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AN *IN-VIVO* STUDY TO ASCERTAIN THE PROPHYLACTIC ACTIVITY OF ACONITUM NAPELLUS 30C, CROTALUS HORRIDUS 30C, MERCURIUS SOLUBILIS 30C, STREPTOCOCCINUM 30C IN *STREPTOCOCCUS AGALACTIAE* INFECTED TILAPIA

BACKGROUND

Streptococcus infection is becoming a major threat to the tilapia culture industry. As India is the second largest country in the aquaculture production, it has very important role in our economy as a flourish sector with much resources and potentials. But the impact of such infectious diseases is adversely affecting the entire industry with a high loss. The use of antibiotic medicines in these diseases results in the development of antibiotic resistance towards the bacterial strain. To resolve this situation there comes a necessity to think about an alternative method of treatment.

KEY WORRDS: *Streptococcus agalactiae*, Tilapia, Homoeopathic medicines

INTRODUCTION

Aquaculture growth around the world is increasing by its social and economic impact through its production due to the increase in fish demand for human nutrition that makes the industry towards intensified culture system. [1] Among this *Oreochromis Niloticus* (Nile Tilapia), an African Freshwater Cichlid is regarded as the second most cultured aquaculture species globally in both developed and developing countries, due to its rapid growth rate, tolerant to a wide range of environmental conditions and ease to culture techniques. [2] Tilapia has become a popular fish food because of its high nutritive value of protein, phosphorous, potassium, selenium, niacin, vitaminB12 and is low in fat and saturated fat, omega-3 fatty acids, calories, carbohydrates and sodium with low expense relative to other fishes. [3] Among the cultured fishes tilapia were considered as the most resistant species to the bacterial, parasitic, fungal and viral diseases when compared to the others. But in recent times they seems to be susceptible with the bacterial and parasitic diseases in which streptococcal infection has become a major problem in tilapia farming industry that results in severe economic loss. Here stresses including

high and low water temperatures, low dissolved oxygen concentration, high salinity and alkalinity, poor water quality like increased ammonia or nitrate concentration, high stocking density and along with this defect in handling and harvesting plays a significant role in the outbreak of this streptococcal infection. [4] But for this antibiotic was the only worldwide implemented treatment to treat the bacterial disease. However these treatment resulted in the appearance of antimicrobial bacteria, and degradation of the cultured environment. So it is important to have a safer and sustainable solutions for tilapia culture. [5]

Streptococcus agalactiae or Group B streptococcus (GBS) is a gram positive, non-spore forming pathogen affecting humans and animals as well as aquatic species. It is the major cause of streptococcosis in fish diseases which is considered as a destructive disease that can result in massive killing of fish and is responsible for heavy economic loss in freshwater and marine fishes worldwide. [6] As a solution to this fish famers use various chemicals and antibiotics like enrofloxacin and penicillin, clindamycin, oflaxacin, doxycycline etc. The wide use of such antibiotics in aquaculture led its resistance towards the bacterial strain mediated by ribosomal protection via the tetM gene. But the recent studies revealed that, these antimicrobial resistant genes and bacteria are transferred to the humans from fishes due to the indiscriminative use of those antibiotics. All these adverse effects that are facing in the aquaculture industry suggest for an alternate mode of treatment. [7]

Homoeopathy, is a safe, gentle and natural health care system treating each animal as a unique individual with an aim to relieve symptoms, restore itself and improve their overall health. Dr. Samuel Hahnemann states that ‘if the laws of nature I proclaimed are true, then they can be applied in all living beings’. This is the major warranty given by the idealizer of homoeopathy for the utilization of homoeopathic science in all living organisms, inclusive of fishes. [8] Homoeopathy works with its basic principles and laws that occurred throughout the development of medicines can be the most mysterious way of disease management. Also these medicines can be used as a preventive for the fish diseases. At present those antimicrobial drugs that used for the treatment of infectious bacterial diseases become unpopular in the field of aquaculture due to its resistance power to such pathogens. So our medical science is improving with its latest and advanced technology for the prevention and management of such disease outbreaks. Here homoeopathy creates the scope for fulfilling the future needs by providing better production without any harmful effects. Likewise homoeopathic medicines can be proven as perfect solution for any kind of diseases, having many beneficial effects however there is less research works conducted in the era. So it is worthful to have such studies in this

field of aquaculture that can favour the farmers along with this industry and our economy by preventing its loss. [9]

SIGNIFICANCE OF THE STUDY

Streptococcus agalactiae is the major cause of Streptococcosis in farmed tilapia that can cause massive killing of large sized fishes and is responsible for heavy economic loss. Also the outbreaks of the disease are reported in the humans through the intake of improperly cooked food and by the handling of infected fishes. So it is necessary to treat the streptococcal infection in the fishes and thus to prevent its further transmission to human beings. Homoeopathy works with all living organisms with the basic principle "like cure like". So it's important to think with an alternate mode of treatment like homoeopathy to the field of aquaculture for a better subsistence.

