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AQUATIC FUNGI AND FISH PRODUCTION IN EGYPT: II - *IN VIVO* STUDIES

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ABSTRACT

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*. It can be easily cultured and its inoculum can survive in water and significantly reduce saprolegniasis severity on the Nile tilapia fish. The fungus *T. viride* is safe and is also used for biological control purposes against plant pathogens. It can also be concluded that the fungus *Alternaria eichhorniae* (Ae5) which did not cause any mortality to the treated fish can be safely used for the biocontrol of water hyacinth with no negative effects on fish.

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

INTRODUCTION

Saprolegnia is ubiquitous in freshwater ecosystems and is the main genus of aquatic fungi responsible for significant fungal infection of freshwater fish and eggs. On fish, *Saprolegnia* invades epidermal tissues, generally beginning on the head or fins and can spread over the entire surface of the body (Willoughby, 1994). 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 the nephritic mycotoxin ochratoxin A, which may produce ochratoxicosis in humans and animals. 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 *in vivo* study the effect of the most effective biocontrol agent, obtained from the fore-mentioned laboratory experiments (Abdelhamid *et al*., 2008), on the average body weight, average length, and mortality rate of Nile tilapia fish in experimental aerated aquaria.

MATERIALS AND METHODS

The use of *Trichoderma viride as a* biocontrol agent for *Saprolegnia* sp. in an aquaria trial:

Nile tilapia fish at two immature stages (small size: ~ 3-g initial body weight, 5.5-cm long and medium size: 24-g initial body weight, 11.5-cm total length) with no history of disease before the onset of the experiment were used in this study. The experiment was conducted at Mansoura University Research Station. The tested fish were kindly obtained from a commercial earthen ponds farm located near Kafr El-Sheikh town (Tolombat No. 7). Fish were transported to the research station in air-pumped large clear polyethylene bags (for the small-size fish) or in uncovered hard plastic tanks (for the medium-size fish), both containing pond water. On arrival to the research station, fish were randomly distributed into 40 L cylindrical plastic aquaria filled with 30 L of de-chlorinated tap water and allowed to acclimate for 10 days. All aquaria were aerated using one air stone/aquarium connected to electrical compressor. The fish did not receive feed for the first two days and then were fed twice daily on 25% protein floating fish fodder (Joe Trade Co., Cairo, Egypt). From these stock-fish aquaria, batches of 12 health-looking fish of the small size or 3-4 fish of the medium size were taken at random and distributed into 40 L cylindrical plastic aquaria filled with 10 L of de-chlorinated tap water and left for one day before the addition of the fungi. Fish for each aquarium were weighed and the length of each one was measured right before distribution process.

Four aquaria (replicates) were prepared for each treatment. For the small-size fish, five treatments were applied: 1) no fungi was added to the aquaria water (Control I), 2) *Saprolegnia* alone was added at 4 x 10^5 zoospores/L (Control II), 3) *T. viride* alone was added at 4.5 x 10^7 conidiospores/L (Control III), 4) both of the pathogen, *Saprolegnia*, and the biocontrol agent, *T. viride* were added together at the same rates as above, and 5) *T. viride* was added first and one week later, *Saprolegnia* was added. For the medium-size fish, treatment 1 (Control I), treatment 2 (Control II), and treatment 4 (*Saprolegnia* and *T. viride* were added together to the

aquaria water) at the same rates as above. The inoculum of the *T. viride* was obtained from still cultures grown in 500-mL Erlenmeyer flasks containing 200 mL of potato dextrose broth (PDB) and incubated at 28 ^{o}C in the dark for two weeks. The inoculum of *Saprolegnia* (zoospores) was made as described above under item 3.1.

All aquaria were received about 1 kg of ice twice a day (at 11:00 AM and 4:00 PM) to keep the water temperature cool to favor the infection with *Saprolegnia*. Also, to induce infection, all fish groups including the negative control (Control I) were subjected to ami-momi (net-shake) treatment (Hatai and Hoshiai, 1994) 12 h before being exposed to fungi and 5 days after exposure to fungi. Fish were then fed at a rate of 1% of fish weight per day. The feeding was kept at this low rate to avoid any feed remain, which may lead to poorer water quality. Thus, changing of aquaria water can be avoided to keep the fungal inoculum at the same level without dilution. In this experiment, fish were starved for 3 days, shaken in the air in a fan-shaped scoop net for 2 minutes, and then rapidly rinsed with water (to eliminate excess mucus) before being returned to their aquaria. All aquaria were covered with fishing nets (4-10 mm knotless mesh) during the tests to prevent fish to jumping out of the aquaria. Five liters of the water in the aquaria (50% of aquarium water) were changed 10 days after the challenge, just to keep the fungal inoculum at a high level as long and as much as it can be.

