Research Paper E-ISSN NO : 2455-295X | VOLUME : 5 | ISSUE : 7 | JULY 2019

"TO STUDY THE EFFECT OF PHYTOCHEMICALS OF ALOEVERA IN THE TREATMENT OF FUNGAL DISEASE IN LABEO ROHITA"

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ABSTRACT:

For this study healthy fishes were collected from Departmental pond of Barkatullah University Bhopal. Fishes were examined with naked eyes and by using magnifying glass to find out any sort of infection .After exanimation of Labeo rohita no infection was found. Fishes were made infected by injecting fungus Aspergillus Fumigatus. Effect of Phytochemicals of Aloe vera was studied in the treatment of fungal disease caused by Aspergillus fuigates. The whole experiment was done in two months. The effect on weight due to infection was also observed. **Saponins** present in the gel of Aloe vera was found effective in the treatment of fungal disease in Labeo rohita.

KEYWORDS:

ASPERGILLUS FUMIGATUS , SAPONINS, PHYTOCHEMICALS, HAEMOCYTOMETER ALOE VERA, LABEO ROHITA.

INTRODUCTION :-

Fresh water fishes are an important protein source for people of many countries (Hussain et al, 2011: Rubbani et al.2011). However, globally fish from freshwater and marine sources are in severe decline, driven in large part by economic and human population growth (Limburg et al., 2011). Fish farming in various parts of the world has increased many folds in the last decade. As a result, fish culture has now become commercially an important industry worldwide. Diseases in fresh water fishes are a great threat to achieve optimum production and become a limiting factor to economic success of aquaculture. Fungi which are responsible for a number of diseases are present in fresh water. Fungal infections are mainly caused due to immune suppression. Fungi can attack fishes of all the ages and it can also prevent successful hatching when it invades fish eggs.

As fishes are placed in lower levels of evolutionary system, non-specific immunity has more important role against pathogens in fishes, so the use of immune stimulatory agents seems to have a good result in fish immune response (Zapata et al., 2006 Swain et al., 2007). Herbal immune stimulants have numerous potential benefits in comparison to vaccines and drugs like antibiotics. They are not expensive and are available almost all around the world in contrast vaccines are generally expensive and are not available for all kinds of disease (Raa et al(1992) and Rojhan, (2007). Present work is carried out to examine the effects of Phytochemicals of *Aloe vera* in the treatment of Fungal disease in *Labeo rohita* , The Fungus *Aspergillus fumigates* was injected in *Labeo rohita*. ***Aspergillus fumigatus*** is one of the most prevalent *Aspergillus* species found in most environments. It can survive at high temperature *A. fumigatus* also exists as a saprophyte that plays an important role in the cycle of carbon and nitrogen in nature. Because it is very prevalent, the spores of *A. fumigates* are also in high concentration in air, which presents a serious health issues for those with poor immunity. *Aloe vera* has a long association with herbal medicine, Three distinct preparations of aloe plants are mostly used in a medicinal, aloe latex (aloe); aloe gel (Aloe vera); and, aloe whole leaf It has been investigated that the gel extract of Aloe vera presents various Pharmacological properties such as promoting and healing wound and burn, frostbite healing, with addition to having anti inflammatory, antifungal, hypoglycemic and gastro protective properties.

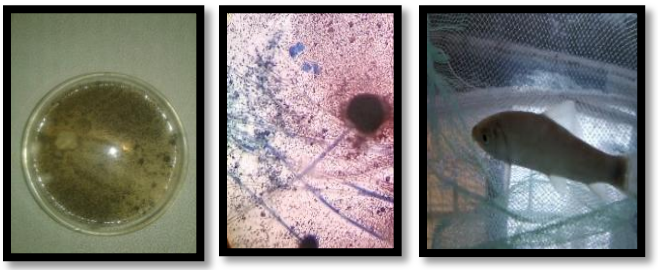
MATERIAL AND METHODS :-

After collecting the fishes the fishes were put in aquarium kept in labouratory. Water samples were collected to grow the fungus in laboratory. For the growth of fungal culture P. D. A. (Potato Dextrose Agar) was used as medium.