AIMS AND OBJECTIVES

AIM:

To study the effectiveness of homoeopathic medicines in the treatment of streptococcal infection in tilapia fish

OBJECTIVE:

- An in-vitro study to evaluate the effectiveness of homoeopathic medicines in bacterial culture
- A comparative study to access the efficacy of homoeopathic drugs with antibiotic drugs
- To evaluate the growth rate of infected fishes after the administration of homoeopathic medicines
- To study the histological changes in streptococcal infection of tilapia before and after treatment
- To study the prophylactic action of homoeopathic medicines in the bacterial diseases of fishes

REVIEW OF LITERATURE

Streptococcosis is one of the fish diseases that is reported in intensive aquaculture systems and causes high economic loss to fish farmers. This disease may yield high mortality rates of more than 50% over a period of 3-7 days. Streptococcus spp. reported to cause disease in fish include Streptococcus iniae, *Streptococcus agalactiae* and Streptococcus difficile. The presence of the pathogen in the environment of the fish is inadequate to cause a disease outbreak. Other main factor is the compromised immune system of the host increasing its susceptibility to the pathogen. Also some stressors plays a significant role in the outbreaks of disease in fish include high and low water temperatures, high salinity and alkalinity (pH>8), low dissolved oxygen concentration, poor water quality (such as high ammonia or nitrite concentrations), high stocking densities, as well as harvesting and handling effects. [10]

EPIDEMIOLOGY

Streptococcosis is the most significant disease that affecting the tilapia production industry. There is a variety of streptococcus species which can cause streptococcosis around the worldwide in which the *S. agalactiae* causing significant mortality and morbidity of the fish species. In addition, it has been isolated from the 17 species of fish including tilapia. [11] The outbreak of the disease usually take place when the fish gets exposed to certain stress factors like increased temperature in water, suboptimal oxygen levels in water and overcrowding for long period. [12] Bacteria are excreted through the faeces of infected fishes and survive in the water that are infective to other animals. The use if dead fish as food for other animals and the cannibalistic behaviour is being responsible for the outbreak of streptococcus disease in other animals. It is said that horizontal transmission of pathogen is the most common mechanism of bacterial spread. [13] The outbreak of the disease can reoccur in the same area or can occur from a new area due to the inappropriate farm management and biosecurity system. The severity and disease pattern are different in different place depending on the bacterial strain and other management system. [14]

ETIOLOGY

S. agalactiae or group B streptococcus is a gram positive, β-haemolytic organism that carries Lancefield group B antigen of the streptococcus genus, [15] which is the most relevant organisms that cause streptococcal infection in the tilapia farming. Along with this *S. iniae* and *S. dysgalactiae* are also a major bacteria that cause disease in tilapia fish. The virulence of the streptococcosis is induced by the high and low bacterial temperatures. [16] Also the factors like

surface antigen, toxins it produced and the enzymes helps the bacterial entry to the host. But these bacteria's are protected from the immune system of host environment by building a sessile colony in the extracellular matrix of polysaccharides. [17]

PATHOLOGY AND PATHOGENESIS

The group B streptococci and particularly *Streptococcus agalactiae* cause significant mortality and morbidity in a wide variety of fresh water and salt water fish species worldwide. The main route of entry of *Streptococcus agalactiae* in tilapia is the gastrointestinal tract. It causes severe mucus secretion in the gastric and intestinal lumen of tilapia that indicates the immediate host defence response against the pathogen. These mucus substance forms a biological mesh which may trap large amounts of bacteria. Also these bacteria can replicate in the lumen of gastrointestinal tract as a major pathogenic mechanism for the permanent colonization of the mucosal epithelium. [18]

The pathogen can enter into the fish through mucous membrane, cannibalism or mucous membrane. Then the fish react to the disease by initiating inflammatory process, to inactive the pathogen by white blood cells such as macrophages and polymorphonuclear leukocyte. Cell mediated immunity and humoral immunity plays an important role in preventing the bacteria to enter the blood circulation and internal organs. Even so some bacteria invade the immune system using some of its properties to cause systemic infection. The fish cell surface has an affinity towards the surface antigen of the bacterial to enter the body and replicate in lymph and blood causing septicaemia and spread to internal organs like liver, kidney, spleen and brain. Streptolysin S and streptolysin O are the toxins produced in streptococcus that can rapidly damages the cells and tissues including white blood cells, liver and heart. Likewise the enzyme produced by the streptococcus can digest large molecules which helps the bacteria to penetrate the skin to enter the body. [16]

However, some may not show any clinical signs before death. Macroscopically there will be skin haemorrhage, splenomegaly, hepatomegaly with organ paleness and visceral adherent are observed. Microscopically, it develops a primary inflammatory response of mononuclear cells with the subsequent formation of granulomatous nodules. Lesions include severe meningo encephalitis that can be haemorrhagic or granulomatous in nature with large areas of encephalomalacia that is similarly with choroid, sclera and eyeball. Also there will be deposition of a fibrinoid material on the pericardium with varying degrees of inflammation. [18, 19]

SIGNS AND SYMPTOMS

The main clinical signs observed in tilapia infected with *Streptococcus agalactiae* are loss of appetite, unilateral or bilateral exophthalmoses, eye haemorrhages, corneal opacity, distended abdomen, curvature of the spinal cord, stiffness, erratic swimming and bleeding at the base if the fins. All together the symptoms can differentiated into external signs and internal signs.

