Green tea extract Role in removing the Trichodina sp. on *Oreochromis niloticus* fry in the Egyptian fish hatcheries

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Abstract: Trichodina sp. are the most common ciliates parasite present on the skin of pond-reared fish. The green tea extract (GTE) can be economical valuable and effective to remove the ectoparasitic ciliate Trichodina sp. from the skin and fins of infested *Oreochromis niloticus* (*O. niloticus*) fry . Bath of infested *O.niloticus* fry (body weight 0.2–0.9 g) exposed to 0.05% GTE for 15 minute (min) or 0.9% GTE for 5 min resulted in a decrease of 80 % and 95 % of the number of parasites on the skin (dorsal and anal) and fins in comparison to 70 % untreated controls (statistically significant, $p < 0.01$).No parasites were present on the skin surface of infested *O.niloticus* 24 hours after baths . Neither the long (0.05 % GTE for 15 min) or short bath treatment (0.9% GTE for 5 min). No mortality was observed in the normal fry. Large-scale application of the treatments with GTE (60,000 *O.niloticus* fry, 0.2–0.4 g) infested with the parasite in hatchery showed significant decreases of 80 % or 95 %, 0.05 % GTE for 15 min or 0.9 % GTE for 5 min respectively. Cumulative mortalities for 48 h after the treatments were 0.08–0.15 %. The 5 min bath with 0.9% GTE was more efficient than the 15 min bath with 0.05% GTE, as estimated from the results at ponds. This study concluded that GTE treatment of infested fry of *O. niloticus* is economic, safe, effective, and applicable to treatment of large numbers of fish in hatchery. [Report and Opinion 2010;2(8):77-81]. (ISSN: 1553-9873).

Key words: Nile tilapia, Green tea extract ;hatchery ; *Trichodina sp; O.niloticus*; fry

1-Introduction:

Tilapia (*O. niloticus*) is one of many economical freshwater fish that are cultured worldwide. The especially heavy infection of Trichodina sp. in small fish has caused gigantic financial losses. **Pinkate et al.,2003** reported that every single tilapia raised by farmers in Chiang Mai, Thailand has a Trichodina infection. This problem was significantly related to the high organic matter in water. Trichodiniasis used to be effectively controlled with formalin, but it now appears insufficient to control this parasitic infection **Madsen et al., 2000b.** This leads to increased needless cost and possibly causes undesired toxic residues in fish flesh and in the environment **Jung, 2001.**There is a fast growing interest in screening antiparasitic substances from plants to replace chemical and antibiotic alternatives.

Trichodina sp. are the most common ciliates parasite present on the skin of pond-reared fish . Heavy infection of the parasite occur frequently in hatchery-reared *O.niloticus* and fry in fresh water and causes a serious problem in Egypt **Abd El-Megiuid 1989.** The treatment of ectoparasitic diseases in freshwater fish with formalin seems at present to be insufficient. For this reason it is evidently a useless cost. In addition, formalin possibly leaves toxic residues in fish flesh and harmful to consumers. A freshly prepared potassium permanganate 3 ppm for infested tilapia was the more common and used safely to control ectoparasitic infestation in Egypt **Eissa, 2002**. However, because the pharmaceutical law in Egypt prohibited the therapeutic use of potassium permanganate, alternative methods for controlling the infection are needed. At present time many chemicals effective against *Trichodina sp.* are approved for uses in aquaculture in Egypt with high risk due to presence of organic matter .The alternative way are adoptive to solve this problem by using traditional medicinal plants. Several extracts and pure compounds obtained from plants have been reported to

antiprotozoan activities without side effects **Asres et al., 2001. Madsen et al., 2000a** reported that raw and squeezed garlic (Allium sativum) at 200 mg/l had potential to treat *trichodiniasis* in eel. Also, Kunio Suzuki et al.,2006 reported the efficacy of green tea extract (GTE) on removal of the ectoparasitic flagellate *Ichthyobodo necator* from the fins and skin surface of infected chum salmon, Oncorhynchus keta, and masu salmon, O. masou, fry. Epigallocatechin gallate (EGCg) and gallocatechin gallate purified from the aqueous extract of green tea (the dried leaf and bud of Camellia sinensis) have shown killing activity against a parasitic flagellate, Trypanosoma cruzi, the causative agent of Chagas' disease in humans **Paveto et al.,2004**. The polyphenols mangiferin and (–)-epigallocatechin-3-gallate (EGCG)have additionally been shown to have potent effects against protozoan and helminth parasites infesting fishes **Meckes et al., 1999, Iglesias et al., 2002 and López-Vélez et al.,2003.**

The present study investigates the efficacy and safety of green tea extract, GTE for control of Trichodina sp. infested fry of *O. niloticus* in hatchery.

