J. Agric. Sci. Mansoura Univ., 33 (7): 4887 - 4899, 2008

AQUATIC FUNGI AND FISH PRODUCTION IN EGYPT: - *IN VITRO* STUDIES

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ABSTRACT

Survey for microorganisms associated with the Nile tilapia fish revealed that more than 20 fungal isolates belonging to different genera and species including *Saprolegnia*, *Trichoderma*, *Alternaria* spp., *Penicillium*, *Fusarium* sp., *Fusarium semitectum* (= *F. incarnatum*), *Cladosporium*, *Phoma*, *Nigrospora*, *Aspergillus niger* and *Aspergillus flavus* in addition to five bacteria were isolated from naturally diseased fish. In the winter season of 2002, an attempt was made to isolate *Saprolegnia* sp. from moderately infected fish, water, and feed sampled from the saprolegniasis-affected pond. *Saprolegnia* sp. was successfully recovered from the infected fish and pond water samples but was not found in the feed samples. However, many saprophytic fungi and bacteria were found contaminating the fodder samples. In the mid summer of 2002, *Aspergillus ochraceus* was found associated with a syndrome of eye dropping on Nile tilapia appeared in ponds of a commercial intensive fish culture located in the area close to Kafr El-Sheikh governorate. This is the first report of using the fungus *Trichoderma* spp. for biological control of fungal diseases of fish.

Keywords: *Oreochromis niloticus* – Nile tilapia – Fungal diseases of fish –Biological control - Antibiosis – *Saprolegnia* – *Aspergillus ochraceus* – *Trichoderma* – *Alternaria eichhorniae*.

INTRODUCTION

Fish is considered a significant source of protein not only in Egypt but also worldwide (Abdelhamid, 2003). The total fish production in Egypt for the year 2002 was 801466 Ton. Of this yield, 38.2% were of Nile tilapia (GAFRD, 2003). *Saprolegnia* is ubiquitous in freshwater ecosystems and is the main genus of water molds responsible for significant fungal infection of freshwater fish and eggs. Almost every freshwater fish is exposed to at least one species of fungus during its lifetime (Noga, 1996). On fish, *Saprolegnia* invades epidermal tissues, generally beginning on the head or fins (Willoughby, 1994) and can spread over the entire surface of the body, visible as white or gray patches of filamentous mycelium (Bruno and Wood, 1999). *Saprolegnia* is characterized by an external, cotton-like appearance that radiates out in a circular, crescent-shaped or whorled pattern. Saprolegniasis in fish can be prevented in laboratory challenge experiments using formalin or diquat, both FAD -approved chemical for use in catfish ponds (Bly *et al.*, 1996). However, the use of such chemical in pond water containing fish destined for human consumption is not particularly desirable, i.e., it can lead to the emergence of resistant strains which are consequently more potent pathogens for man as well as for fish. Yet, while attempts are being made to identify new chemical against *Saprolegnia*, biological control of this organism has received little attention. The agricultural importance of the genus

Trichoderma is that some of its members possess mycoparasitic abilities against plant pathogenic fungi, which allows for the development of biocontrol strategies based on *Trichoderma* strains (Manczinger *et al.*, 2002).The observation that some *Trichoderma* species inhibited *Saprolegnia* hyphal growth has added to the potency of this fungal genus as a promising biocontrol agent for fish pathogenic fungi as well. *Aspergillus ochraceus* is reported to be allergenic and can also produce ochratoxin A, which may produce ochratoxicosis in humans and animals. Other toxins which can be produced by this fungus include penicillic acid, xanthomegnin and viomellein. These are all reported to be kidney and liver toxins. This fungus is widespread in cultivated soils, but has also been documented in uncultivated soils, grains, and food products (Abdelhamid, 2000). *Alternaria eichhorniae* Nag Raj & Ponnappa, a fungal pathogen of water hyacinth, *Eichhornia crassipes* (Mart.) Solms.; Pontederiaceae, has been reported on water hyacinth in many countries in the world (Shabana, 2002). This fungus has shown to be host-specific to water hyacinth and capable of severely damaging and suppressing the weed (Shabana *et al.*, 1995a, b & c). A good understanding of the biology and pathology of this fungus has been gained (Shabana, 2002). Therefore, in order to confirm the feasibility of using *A. eichhorniae* as a mycoherbicide for water hyacinth, it must be tested on fish to verify the safety of using this fungus as a bioherbicide for water hyacinth. Therefore, the present investigation was undertaken to isolate, purify, and identify the causal agents of two fungal diseases found on the Nile tilapia fish culture in earthen ponds around Kafr El-Sheikh governorate. In addition, to study the efficacy of some fungal biocontrol agents namely, *Trichoderma viride*, *T. harzianum*, *T. hamatum*, and *Coniothyrium* sp. in suppressing the mycelial growth of *Saprolegnia* sp. and *Aspergillus ochraceus in vitro*, causal organisms of fish diseases.