External Signs: It shows an abnormal behaviour pattern due to central nervous system affection that includes swirling behaviour, lethargy, bent bodies and disoriented fish. The diseased fish have eye affections such as endophthalmia or exophthalmia, unilateral or bilateral opacification and eye haemorrhages. Abscess are a common feature of streptococcus infected fish. It is symmetrically visible on the inferior jaw; base of the tail and pectoral fins that quickly burst and become a haemorrhagic ulcer. The abscess contain purulent material with a size from 2mm to 20mm according to the site of appearance. Haemorrhages are a general feature of streptococcosis. Multifocal pinpoint haemorrhages are visible around the mouth and at the base of fins. Ascites often with protruded anus is common during the outbreak of streptococcosis.

Internal Signs: The internal signs of streptococcus infection is related with the septicaemia. Here the bacteria reaches the circulatory system and disseminated to all the internal organs. So the septicaemia relates with the haemorrhages and inflammation in liver, spleen, kidney, heart, brain, eye and intestinal tract. In case of severe infection there will be adhesion of internal organs together and with peritoneal cavity walls. [12]

DIAGNOSIS

A streptococcal infection can be highly suspected if the affected fish exhibit abnormal swimming behaviour, pop eye, haemorrhages, and rapid severe mortalities. But for a confirmed diagnosis requires culture of internal organs followed by identification of the bacterium. So the proper diagnosis should be made if both the clinical signs and gross findings correlate with each other. Gross examination is performed by tissue sampling of the organs such as the brain, retro-orbital region, heart, kidney, spleen and liver. Also immunohistochemistry and molecular identification from culture is done. So for the proper diagnosis of the infection can confirmed by considering the clinical signs with histopathological lesions and microbiological isolation. [20]

PREVENTION AND TREATMENT

A decrease in the feed during streptococcal infection can reduce the mortality rate partially or completely. It is because the bacteria present in the water and their uptake by the fish is facilitated by feeding.

Reducing the water temperature can be an approach in recirculating system where the temperature is controllable with various methods like sunscreens, water sprinklers etc.

Antibiotics treatment are only effective when it is administered early during the course of disease. Oral antibiotics are ineffective in most cases as the diseased fishes have a reduced appetite. Also the mortality rate of the fishes are increased when action of the antibiotic is over. So the farmers increase the dosage of antibiotic which results in the antibiotic resistance of bacterial strain. [21]

SELECTION OF HOMOEOPATHIC REMEDY

The external and internal manifestations of the streptococcosis disease in tilapia fish were considered for the selection of medicine. Aconitum napellus and Mercurius solubilis are the medicines that cover the totality of the symptoms for remedy selection. Here the remedy was selected by collecting all the symptoms in its external and internal appearance and through other examinations. Hahnemann stated the totality as; *"the symptoms are the outwardly reflected picture of the internal essence of the disease that is the affection of the vital force"*. [22] The other two drugs, Crotalus horridus and Streptococcinum were taken on the basis of 'Law of similia'. In this selection the signs and symptoms presented by fish corresponds with symptoms in the Materia Medica. These are the two major principle for the selection of medicine in homoeopathy.

ACONITUM NAPELLUS

- the common name of this drug is Monkshood from the family Leguminosae
- acts in the beginning of an acute disease with sudden origin
- physical restlessness is one of the most characteristic manifestation of aconite
- aconite has a wide sphere of usefulness in affections of eye(exophthalmos, eye haemorrhage)
- eyes red and inflamed, with deep redness of the vessels
- red, swollen appearance of the skin
- indicated for haemorrhages

- loss of appetite
- inflammation and soreness in the liver
- swelling of the abdomen as in ascites
- peritonitis [23, 24, 25,26]

CROTALUS HORRIDUS

- it is a medicine prepared from the venom of rattle snake; a remedy of Ophidia group
- it causes bleeding from all orifices and surfaces, and it corresponds to the haemorrhagic diathesis
- for absorption of intra-ocular haemorrhages, into the vitreous, but particularly for non-inflammatory retinal haemorrhages
- it is directly indicated for cerebro-spinal meningitis, intestinal haemorrhage, peritonitis
- ulceration of the stomach
- distended abdomen
- swelling and discolouration with great sensitiveness
- haemorrhage from all part of the body and even from the pores of the skin
- haemorrhage- slow oozing, dark thin fluid, excessive flow, involuntary evacuations, great debility
- Skin lesions like petechiae (pin point haemorrhage), ulcers, abscess [23,24,25,26]

MERCURIUS SOLUBILIS

- The potencies are prepared from the soluble black oxide mercury or from pure metallic mercury
- Merc affect more or less every organ and tissues of the body
- Ulcerations of the mucous membranes especially of mouth
- Swelling oedematous, continued exudations
- Suppurations, pus, bloody with offensive discharges
- Trembling, twitching with disordered motions
- Face pale, yellow, dirty looking, puffy under eyes
- Painful, swollen, bleeding from gums, ulcers in the mouth
- Liver enlarged, sore, indurated (hepatomegaly)
- Prolapse of anus (anus protruded)