2- Materials and Methods

Green tea extract (GTE)

Green tea leaves was obtained from, ISIS Co. for food products, Heliopolis, Cairo, Egypt, water extract of green tea leaves was one at 80 °C after 20 min (97%) according to **Amra Perva-Uzunalić et al.,2005**.

Fish

Atotal of 40000 *O.niloticus* fries (0.2 - 0.9 g) experimentally infested with Trichodina sp. and 20000 *O .niloticus* fries (0.2 - 0.9 g) parasitic free was obtained from Kafr El Sheikh governorate fish hatchery, were divided into equal 3 group in 3 ponds, each one 3×8 square meter . Infestation was determined by light microscopy as described below. Approximately fries in each group in the hatchery ponds were supplied with well water and fed daily with commercial dry pellets (25% protein) at 10 % body weight per day. The water temperature was constant at 25°C. Aeration was supplied to each pond.

Efficacy tests of GTE

At the beginning of the study, A safety of GTE bath to 200 normal *O.niloticus* fries (0.2 - 0.9 g) in four a aquariums (50 fries in each) contain, 0.05%,0.5%,0.9% and 1% GTE for 5,15,30,60 min at 24 h after the treatments were recorded a cumulative mortality **(Table 1)** .

For long baths, group one (20000 *O. niloticus* fries) experimentally infested with Trichodina sp. were immersed in well water containing 0.05 % GTE (w/v) for 15 min with aeration in a pond water. For short baths, group two (20000 *O. niloticus* fries) experimentally infected with Trichodina sp. were bathed in well water containing 0.9 % GTE for 5 min with aeration in still water, at 25°C with aeration. The number of parasites was counted on thirty of the surviving fry.

Parasitological examination

In order to confirm the efficacy of the treatments in removing the parasites from the skin and fins surface, 10 *O.niloticus* fries infested with Trichodina sp. ,24 h after bath in 0.05% GTE for15 minute and 0.9% GTE for 5 minute in treated group one and two respectively were examined. Ten infected *O.niloticus* fry infected with *Trichodina sp* were examined in the control group under binocular microscope, magnification of 40×. The mortality for 48 h before and after the treatment was recorded.

3- Results and Discussion

Among Tilapia species produced in Egypt, is the most abundant: more than one billion fry are reared in hatcheries and annually released into farms and Lakes in Egypt (GAFRD), 2008.The clinical examinations of skin and fins of heavily infested fries with Trichodina sp. (Fig. 1), revealed, anorexia, debilitated lose condition, and mortality can be much higher, Noga 1996. The remove of the parasite from host fish is thus important for Nile tilapia fry production .The medicinal plants are more economical than formalin and, for this reason, the cost of treatments would be reduced. However, Trichodina sp. became re-apparent after two weeks. They are possibly able to cause water deterioration and would be exchange the water a day after treatment as shown in Madsen, et al.,2000b to eradicate Trichodina sp. infestation. The active ingredient for this treatment is needed to identify and find out the effective dosage before commercial application. Additionally, it is difficult to eradicate all Trichodina sp. infestation from the system. Trichodiniasis is primarily a problem of overstocking and poor water management; for this reason, the proper stocking density and water quality management is

strongly required to relieve this problem. Madsen et al., 2000a suggested that the infection pressure from Trichodiniasis in farms with a relatively high load of organic matter may be relieved by reducing the content of organic dry matter in the processed water.

The optimal concentration of GTE and durations of exposure for removal of Trichodina sp. from the skin surface and fins of O.niloticus fries was 0.9% for 5 min and the minimum concentration was 0.05% for 15 min Kunio Suzuki., 2006 .

A bath of infested O.niloticus fries in 0.9 % and 0.05 % GTE for 5 and 15 min resulted in a decrease of Trichodina sp infest O.niloticus fries (0.2 - 0.9 g) were 90 % and 80 % , respectively. The cumulative mortalities of the treated O.niloticus fries in GTE 0.9 % and 0.05 % GTE for 5 and 15 min decreased to 5 % and 20 %, respectively. The fish untreated with GTE showed 30% cumulative mortalities **(Fig. 2 and Table 2**). The field results suggest the efficacy of the treatments for a large number of fish in hatcheries. The baths with higher concentrations of GTE and shorter exposure times would be more efficient, less time-consuming, and less labor-intensive than long baths with lower GTE concentrations and longer exposure times. The long baths in a pond lead to stress to fries, whereas the short baths in ponds lead to death of Trichodina without stress on fries. Nevertheless, the short bath treatments are more efficacious for removal of the parasite and thus would be useful when heavy infection occurs. In consideration of efficiency, and safety, it was predicted that bath of 0.9% GTE for 5 min were the optimal conditions for the practical treatments in hatcheries.