Survey for microorganisms associated with the Nile tilapia fish:

Eleven Nile tilapia (*Oreochromis niloticus*) commercial earthen pond farms situated in Kafr El-Sheikh governorate, where semi-intensive culture ponds were used for this survey. The survey was performed throughout the winter season of 2002. Samples of individual fish varied in size (113-216 g) showing diverse types of disease symptoms were freshly collected from each pond, transported on ice to the laboratory, and processed within 3 to 4 hours of collection. For each sample, isolation of microbes associated with a disease/disorder symptom was conducted from the scales, epidermal tissues, and/or gills. In some cases, isolation was also carried out from eyes, as needed. A single-spore or hyphal-tip isolation technique was employed to obtain the fungus in pure culture according to Booth (1971), Barnett and Hunter (1972) and Ellis (1976).

Microorganisms used:

One isolate of *Saprolegnia* sp. that was recovered from the Nile tilapia fish expressing saprolegniasis in commercial ponds located at Abu-Sekkein, Al-Hamool, Kafr El-Sheikh governorate, was used in this study. An isolate of *Aspergillus ochraceus* that was associated with the eye dropping syndrome on the Nile tilapia obtained from commercial ponds near El-Mansoura, Dakahlia governorate was also used in the present investigation.

Three *Trichoderma* species, *T. harzianum*, *T. hamatum*, and *T. viride* as well as an isolate of *Coniothyrium minitans* and *Alternaria eichhorniae* 5 (Ae5) obtained from the fungal collection of Dr. Yasser Shabana were used in this study to determine their efficacy as biocontrol agents for *Saprolegnia* sp. and *Aspergillus ochraceus*. Three strains of bacteria, *Pseudomonas fluorescens*, *Bacillus subtilis*, and unidentified strain obtained from Dr. Yasser Shabana were also tested for their antagonistic activity against *Aspergillus ochraceus*. A hypha!-tip and/or single- spore cultures of all fungal isolates were grown on PDA, except for *Saprolegnia* sp. that was grown on Sabouraud's dextrose agar (Rosenthal and Furnari, 1957). Pure cultures of bacterial strains were grown on Difco nutrient agar (NA).

