PRELIMINARY STUDY OF ANTIMICROBIAL ACTIVITY OF PORPHYRINS AGAINST THE WHITE SPOT DISEASE CAUSED BY *Ichthyophthirius multifilis* IN BLACK MOLLY (*Poecilia saphenous*)

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INTRODUCTION

The international trade of ornamental fishery industry increases at an annual rate of 14%. Sri Lanka's share in the world trade, at over 1 %, is projected to increase to 10 percent in the medium term. The tropical fish industry has been unsuccessfully dealing with the imported disease problems for at least 25 years.

White spot disease is the wide spread protozoan disease of fish caused by *Ichthyophthirius multifilis*. It is a major threat to aquaria as the parasite can produce higher number of tomites from one cyst and also can easily spread and infect the fish but (Andrew *et al.,* 1998). The most characteristic external sign is the presence of white spots on the skin and gills, due to parasite trophonts located under the upper layer of the skin. The mortality of affected fish is mostly dependent on fish size and infection intensity. The commonly used chemicals in controlling the white spot disease are Copper sulfate, Potassium permanganate and Formalin (Peggy and Ruth 1991). Pophyrins are a ubiquitious class of deeply coloured fluorescent organic compounds with synthetic or natural origin. The photoreactions of porphyrins in the presence of light and molecular oxygen are of considerable importance due to their likely role in photodynamic therapy of cancer, photodecantimination of microbial and chemically polluted water. Upon visible light excitation of porphyrins in the presence of ground state of oxygen, it generates electronically excited molecular oxygen (singlet oxygen) or superoxide radical anion. Singlet oxygen is a powerful fairly indiscriminant oxidant reacts with bio molecules such as unsaturated lipids, cholesterol and amino acid side chains (Amarasinghe *et al.,* 2005). The combination of porphyrins and visible light could represent a viable and environmentally friendly alternative for the control of the population of potential pathogens in waters from fish farming ponds (Koji *et al.,*2004).

Objectives of this study were to investigate the photodynamic action of porphyrin derivatives against the parasite and to observe behavioral changes of fish during the treatment period.

MATERIALS AND METHODS

The experiments were conducted in indoor glass tanks. Randomized Complete Block Design was used as the experimental design for three treatments (T1, T2 and T3) and each with 3 replicates. *Meso*-5-(4-hydroxyphenyl)-10,15,20-trisphenyl porphyrin (MHPP) was dissolved in acetone and diluted with distilled water to prepare a 5.0 ppm of standard solution from which concentration of 1.00 ppm (T1), 2.00 ppm (T2), 3.00 ppm (T3) were prepared. Healthy black molly fish (*Poecilia saphenous*) were treated with methelene blue (5ml/l) and salt (1g/l) to remove any existing pathogens before the experiment. The healthy molly fish were infected artificially by mixing with white spot disease infected fish. Ten infected black molly fish were introduced separately to each replicate of three treatments *viz.* DT1 (1.0ppm), DT2 (2.0ppm) and DT3 (3.0ppm). The same set of treatments *viz.* HT1, HT2 and HT3 were experimented for healthy fish. In addition an untreated tank NT for diseased fish was used as a control. The fish were fed twice a day with artificial feed at a rate of 5% of body weight. Number of parasites in the water was counted using a counting chamber for three days (before applying the treatment, after 24hrs and after 48hrs of applying the treatment). Water quality parameters such as temperature, pH, dissolved oxygen (DO) level as well as behavioral changes of fishes were monitored during the treatment period. One way ANOVA was used to compare the significance differences among the treatments.

RESULTS AND DISCUSSION

Water quality parameters

The water quality parameters of the treatment tanks were within the favorable range (pH 6.5-8.5, Temperature 25-30 ^{0}C and DO >5 ppm). There was no significant difference ($p > 0.05$) among the treatments for water quality parameters.

Change of behavior pattern of Black Molly fish

Abnormal swimming behavior, reduction of feeding rate and disappearance of social behaviors were observed in the infected fish. However they recovered their behavior patterns such as feeding, swimming during the period of treatment.