- Tendency to ulcerate runs through the remedy; ulcers sting and burn and have a lardaceous base with an ashy white appearance looking as if spread over with a coating of lard [27]
- Ulcers over skin; irregular in shape, undefined edges, spreading, shallow, bleeding, with cutting and proud flesh; also boils and abscess over skin
- Abscess form when the life force is so slow that there is no tendency to repair, a slow and prolonged pus formation, no irritability in the abscess, no tendency to granulate, it opens and keeps on discharging and seems dead. Merc will warm it up and favour granulation
- Extreme restlessness with anxious, impulsive disposition [23,24,25,26]

STREPTOCOCCINUM

- It's says that when animals experimentally infected with a strain of streptococcus, the germ is apt to invade the same tissues in the new host that it invaded in the old [29]
- Streptococcinum are more to be indicated in acute infections
- Anti-febrile action; septic symptoms in infectious diseases. Rapid in its action
- Eczema dry desquamating, repeated eczema
- Skin cellulitic patches, fibrinous dermatitis [28]
- Having action on nervous system, eyes, circulation, digestive organs, skin [30]

SELECTION OF POTENCY

Maupertius, a French mathematician stated; *"the quantity of action necessary to effect any change in the nature is least possible, the decisive movement is always a minimum, an infinitesimal".* The potency is selected on the basis of the susceptibility of the organism. So in lower organisms like fishes are always suited with low potencies. Also in diseases with increased pathology show needs a low potency. While treating fish diseases it will not be having much general symptoms like that of humans and also in cases where the complete picture of the disease is not available. So all these exhibits the need for the selection of lower potency. [31]

REPETITION OF MEDICINE

After the administration of the homoeopathic medicine, observe the changes for every few hours. Repetition of medicine can only be done when it is strictly indicated. According to the teaching of Stuart Close "repeat the dose only when the improvement ceases". Apart from this,

multiple doses are indicated only when the single dose fails to bring out the expected results. Once the improvement is observed the medicine can be withdrawn. [32]

REVIEW OF RELATED LITERATURE

1. **EFFECTS OF STREPTOCOCCINUM, HEPAR SULFUR, ROSMARINUS OFFICINALIS AND ERYTHROMYCIN ON CULTURED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) WITH EXPERIMENTAL STREPTOCOCCOSIS:** This is an experimental study to evaluate the effectiveness of Streptococcinum, Hepar sulphur and Rosmarinus officinalis compared with that of Erythromycin. There were four treatments and two controls; one without any injection and therapy and other with *Streptococcus iniae* injection without any therapy. Here the survival rate of the control with other treatment was significant. There was a survival rate of 56.7% in Erythromycin, 42.5% in Streptococcinum, 32.5% in Rosmarinus officinalis and 49.2% in Hepar sulphur. Similarly 86.7% survival rate in control 1 and 21% in control 2. This shows that all the extracts taken were effective for inhibiting the growth. [33]
2. **ANTIMICROBIAL ACTIVITY OF DIFFERENT HOMOEOPATHIC DRUGS AND THEIR POTENCIES AGAINST *ASPERGILLUS NIGER* IN VITRO:** This study deals with an in-vitro study on confirmation of the antifungal activity of homoeopathic remedy which inhibits the further growth of fungal hyphae. Here, the mother tincture and decimal scale potencies of 10 different homoeopathic remedies are taken along with Ketoconazole as standard antifungal drug and 90% alcohol, double distilled water as control group. A marked anti-fungal activity was observed in Zingiber officinalis mother tincture followed by other remedies. Thus a new alternative method of treatment for aspergillosis had been proved. [34]
3. **A REVIEW ON SCOPE OF HOMOEOPATHY IN AQUACULTURE FOR A NOURISHING FUTURE:** This literature shows the importance of aquaculture in ancient times as well as in the present days as the basic requirement for survival. Here the homoeopathy is providing adequate treatment and support for any kind of disease management and prevention. Medical science has improved with the latest and advanced technology for the prevention and management of any kind of major disease outbreaks. Increased use of antibiotics causes the development of resistant strains of bacteria in worldwide resulting in large loss, which urges the farmers to adopt the

homoeopathic treatment. Here the homoeopathy creates the scope for fulfilling the future needs by providing better production without any harmful effects. [9]

4. **PROPHYLACTIC EFFECT OF *ANDROGRAPHIS PANICULATA* EXTRACTS AGAINST *STREPTOCOCCUS AGALACTIAE* INFECTION IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*):** This study is to demonstrate the antimicrobial activity of *A. paniculata* against the *S. agalactiae*. Six herbs was extracted with water, 95%alcohol and methanol as solvents. Although antibiotics are used to control the bacterial infections, it is been banned due to the bad effects. So here it is necessary to find an alternative antimicrobial substance to replace such adverse drugs. Thus in this study *A. paniculata* shows a highest antimicrobial activity against *S. agalactiae* from the result on inhibition zone size and MIC value. So this study suggest that different fish pathogenic bacteria have different sensitivity to the herb extracts, which is to be confirmed for different pathogens like that of *S. agalactiae*. [35]
5. **ASPECTS OF THE NATURAL HISTORY AND VIRULENCE OF *S. AGALACTIAE* INFECTION IN NILE TILAPIA:** This study was done to investigate the epidemiology, transmission and virulence of this bacterial infection. For this nine outbreaks of meningocephalitis and septicaemia in Nile tilapia farms were analysed. It shows that there is a large variation in the weight of affected fish, high mortality and disease occurrence at water temperature above 26^0C. From the isolated bacteria 29 strains were identified with by phenotypic test and rRNA gene sequencing. The transmission and route of infection were identified as by direct contact or through water. So we can conclude that *S. agalactiae* is a major pathogen of Nile tilapia exhibiting high virulence, regardless of the geographic origin of the isolated strains. [36]