Isolation of the causal organisms

Isolation of *Saprolegnia* sp.: In the winter season of 2002, an epidemic/outbreak of saprolegniasis occurred in a commercial Nile tilapia semi-intensive culture system located close to Kafr El-Sheikh town, Kafr El-Sheikh Governorate. Died Nile tilapia fish with numerous fungal lesions were found floating on the pond surface within one week of a recorded drop in water temperature (17 to 12 °C in 4 days). Mortalities continued to rise as more fish became affected with this condition and lots of dead fish were seen on the pond shoreline. An attempt was made to isolate *Saprolegnia* sp. from moderately infected fish, water, and fodder sampled from the saprolegniasis-affected pond. Samples of infected fish were collected from ponds and transferred on ice to the laboratory at Mansoura University. Isolation from the skin lesions was conducted within 3-4 hours of collection. A hyphal-tip isolation technique was employed to obtain the fungus in pure culture. Pure culture was identified as described by Lategan and Gibson (2003). Pond water sample was taken by submerging sterile 500-mL glass bottle and opening it 5 cm under the water surface. A representative sample was taken from the fodder used to feed fish in the saprolegniasis-affected pond. Samples were transported to the laboratory in an ice box containing ice cubes and were processed within 3 to 4 hours of collection. Microbes recovered were counted 6 days after dilution plating. Calculations were made to express the numbers of colonies as colony forming unit per milliliter (CFU/mL) for water samples and CFU/g for fodder samples. Working cultures of fungi and bacteria were transferred to 9-cm PDA and NA plates, respectively, and exposed to diurnal black light (12-hour cycle) to enhance sporulation. Identical-looking colonies of the recovered microbes were considered the same microbe. Fungal isolates were identified by cultural characteristics and microscopic examination according to Ellis (1976). Bacterial strains were tested for Gram reaction. For production of asexual zoospores, cysts and vegetative hyphae by *Saprolegnia*, Petri plates containing corn meal glucose agar were inoculated with *Saprolegnia* sp. and incubated at room temperature for one week. Two to three mm plugs of agar were cut from the growing front of mycelium and placed in 30 mL of Griffin's glucose yeast extract (GY) broth (Griffin, 1978) in 250-mL Erlenmeyer flask and placed on a rotary shaker at 100-120 rpm at room temperature for three days. After 3 days, agar plugs that were covered with fuzzy mycelial growth were removed and rinsed several times in Griffin's (1978) SM medium. Then

these mycelial wefts were transferred to 20 mL of fresh SM in 10-cm-diam Petri dishes and left without shaking at room temperature. After 16 and up to 48 hours, these mycelial plugs were repeatedly examined using stereomicroscope and/or compound microscope for the formation/production of zoosporangia and the releasing, encysting and germination of zoosporangio-spores.

Isolation of *Aspergillus ochraceus:* In the mid summer season (25th June 2002), a syndrome of eye dropping on Nile tilapia was found in commercial Nile tilapia semi-intensive culture ponds located in the area close to the city of Kafr El-Sheikh, Kafr El-Sheikh governorate. An attempt was made to isolate the microorganism associated. The naturally infected fish were collected from ponds and kept cool until processed, within 3-4 hours of collection, at the laboratory. The damaged fish were surface sterilized with 10% sodium hypochlorite for 1 min, while some were left without surface sterilization. Both groups were washed thoroughly with sterile distilled water and dried on sterile filter paper. Bits of the soft tissue were pulled out from the eye chamber with sterile forceps and placed on water agar supplemented with streptomycin sulphate (0.3 g/L) and chloramphenicol (0.3 g/L) in Petri plates. After seeding, plates were incubated in the dark at 28 °C for 4-6 days. A single-spore isolation technique was employed to obtain the fungus in pure culture. Pure culture was identified and maintained at 5 °C on slants containing potato carrot agar.

Laboratory studies:

Antagonistic effects of selected fungal isolates against *Saprolegnia* sp.: Discs (4-mm in diameter) from pure cultures of *Saprolegnia* sp. and/or *T. harzianum, T. viride, T. hamatum,* and *Coniothyrium minitans* were oppositely seeded at the periphery of Petri plates containing PDA and/or Sabouraud's agar. Culture plates were incubated in the dark at 20 °C. When biocontrol activity was tested, both *Saprolegnia* sp. and the test biocontrol agent were seeded in the same dish at opposite sides (dual cultures). Controls were performed by seeding each fungus alone at periphery of the plate. Seven replicates (plates) were used for each *Saprolegnia*/biocontrol-agent combination. After the *Saprolegnia* almost covered the medium surface in the control plates, the mean linear mycelial growth of *Saprolegnia* occurred in the direction to the test biocontrol agent was measured and the inhibition rate of the pathogen growth due to the presence of the test biocontrol agent was calculated.

