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A study of herbal plant extracts on fungal infections in fresh water ornamental fish *Poecilia reticulata*

M. Umamaheswari and Dr.R.Krishnamurthy

Govt Arts College for Men,Nandanam,
Chennai -600 035, India

Abstract

Different plant parts from 6 medicinal plant species were collected from Coimbatore District, Tamilnadu. Infected ornamental fishes were collected from local fish Market. Collected samples were aseptically taken to the laboratory and kept in the freezer until mycological examinations are carried out. The collected plant materials were shade dried at 31 ^{0}C for about 10 days. Then the samples were cut into small pieces and powdered in a stone mortar and pestle. Cold extraction was carried out with chloroform solvent for the extraction of bioactive compounds. After 7 days of percolation, the extract was filtered, evaporated and the residue obtained was stored in a sterile container for further use. All the extracts were tested against 3 fungal pathogens isolated from the infected ornamental fish species. Out of which, no plant species showed sensitivity against one or more fungal pathogens.

Key Words: *Fish culture, fungal diseases, herbal extracts, isolation and identification of pathogens.*

1.Introduction

Many tropical countries in Asia have a long tradition of aquaculture, over 80% of fish produced by aquaculture comes from Asia. Aquatic animal disease and environmental related problems may cause annual losses of more than US$ 3 billion dollars among Asian countries (FAQ, 1996). Fish in fresh water environments are susceptible to several bacterial, fungal, viral and parasitic diseases.

2.Review of literature

Herbs have been widely used in veterinary and human medicine. They are natural products that are not only safe for consumers but also widely available throughout Asia. Now a days herbs or herbal products also have a significant role in fish culture. Many kinds of herbal medicine have been used in China to control fish disease and have produced satisfactory results (Rajandra, 1990). In Vietnam, the Institute of Ecology and Bioresources has undertaken applied research on some medicinal herbs for prophylaxis and treatment of fish and shrimp diseases such as ulcer, intestinaldiseases, white mouth, white head, red skin and red spot in fish, and luminescence and brown spot disease in shrimp (Dung, 1990).

Allan (1985) reported that, *Fusariuni sps, Penicillium sps, and Aspergillus sps* were the predominant fungal pathogens in fish.Richards et al. (1978) reported that, Fusarium sps was the predominant pathogen in salmonids. Hendricks and Bailey (1989) and Tacon (1992) reported that, *Aspergillus sps, Fusarium sps and Penicillium sps* were the predominant forms. Singh (1996) reported that A*spergillus flavus* and *A. carbolarius* were the predominant pathogens in Satpati fishing centre, Paighar, India. Branislav Rankovie (2005) reported that *Penicillium, Aspergillus, Cladosporiuni, Fusarium, Rhizo pus, Mucor, Phoma and Verticilliuni* were the predominant forms. In Egypt, *Aspergillus, Penicillium and Trichodernia* were found to be the most common genera among Nile fishes (Bagy et al., 1993).

Table.1Taxonomic position of Poecilia *reticulata*

Order	Cyprinodontiformes
Family	Poecilidae
Genus	***Poecilia***
Species	***reticulata***

Poecilia reticulata is commonly called as 'guppy', is one of the most popular fresh water aquarium fish species in the world. It is a member of the Poecilidae family. (Females: 3 centimetres long, male: 2-centimetre-long) and a live-bearer. The female guppy is dark brown in colour. The smaller male naturally has a colourful caudal fin (tail fin), which has been considerably enhanced in shape and colour by selective breeding.

3. Materials and Methods

3.1 Isolation of fungal pathogens

To isolate the fungi, the infected part from chosen fishes were cut into small pieces and ground with 10 n-il of distilled water. Serial dilutions were made up to 10-8. 1 ml of diluted samples from each dilution were inoculated on already prepared Rose Bengal agar plates, supplemented with 200 ppm of streptomycin to suppress the growth of bacterial contamination.

All the inoculated plates were incubated at 24 ± 2°C for 72 hrs. After that, the colonies appeared on the media were counted and expressed as Colony Forming Unit (CFU). Then the colonies with different morphology were selected, streaked on Potato Dextrose agar slant and stored at 40C for further studies.

Table.2 Media composition of Rose Bengal agar ($g.l^{-1}$)

Peptic digest of soya bean meal	5
Dextrose	10
Mono potassium phosphate	1
Magnesium sulphate	0.5
Rose bengal	0.05
Agar	15
pH	7.2+_0.2

3.2 Media composition of potato dextrose agar ($g.1^{-1}$)

Potato infusion - 200

Dextrose - 20.00

Agar - 18.00

Ph - 6.50

3.3 Identification of fungal pathogens

Isolated fungal strains were identified with the help of Smith's introduction to Industrial Mycology, Seventh Edition, Onions et al., (1981). Permanent preparation was made by incorporating poly vinyl alcohol in place of glycerine into the mounting medium. For rapid and routine examination of almost all types of fungi and spores, they were tested out on a clean slide in a drop of stain and a cover glass was placed over preparations which was then used for microscopic examinations. Lactophenol cotton blue is a stain commonly used for making semi-permanent microscopic preparations of fungi. It stains the fungal cytoplasm and provides the light blue background against the wall of hyphae so that it can be readily seen.

3.4 Preparation of stain for microscopic identification of fungi

Cotton blue (Aniline blue) - 0.05g

Phenyl crystals (C6HSO4) - 20.00g

Glycerol - 40.00ml

Lactic acid (CH3 CHOH) -20.00ml

Distilled water -20ml

3.5 The stain was prepared as follows

1. The cotton blue was dissolved in distilled water and left overnight to eliminate insoluble dye.

2. The phenol crystal was dissolved in lactic acid in a glass beaker and placed on magnetic stirrer until phenol is dissolved.