The aquaria were checked each day after the challenge (defined as day 0) for 15 days, and dead and moribund fish were removed for examination. Air and water temperature were daily recorded every one hour from 8:00 AM to 8:00 PM. Dissolved oxygen (DO) (measured by using Lutron® DO-5509 dissolved oxygen meter), pH, mill volt (mV, measured by using a pH-mV meter), conductivity (CD, determined by using Lutron® CD-4301 conductivity meter), non-ionized ammonium (NH_3, determined as mg/L using Viscolor® ECO kits, Macherey-Nagel, Düren, Germany), and nitrites (NO_2, determined as mg/L using Viscolor® ECO kits, Macherey-Nagel, Düren, Germany) were measured four times during the experiment (at day 0, 1, 3, and 10). Number of the dead fish was recorded throughout the course of the experiment. All fish remaining at the end of the 15-day period were measured for weight and total length and removed for examination. Percent weight and length gain (or loss) and percent mortality and corrected mortality were calculated at the end of the experiment according to Abdelhamid (2009) and Abbot (1925), respectively.

The use of *Trichoderma viride as a* biocontrol agent for *Aspergillus ochraceus* in an aquaria trial:

The experiment was sat up as previously described under item 5. However in this experiment only the small-size Nile tilapia fish were used. Five treatments were applied: 1) no fungi was added to the aquaria water (Control I), 2) *A. ochraceus* alone was added at 4 x 10^7 conidia/L (Control II), 3) *T. viride* alone was added at 4.5 x 10^7 conidia/L (Control III), 4) both of the pathogen, *A. ochraceus*, and the biocontrol agent, *T. viride* was added together at the same rates as above, and 5) *T. viride* was added first and one week later, *A. ochraceus* was added. Fungal inocula of *A. ochraceus* and *T. viride* were obtained from still cultures grown in 500-mL Erlenmeyer flasks

containing 200 mL of potato dextrose broth (PDB) and incubated at 28 ^{o}C in the dark for two weeks. Number of the dead fish was recorded throughout the course of the experiment. All fish remaining at the end of the 15-day period were measured for weight and total length and removed for examination. Percent weight and length gain/loss and percent mortality and corrected mortality were calculated at the end of the experiment according to the fore-mentioned equations.

Effect of *Alternaria eichhorniae* 5 (Ae5) on the Nile tilapia fish in an aquaria trial:

In order to prove the possibility of using *A. eichhorniae* as a mycoherbicide for waterhyacinth in Egypt, the present study was conducted to verify the safety of using this fungus as a bioherbicide for waterhyacinth. The experiment was sat up as previously described under item 5. However, in this experiment only the small-size Nile tilapia fish were used. Three treatments were applied: 1) no fungi was added to the aquaria water (Control), 2) Ae5 prepared in aqueous suspension was added at 6.6 x 10^5 propagules/L, 3) Ae5 formulated in cottonseed oil emulsion was added at 6.6 x 10^5 propagules/L. Mycelia were obtained from still cultures grown on potato dextrose broth (PDB) at 28 ^{o}C in the dark for two weeks. Mycelial fragments and not spores were used because it was found in a previous study (Shabana *et al*., 1995a) that the efficacy of spore inoculum was equal to that of mycelial inoculum of Ae5; yielding a similar level of disease severity (DS) on water hyacinth plants. Number of the dead fish was recorded throughout the course of the experiment. All fish remaining at the end of the 15-day period were measured for weight and total length and removed for examination. Percent weight and length gain/loss and percent mortality and corrected mortality were calculated at the end of the experiment according to the fore-mentioned equations.