METHODOLOGY

Place of Study: Microbiology Lab, Department of Aquatic Biology and Fisheries; University of Kerala; Kariavattom; Thiruvananthapuram; Kerala

REQUIREMENTS

HOMOEOPATHIC MEDICINES: Aconitum napellus 30C

Crotalus horridus 30C

Mercurius solubilis 30C

Streptococcinum 30C

PATHOGEN: *Streptococcus agalactiae*

MEDICINES

For this study; Aconitum napellus and Mercurius solubilis was selected on the basis of totality of symptoms; Crotalus horridus and Streptococcinum on the basis of symptom similarity. The 30th potency of this medicines were selected on the basis of posology. [31] The medicines were manufactured by Dr. Reckeweg & Co. GmbH and purchased on 31 January 2020.

PATHOGEN

The active microbial culture of *S. agalactiae* (CMFRI/SA-01) slant was obtained from Central Marine Fisheries Research Institute, Kochi; Kerala on 20th January 2020. The isolate was further streaked on fresh Tryptic soya agar (Himedia) plates to test the purity and pure isolates were selected for the study. The identity of the strain was further tested by amplification and sequencing of the 16S rRNA gene. [37] The isolates were stored in respective slants for future use.

PREPARATION OF DISC FOR ANTIBACTERIAL ASSAY

For determining antibacterial activity of different homoeopathic medicines agar disc diffusion method was used Bauer, et al., 1966. [38] Filter paper (Whatman no. 1) was used to prepare discs approximately 5 mm in diameter, which were placed in a petri dish autoclaved at 15lbs pressure and dried in a hot air oven. Sterilised discs of filter paper were soaked in respective homoeopathic medicines allowed to stand for 30 min. Drug-impregnated discs were used for further drug sensitivity assay.

ANTI MICROBIAL ASSAY OF HOMOEOPATHIC MEDICINES BY DISC DIFFUSION METHOD

Nutrient agar plates were inoculated with 0.1 ml of *Streptococcus agalactiae* in triplicates and spread well with sterile swabs. Autoclaved and air dried Filter paper discs (Whatman's 1) were soaked with Aconitum napellus 30, Crotalus horridus 30, Mercurius solubilis 30 and Streptococcinum 30 were aseptically placed apart from each other on each agar plate. Standard drug, Tetracycline 10mcg (HIMEDIA) was used as positive control and 90% ethanol was used as vehicle control. The plates were incubated at 37ºC for 18 - 24 h. The zones of inhibition was measured and expressed in millimetre.

MEASURING ACTIVITY INDEX

According to Haniffa and Kavitha (2012), the following formula was used to measure the Relative Inhibition Zone Diameter (RIZD %),

% RIZD = (IZD of sample / IZD of antibiotic) * 100

STATISTICAL ANALYSIS

Data were represented as mean ± standard deviation of mean. The mean differences between the groups were assessed using the 1-way analysis of variance (ANOVA). A 'P value' less than 0.05 was considered significant. Statistical analysis was performed using the Microsoft Excel (2006).

RESULTS

ANTI MICROBIAL ASSAY OF HOMOEOPATHIC MEDICINES

MEDICINE	Zone of inhibition (mm) Day 1	SD	Zone of inhibition (mm) Day 2	SD
Aconitum napellus	6.67	0.57	10.33	0.57
Mercurius solubilis	6.5	0.5	6.33	5.68
Crotalus horridus	7.83	0.76	11	1.73
Streptococcinum	7.6	0.36	7	6.24
Ethanol	6.1	028	6.9	0.36
Tetracycline	21.3	0.31	26.2	0.5

Table 1: Anti-Microbial effect of homeopathic medicines against *S. agalactiae*

Table 1 shows the antimicrobial activity of homoeopathic medicines against the *S. agalactiae* (Figure 1). The observations has done every 12 hours and after 24 hours when an evident zone of inhibition was observed the reading has taken in mm measurement. So in the first day, Crotalus gave maximum zone of inhibition 7.83$\pm$0.76 mm and minimum with Mercurius solubilis (6.5$\pm$0.5 mm). On day 2, the lowest activity was shown by Mercurius solubilis with 6.33$\pm$5.68 mm and maximum with Crotalus (11$\pm$1.73 mm).