Antagonistic effects of selected fungal isolates against *Aspergillus ochraceus:* Discs (4-mm in diameter) from pure cultures of *A. ochraceus* and/or *T. harzianum, T. viride, T. hamatum,* and *A. eichhorniae* were oppositely seeded at the periphery of Petri plates containing PDA. Culture plates were incubated in diurnal light (12 h light cycle) at room temperature (25 ± 3°C). When biocontrol activity was tested, both *A. ochraceus* and the test biocontrol agent were seeded in the same dish at opposite sides (dual cultures). Controls were performed by seeding each fungus alone at periphery of the plate. Eight replicates (plates) were used for each *A. ochraceus*/biocontrol-agent combination. After the *A. ochraceus* almost covered the medium surface in the control plates, the mean linear mycelial

growth of *A. ochraceus* occurred in the direction to the test biocontrol agent was measured and the inhibition rate of the pathogen growth due to the presence of the test biocontrol agent was calculated.

Antagonistic effects of selected bacterial strains against *Aspergillus ochraceus*: Three strains of bacteria, *Pseudomonas fluorescens*, *Bacillus subtilis*, and unidentified strain were also tested for their antagonistic activity against *A. ochraceus*. Discs (4-mm in diameter) from pure cultures of *A. ochraceus* were seeded at the periphery of Petri plates containing PDA. At the same time, bacteria were streaked on the opposite side to the fungal discs. Culture plates were incubated in diurnal light (12 h light cycle) at room temperature (25 ± 3°C). Controls were performed by seeding *A. ochraceus* alone at periphery of the plate. Eight replicates (plates) were used for each *A. ochraceus*/bacterial strain combination. After the *A. ochraceus* almost covered the medium surface in the control plates, the mean linear mycelial growth of *A. ochraceus* occurred in the direction to the test biocontrol agent was measured and the inhibition rate of the pathogen growth due to the presence of the test biocontrol agent was calculated.

Antagonistic activity of the non-volatile metabolites from *Trichoderma* species against *Saprolegnia* sp. and *Aspergillus ochraceus*: The effect of non-volatile metabolites from *Trichoderma* species against *Saprolegnia* sp. and *A. ochraceus* was tested by the method described by Dennis and Webster (1971).

Statistical Analyses:

The data were subjected to ANOVA using SAS software package (SAS Institute, 1996). Significant differences among treatment means were determined with Tukey's studentized range test or Duncan's new multiple range test as appropriate.

RESULTS AND DISCUSSION

1. Survey for microorganisms associated with the Nile tilapia fish:

Eleven sites of the Nile tilapia commercial earthen pond farms situated within Kafr El-Sheikh governorate (Egypt), were used for this survey. Surveys for microorganisms associated with naturally diseased Nile tilapia fish were continued during the winter of 2002. Results revealed that 20 fungal isolates belonging to different genera and species including *Saprolegnia*, *Trichoderma*, *Alternaria* spp., *Penicillium*, *Fusarium* sp., *Fusarium semitectum* (= *F. incarnatum*), *Cladosporium*, *Phoma*, *Nigrospora*, *Aspergillus niger* and *Aspergillus flavus* were isolated from naturally diseased fish (Table 1). In addition, five bacterial species were recovered (Table 1). These surveys revealed that wide variety of fungi belonging to more than 11 genera was associated with fish in nature. From these fungi, only *Saprolegnia* and *Aspergillus* are known to cause diseases to fish. This is in agreement with the findings of Mohamed (2003) who reported that the most common fungi affecting fresh water fish in Egypt are *Saprolegnia* and *Aspergillus* species. The other fungi are either saprophytes or known as plant pathogens. The presence of these fungi on the fish surfaces may play a significant role in

protecting fish against infection by pathogens or lessen the disease severity if the disease was initiated. This may explain in part the obtained results that *Trichoderma viride* has reduced the mortality in fish that exposed to *Saprolegnia*.