Monitoring the longevity of fungi used in the aquaria trials:

The viability of *Saprolegnia* sp., *A. ochraceus*, *T. viride*, and *A. eichhorniae* after using in the aquaria trials was monitored throughout the experiment. Samples of aquaria water (20 mL) were withdrawn (by sterile pipettes) from each treatment and dispensed in sterile 100-mL glass bottles. Samples were transported to the laboratory and processed within 2 to 3 hours of collection. Aqueous serial dilutions of aquaria water samples were plated on 9-cm agar plates containing PDA (for aquaria treated with *A. ochraceus*, *T. viride*, and *A. eichhorniae*) or containing Sabouraud's agar (for aquaria treated with *Saprolegnia* sp.). Each diluted sample was pipetted at 0.50 mL per agar plate and spread with a flame-sterilized glass rod. Two serial dilutions were made for each sample. Three replicates (plates) were used per sample dilution. Inoculated plates were incubated in the dark at 28 ^{o}C. Microbes recovered were counted 4 days after dilution plating. Calculations were made to express the numbers of colonies as colony forming unit per milliliter (CFU/mL). Working cultures of fungi were transferred to 9-cm PDA and Sabouraud's agar plates to allow development of mature and characteristic colonies of the recovered fungi to facilitate identification.

Statistical analyses:

The data were analyzed using SAS software package (SAS Institute, 1996). All multiple comparisons were first subjected to ANOVA. 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

The use of *Trichoderma viride* as a biocontrol agent for *Saprolegnia* sp. in an aquaria trial:

Trichoderma viride was used in this study because it provided the highest level of antagonistic activity against *Saprolegnia* in the *in vitro* studies. Water quality measurements including DO, pH, mV, CD, NH_3, and NO_2 during the experiment are presented in Table 1.

Table 1: Measurements of water characteristics in all treatment groups of the aquaria experiments at 0, 1st, 3rd, 10th day.

Treatment	DO (mg/L) after days				pH after days			
	0	1	3	10	0	1	3	10
Check (no fungi)	8.8	7.7	7.1	9	7.9	8.5	8.8	8.4
Aspergillus ochraceus	8.8	7.6	6.2	8.3	7.9	8.5	8.5	8
Trichoderma viride	8.8	8.0	6.3	7.5	7.9	8.4	8.3	8
Aspergillus + Trichoderma	8.8	NM	5.4	9	7.9	NM	8.5	8.5
Saprolegnia (small fish)	8.8	NM	7.8	9	7.9	NM	8.4	8.3
Sapro + TV (small fish)	8.8	NM	6.7	9.2	7.9	NM	7.9	8.8
Saprolegnia (medium fish)	8.8	NM	9	7	7.9	NM	8.4	8
Sapro + TV (medium fish)	8.8	NM	6.7	7.2	7.9	NM	7.8	7.7
Ae5 (aqueous formulation)	8.8	8.0	7.3	9	7.9	8.0	7.9	8.5
Ae5 (emulsion formulation)	8.8	8.2	8.7	9.1	7.9	8.6	8.4	8.6

Optimum DO and pH levels for the aquatic life are ≥ 5 mg/L and 6.5 – 9, respectively (Abdelhamid, 2009). NM = Not measured.

Table 1. Continued

Treatment	mV after days				CD (mS) after days			
	0	1	3	10	0	1	3	10
Check (no fungi)	- 64	NM	- 78	- 60.5	0.26	0.29	0.46	0.50
Aspergillus ochraceus	- 64	NM	- 67.5	- 51	0.26	0.35	0.46	0.48
Trichoderma viride	- 64	NM	- 60	- 39	0.26	0.29	0.47	0.48
Aspergillus + Trichoderma	- 64	NM	- 65.5	- 61.5	0.26	NM	0.46	0.49
Saprolegnia (small fish)	- 64	NM	- 57.5	-74	0.26	NM	0.44	0.41
Sapro + TV (small fish)	- 64	NM	- 31	- 75.5	0.26	NM	0.44	0.43
Saprolegnia (medium fish)	- 64	NM	- 65.5	- 38	0.26	NM	0.46	0.46
Sapro + TV (medium fish)	- 64	NM	- 37	- 31.5	0.26	NM	0.49	0.49
Ae5 (aqueous formulation)	- 64	NM	- 39	- 62	0.26	0.26	0.40	0.45
Ae5 (emulsion formulation)	- 64	NM	- 62.5	- 63	0.26	0.32	0.43	0.47

