

FINAL REPORT

MINOR RESEARCH PROJECT (UGC)

Assessment of the Effect of Aloe vera (Ghritkumari) Extracts on Pathogenic Fungi Saprolegnia causing Water-mould disease among Fresh water Fishes

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Project Title-

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Introduction

This project work pertains to a very relevant problem associated with Aquaculture and Fisheries. In the practice of aquaculture / fish farming, whenever fishes are subjected to some physical shocks or abrasion stress, owing to some prevailing and commonly occurring factors, they become highly vulnerable to the infection of the fungus *Saprolegnia*. These infections are responsible for devastating implication on fish in aquaculture, fish farms and hobby fish tanks. This problem exists with all kinds of fish culture all over the world. Traditional or other generally recommended methods are there but not devoid of some associated hazards. The chemicals used for this purpose are toxic, bioaccumulative, carcinogenic and detrimental to the environment. Hence, some alternate regimen for the control of this fungal problem is warranted.

This work pertains to seeking a herbal cure against the water-mould disease caused by *Saprolegnia* among fish fauna. The plant that was selected for this study, Aloe vera has been reported to possess anti-inflammatory, immuno-modulatory, wound healing, anti-bacterial and many other medicinal properties, and authenticated for their fungicidal actions.

An extract of the Aloe vera plant that can be used to promote healing of damaged fish tissue has been patented at US and European patent office (U.S. Pat. No. 4,500,510. [1985 > February 19, 1985](#) or www.ptodirect.com/ and European Patent CA 1220721. <http://ep.espacenet.com/>). But, in the patent statement nowhere *Saprolegnia* has been mentioned as causative factor or that the methodology is directed against *Saprolegniasis*. If fungistatic property of this plant against *Saprolegnia* infection is established along with the protocol of the disease treatment, that may also merit a patent application. Besides, the research may further pave the way to study of isolating the active ingredients in the plant material used and immuno-modulation mechanism among the experimental animals.

Taking the cue from the papers reporting fungicidal property, and as there is no report of this plant being used to mitigate or prevent the menace of *Saprolegniasis* on fish fauna, this study was planned.

Objective

This investigation was intended to examine the protective potentials of the selected plant *Aloe vera* against the infection of fungus *Saprolegnia parasitica* on culturable fish species. It was assumed that if the considerably harmless and easily accessible herbal or plant ingredients can be effectively used for this purpose, it will be favorably appreciated in the practice of fish trade and aquaculture. The herb *Aloe vera* has been indicated to possess fungistatic activity on literature search. Therefore, the various extracts of this plant was planned to tested for their potential use against Saprolegnia infection on two selected species of fish important from aquaculture point of view.

In the first part of study plant / herbal extracts was planned to put to *in vitro* testing for their anti-fungal potential against the targeted fungus *Saprolegnia parasitica*. Some studies in this regard was found to exist in literature and some preliminary investigation detecting fungistatic study of this plant extract was conducted in our laboratory also.

Second phase of investigation (*in vivo*) was designated for the use of this plants against the fungal disease *Saprolegniasis* (water moulds) on selected fresh water fish species reared in fish tanks.

Earlier works on this problem

Fungi are hetero-morphic plants lacking chlorophyll. Fungal species are pathogenic to human beings and animals. The mycota pertinent from aquaculture point of view belongs to Genus *Saprolegnia*. Pathogenic *Saprolegnia* sp. produces an easily recognizable cottony white growth on the epidermis of the affected animal the fish. Infection is normally restricted to the superficial tissues, resulting in a breakdown of the fish osmo-regulatory mechanism, and unless fish can be treated, infection reaches to every external parts of the body and the condition is usually lethal (Bruno and Wood 1999). This infection spreads with rapid pace in all the fish present in the vicinity. In aquaculture industry and hobby fish aquarium, this fungal infection is real cause of concern (Bly et. al., 1994; Hatai and Hoshiai, 1994)

Previously, *Saprolegnia* infections were advised to be kept under control with malachite green, an organic dye that is very efficient at killing the pathogen. However, the use of malachite green has been banned around the world, due to its carcinogenic, teratogenic, bioaccumulative and other toxicological effects (Srivastava et al. 2004). And, the aquaculture products containing the residues of this chemical is quarantined. Other prevailing alternatives are the uses of hydrogen peroxide, formalin, Potassium permanganate, Copper sulphate, methylene blue and sodium chloride etc. But, these chemical regimens are either less effective or not free from some other hazards (Meng et. al. 1996). Therefore, there is an urgent need of a novel alternative method for management of Saprolegniasis.