Table 1: Microorganisms associated with naturally diseased Nile tilapia fish collected from eleven commercial earthen pond farms situated within Kafr El-Sheikh Governorate

Sample site	Fish length (cm)	Fish fresh weight (g)	Disease symptoms	Isolation from	Microbes recovered
1	19.5	168.77	Slight reddening in the eyes	Eyes	-
				Scales	*Fusarium* sp. (F1)
				Flesh	Orange, coarse bacteria (G^+) (B1) + Bright beige bacteria (G^+) (B2)
2	18.5	131.41	Slight reddening in the eyes	Eyes	*Trichoderma* sp. (F2), *Penicillium* sp. (F3)
				Scales	-
				Flesh	-
3	19.5	167.05	Bleeding under the gills + black spots on the body surface	Gills	*Alternaria* sp. (F4)
				Scales	F4 + B2
				Flesh	B2 + B3
4	21.1	184.38	Bleeding under the front fins	Gills	F4
				Scales	*Fusarium* sp. (F5)
				Flesh	F4
5	17.5	114.33	Epidermal black spots	Gills	F2
				Scales	*Saprolegnia* sp. (F6)
				Flesh	-
6	16.5	101.85	Bleeding around the epidermal black spots	Gills	F4 + F5
				Scales	F4 + B2
				Flesh	-
7	21.3	215.59	Epidermal black spots	Gills	*Fusarium* sp. (F7)
				Scales	*Nigrospora* sp. (F8)
				Flesh	-
8	19.4	144.34	Epidermal black spots	Flesh	*Fusarium* sp. (F9) + *Cladosporium* sp. (F10
9	19.0	142.40	Bleeding from under the front fins	Flesh	*Fusarium semitectum* (F11)
10	18.5	133.14	Bleeding around the epidermal black spots	Flesh	*Phoma* sp. (F12) + F4 + B2
11	17.4	113.45	Bleeding from under the front fins	Flesh	*Aspergillus niger* (F13)

2. Isolation of the causal organisms:

2.1. Isolation of *Saprolegnia* sp.: In the winter season of 2002, an attempt was made to isolate *Saprolegnia* sp. from moderately infected fish, water, and feed samples from the saprolegniasis-affected pond. *Saprolegnia* sp. was successfully recovered from the infected fish and pond water samples but was not found in the feed samples. However, many saprophytic fungi and bacteria were found contaminating the fodder samples (Table 2).

Table 2: Microorganisms associated with fodder samples plated on three types of culture media.

Medium	Microbes recovered	Colony forming unit (cfu) / g
Rose bengal potato dextrose agar	*Penicillium* sp.	1×10^6
	Aspergillus niger	2.5×10^5
	Aspergillus flavus	1×10^4
	Beige bacteria	1×10^4
Sabouraud's agar	*Penicillium* sp.	4.5×10^5
	Aspergillus niger	2×10^4
	Aspergillus flavus	2.5×10^3
	Fusarium sp.	1.5×10^4
	Reddish bacteria	4.5×10^5
Nutrient agar (NA)	*Penicillium* sp.	8×10^4
	Aspergillus niger	5×10^3
	Aspergillus flavus	2×10^4
	Fusarium sp.	2.5×10^3
	Reddish bacteria	2.5×10^3
	Yellow bacteria	1×10^4
	Beige bacteria	2.5×10^3

2.2. Production of asexual zoospores, cysts and vegetative hyphae by *Saprolegnia:* Within 12-16 hours after the mycelium was transferred from GY, to SM medium, zoosporangia started to develop at hyphal tips (the darkened area of the hyphal tip). After 16-24 hours, zoospores were readily evident emerging from zoosporangia. In addition, encysted zoospores were scattered across the bottom of the Petri dish and attached to hyphae. After 24-48 hours, cysts germinated by producing delicate hyphae.

