Application of some medicinalPlants 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 Trichodinaiasis and Aeromonus 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. 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 in cement ponds (3×8 metre) and sex diets were formulated to contain different levels of Allium sativum extract (1, 4 and 8 g/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. [Report and Opinion 2010;2(8):54-59]. (ISSN: 1553-9873).

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, 2003). Today plant materials are present in, or have provided the models for 50% of western drugs (Robbers et al., 1996). At first, tilapia were 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 (Philip 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. It has been used in developing countries as well as using extended to developed countries (Lanfranco, 1999). Plant extracts decrease the selective pressure for developing antibiotic resistance (Lewis & 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 Trichodinaiasis 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 (Corzo-Martinez et al, 2007). Madsen et al. (2000b) reported that raw and squeezed garlic (Allium sativum) at 200 mg/l had Report and Opinion 2009, Noor El Deen et al, Application of some Medicinal plant potential to treat Trichodinaiasis 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 Trichodinaiosis treatment. The aim of this present research was to (1) determine the efficacy of garlic and Artemisia vulgaris as cheap antibacterial medicinal plants alternatives to control Trichodinaiosis epizootic in tilapia.

2- Materials and Methods

2-1-Fish

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 in cement ponds (3×8 metre) and supplied with well-aerated freshwater using compressed air. Cement ponds were daily cleaned, and the water exchange rate per day, 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, with high density for 20 days.

2-1-Diets

Sex 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 (Chehregani et al.,2007). In the same way control disc was also prepared by using acetone according to Asha ,et al (2008) and Abdul Mannan, et al (2008).A commercial pellet feed was given once a day.

2-4-Parasitic Examination

Mucus was scraped from total surface of skin and two gill arches were removed from experimental tilapia. 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. Experimental fish were then randomly checked for parasite infections in the next two and four weeks.

2-5-Challenge experiment

After 14 days, 350 *O. niloticus* (50 in each 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 (1989). 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 sides, trunk region, fins and scales. The postmortem lesion was congestion in the gills and internal organs. As well as, enlarged of gall bladder and engorged with bile Fig 1.

3-1-Parasitological results

Out of two plant extracts screened, *garlic* and *Artemisia vulgaris* had antibacterial compound

against *Trichodina sp* . Showed antimicrobial activity against *Trichodina sp* as seen in Table 1.

3-2-Survival rate

Survival decreased in control group (30 %) up to 10 days after challenge infection. However, this was increased in the garlic treatment group, i.e. 60,70 and 80 % survivability in the 1,4 and 8 g garlic kg−1 respectively and 60,72 and 85 % survivability in the 1,3and 4.5 g *Artemisia vulgaris* kg−1 respectively Table 2 & 3.

Fig. 1: Showing Oreomonis niloticus infested with trichodina and Aeromonua sp.

Table1.Antiprotozo a activity of plant extracts against *Trichodina sp* in tilapia (O.niloticus) .

Pond fish	**15-day during treatment**	**30-day during treatment**	**45-day during 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	……..
4.5 g 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	10	15	35	70
1 g. Garlic	50	12	5	3	20	40
4 g garlic	50	10	3	2	15	30
8 g garlic	50	6	4	……..	10	20
1 g Artemisia vulgaris	50	12	5	2	19	38
3 g Artemisia vulgaris	50	10	5	……..	15	30
4.5g Artemisia vulgaris	50	7	1	……..	8	16

4-Discussion

The clinical signs of heavy infested tilapia *O.niloticus* with Trichodina sp has caused gigantic financial losses, letharagic, generate excessive mucus and become off feed 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. It is used as food for humans as well as some animals and as remedy for several diseases, as reported in folk medicine. Artemisia vulgarism might provide a suitable basis for new antimicrobial 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 were able to remove all *Trichodina sp*. from tilapia after 2-day treatment (Table 1). All treatments were significantly different from control groups. Both garlic and Artemisia vulgaris are more economical and effective in the presence of organic matter for along time than freshly prepared Potassium permanganate 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 working concentration for *Trichodina and Aeromonus sp* treatment is much less than the concentration that causes fish deaths. Based on this outcome, either garlic or Artemisia vulgaris could be developed for safer treatment. 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 garlic extract(200 mg/L) as shown in Madsen *et al.* (2000b) to eradicate *Trichodina* infection in eels. The possible explanation

might be due to the variation of method of application and type of fish. This is one of the drawbacks of crude extract plant application. 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. Some other 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 38,25 and 15 % mortility 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 Allium sativum and *Artemisia vulgaris* showed the same effect on the mortality rate of O. niloticus challenged intraperitonealy with A. hydrophila. 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) who reported that methanolic plant extract from Artemisia vulgarius posses strong in vitro antimicrobial against Aeromonas salmonnicida..

5- conclusion

Garlic and Artemisia vulgaris can be used as an alternatives to chemicals to treat *Trichodina and Aeromonus* sp. infections in tilapia in laboratory trials. Further studies, including the chronic effect on growth, survival rate, and reproduction need to be investigated. These results indicate that *Allium sativum* and Artemisia vulgaris has antiparasitic and antibacterial affect and makes tilapia more resistant to infection by *Trichodina sp* and *A. hydrophila*.

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