Aloe vera is reported to possess anti-inflammatory, immuno-modulatory, wound healing, anti-bacterial and many other medicinal properties (Davis, 1997; Reynolds, 1999). It has also been authenticated for their inhibitory actions on some species of fungi, particularly against *Candida* (Ali, et. al. 2002; Perumal et. al., 2004; Agarry et al., 2005). Growth of dermatophyte *Trichophyton* has been shown to be checked by the leaf extract of *Aloe arborescens* (Fujita et. al.

1978), and gel extract of *Aloe vera* (Agarry et. al., 2005). Work on protective action of *Aloe* species against *Saprolegnia* species of fungi appears to be lacking in the literature.

Use of herbs to prevent or mitigate the fungal pathogens on fish fauna is rather lacking in the literature. Still, few references of the same pursuit are reported from India also. Rath (1993) has described the use of medicinal herbs to prevent fish diseases but modules of treatment and plant species are different. Report of controlling certain fungi on fishes using *Azadiracta* has been posted few years back (Harikrishnan et al., 2005). Though, it has not been carried out with *Saprolegnia*.

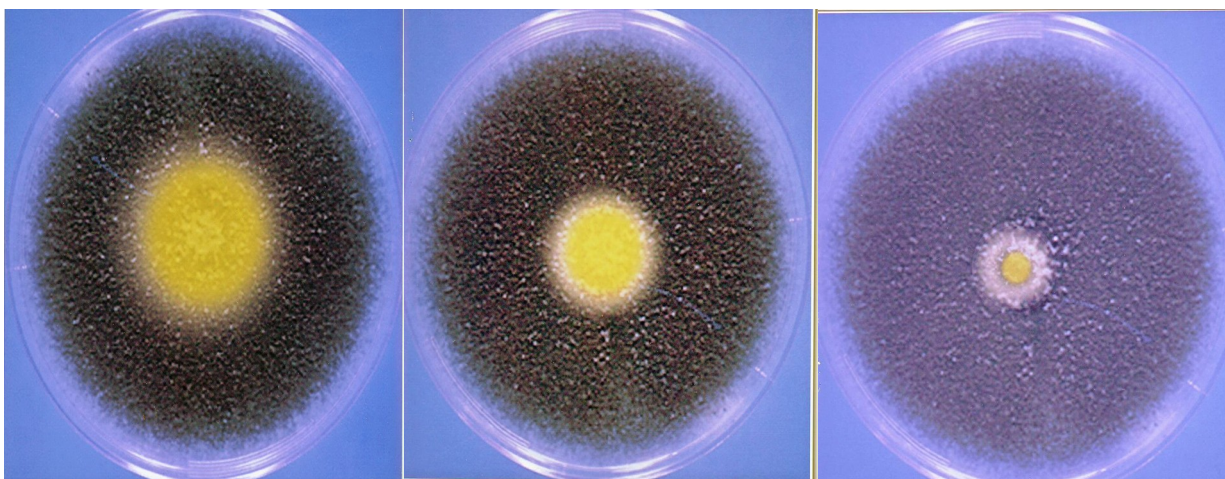
Many papers has been published on the infextion of Saprolegnia on fresh water fish fauna in India (Das et al. 2012; Chauhan, 2012; Mastan et al., 2012, 2015 and others). But, in all these studies were field surveys and in none of these any remedial measures were investigated.