2.3. Isolation of *Aspergillus ochraceus:* In the mid summer season (25th June 2002), a syndrome of eye dropping on Nile tilapia was found in commercial Nile tilapia intensive culture ponds located in an area close to Kafr El-Sheikh town, Egypt. An attempt was made to isolate the microorganisms associated. *Aspergillus ochraceus* was recovered from the collected fish samples showing eye dropping. The fungus was purified in a single-spored culture. Out of the isolation experiments, it was noticed that from saprolegniasis-affected ponds, *Saprolegnia* sp. was successfully recovered from the infected fish and pond water samples but was not found in the feed samples. This finding is logical since *Saprolegnia* is an aquatic fungus. However, toxigenic fungi such as *Aspergillus flavus* and *Penicillium* were found contaminating the fodder samples. Results showed that *Saprolegnia* was isolated in the winter but *A. ochraceus* was found in the summer season, indicating that each one favors different temperature

conditions. Thus, saprolegniasis is a low temperature disease confirming the finding reported by Neish (1977) and Willoughby (1994), whereas eye-dropping is a high temperature disease.

3. Laboratory Studies:

3.1. Antagonistic effects of selected fungal isolates against *Saprolegnia* sp.: The antagonistic effect of three *Trichoderma* species (*T. harzianum, T. viride, T. hamatum*) and *Coniothyrium minitans* against *Saprolegnia* sp. was measured on two types of culture media (PDA and Sabouraud's dextrose agar). Results showed that *T. viride* was the most suppressive agent for the growth of *Saprolegnia* sp. on both media, PDA and Sabouraud's dextrose agar, causing 77 and 66% inhibition, respectively (Table 3). The *T. hamatum* had the least inhibitory effect on *Saprolegnia* growth. *Coniothyrium* sp. was highly inhibitive to *Saprolegnia* growth only on PDA, but caused the least antagonism to *Saprolegnia* on Sabouraud's dextrose agar (Table 3). In general, inhibition of *Saprolegnia* growth caused by the biocontrol agents was stronger on PDA than on Sabouraud's dextrose agar medium. Three weeks after inoculation, it was apparent that all *Trichoderma* species overgrew *Saprolegnia* growth.

Table 3: Effect of *Trichoderma* spp. and *Coniothyrium* sp. on the mycelial growth of *Saprolegnia* sp. on two culture media five days after inoculation.

Antagonist	Inhibition of *Saprolegnia* growth (%)	
	On PDA medium	On Sabouraud's medium
Trichoderma viride	77 a[1]	66 a
T. harzianum	65 ab	56 ab
T. hamatum	47 b	43 b
Coniothyrium sp.	70 a	42 b

1 Values represent means of seven replicates. Means within a column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range test ($P = 0.05$).

3.2. Antagonistic effects of selected fungal isolates against *Aspergillus ochraceus*: The antagonistic effect of three *Trichoderma* species (*T. harzianum, T. viride, T. hamatum*) and *A. eichhorniae* against *A. ochraceus* was measured on PDA. Results showed that *T. harzianum* and *T. viride* were the most suppressive agents for the growth of *A. ochraceus*, causing 95 and 93% inhibition, respectively (Table 4). *T. hamatum* was the second best with regard to its inhibitory effect against *A. ochraceus* growth. However, *A. eichhorniae* had the least inhibitory effect or even did not inhibit the growth of *A. ochraceus* at all. These results are slightly different from that of Calistru *et al.* (1997), who found that *T. viride* had stronger inhibitory effect against *A. flavus* than *T. harzianum*.

3.3. Antagonistic effects of selected bacterial strains against *Aspergillus ochraceus*: Three strains of bacteria, *Pseudomonas fluorescens*, *Bacillus subtilis*, and unidentified strain were tested for their antagonistic activity against *A. ochraceus*. It was observed that *P. fluorescens* was the most suppressive strain against the growth of *A. ochraceus*, followed by *B. subtilis*, while the unidentified strain was the least effective one.

Table 4: Effect of *Trichoderma* spp. and *Alternaria eichhorniae* 5 (Ae5) on the growth of *Aspergillus ochraceus* on PDA five days after inoculation.

