructed based on the sequenced 16S rRNA genes of *P. fluorescens* was grouped with their relevant *P. fluorescens* sequences which were genetically apart from other related species *P. aeruginosa* (99%) and *P. putida* (97%) depending on their degree of similarities (Figure 4).

Stressed fish frequently lose their ability to defend themselves against pathogenic agents. *Saprolegnia* species are frequently implicated in fungal infections; nevertheless, it is believed that they are secondary invaders arising from physical damage resulting from rough handling or invasion by primary pathogens [8, 19]. Fish immune systems can be rendered ineffective by physical stress, inadequate nutrition, poor water quality, and overcrowding. Yet, instead of really killing the fish, they cause outbreaks of dangerous fungi.

It is generally accepted that Saprolegnia spp. is a saprotrophic and necrotrophic opportunistic pathogen that causes saprolegniasis, or winter deaths. *Saprolegnia delica*, *S. ferax*, *S. australis*, *S. dicilina*, and *S. parasitica* have all been linked to dermal diseases in freshwater fish, according to literature [19]. However, it soon became clear that some *S. parasitica* strains are incredibly virulent and can infect freshwater fish initially. One of the most harmful oomycetes is *S. parasitica*. The most common sign of saprolegniasis in fish that resembles the signs reported in this study is a superficial "cotton-like" growth with a white growth of mycelia on the fish skin, especially on the head, dorsal and caudal fins, gills, and mouth [19]. Saprolegnia hyphae can penetrate epidermal tissues and infect the entire surface of the body. It results in cellular necrosis, dermal and epidermal damage, and ultimately osmoregulatory failure-induced death [19, 31].

Proteases are thought to be a crucial component of dermal fungi pathogenicity [37]. Aquatic organisms' health has long been known to be impacted by *Saprolegnia* spp [38]. Such infections have high levels of extracellular protease activity, which promotes pathogenesis. Severe Saprolegnia infections cause osmoregulatory / respiratory failure, lethargy, loss of balance, and typically fish mass kills, as demonstrated by the recent mass killings of Nile tilapia fingerlings in the current study [19]. It has been hypothesized that certain *S. parasitica* cDNAs encode secreted proteins, including glycosyl hydrolases, proteases, and protease inhibitors, which may have virulence properties. SpHtp1, a particular host-targeting protein, binds to a fish receptor ligand that is tyrosine-O-sulfated in order to enter the host cell [39]. SpHtp1 translocates within the host cell, and additional factors are secreted as extracellular proteases, toxins, or cell surface lectins that may target the host membrane and nucleases [39]. According to literatures, the metalloprotein and serine protease (SPRG_14567) that *S. parasitica* breaks down the immunoglobulin M (IgM) of fish, indicating that the organism is virulent and can inhibit the host immune response [40].

Hyalinization, necrosis, inflammatory cells infiltration, and melanophores activation together with the detection of mold spores and hyphae of skin, fins and muscles histopathological sections are typically consistent with the *S. parasitica* histopathological picture reported in previous clinical studies [41]. The hyalinization and necrosis could be attributed to the proteolytic effects of glycosyl hydrolases and proteases secreted by *S. parasitica* together with the exotoxins produced by *P. fluorescens* [39].

Identification of *Saprolegnia* species can be difficult and perplexing at times. However, the conventional identification of Saprolegnia is based on a number of typical morphological features involving both asexual and sexual reproduction [28]. In the current study, using the criteria outlined by [33], the water mold responsible for mass kills among Nile tilapia fingerlings has been recognized as *S. parasitica* [33]. Presumptive identification of asexual stages of the retrieved water mold was based on lactophenol blue- stained slides made from

colonies obtained on SDA plates [35]. The detected mycelia and hyphae were non-septate with club-shaped conidiospores divided into several parts at the end of the hyphae which coincides with Saprolgenia criteria described by [33]. However, the difficulty of obtaining hemp seed to incorporate into the isolation media for the production of oogonia (sexual production) mandates using an alternative confirmative tool to confirm the full identity of *S. parasitica* isolates. Thus, using molecular tools such as PCR coupled with partial sequencing of inter-transcribed spacer (ITS) gene was one of the most important choices to distinguish *S. parasitica* from other water molds (Oomycetes) [19, 33]. Depending on sequence analysis of the assembled ITS rDNA gene sequence submitted to GenBank; the obtained sequences were confirmed to be *S. parasitica* which was concordant with results reported by [19]. The phylogenetic analysis using the neighbor-joining method concluded that the retrieved water mold was *S. parasitica* comparable to those reported in previous studies such as [19].