Methods and Results

I. In Vitro Studies

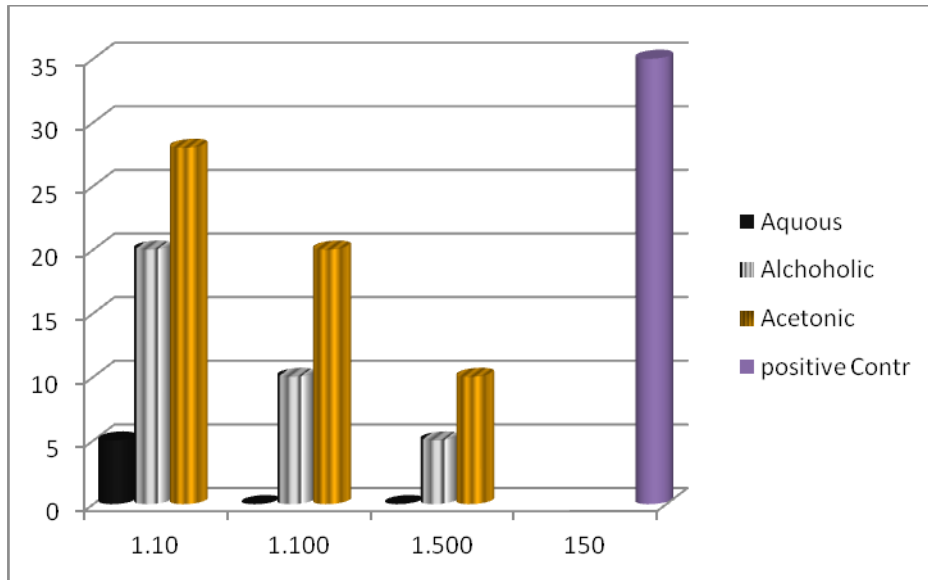
For in vitro study Pure culture of *Saprolegnia parasitica* was procured from IMTECH, Chandigarh and maintained in Sabouraud dextrose agar. Aqueous and organic solvent extracts of *Aloe vera* (that was procured locally) leaf gel & juice were tested for antifungal property as per the method of Khan et al. (2006).

Very promising results of fungistatic/ antifungal activities were exhibited by *Aloe vera* extracts. Dried extracts dissolved (100 mg/ ml) were tested at the dilution of 1:10, 1:100 and 1:500 in sterile culture plates of *Saprolegnia* and zone of inhibition was noted after incubation at 37°C for 48 hours.



Plates photo- Inhibition of *Saprolegnia* pur culture on treatment of Acetonic, Alcoholic and Aquous extracts

Inhibition score of Saprolegnia culture after treatment of various extracts of Aloe vera is being shown below as graphical representation.



As evident from the graph acetonic extract of the Aloe vera gel/ juice has been proved to be a strong fungistatic compound against this fungal species. And, it certainly indicates its potential to be used in prevention/ treatment of saprolegniasis

In vivo studies

For this investigation we surveyed various major water bodies of Dungarpur and Udaipur districts. We could not collect saprolegnia infected mature Catla and Mystus species. Though, some other fish infected with cotton wool disease were recorded. Using the Saprolegnia culture procured from IMTECH Chandigarh we infected some fingerlings of Catla and Mystus seenghala fishes in lab aquarium.



Photo- Catla fingerling exhibiting saprolegniasis infection

In the laboratory fish infected with saprolegnia were first examined carefully for external tissue damage. Mostly it was abrasion and inflammation of skin, operculum, gill and fin area. Tissues of infected fish (Skin and gill) were fixed for histopathological studies. Severe damage in gill filaments and skin epidermis and muscle fibers were noticed in histological preparations.



