Application of some medicinal Plants to eliminate *Trichodina sp* in tilapia (*Oreochromis niloticus*)

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Abstract: Medicinal plants are important elements of traditional medicine in virtually all cultures and product promise a cheaper source for therapeutics, greater accuracy than chemotherapeutic agents and a viable solution for all problems which groupers culture faces today. The control of *Trichodiniosis* and *Aeromonas hydrophila* in ponds of stocked tilapia with any antiprotozoal and antibacterial agent at present is evidently a cost. In addition, possibly leaves toxic residues in tilapia and mortality. Also, affect for a short times. For the previous reasons, the medicinal plants instead. Garlic (*Allium sativum*) and *Artemisia vulgaris* as optional medicinal plants to treat fish *Trichodina* sp and *Aeromonas hydrophila* was done. 350 Tilapia *Oreochromis .niloticus* (average weight 100 ±20 g) derived from a private fish farm in Kafr El Sheikh governorate infested with Trichodina sp, were kept in cement ponds (3×8 metre) and six diets were formulated to contain different levels of *Allium sativum* extract (1, 4 and 8g/kg diet) and *Artemisia vulgaris* extract (1, 3, and 4.5g/kg diet) added. The results showed that crude extracts of either garlic or *Artemisia vulgaris* at 800 mg/l significantly ($P < 0.05$) eliminated *Trichodina* sp and *Aeromonas hydrophila* infections in tilapia. Garlic and *Artemisia vulgaris* will be used as an alternatives to chemicals to treat of infected tilapia with *Trichodina and Aeromonus* sp. [Ahmed I.E. Noor El Deen and Razin, A.Mohamed. **Application of some medicinal Plants to eliminate Trichodina sp in tilapia (Oreochromis niloticus).** *Report and Opinion*. 2009;1(6):1-5]. (ISSN 1545-4570).

Key words: *Trichodina* sp, *Aeromonas hydrophila* , tilapia, medicinal plant, garlic, *Artemisia vulgaris*

1. Introduction:

Tilapia (*Oreochromis niloticus*) is one of many economical freshwater fish that are cultured worldwide and the third most commonly farmed fish after carp and salmon with global production of 1.49 million metric tonnes (mmt) in 2002, and Farmed tilapia is exceeded two million metric tons in 2004 worldwide (Fitzimmons, 2005). Today plant materials are present in, or have provided the models for 50% of western drugs (Robbers et al., 1996). At first, tilapia was considered to be more resistant to bacteria and parasitic diseases than other species of cultured fish. However, in more recent times, tilapias have been found to be most susceptible to both bacterial and parasitic diseases (Pereira et al., 2008). Common tilapia pathogens are *Aeromonas hydrophila* and *Trichodina* sp. *Aeromonas* and *Trichodina* sp in tilapia has become an increasing problem and are leading to diseases that cause severe economical impact. Chemotherapy is widely used to control infectious bacterial and parasitic diseases. The use of chemicals in treating health problems has also been complicated by the misleading advice provided to the farmers by feed and chemical companies regarding the use of antibiotics and other therapeutic drugs and not be used in developing countries as well as using extended to developed countries (Kruse and Soram ,1994) . Plant extracts decrease the selective pressure for developing antibiotic resistance (Lewis and Ausubel, 2006). The screening of plant extracts and natural products for antimicrobial activity has shown that higher plants represented a potential source of new anti-infective agents (Press, 1996). Controlling of *Trichodiniosis* with freshly prepared Potassium permanganate (Eissa, 2002), but it now very expensive and of no effect in the presence of organic matter. In correspond; there is a fast growing interest in screening antiparasitic and antibacterial substances from plants to replace antiparasitic and disinfectant alternatives. Two such plants are garlic and *Artemisia vulgaris*. Garlic is one of the edible plants that had a strong interest to scientists and recognized as an important medicinal plant which has a wide spectrum of actions; not only antibacterial and antiprotozoal, but also has beneficial effects on the immune systems (Harris et al., 2001). In addition to their effects, activities against the variety of Gram-negative and Gram-positive were and continue to be extensively investigated (Whitemore & Naidu, 2000). A wide range of microorganisms including bacteria and protozoa have been shown to be sensitive to crushed garlic preparations and can help in the control of pathogens, especially bacteria, and increase the welfare of fish (Sivam,,2001 and Corzo-Martinez et al, 2007;). Also, Madsen et al. (2000b) reported that raw and squeezed garlic (*Allium sativum*) at 200 mg/l had potential to treat *Trichodiniosis* in eel. *Artemisia vulgaris* is another

plant that is promising to prevent fish diseases (Shagnliang et al, 1990) and (Direkbusarakom, 2004), thus their attention to plants for medicinal use. Using the crude extract from either garlic or *Artemisia vulgaris* are one of the new challenging methods for *Trichodiniosis* treatment. The aim of this present research was to determine the efficacy of garlic and *Artemisia vulgaris* as cheap antibacterial medicinal plants alternatives to control *Trichodiniosis* epizootic in tilapia.