Distribution of number of parasites and effectiveness of porphyrin

After 24 hr of treatment, number of parasites significantly reduced by 29.29% in 1.00ppm (DT1), 4.14%in 2.00 ppm (DT2) and 19.13% in 3.00ppm (DT3) when compared to the initial amount of parasites. There was a significant difference ($p < 0.05$) between the numbers of parasites among the treatments. On the other hand number of parasites was reduced when porphyrin concentration was increased (Figure 1). Therefore, comparatively high amount of parasites were destroyed at the high MHPP concentration (3 ppm). However after 48 hrs of treatment, number of parasites was increased by 433.28%, 115.86% and 24.98% in concentration of 1.00ppm (DT1), 2.00ppm (DT2) and 3.00 ppm (DT3) respectively. Among the treatments the increase of parasites was comparatively highest in porphyrin with a concentration of 1ppm (DT1). In general only theront and tomant stages in water are sensitive to chemicals. In contrast, trophont stage attacks on fish and matures while protected from chemical treatment being under the fish mucus and skin. After 48 hrs the action of porphyrin was reduced, presumably due to photodegradation of MHPP as a function of time. Hence the tomants which were shed from the fish to the water were not killed by the action of porphyrin. Therefore multiple treatments should be practiced to attack individuals of itch organisms in the vulnerable stages of their life cycles. Numbers of parasites were continuously increased in the infected non treated tank (NT). There was no phototoxic effect of MHPP on healthy fish (HT1, HT2 and HT3).

Importance of porphyrin as a treatment

There are several chemicals already used for the treatment of white spot disease such as Copper sulfate, Potassium permanganate at a concentration of 2 mg/l and Malachite green (Peggy and Ruth 1991). However, those are not suitable to treat food fish as they are toxic and therefore it will directly affect the human health. However, porphyrin does not produce any toxic material and it is environmentally friendly alternative chemical (Koji *et al.* 2004) for treating the white spot disease.

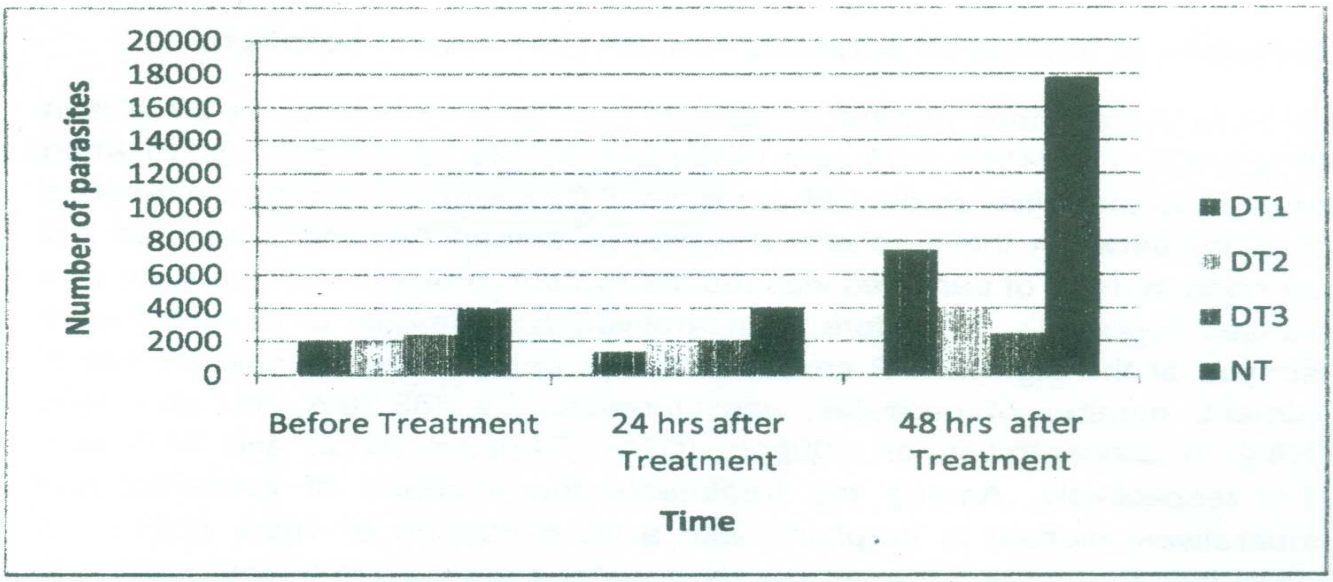

Figure 1- Distribution of number of parasites

CONCLUSIONS AND RECOMMENDATIONS

The fact that porphrin does not leave any residues in the fish body it is more suitable for the treatment of white spot disease on food fish. MHPP at a concentration of 3ppm is the best treatment for prevention of white spot disease. Since the efficiency of photosensitization of MHPP decreases with time, after 48 hrs, replacement of tank water with fresh water and addition of 3 ppm of MHPP to the water in every 48 hrs are recommended. The treatment should be continued at least for one week in order to kill all the itch parasites.

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