Photo- Signs of Abrasion and inflammation in infected Catla catla

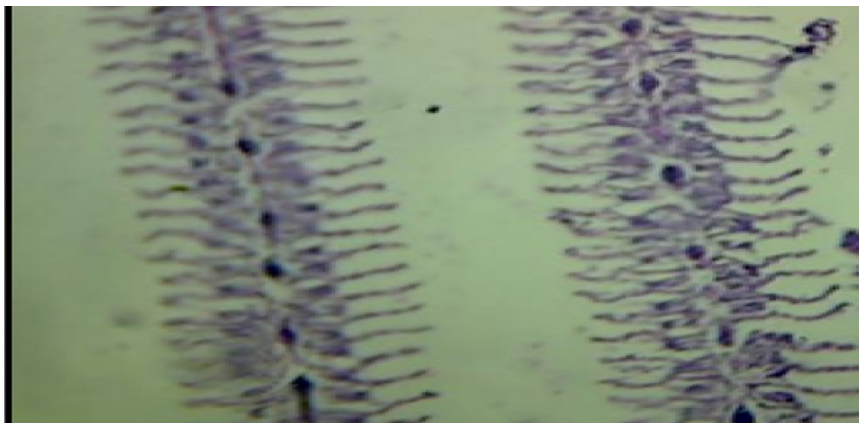


Photo- Normal gill section of Catla

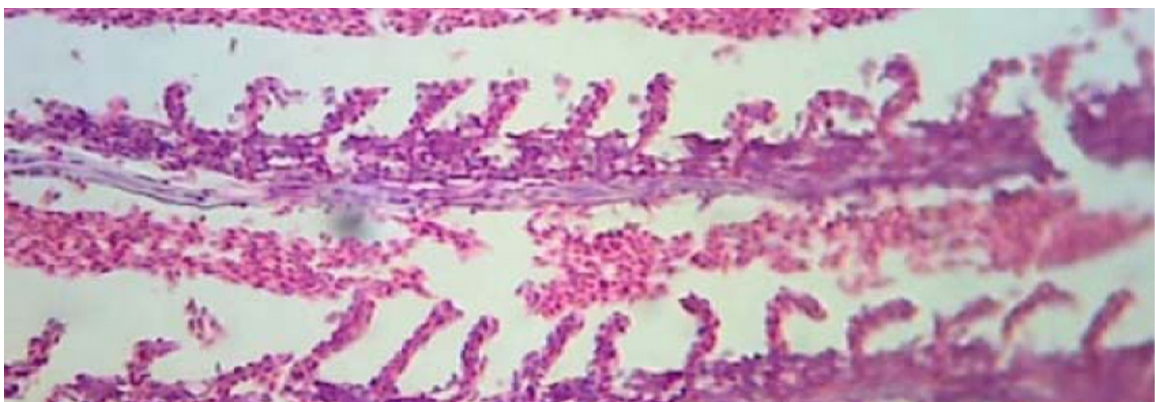


Photo- Slide of Gill *Mystus* displaying clubbing of filaments, hypertrophication and necrosis

Moderately infected fish were given the dip treatment in Aloe extract mixed water tank for 5 minutes twice a day for seven days. External signs of infections were seen to be subsiding. Histological slides of the fish treated for seven days were also prepared to evaluate the sign of healing.

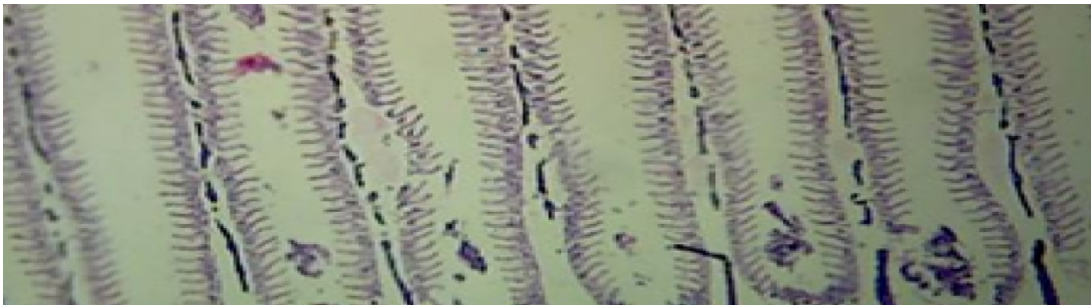


Photo- In the section of the gill of treated fish sign of recovery is being seen but still some hypertrophied filaments and necrosis is observed.