Moreover, the infested fish showed relatively low cumulative mortalities (5–20%) due to the treatments except for the infested *O.niloticus* fry treated with 0.05% and 0.9% of GTE for 15 and 5 min respectively. These results indicate the safety of the treatments under the optimal conditions described above studies will be necessary to evaluate the other effects of the treatments on the physiological status of *O.niloticus* fries.

The results of field trials showed that both the long (0.05% GTE for 15 min) and short (0.9% GTE for 5 min) bath treatments were applicable to control Trichodina sp. infestations in 20,000 *O.niloticus* fry reared in pond of hatcheries. The 5 min bath with 0.9% GTE was more efficient than the 15 min bath with 0.05% GTE, as estimated from the results at ponds. Low cumulative mortalities within 48 h after treatment (5-15%) confirmed the safety of both treatments **Asres et al ., 2001**. Moreover, adaptability of *O.niloticus* fry treated in Hatchery, just before release into farms confirmed its efficacy in hatcheries.

In conclusion， GTE can be used as an alternative to chemicals to treat and control of Trichodina sp. infestations of *O.niloticus* fry in hatchery and applicable to large numbers of fries in hatchery under the natural conditions.

Further studies, including the chronic effect on growth, survival rate, and reproduction need to be investigated. The mechanism of these plants on the non-specific immune responses must be conducted before acceptance for field treatment.

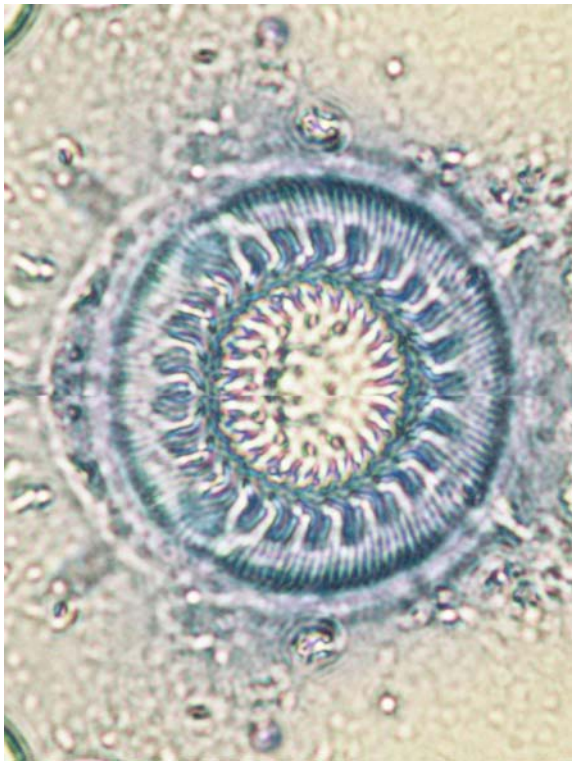

Fig (1): Wet mount of *Trichodia sp.* affect *O.niloticus* fry.

Table 1: Safety of green tea extract (GTE) on fry of O. niloticus

Concentration of GTE in ponds %	Times of bath/ minute	Mortality rate %	Times of bath/ minute	Mortality rate %	Times of bath / minute	Mortality rate %	Times of bath / minute	Mortality rate %
0.05	1	4	5	18	15	6	30	36
0.5	1	3	5	8	15	9	30	22
0.9	1	2	5	5	15	18	30	24
1	1	5	5	21	15	23	30	39

Table 2: Effects of green tea extract (GTE) on infested fry of O.niloticus with Trichodina sp

Ponds	Green tea extract concentration	Period of immersion/minute	No. of fries infected with Trichodina sp	No. of mortality 48 hours before treatment	% of mortality 48 hours before treatment	No. of mortality 48 hours after treatment	% of mortality 48 hours after treatment
1	0.05%	15	2000	480	24 %	114	5.58 %
2	0.9%	5	2000	490	24.5 %	132	6.60 %
3	----------	--------	2000	450	22.5 %	560	28 %

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