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Bioprophylaxis of Saprolegniasis in Incubated Clarias gariepinus Eggs Using Pyocyanin Extracted from Pseudomonas aeruginosa

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Abstract : Saprolegniasis is a major pathogenic infection that contributes significantly to poor hatching rates in incubated fish eggs in the Africa catfish hatchery in Nigeria. Malachite green known to be very effective against this condition has been banned because it is carcinogenic. There is, therefore, the need for other effective yet safer methods of controlling saprolegniasis in incubated fish eggs. A total of 50 ml crude, chloroform extract of pyocyanin from which solvent was removed to attain 30 ml, having a concentration of 12.16 ug/ml was produced from 700 ml broth culture of Pseudomonas aeruginosa isolated from a previous study. In-vitro susceptibility of the fungus was investigated by exposing fungal infected eggs to two different time-concentration ratios of pyocyanin; 0.275 ug/ml and 2.75 ug/ml for 1 and 24 hours, and 5 mg/L malachite green as positive control while normal saline was the control. The efficacy of pyocyanin was evaluated using the degree of mycelial growth inhibition in different treatments. Fertilized Clarias gariepinus eggs (between 45 to 64 eggs) were then incubated in 20 ml of medium containing similar concentrations of pyocyanin and malachite green, with freshwater as a control for 24 hours. Hatching rates of the incubated eggs were observed. Three samples of un-hatched eggs were taken from each medium and observed for the presence of fungal pathogens using microscopy. Another batch of three samples of un-hatched eggs from each treatment was also inoculated on Sabourand dextrose agar (SDA) using Egg-Agar Transfer Technique to observe for fungal growth. Mycelial growth was inhibited in fungal infected eggs treated with 2.75 ug/ml for 24 hrs and the 5 mg/L malachite green for both 1 hr and 24 hrs. The mortality rate was 100% in fertilized C. gariepinus eggs exposed for 24 hrs to 0.275 and 2.75 ug/ml of pyocyanin. The mortality rate was least in malachite green followed by the control treatment. Embryonic development was observed to be arrested in the eggs treated with the two pyocyanin concentrations as they maintain their colour but showed no development beyond the gastrula stage, whereas viable eggs in the control and malachite green treatments developed fully into healthy hatchlings. Furthermore, microscopy of the un-hatched eggs revealed the presence of a protozoan ciliate; Colpidium sp, (Tetrahymenidae), as well as a pathogenic fungus; Saprolegnia sp. in the control but not in the malachite green and pyocyanin treatments. Growth of Saprolegnia sp was also observed in SDA culture of un-hatched eggs from the control, but not from pyocyanin and malachite green treated eggs. Pyocyanin treatment of incubated eggs of Clarias gariepinus effectively prevented fungal infection in the eggs, but also arrested the development of the embryo. Therefore, crude chloroform extract of pyocyanin from Pseudomonas aeruginosa cannot be used in the control of Saprolegniasis in incubated Clarias gariepinus eggs at the concentration and duration tested in this study.

Keywords : African catfish, bioprophylaxis, catfish embryo, Saprolegniasis
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