Antagonist	Inhibition of *Aspergillus ochraceus* growth (%)
Trichoderma viride	93 a[1]
T. harzianum	95 a
T. hamatum	80 b
A. eichhorniae 5 (Ae5)	57 c

1 Values represent means of eight replicates. Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range test ($P = 0.05$).

3.4.Antagonistic activity of the non-volatile metabolites from *Trichoderma* species against *Saprolegnia* sp. : The effect of non-volatile metabolites produced by *Trichoderma* species against *Saprolegnia* sp. was studied on Sabouraud's dextrose agar medium. The results revealed that *T. viride* grown on Sabouraud's agar medium produced bigger amounts of the non-volatile metabolites of antagonistic activity against *Saprolegnia* sp. than *T. harzianum*. Four days after seeding *Saprolegnia* sp. discs on Sabouraud's agar medium, where previously there was a cellophane disc carrying *T. viride* or *T. harzianum* grown for 72 hours, the growth of *Saprolegnia* was inhibited by 76 and 24% due to the non-volatile compounds produced into the culture medium by *T. viride* and *T. harzianum*, respectively in comparison with the control treatment. It was noticed also that the inhibition rate caused by the non-volatile compounds produced by *T. viride* was the same at day 2 and day 4 after inoculation of *Saprolegnia*, while the inhibition rate caused by the non-volatile compounds produced by *T. harzianum* double increased between day 2 and day 4 after inoculation of *Saprolegnia* (Table 5). The finding of Cherif and Benhamou (1990) that some aggressive *Trichoderma* spp. used as biocontrol agents are capable of producing either antibiotics or extracellular enzymes, or both, has provided crucial information in understanding the events associated with parasitism.

Table 5: Effect of non-volatile cultural metabolites of *Trichoderma viride* and *T. harzianum* on the mycelial growth of *Saprolegnia* sp. on Sabouraud's agar medium.

Antagonist	After 2 days		After 4 days	
	Average colony diameter (cm)	Inhibition of *Saprolegnia* growth(%)	Average colony diameter (cm)	Inhibition of *Saprolegnia* growth(%)
Check (no antagonist)	1.7 a[1]	-	2.1 a	-
Trichoderma viride	0.4 b	76 a	0.5 b	76 a
T. harzianum	1.5 a	12 b	1.6 a	24 b

[1] Values represent means of six replicates. Means within a column followed by the same letter are not significantly different according to Tukey's studentized range test ($P \leq 0.05$).

3.5. Antagonistic activity of the non-volatile metabolites from *Trichoderma* species against *Aspergillus ochraceus*: The effect of non-volatile metabolites from *Trichoderma* species against *A. ochraceus* was studied on PDA medium. The results revealed that four days after seeding *A. ochraceus* discs on the PDA medium, where previously there was a cellophane disc carrying *T. viride* or *T. harzianum* grown for 72 hours, the growth of *A. ochraceus* was inhibited by 29% due to the non-volatile compounds produced into the culture medium by *T. viride* and *T. harzianum* in comparison with the control treatment. It was noticed also that the inhibition rate caused by the non-volatile compounds produced by *T. viride* was approximately at the same level 4 and 6 days after inoculation of *A. ochraceus* while the inhibition rate caused by the non-volatile compounds produced by *T. harzianum* more or less double-increased between day 4 and day 6 after inoculation of *A. ochraceus* (Table 6).

Table 6: Effect of the cultural metabolites of *Trichoderma viride* and *T. harzianum* on the cultural growth of *Aspergillus ochraceus* on potato dextrose agar (PDA) medium.

Antagonist	After 4 days		After 6 days	
	Average colony diameter (cm)	Growth inhibition of *A. ochraceus* (%)	Average colony diameter (cm)	Growth inhibition of *A. ochraceus* (%)
Check (no antagonist)	2.5 a[1]	-	3.1 a	-
Trichoderma viride	1.7 b	32 a	2.2 b	29 a
T. harzianum	2.1 ab	16 b	2.2 b	29 a

[1] Values represent means of six replicates. Means within a column followed by the same letter(s) are not significantly different according to Tukey's studentized range test ($P \leq 0.05$).