3. Then glycerol was added

4. Further, the cotton blue and distilled water solution was filtered into phenol / glycerol / lactic acid solution, mixed and stored at room temperature.

3.6 Procedure

1. A drop of lactophenol - cotton blue strain was placed on a clean glass slide.

2. A small tuft of fungus was transferred onto the stain.

3. The cells were mounted by gentle teasing using a needle.

4. After that, the stain with the mould structure was covered with cover slip, with care to avoid dislocation and observed under the microscope (450x).

4. Results

4.1 Observation

The fungal cytoplasm was a lightly stained blue region forming a layer inside the unstained cell wall of hyphae, Conidiophores and conidia that was surrounded by a light blue background on the slide were observed and identified as *Aspergillus niger*, *Penicillium chrysogenurn*, *Penicilliuni restrictun'i* and *Rhizo pus stolonifer*.

4.2 Antimicrobial sensitivity of medicinal plants against identified fish pathogens

Different plant parts from 6 medicinal plant species were collected from Coimbatore District, Tamilnadu.

Table.3: Name of the plant species, local name and parts chosen for the extraction of antimicrobial compounds are given below:

Name of the plant	Local name	Plant part used
Ocimum sanctum L.	Thulasi	Entire plant
Ricinus communis L.	Aamanakku	Leaf
Acalypha indica L.	Kupaimeni	Entire
Leucas aspera (Wild) Link.	Thumbai	Entire plant
Mukia maderaspatana (L.) Roem.	Musumusukkai	Entire plant
Hibiscus rosasinensis Linn.	Chembaruthi	Leaf

4.3 Extraction of bioactive compounds

The collected plant materials were shade dried at 31 ^{0}C for about 10 days. Then the samples were cut into small pieces and powdered in a stone mortar and pestle. Cold extraction was carried out with chloroform solvent for the extraction of bioactive compounds. After 7 days of percolation, the extract was filtered, evaporated and the residue obtained was stored in a sterile container for further use. The percentage of extraction from each sample was calculated using the formula.

$$\% \text{ of extraction } (\%) = \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant material (g)}} \times 100$$

4.6 Antifungal assay (Lehrer et al., 1991)

The antifungal assay was carried out using two-stage radial diffusion assay. 10 ml of molten gel solution (1% agarose and 0.3 mg of Sabouraud dextrose broth powder per ml and 10 mM sodium phosphate buffer, pH 7.4) was mixed with pathogenic fungal spores (102 spores ml-^{1}) viz. Aspergillus niger, Rhizophus stolonifer, Penicillum restrictum and poured into a sterile petriplate and extracts impregnated on sterile discs were placed onto the gel and incubated overnight at 37°C. After incubation, the zone of inhibition was measured. The entire assay was carried out thrice with three replicates.

Infected ornamental fish species, *Poecilia reticulata* were analysed for the bacterial and fungal infections. The isolated pathogens were tested for their antimicrobial sensitivity and *in vivo* studies by the extracts from medicinal plants and the results are given bellow:

4.7 Clinical symptoms observed

Swollen abdomen

4.8 Culture conditions

Water temperature: 29^{0}C ; pH : 7.23 ; bicarbonate : 1.6 mg.H chloride : 0.7 mg.^{1} ; calcium : 1.5 mg.1 ^{1} ; magnesium : 0.6 mg.l' sodium : 1.3 mg.1-^{1} ; potassium : 0.04 mg.1' ; total anions and cations 2.3 mg.1-^{1} ; electrical conductivity: 0.23 ds.m^1.

Table showing Antifungal sensitivity of 6 medicinal plants against isolated fungal pathogens

Name of the plant species	Zone of inhibition (mm dia)		
	Rhizopus stolonifer	*Aspergillus niger*	*Penicillium restrictum*
Ocimum sanctum L.	-	-	-
Ricinus commuinis L.	-	-	-
Acalypha indica L.	-	-	--
Leucas aspera (Wilid) Link.	-	-	-
Mukia maderaspatana (L.) Roem.	-	-	-
Hibiscus rosasinensis Linn.	-	-	-

(-) : No Sensitivity

Among the fungal species,*Aspergillus niger* was reported to have maximum In the present study, an attempt has been made to find the herbal based disease treatment on ornamental fishes in South India.

Table. Showing Fungal pathogens ***identified in ornamental fish***

Fungal pathogens	***% of Pathogens***
Rhizopus stolonifer	***25%***
Aspergillus niger	***56%***
Penicillium restrictum	***19%***

5.Discussion

The incidence of mycotic infections in chosen fishes has also been reported in the present study. Fungi are known to attack eggs,fry, fingerlings and adult fishes. Generally most of the fishery biologists have wrongly assumed that almost all the fungal infections of fish and fish eggs are caused by the members of the genus Saprolegnia. But other fungal species have also been reported in variety of fishes (Srivastava and Srivastava, 1977; 1978)

About 6 plant species subjected for the extraction of bioactive compounds by using Chloroform. All the extracts were tested against 3 fungal pathogens isolated from the infected ornamental fish species. Out of which, no plant species showed sensitivity against one or more fungal pathogens.

It is concluded from the present investigation that, prevention and treatment of ornamental fish diseases could not be done effectively with the supplementation leaf extract.

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