NM = Not measured

Table 1. Continued

Treatment	Ammonium (mg/L) after days				Nitrite (mg/L) after days			
	0	**1**	**3**	**10**	**0**	**1**	**3**	**10**
Check (no fungi)	3	NM	3	3	0.05	NM	0.05	1
Aspergillus ochraceus	3	NM	3	3	0.05	NM	0.05	1
Trichoderma viride	3	NM	3	3	0.05	NM	0.10	2
Aspergillus + *Trichoderma*	3	NM	3	3	0.05	NM	NM	2
Saprolegnia (small fish)	3	NM	3	3	0.05	NM	1	2
Sapro + TV (small fish)	3	NM	3	3	0.05	NM	NM	2
Saprolegnia (medium fish)	3	NM	> 3	> 3	0.05	NM	0.25	2
Sapro + TV (medium fish)	3	NM	3	3	0.05	NM	NM	2
Ae5 (aqueous formulation)	3	NM	0.2	3	0.05	NM	< 0.05	< 0.05
Ae5 (emulsion formulation)	3	NM	3	3	0.05	NM	0.50	1

NM = Not measured.

A significant finding was reported during monitoring the nitrite concentration in all treatments. In this regard, the nitrite concentration increased by the time in all treatments except in the one of *A. eichhorniae* (Ae5) in aqueous suspension, in which the nitrite level did not increase until the experiment was terminated.

For the small-size fish: Results showed that by the end of the experiment (two weeks after releasing fungi in aquaria water), only fish that exposed to *Saprolegnia* alone have lost weight (3.36 % in average per individual fish) while untreated fish (control I) or fish treated with *Trichoderma* alone or *Trichoderma* plus *Saprolegnia* have gained weight (range of increase between 1.51 and 5.4% per individual fish). Fish in all treatments have grown up by the end of the experiment and gained some length ranged from 5.45% (for fish treated with *Trichoderma* plus *Saprolegnia* added at the same time) to 7.27% per individual (for fish treated with *Saprolegnia* alone). The maximum mortality rate was obtained from fish treated with *Saprolegnia* alone (44.4 %), while no mortality occurred in fish group treated with *Trichoderma* plus *Saprolegnia* whether added simultaneously or subsequently (Table 2). This finding confirms the results obtained *in vitro* that *T. viride* markedly inhibits the growth of *Saprolegnia*, thus it can be used as an effective biocontrol agent for saprolegniasis. This is the first report of using *Trichoderma* for biological control of a fungal disease of fish.

For the medium-size fish: Results showed that by the end of the experiment (two weeks after releasing fungi in aquaria water), only fish that exposed to *Trichoderma* plus *Saprolegnia* have gained weight (1.37% in average per individual fish), while untreated fish (control I) or fish treated with *Saprolegnia* alone (control II) have lost weight (2.63 and 14.67% per individual fish, respectively). Fish in all treatment groups have grown up by the end of the experiment and increased in their body length which ranged from 4.28% (for fish treated with *Saprolegnia* alone) to 9.78% (for fish treated with *Trichoderma* plus *Saprolegnia*). The maximum mortality rate was obtained from fish treated with *Saprolegnia* alone (50 %), while 16.75% mortality occurred in the untreated fish group (control I) as well as to the fish

treated with *Trichoderma* plus *Saprolegnia*. By calculating the corrected mortality, it was found that *Saprolegnia* caused 40% of fish to die within the time of the experiment (15 days) (Table 3).

Table 2: Effect of *Trichoderma viride* on growth performance of Nile tilapia fish (3.19 g-initial body weight in average) exposed to *Saprolegnia* sp. in experimental aerated aquaria.

Treatment	Ave. body wt. (g)		Wt. gain or loss (%)	Ave. length (cm)		F/l Length (%)	No. dead fish	Mortality (%)	Corrected mortality (%)
	Initial	Final		Initial	Final				
Control I (No fungi)	3.31 a[1]	3.49 a	+ 5.4	5.6 a	5.8 a	+ 3.57	0.5	4.17 b	0.00
Control II (Sapro. only)	3.27 a	3.16 a	- 3.36	5.5 a	5.9 a	+ 7.27	6.0	44.44 a	42.00
Control III (TV only)	3.07 a	3.22 a	+ 4.88	5.5 a	5.8 a	+ 5.45	0.7	5.58 b	1.50
Sapro. + TV (added together)	3.30 a	3.35 a	+ 1.51	5.5 a	5.8 a	+ 5.45	0.00	0.00 b	0.00
Sapro.+ TV (Sapro. added 1 wk after TV)	3.02 a	3.10 a	+ 2.64	5.4 a	5.7 a	+ 5.55	0.00	0.00 b	0.00

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

Table 3: Effect of *Trichoderma viride* on growth performance of Nile tilapia fish (24.4 g initial body weight) exposed to *Saprolegnia* sp. in experimental aerated aquaria.