Conclusion

Poor water quality, high stocking densities and extreme climatic changes such as stormy weather and heavy rains could be the most obvious triggers of *P. fluorescens* and *S. parasitica* infections among earthen pond - farmed Nile tilapia fingerlings with ultimate mass kills. Such types of mass kills could be responsible for moderate economic losses in both tilapia farms and hatcheries during winter, late winter and early spring seasons. These critical impacts necessitate the development of quick and effective tools for diagnosis, control, and prevention of such disease eruptions among earthen pond - farmed tilapia.

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Declaration of Conflict of Interest

All authors declare that they have no conflict of interest.

Ethical of approval

The current study is ethically approved by the Institutional Animal Care and Use Committee (Vet.CU.IACUC). The ethical approval number is "Vet CU 09092023748".

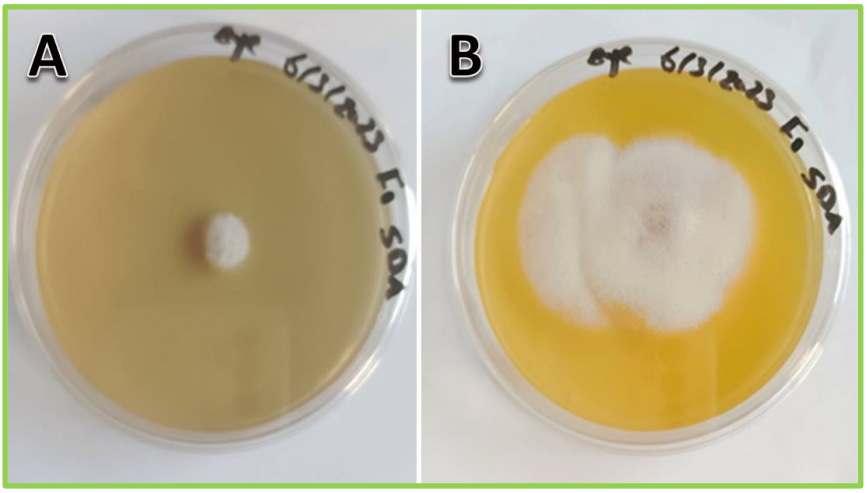

Fig. 1. SDA cultured plate showing the typical whitish / grayish white cottony hyphal growths of Saprolgenia colonies on SDA plates

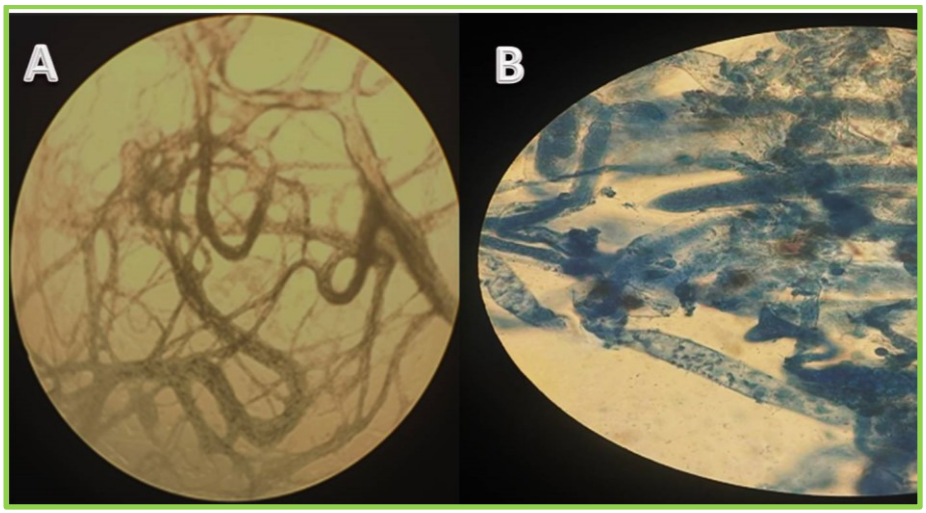

Fig. 2. (A): Non stained smears showing the characteristic appearance of branched non-septated hyphae, (B): Stained smears showing masses of mature and immature sporangia indicative for asexual reproduction