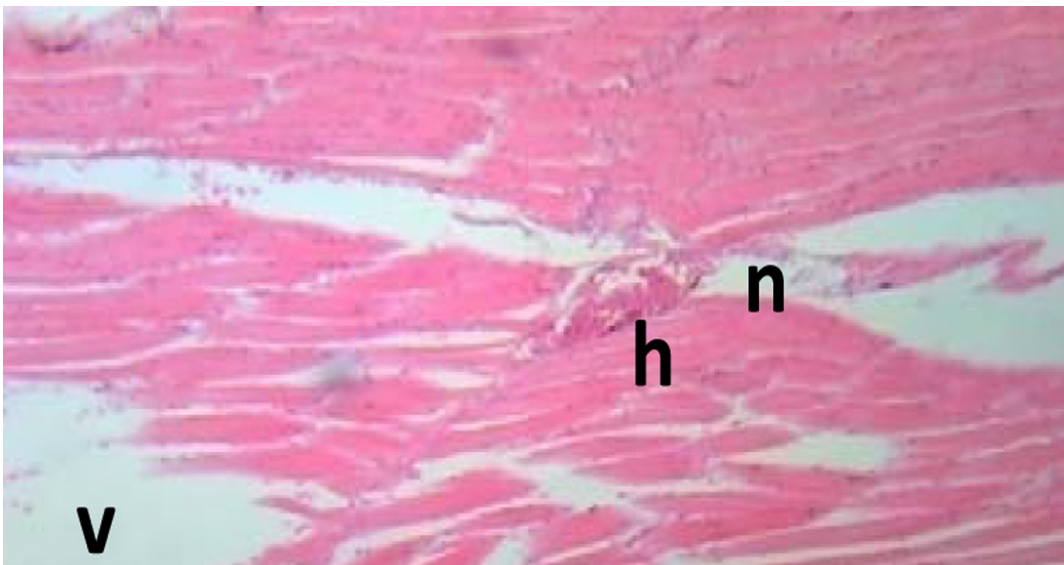


Photo- Splitted muscle of *Catla* showing necrosis, haemorrhage and necrosis after infection

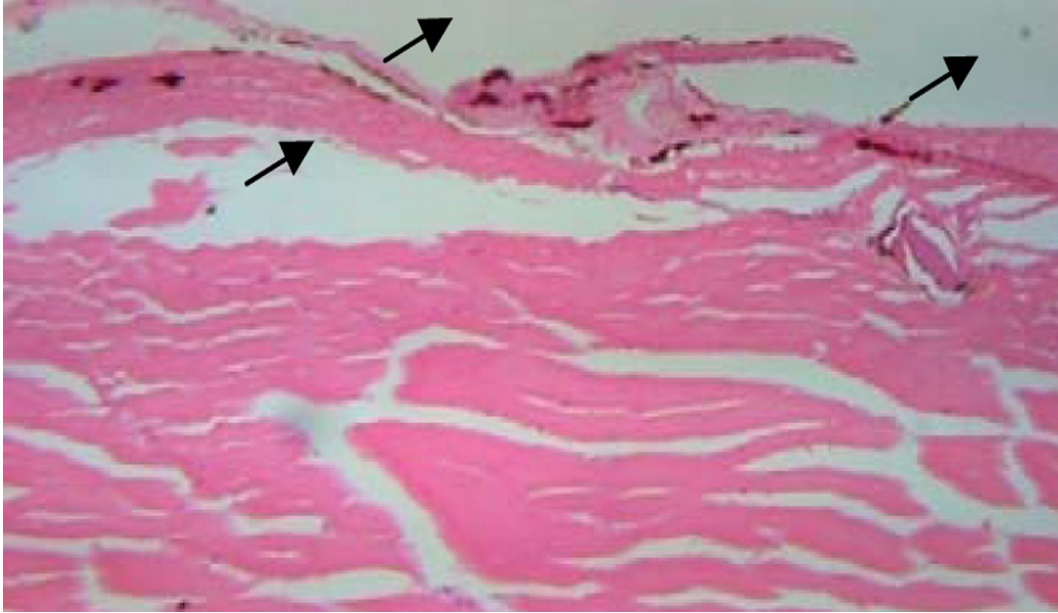


Photo – Skin and muscle of *Mystus* showing loss of epidermal and dermal tissue and separation of muscle

On histological examination in the *in vivo* study prominent tissue damage has been observed in infected fishes. And, after the dip treatment some signs of recovery has also been noted.

Conclusion

As indicated in results definite fungistatic activity of Aloe vera extracts had been found in *in vitro* studies. In *in vivo* studies also recovery of tissue damage is noted, but since during treatment fish were kept in water medium free from saprolegnia or any other infection. Hence, it is still to be ascertained that whether the recovery is due to the immunomodulation/ recovery of the fish on its own or due to the treatment regimen. Therefore some more investigation in the *in vivo* study is warranted.

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