Treatment	Ave. body wt. (g)		Wt. gain or loss (%)	Ave. length (cm)		F/l Length (%)	No. dead fish	Mortality (%)	Corrected mortality (%)
	Initial	Final		Initial	Final				
Control I (No fungi)	23.88 a[1]	23.25	- 2.63	11.31	12.16	+ 7.51	0.67	16.75 b	0.00
Control II (Sapro. only)	22.28 a	19.01	- 14.67	11.19	11.67	+ 4.28	2.00	50.00 a	40.00
Sapro. + TV (added together)	26.91 a	27.28	+ 1.37	11.65	12.79	+ 9.78	0.67	16.75 b	0.00

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

Again, *Trichoderma* used as antagonist against *Saprolegnia* has been suggested to inhibit by three mechanisms: parasitism (deriving nutrients from the host); competition (for space and nutrients) and antibiosis (production of an inhibitory metabolites or antibiotics). While one mechanism predominates, the other two mechanisms may also play a role in the antagonistic behavior. Data obtained in this experiment has shown that medium-size fish were more susceptible to the infection with *Saprolegnia* than the small-size fish confirming the observations of Mr. Salah M. Ibrahim,

the owner of private fish hatchery and production ponds (personal communication).

The Use of *Trichoderma viride* as a Biocontrol Agent for *Aspergillus ochraceus* in an Aquaria Trial:

Trichoderma viride was used in this study because it provided the highest level of antagonistic activity against *A. ochraceus* in the *in vitro* studies. Water quality measurements including DO, pH, mV, CD, NH_3, and NO_2 during the experiment are presented in Table 1. The ammonium and nitrite concentration increased by the time in all treatments except in the one of *A. eichhorniae* (Ae5) in aqueous suspension, in which the nitrite level did not increase until the experiment was terminated. Results showed that fish in all treatment groups have lost weight with being the untreated fish (Control I) and the fish treated with *A. ochraceus* alone the most affected ones (8.28 and 9.81% weight loss, respectively, over the time of the experiment (15 days). Fish that exposed to *Trichoderma* plus *A. ochraceus* that were added simultaneously were significantly less affected (2.16% weight loss). Fish in all treatments, except the untreated one (control I), have grown up by the end of the experiment and increased in their body length ranging from 1.81% (for fish treated with *A. ochraceus* alone) to 11.53% (for fish treated with *Trichoderma* plus *A. ochraceus* that were added subsequently. However, the differences among groups were not significant. There were no significant differences between all treatments with regard to mortality rate. However, maximum mortality rate was obtained from fish treated with *A. ochraceus* alone (5.74 %), while no mortality occurred in fish group treated with *Trichoderma* plus *A. ochraceus* whether added simultaneously or subsequently (Table 4).

Table 4: Effect of *Trichoderma viride* on growth performance of Nile tilapia fish (3.29 g initial body weight in average) exposed to *Aspergillus ochraceus* in experimental aerated aquaria.

Treatment	Ave. body wt. (g)		Wt. gain or loss (%)	Ave. length (cm)		F/l Length (%)	No. dead fish	Mortality (%)	Corrected mortality (%)
	Initial	Final		Initial	Final				
Control I (No fungi)	3.74 a[1]	3.43 a	- 8.28 a	5.8 a	5.8 a	0.00 c	0.33	2.75 a	0.00
Control II (Asp. only)	3.26 ab	2.94 ab	- 9.81 a	5.5 ab	5.6 a	+ 1.81 c	1.00	8.33 a	5.74
Control III (TV only)	3.32 ab	3.14 ab	- 5.42 ab	5.6 ab	5.9 a	+ 5.35 bc	0.33	2.75 a	0.00
Asp. + TV (added together)	3.24 ab	3.17 ab	- 2.16 b	5.6 ab	6.0 a	+ 7.14 ab	0.00	0.00 a	0.00
Asp.+ TV (Asp. added 1 wk after TV)	2.89 b	2.70 b	- 6.57 ab	5.2 b	5.8 a	+ 11.53 a	0.00	0.00 a	0.00

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

These data show that there are no apparent negative effects caused by *A. ochraceus* on the treated fish during the course of the experiment (15 days). However, longer time of exposure may result in some harmful effects in terms of some histopathological signs that should have been examined.