2- Materials and Methods

2-1-Fish

350 Tilapia *O.niloticus* (average weight 100 ±20 g) derived from a private fish farm in Kafr El Sheikh Governorate infested with *Trichodina sp*, were kept equally in seven cement ponds (2×3 meters), and supplied with well-aerated freshwater using compressed air. Cement ponds were daily cleaned, and the water exchange daily, including fish feces and remaining food, was approximately 25% of the total volume. The water temperature was adjusted (26-27°C) by a thermostat column heater in each pond, for the time of study.

2-1-Diets

Sex groups of diets were formulated to contain different levels of *Allium sativum* extract (1, 4 and 8g/kg diet) and *Artemisia vulgaris* extract (1, 3, and 4.5g/kg diet). Control diet was free from both *Allium sativum* and *Artemisia vulgaris*. Diets were formulated from ingredients commercially available in Egypt.

2-3-Extracts

Garlic and *Artemisia vulgaris* collected and dried in darkness. The air-dried and finely ground sample of each samples were extracted (Lee et al.,2004) .A500 g dry weight sample of each samples was washed, mined and added adequate amount of water to concentration of 12.5% (w/v), respectively, the ground in a blender. The extracts were passed through a 0.2 um filter. The procedures of extraction and filtration were operated at room temperature and then the sterilized filtrates were stored at 4 °C and used in antibacterial assay (Chechregani et al., 2007). Also, the same the extracts was used as antiprotozoal according to Asha, et al (2008) and Abdul Mannan, et al (2008). A commercial pellet with different types and dose of extracts were given for 15 days.

2-4-Parasitic Examination

Mucus was scraped from total surface of skin and gills for all examined tilapia groups after 5, 10 and 15 days of treatment for parasite infections in the next two and four weeks.. The degree of infestation of *Trichodina* sp. light (less than 10), moderate (10-20) and heavy (over than 20) from mucus and gills was subsequently counted under a microscope.

2-5-Challenge experiment

After treatment, five fishes from each treated group and five fish from the control were examined and determined to be free from bacterial infection ,were then artificially infected by interaperitonal injection with 0.5 ml of culture suspension of pathogenic *Aeromonas hydrophila* containing 109 bacteria ml-1 that were previously isolateted from moribund fish. A culture suspension of *Aeromonas hydrophila* was prepared by culturing in agar for 24h, washed and suspended in saline (0.85%) and counted using McFarland standard tubes (No.1). The mortality % was recorded up to day 10 post-challenge.

2-6-Statistical analysis

Data were analyzed by analysis of variance using the SAS program . Duncan's multiple-range test (1955) was used to verify significance of the mean differences among treatments.

3-Results

The examined *O. niloticus* are suffered from peticeal hemorrhage on the head, trunk region, fins and ulcers on skin Fig 1. The postmortem lesion was congestion in the gills and internal organs. As well as, enlarged of gall bladder and engorged with bile.

3-1-Parasitological results

The plant extracts, garlic and *Artemisia vulgaris* had antiparasitc affecting against *Trichodina sp*. Showed antiprotozoal activity against *Trichodina sp* as seen in Table 1.

3-2-Survival rate

Survival decreased in control group (30 %) up to 45 days after challenge infection. However, this was increased in the garlic treatment group to 60,70 and 80 % survivability in treated groups with 1,4 and 8 g garlic/ kg diet respectively and 62, 70 and 84 % survivability % in treated groups with 1,3and 4.5 g *Artemisia vulgaris* / kg diet respectively as in Table 2 .

Fig 1. Showing peticeal hemorrhage and ulcers *Oreochomonis niloticus*.

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Table 1. Antiprotozo a activity of plant extracts against *Trichodina sp* in tilapia (O.niloticus)

Pond fish	**day -5 after treatment**	**day- 10 after treatment**	**day –15 after treatment**
Control	Slight	Moderate	Heavy
1g garlic	Slight	Slight	Slight
4 g garlic	Slight	Slight	……
8 g garlic	…….	…….	……
1 g Artemisia vulgaris	Slight	Slight	Slight
3 g Artemisia vulgaris	Slight	Slight	…….
4.5g Artemisia vulgaris	……	…….	…….