PREPARATION OF PURE CULTURE:

To avoid bacterial contamination all the glass wares, instruments and media were sterllized along with all aseptic conditions. Inoculation was done in Laminar flow in sterilized condition. 13.5 gm of Potato Dextrose Agar (P D A) was mixed in 250 mg of sterilized water and the mixture was put in six Petri-dishes. The mixture was centrifuged for about 15 minutes after that the agar plates were incubated at 28-30 °c for the growth of cultures. Growth of colony was observed in 5-6 days. For full growth of the colony plates were kept for (8-10) days. For identification, slides were prepared from each colony by taking small tuft of mycelium and stained with Lacto-phenol cotton blue. Mycelium can be stained with the help of cotton blue for Permanent or temporary mount.

Neutral red is useful to stain living cultures for study and photography.



A B C

Fig (A) Colony of *Aspergillus fumigates* grown on Potato Dextrose Agar.

***(B)* Conidia of seen under compound microscope.**

(C) Symptoms of Disease

HOW TO EXTRACT ALOE VERA GEL :-

Aloe vera extract is well-known for its antioxidant, antifungal and antibacterial properties and is widely used to treat burns.

- Assess your Aloe vera plant.:
- Cut off the top third of the leaves:
- Remove the spines:
- Remove the top and bottom layers.:
- Remove and store the gel:

PREPARATION OF POTATO DEXTROSE AGAR MEDIUM :-

Potato-dextrose agar (13.5) g. Water 250 ml was taken. Aseptically

Streptopencilline, was added 10µg per ml, to the sterile, molten and cooled medium.

EQUIPMENTS :-

- Sterile Petri dish
- Filter paper (9cm diameter)
- U-shaped glass rod
- Microscope slides and coverslips (Sterile)
- Agar plate with mixed culture of fungi
- Sterile agar plate
- lactophenol cotton blue stain
- Glass capillary tube
- Scalpel
- Inoculating needle
- Sterile distilled water
- 95% ethanol
- Forceps

FEED : -

The fishes were fed with commercial feed brought from the market consisted of 30% crude protein ingredients consist of rice bran and cod liver oil

FEEDING FREQUENCY: -

Fish are of varying feeding habits in nature .As a general rule fish should be fed twice a day , first in the morning and evening at the rate of 1/5 of the body weight per/day which will ensure the appropriate supplementary feeding of fish .As the fish grows older , changes occur in the physiology and body structure and these changes result in change in feeding behavior of fish. These changes include increase in the length of alimentary canal and gap size of the mouth (Mc Cormick,1997).

EXPERIMENTAL SET UP : -

Healthy fishes were collected and kept in aquarium of 55L capacity with average weight of 8-10gm under observation for seven days with continuous aeration and fed with artificial feed. For experimental purpose the agar plates were inserted in the aquarium to infect the fishes with conidial spore suspension was taken gently from 8-10 days old colony. The conidial suspension was counted by haemocytometer and suspension was diluted to reach 8*105 spores /ml. For experimental purpose fishes were injected intermuscularly with 0.2ml conidial suspension. The aquarium was aerated continuously .For the treatment of Fungal disease the effectsof phytochemicals of aloe vera were studied .

RESULTS :-

During the present study a total number of 12 healthy fishes were collected i.e. *Labeo rohita* and were made infected by conidial fungi of Ascomycete group viz. Aspergillus fumigates and for the treatment of disease Phytochemicals of *Aloe vera* were studied.

SYMPTOMS :-

Small white patches of fungus appeared on head, fins. Darkening of skin was also observed on the body of Labeo rohita. Fungal lesions occur on the body of the fish, most commonly on the dorsal surface of the head, in front of the dorsal fin, in the region of adipose fin, and on other fin giving fish a characteristic appearance.

TABLE -1: PATHOGENICITY OF ASPERGILLUS FUMIGATES ON LABEO ROHITA:

S.No.	Injected fungi	Experimental fish	No. of fishes	Concentration of Spores	Dose (ml)	No of infected fishes	Infection within 7 days	Mortal-ity %
01	*A.fumigatus*	*Labeo rohita*	6	$8*10^5$	0.2	6	2nd day	100%

ARTIFICIAL INFECTION STUDIES WITH ASPERGILLUS FUMIGATES:-

Results of artificial infection studies with Aspergillus fumigates showed that it is pathogenic to Labeo rohita. Within the period of seven days infection was observed resulting in 100% mortality. These findings are compared with the reports of (Shrivastava 1996) who reported the pathogenicity of Aspergillus on fresh water fishes and death within 10 days.