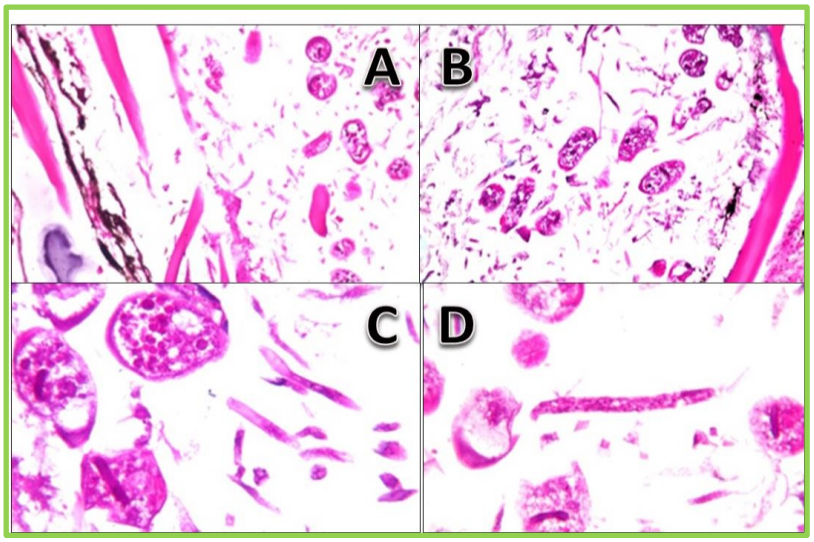

Fig. 3. Histogram of HandE stained skin, muscles and fins sections from autopsied Nile tilapia fingerlins exihibiting complete hyalinization and necrosis of fin tissues, melanophore activation and mold spores / hyphae embedded within the fin tissues

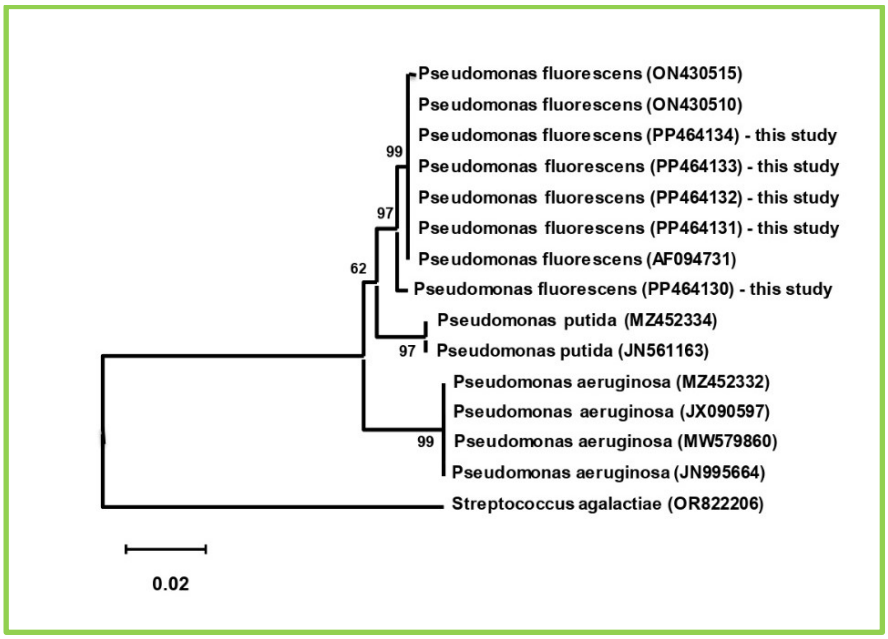

Fig. 4. Phylogram of the retrieved *P. fluorescens* isolates grouped with their relevant *P. fluorescens* sequences based on the sequenced 16S rRNA genes of *P. fluorescens*