Effect of *Alternaria eichhorniae* 5 (Ae5), a biocontrol agent for waterhyacinth, on the Nile tilapia Fish in an Aquaria Trial:

In order to use *A. eichhorniae* as a bioherbicide for waterhyacinth, this fungus was first tested on fish to confirm its safety on fish. Water quality measurements including DO, pH, mV, CD, NH_3, and NO_2 during the experiment are presented in Table 1. significant finding was reported during monitoring the nitrite concentration in all treatments. In this regard, the nitrite concentration increased by the time in all treatments except in the one of *A. eichhorniae* (Ae5) in aqueous suspension, in which the nitrite level did not increase until the end of experiment. This may indicate that the fungus *A. eichhorniae* can use up the nitrite as a nitrogen source, which will lead to the improvement and upgrading of water quality. This criterion will add to the advantages of using Ae5 for the biocontrol of water hyacinth. Results showed that fish treated with Ae5 as aqueous suspension have gained weight (5.91% per individual fish, in average) whereas the untreated control fish or fish treated with Ae5 formulated in oil emulsion have lost weight (2.02 and 2.07% weight loss, respectively), however, there were no significant differences among all treatment groups in this regard. Fish in all treatment groups have grown up by the end of the experiment and increased in their body length ranging from 5.35% (for fish treated with Ae5 formulated in oil emulsion) to 8.92% (for fish treated with Ae5 in aqueous suspension). However, there were no significant differences among all treatment groups in this regard. The fungus Ae5 in both formulations (in aqueous suspension or in oil emulsion) had no adverse effects on the populations of the tested fish whether from the actively growing mycelium or the fungal metabolites (pigment). It did not cause any mortality to the treated fish during the course of the experiment (15 days). This finding is of great importance providing evidence for the safety of Ae5 on fish. Thus, Ae5 can be successfully used for the biocontrol of water hyacinth without any harm effects on fish performance.The inoculum concentration used for Ae5 in this test was equivalent to the rate used in the field (on water hyacinth). In actual usage the inoculum is applied to the leaves of the water hyacinth and is not injected into the water. Therefore, it is highly unlikely that the inoculum concentration of the fungus in water would reach the concentration level of the one applied on the weed leaves. Therefore, at the rates of inoculation currently used in the field trials, it is evident that the use of Ae5 as a biological control of water hyacinth would not adversely affect populations of the Nile tilapia in waters where water hyacinth were being treated. However, some considerations may have to be included in future tests of fungal biocontrol organisms used in the aquatic environment concerning the possible consumption of inoculum by fish or other consumers (Table 5).

Table 5: Effect of *Alternaria eichhorniae* (Ae5), a biocontrol agent for water hyacinth on growth performance of Nile tilapia fish (3.18 g- initial body weight in average) in experimental aerated aquaria.

Treatment	Ave. body wt. (g)		Wt. gain or loss (%)	Ave. length (cm)		F/I Length (%)	No. dead fish	Mortality (%)	Corrected mortality (%)
	Initial	Final		Initial	Final				
Control (No fungus)	2.96 a	2.9 a	- 2.02 b	5.4 a	5.8 a	+ 7.40 a	0.33 a	2.75 a	0.00
Ae5 - Aqueous suspension	3.21 a	3.4 a	+ 5.91 a	5.6 a	6.1 a	+ 8.92 a	0.00 a	0.00 a	0.00
Ae5 – Oil emulsion	3.37 a	3.3 a	- 2.07 b	5.6 a	5.9 a	+ 5.35 a	0.00 a	0.00 a	0.00

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

Monitoring the longevity of fungi used in the aquaria trials:

Trichoderma sp. was the dominant fungus that was recovered from water samples of all treatments, except those treated with *Saprolegnia* alone or *Aspergillus* alone. It was recovered in concentrations ranged from $1x10^4$ cfu/L (in the untreated controls) to $1x10^8$ cfu/L (in aquaria treated with *Aspergillus* + *T. viride*). One week after inoculation, the concentration of *Saprolegnia* in water treated with *Saprolegnia* alone or *Saprolegnia* + *T. viride* was higher than its initial concentration (at zero time) (4×10^5 cfu/L initial concentration at zero time versus $1x10^7$ cfu/L one week later). This finding is logical since *Saprolegnia* is a water mold that can reproduce and proliferate in aqua. n the other hand, *Aspergillus ochraceus* viability decreased by 40% one week after its addition to water (4×10^7 cfu/L initial concentration at zero time versus $1.6x10^7$ cfu/L one week later). This finding may indicate that *A. ochraceus* can survive in water for a period of time during which it may directly harm fish or contaminate water with its hazardous toxins (ochratoxin). Normally *A. ochraceus* can get into pond water by offering contaminated feed to fish. *lternaria eichhorniae* (Ae5) whether prepared in aqueous suspension or in oil emulsion was added at 6.6×10^5 propagules/L at the zero time, but could not be recovered from aquaria water one week after its addition. Instead, *Trichoderma* was recovered from the same aquaria after one week in a concentration of 1×10^6 cfu/L (from water treated with Ae5 in aqueous suspension) or 1×10^7 cfu/L (from water treated with Ae5 in oil emulsion) (Table 6). The finding that Ae5 was not recovered from aquaria water that was treated with it one week earlier and the recovery of *Trichoderma* instead can be interpreted by the fact that *Trichoderma* [which coincidently cross contaminated the Ae5-treated water due to moving devices (pH meter and conductivity electrodes) between treatments, from an aquaria to another, without surface sterilization] is a fast growing fungus which can vigorously compete for the space and nutrients especially with a slow growing fungus such as Ae5, resulting in covering the whole surface of the culture plate not allowing Ae5 to even grow or emerge. This

interpretation is supported by the finding that the nitrite level in Ae5-treated aquaria continued to be low up to the last reading 10 days after treatment in comparison with the control treatments, which indicates the existence of Ae5 viable (alive) in water.

Table 6: Viability of fungi utilized in the aquaria trials one week after release in aquaria water.

Treatment	Fungi recovered from aquaria water samples	Fungal concentration (cfu/L)	
		At 0 time	After 1 week
Control (no fungi) with icy water	*Trichoderma*	0	$1x10^4$
Control (no fungi) with non-icy water	*Trichoderma*	0	$1x10^4$
Saprolegnia only	*Saprolegnia*	4×10^5	$1x10^7$
T. viride only	*Trichoderma*	4.5×10^7	$1.5x10^7$
Aspergillus only	*Aspergillus*	4×10^7	$1.6x10^7$
Saprolegnia + T. viride	*Saprolegnia* *Trichoderma*	4×10^5 4.5×10^7	$1x10^7$ $1x10^7$
Aspergillus + T. viride	*Aspergillus* *Trichoderma*	4×10^7 4.5×10^7	$1x10^7$ $1x10^8$
Alternaria eichhorniae in aqueous suspension[1]	*Trichoderma*	0	$1x10^6$
Alternaria eichhorniae in oil emulsion[2]	*Trichoderma*	0	$1x10^7$

[1]Ae5 prepared in aqueous suspension was added at 6.6×10^5 propagules/L at the zero time but could not be recovered from aquaria water one week after its addition. Instead, *Trichoderma* was recovered from the same aquaria after one week.

[2]Ae5 formulated in cottonseed oil emulsion was added at 6.6×10^5 propagules/L at the zero time but could not be recovered from aquaria water one week after its addition. Instead, *Trichoderma* was recovered from the same aquaria after one week.

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الفطريات المائية وإنتاج الأسماك فى مصر :
٢- دراسات حيوية

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فى دراسة معملية على أسماك البلطى النيلى، تم تجريب استخدام المكافحة البيولوجية لبعض مسببات الأمراض الفطرية للأسماك (سابرولينا و الأسبرجيلس أوكراشيوس)، فاستخدم فطر التريكوديرما فيريدا الذى أثبت حياتية عالية فى الماء وخفّض بشدة من الإصابة السمكية بالسابرولينا، فالتريكوديرما فيريدا فطر آمن كما يستخدم فى المكافحة البيولوجية لمسببات الأمراض النباتية. كما أثبت فطر الألترناريا أيكورنيا أمانه على الأسماك، فلم يسبب أى نفوق سمكى، وهو مستخدم فى المكافحة البيولوجية لنبات ورد النيل بدون أضرار على الأسماك فى البيئة المائية.