WEIGHT MEASUREMENT :-

The weight of fishes was measured thrice i.e when the fishes were healthy, during infection and after treatment .During the course of my experiment the fishes were considered as Smallest in size, Medium sized and the Biggest in size. A slight decrease in growth rate was observed during infection and when I started the

treatment increase in the body weight of fishes was observed.

FIRST MEASUREMENT:- First measurement of weight measurement was done on 20 /09/ 2018

S. No.	Fish Size	Weight (gm)
1	Smallest	7.5
2	Medium	8.3
3	Biggest	11.5

TABLE: WEIGHT OF FISHES AS MEASURED ON 20/09/2018 BEFORE INFECTION.

SECOND MEASUREMENT :- Second measurement of weight was done on 20/10/2018 .During the time when fishes were infected by the fungal disease. A slight decrease in weight was observed

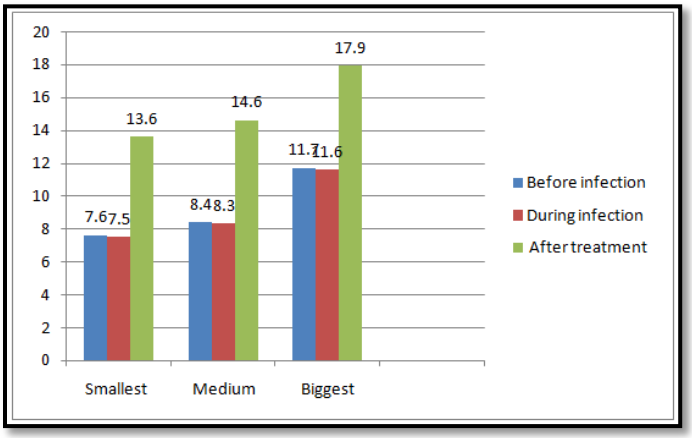
S No.	Fish Size	Weight (gm)
1	Smallest	13.6
2	Medium	14.6
3	Biggest	17.9

TABLE: WEIGHT OF FISHES AS MEASURED ON 20/10/2018

THIRD MEASUREMENT :- Third measurement of weight was done when the fish regained the healthy status back.

S No	Fish Size	Weight (gm)
1	Smallest	13.6
2	Medium	14.6
3	Biggest	17.9

TABLE: WEIGHT OF FISHES AS MEASURED ON 20/11/2018 AFTER TREATMENT.

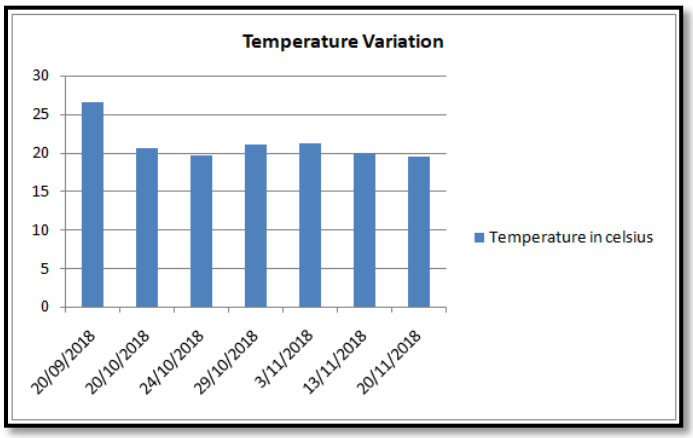


WEIGHT MEASUREMENT DURING THE WHOLE EXPERIMENT

TEMPERATURE:-

Temperature affects all chemical and biological processes. The metabolic rate of fish doubles for every rise of 10°C. Therefore, temperature has a direct effect on important factors such as growth, oxygen demand food requirements & food conversion efficiency The higher the temperature, the greater the requirement f oxygen and food and the faster the growth rate. Temperature partly determines the concentration of oxygen in water. The solubility of oxygen decreases with increasing temperature and so concentrations are usually lower in summer. Temperature also has a crucial role in stimulating fish gonad maturation and spawning activity.