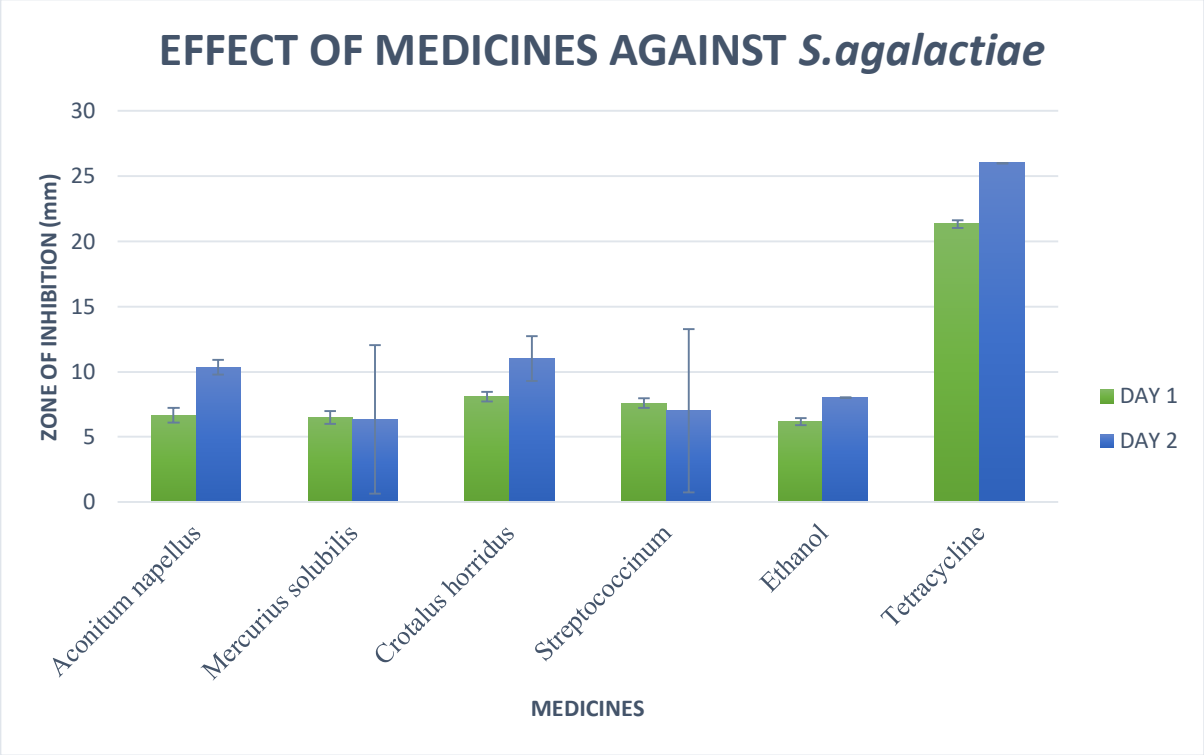

Fig. 1: Graph showing the Zone of inhibition of medicines against *S. agalactiae*

RZID (%) showed maximum value of 38.57 % with Crotalus while the lowest was 30.95% with Mercurius solubilis on Day 1. On Day 2, the values were highest with 42.3 in Crotalus and lowest with 24.35 with Mercurius solubilis (Fig. 2).

MEDICINE	RZID% DAY 1	RZID% DAY 2
Aconitum napellus	31.74	39.74
Mercurius solubilis	30.95	24.35
Crotalus horridus	38.57	42.3
Streptococcinum	36.19	26.92

Table 2: Relative Inhibition Zone Diameter (RIZD %) of medicines with respect to Tetracycline.

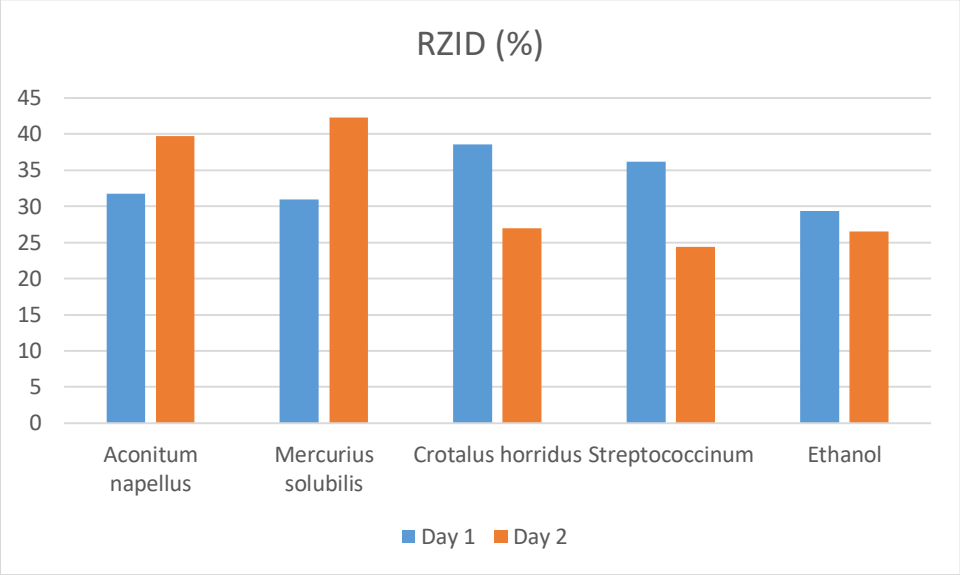

Fig. 2: Graph showing the RIZD% of medicines with respect to Tetracycline (10mcg)

RIZD (%) of the drugs are shown in Fig. 2 and Plate 1. On day 1, the maximum activity was with Crotalus horridus (38.57) while the lowest was with Mercurius solubilis (30.95). Also in the second day results showed maximum activity with Crotalus horridus (42.3) while the lowest was with Mercurius solubilis (24.35).