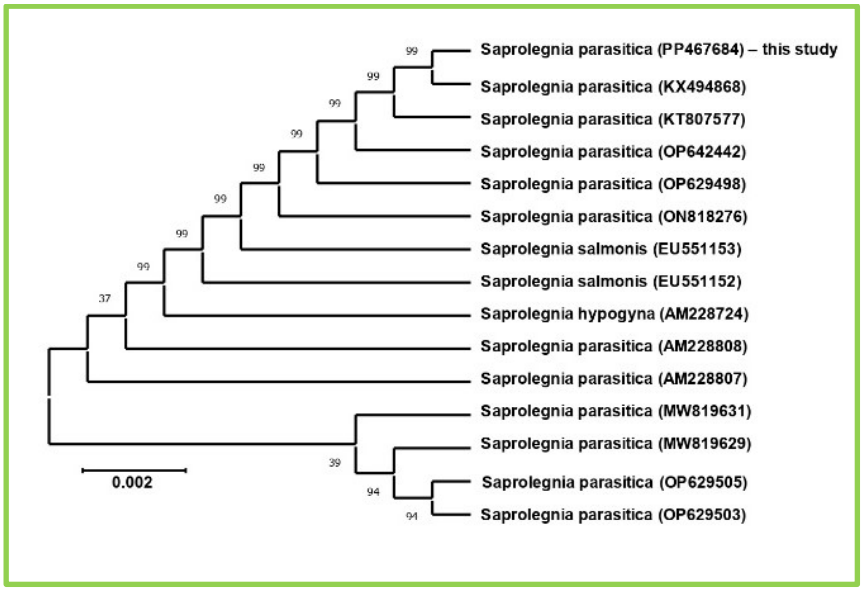

Fig. 5. Phylogram of *S. parasitica* isolate retrieved in the current study grouped with their relevant *S. parasitica* sequences based on the sequenced ITS rDNA of S. parasitica

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النفوق الجماعي المرتبط بالعدوى المتزامنة لـ Pseudomonas Fluorescens وSaprolegnia parasitica في أسماك البلطي النيلي المستزرعة في الأحواض الترابية

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الملخص

يعتبر سوء نوعية المياه من أهم العوامل المؤدية لظهور الأمراض في منشآت الاستزراع السمكي ويعد الانخفاض المفاجئ أو التقلب في درجة حرارة الماء عاملاً حاسما في حدوث العدوى ببعض الميكروبات المرتبطة بدرجات الحرارة المنخفضة. و يعتبر الميكروب السودوموناس واسع الانتشار وعفن الماء الريمي *S* طفيل السابرولجنيا من النماذج المثالية لمسار هذا المرض. في الدراسة الحالية، تم عزل الميكروبين واسعى الانتشار فى المياه من أسماك البلطي النيلي المرباه في بركة ترابية بحجم فدان والتي كانت مكتظة بأكثر من 25000 إصبعية خلال فترة النفوق الجماعي في منتصف فبراير 2023. وقد وصل النفوق الجماعي الى ذروته بعد هطول الأمطار الغزيرة والعواصف والرياح التى ضربت البلاد في هذا الوقت من العام. وكانت معظم الأسماك النافقة وتلك المحتضرة تعاني من تقرحات جلدية على سائر انحاء الجسم ، وتأكل الزعانف، واحتقان الخياشيم، وخيوط بيضاء رمادية تشبه القطن مرتبطة بشكل وثيق بتقرحات الجلد والزعانف. و داخليًا، كانت جميع الأعضاء الداخلية تقريبًا بما في ذلك الكلى والطحال والكبد محتقن. وقد أظهر الفحص الهستوباثلوجى للأسماك المصابة تبلور ونخر أنسجة الزعانف مع انتشار للخلايا الالتهابية. علاوة على ذلك، تم اكتشاف خيوط فطرية داخل الحزم العضلية. وقد تم تأكيد الهوية النهائية لبكتيريا السودوموناس المعزولة باستخدام تحليل تسلسل الجينات 16SrRNA، في حين تم تأكيد عزلات طفيل السابرولجنيا باستخدام تحليل تسلسل الجينات ITS rDNA وقد تم نشر جميع أرقام الدخول الداعمة للعزلات المعزولة من خلال موقع بنك الجينات الرسمي Genbank .

الكلمات الدالة: النفوق شتاءً، السودوموناس، السابرولجنيا، العدوى المتزامنة، نوعية المياه، والإجهاد المناخي.