S No.	Date	Temperature (c^0
1	20/09/2018	24.6
2	20/10/2018	20.5
3	24/10/2018	19.6
4	29/10/2018	21.0
5	03/11/2018	21.2
6	03/11/2018	20.0
7	20/11/2018	19.5



CLUSTERED COLUM SHOWING VARIATION IN TEMPERATURE

TREATMENT OF DISEASE:-

For the treatment of fungal disease in *labeo rohita* the gel extracted from vera was found to cure the fungal disease in fish The gel was put in aquarium twice a day for 25 days ,and the fish was brought to normal health condition .All the 6 fishes were cured , the phytochemicals present in the gel show their effect in the treatment of fungal disease in *labeo rohita* .

DISCUSSION: -

During the present study a shoal of fresh water fishes containing *Labeo rohita* 12 in number were collected from the Department pond, Department of Zoology and Applied Aquaculture, Barkatullah University Bhopal. These healthy fishes were infected by conidial fungi of Ascomcetes group viz. *Aspergillus*. Small white patches of fungus appeared on head, fins darkening of skin was also observed on the body of *Labeo rohita*. Fungal lesions occur on the body of the fish , most commonly on the dorsal surface of the head , in front of the dorsal fin the region of adipose fin, and on other fin giving fish a characteristic appearance. Loss of brig-htness of the body color for some time. During the infection the fishes were unable to take food properly due to less intake of food there was a decrease in the body weight of fishes, and during the infection the waste product was excreated in very less amount. It was found that the fungus *Aspergillus fumigates* was highly

pathogenic to *Labeo rohita* and fishes were infected within seven days and resulted in 100% percent mortality of fish.

When the fishes were treated with the gel extracted from Aloe vera it was found that the Phytochemical like **Terpenoid, Flavinoid, saphonin, Tenin,** and **Steroid** were found effective inthe treatment of fungal infection in the fish. Herbal immunestimulants have numerous potential benefits in comparison to vaccines and drugs like antibiotics. They are not expensive and are available almost all around the world; in contrast vaccines are generally expensive and are not available for all kinds of disease Although herbal remedies have been with us for human therapy for millennia, there has been relatively little research on the medicinal plants to be used against fish diseases. Herbal drugs can be used not only as remedies but even more so, as growth promoters, stress resistance boosters and preventatives of infections. Hence, herbal drugs in disease management are gaining success, because they are cost effective, eco-friendly and have no side effects. A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred plants are vital source for potent and powerful drugs. Plants rich in a wide variety of secondary metabolites of Phytochemical constituents such as tannins, alkaloids and flavonoids, which act against different diseases.

CONCLUSIONS:-

For carrying out the experiment in order to study the fungal disease caused due to fungus *Aspergillus fumigates* the fish *Labeo rohita* was selected because it is used as food and has a good amount of protein and help a lot to fulfills the need of proteins necessary for a person. But due to the outbreak of fungal infection the production of fish decreases and results in less amount fish production. Fungal infections are among the most common diseases seen in tropical fish. Because fungal spores are found in all fish aquariums, they can quickly colonize and create problems in stressed, injured, or diseased fish. Poor water quality can exacerbate the situation and lead to an increase in fungal infections in a seemingly healthy fish population. From the above piece of work that the conidial fungus *Aspergillus fumigates* causes mycosis in *Labeo rohita.* It was also observed that *Aspergillus fumigates* was highly pathogenic to *Labeo rohita* causing mortality of fish. Further the effects of Phytochemicals of *Aloe vera* were studied which seems to control the fungal disease and brought back the fish in normal condition. Phytochemical like Terpenoid , Flavinoid ,saphonin, Tenin, and Steroid were found effective in the treatment of fungal infection in the fish The conidia of *Aspergillus* spread very fast leading to the mortality of fishes these fungi grow and can spread disease in humans so control measures should be taken to avoid the spread of disease.

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