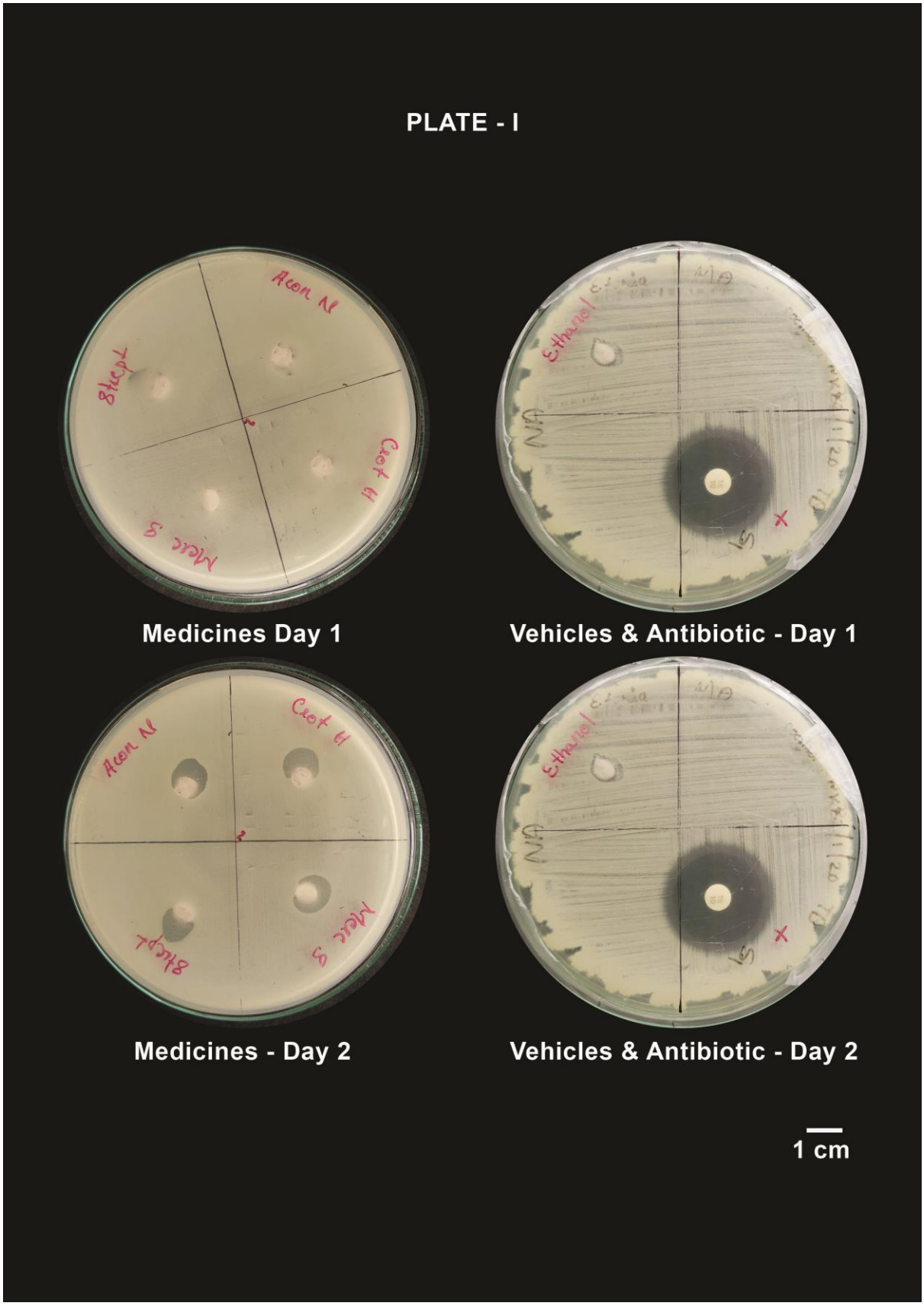

Plate 1: Antimicrobial activity of homoeopathic drugs against *S. agalactiae*

DISCUSSION

Streptococcus agalactiae is a serious pathogen affecting the major farmed fishes and shellfishes causing serious loss to the farmers across several continents covering tropical and temperate areas. Thus developing a cheap alternative to antibiotics is a serious concern. Due to bioaccumulation, antibiotics are of least interest for way forward, giving way to research and application of traditional medicines and homeopathic medicines.

The effect of homeopathic medicines applied in fish and other livestock is increasing day by day. The promising results obtained from the current study indicate acceptability of drugs such as Aconitum napellus, Crotalus horridus, Mercurius solubilis and Streptococcinum for tilapia and thus can be applied in other aquatic organisms.

Tilapia commonly referred as "Aquatic chicken" has been an important target fish for several disease and other drug related therapies/experiments across several disciplines. There has been several attempts looking at studies on bacterial, fungal and other parasitic infections on tilapia. Antimicrobial activity of homeopathic drugs have been studied in-vitro and in-vivo, but found to be effective in tilapia, carp, shellfishes, etc.