No research has been carried out so far on using fungi as biocontrol agents for the fungal fish pathogens. Therefore, three *Trichoderma* species (*T. harzianum*, *T. viride*, *T. hamatum*) and *Coniothyrium minitans* were selected in our studies for testing their antagonistic activities against *Saprolegnia* sp. and/or *Aspergillus ochraceus* upon the reports of many researchers indicating their potential biocontrol against plant pathogenic fungi (O'Neill *et al.*, 1996 and Küçük and Kivanç, 2003). The finding that *Trichoderma* species inhibited *Saprolegnia* sp. and *A. ochraceus* growth has added to the potency of this fungal genus as a potential biocontrol agent for fish fungal pathogens as well. In order to attain practical and applicable results out of the antagonistic studies, conditions that are favored by the fish pathogen should be offered. Thus, Sabouraud's dextrose agar medium and low incubation temperature were provided for assessment of the antagonism against *Saprolegnia* growth, while PDA medium and warmer incubation temperature were furnished for determination of the antagonism against *A. ochraceus* growth. For this reason, *T. viride* was more suppressive against *Saprolegnia* sp. on PDA (less preferred medium by *Saprolegnia*) than on Sabouraud's dextrose agar (more preferred), causing 77 and 66% inhibition,

respectively. In general, various mechanisms have been proposed to explain the antagonistic activity of *Trichoderma* species against *Saprolegnia* sp. and *Aspergillus ochraceus*, namely production of antibiotics (Faull *et al.*, 1994) and hydrolytic enzymes (Lorito *et al.*, 1993) and mycoparasitism and hyphal disruption (Elad *et al.*, 1987). In the present study, one mechanism has been proven, i. e., the production of non-volatile metabolites by *Trichoderma* species against *Saprolegnia* sp. and *A. ochraceus*. In this regard, *T. viride* was more effective than *T. harzianum*, producing larger amounts of the non-volatile metabolites in the culture media.

Conclusion

Out of the present study, it can be concluded that *Trichoderma viride* is a promising biocontrol agent for the fish pathogens, *Saprolegnia* sp. and *Aspergillus ochraceus*. Further research and development of this biocontrol agent is required to establish culture parameters for scaled-up production for large scale applications and to develop it as a bio-fungicide in a form of granules, wettable powder or other suitable form.

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الفطريات المائية وإنتاج الأسماك فى مصر:
١ – دراسات معملية

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أثبت المسح الميكروبى المرتبط بأسماك البلطى النيلى أثبت وجود أكثر من ٢٠ عزلة فطرية تنتمى لأجناس وأنواع مختلفة، إضافة الى عزل ٥ أنواع بكتيرية من الأسماك المريضة بشكل طبيعى. ولقد أجريت محاولة لعزل فطر السابرولجنيا من الأسماك المصابة نسبيا ، وكذا من الماء والعلف من الأحواض السمكية المتأثرة بالسابرولجنيا وذلك فى شتاء ٢٠٠٢م، وأكتشفت السابرولجنيا بنجاح من الأسماك المصابة وماء الأحواض السمكية، لكن ليس من العلف. وفى صيف ٢٠٠٢م وجد فطر الأسبرجيلس أوكراشيوس مرتبطا بمرض فقع العين فى أسماك البلطى النيلى من الأحواض التجارية مكثفة الاستزراع فى محافظة كفر الشيخ. وهذا هو أول تقرير يسجل استخدام فطر التريكودرما فى التحكم البيولوجى فى الأمراض الفطرية للأسماك، ومن هذه الدراسة يمكن استخلاص أن فطر التريكودرما فيردى يعد وسيلة تحكم بيولوجية واعدة لمسببات أمراض الأسماك مثل السابرولجنيا والأسبرجلس أوكراشيوس.