Table 2. Antibacterial activity of herbal plant extracts against *Aeromonas hydrophilla*

Pond fish	**No. of fish in pond**	**15-day post treatment**	**30-day post treatment**	**45-day post treatment**	**Total No.**	**Total %**
Control (water)	50	20±4.10	10±1.10	15±1.50	35	70
1 g. Garlic	50	12±2.10	5±0.50	3±0.30	20	40
4 g garlic	50	10±1.10	3±0.3	2±0.2	15	30
8 g garlic	50	6±0.55	4±0.40	……..	10	20
1 g Artemisia vulgaris	50	12±1.2	5±0.50	2±0.02	19	38
3 g Artemisia vulgaris	50	10±1.0	5±0.50	…….	15	30
4.5 g Artemisia vulgaris	50	7±0.7	1±0.1	…….	8	16

$Chi^2 = 13.55^*$

4-Discussion

Clinical signs of a heavy infested tilapia *O.niloticus* with *Trichodina sp* has caused gigantic financial losses, letharagic, generate excessive mucus may be attributed to dilute the irritation as a defensive mechanism against pathogens as recorded by Chitmanat et al,2005. The clinical infestation of challenged tilapia *O.niloticus* with *Aeromonas hydrophila* as peticeal hemorrhage on the peduncle region, fins, trunk and scales due to reaction of bacterial toxin and postmortem finding were congesion in gills, internal organs, these findings recorded by Cipriano,2001 who reported that *A.hydrophila* is highly pathogenic bacteria in the cultured and wild fish. Garlic is an important vegetable extensively cultivated in many countries. Artemisia vulgarism might provide a suitable basis as a new antiparasitic action (Navarro et al.,1996). Otherwise the plant extracts have antimicrobial activity against fish pathogenic bacteria. Jinist,2002 . Either garlic 800 mg or *Artemisia vulgarism* at 450 mg/kg was able to remove all *Trichodina sp*. from tilapia after 2 days treatment. All treated group were significantly different from control group. Both garlic and *Artemisia vulgaris* are more economical and effective in the presence of organic matter for along time than freshly prepared Pot.permanganate .These may be attributed to chemical reaction between Pot.permanganate and organic matter. For this reason, the cost of treatments would be reduced. However, *Trichodina sp*. became re-apparent after two weeks and act as predisposing to bacterial infection. Both garlic and Artemisia vulgarism had low acute toxicity to tilapia at the working concentration to treat *Trichodinaiasis*. It was found that a heavy suspension of solids adhered to the gills. However, the concentration of *Trichodina and Aeromonus sp* treatment is much less than the concentration that causes fish deaths but either garlic or *Artemisia vulgaris* could be considered the effective treatment on pathogens. As it is extremely desirable to reduce the use of hazardous therapeutics for *Trichodinaiasis* and *Aeromoniasis* control, there is a great potential of using garlic and *Artemisia vulgaris* for this treatment. All *Trichodina* sp. and signs of *Aeromoniasis* were disappeared two days after treated with either 800 ppm garlic or 450 mg *Artemisia vulgaris***.** The acute toxicity response of garlic and *Artemisia vulgaris* to tilapia was much lower than that of Pot.Permanganate. In this study, we could not use the same amount of extracts in field to eradicate *Trichodina* infection. These may be

attributed to the variation of method of extract and type and size of fish; is one of the drawbacks of crude extract plant application as shown in Madsen *et al.*, 2000b. Referring to this evidence, 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* infection from the system. We found some *Trichodina* reoccurred after two week of treatment. *Trichodinaiasis* 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 *Trichodinaiasis* 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. These medicinal plants have been used as antibiotic and chemical alternatives as reported by Chitemanat t al., 2007. Results of the challenge test shown in Table 2 revealed that the mortality rate was 40, 30 and 20 % with 1, 4 and 8 gm/kg doses of *Allium sativum* and 28, 20 and 15 % mortality rate with 1 ,3 and 4.5 gm/kg doses of *Artemisia vulgaris* respectively. On the other hand, the mortality rate of control was 70%. Diets with *Artemisia vulgaris* showed the less effective than Allium *sativum* on the mortality rate of *O. niloticus* challenged intraperitonealy with *A. hydrophila*. These may be attributed to plant extract from Artemisia vulgarius posses strong in vitro antimicrobial against Aeromonas. *Allium sativum* had antibacterial activity antagonized by *A. hydrophila* in fresh water as reported by Diab ,2002, Pereira et al. (2008) and Mesalalhy et al.(2008) and Artemisia vulgaris has antiparasitic and antibacterial affect as report by Shagnliang et al, 1990 and Navrro et al.(1996).

5- Conclusion

Garlic and Artemisia vulgaris can be used as alternatives to chemicals disinfectant and antimicrobial drugs against *Trichodina and Aeromonus* sp. infections in tilapia. These results indicate that *Allium sativum* and *Artemisia vulgaris* have antiparasitic and antibacterial effect and make tilapia more resistant to infection by *Trichodina sp* and *A. hydrophila*.

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