From the current study, it is very clear that the homeopathic drugs such as Crotalus Horridus and Aconitum Napellus has given positive results showing anti-bacterial effect against fish pathogenic bacteria, *S.agalactiae sp.* The homeopathic medicines got a chance of activity against pathogen can be expected in fishes rather than in petri dishes due to the possible symptom expression in fishes alone. This is the reason why there is lower expression activities in the current study (antibacterial activity).

Drugs selected for the current study include Aconitum napellus 30C, Crotalus horridus 30C, Mercurius solubilis 30C and Streptococcinum 30C showed interesting results against the bacteria in the current study indicating wide application in fishes and other livestock management. The results gave promising results while after the repetition of dose, suggested by the Stuart close in the posology. Thus, higher effect can be expected in fish by giving repetition of dosage.

Potency of a drug is the most crucial part validating the principles of homeopathy. Lower potency shows a good result in the lower organisms, better the activity is expected. The current study showed positive results at a potency of 30C. More dilutions can be tried further to see improved effect on the target fish. Similar results have been observed in several other studies with homoeopathic medicines.

Drugs such as Hepar sulphur, Streptococcinum, Arsenicum album, Sulphur etc. have shown positive effects on growth, immune enhancement, disease resistance, etc. in tilapia, zebra fish, gold fish, etc. Thus the applications of homeopathic medicine needs further exploration to conclude the positive impacts on fishes and other livestock. The loss of stock due to several pathogens can be limited thereby reducing the monetary loss for farmers by application of these drugs via feed, culturing water, etc. Delivery of drugs via different modes also need optimisation.

LIMITATION

1. Due to pandemic Covid-19, all the educational and research institutions were under lock down affected the study very badly. Thus no research activities were able to be conducted within the scheduled plan.
2. Due to lockdown, fish was also not available for the experiment
3. Thus the exposure studies in fish was not able to be performed as planned earlier in the synopsis, but antimicrobial studies alone.

CONCLUSION

The result can be concluded as Homoeopathic remedies being an efficient, safe, economical, alternative antibacterial treatment for *S.agalactiae sp*. over various hazardous chemicals and antibiotics in use. Among homoeopathic remedies used the most effective is, Crotalus horridus 30^{th}C potency. Through this the Law of Similia being reproved and the most effective potency among freshwater fish for streptococcosis is 30^{th} potency. The efficacy of Homoeopathic medicines in the field of Aquaculture over the Allopathic antibiotics is been proved in this study.

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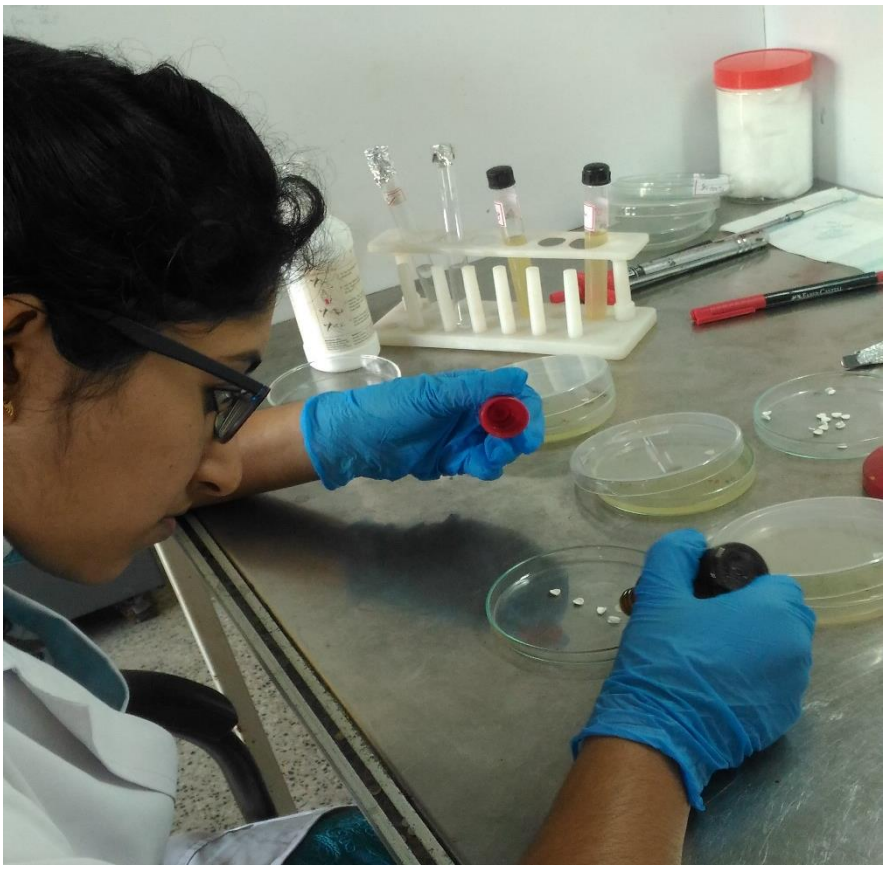
APPENDIX



Disc preparation

Sub-culturing Pathogen, *S. agalactiae*