



## Efficacy of certain Compounds in Controlling major Fish pathogens and Physio-chemical Parameter analysis from two ponds at Alwarkurichi village, Tirunelveli district in Tamilnadu, India

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### Abstract

The present study was aimed to investigate the two aquaculture pond water samples physio-chemical parameters and microbial analysis at Alwarkurichi village, Tirunelveli district. The physio-chemical parameters such as pH, conductivity, dissolved oxygen, BOD (Biological oxygen demand), calcium, magnesium, total hardness, chloride, phosphate, nitrate, and total dissolved solid were tested at Tamilnadu water supply and Drainage broad District water testing laboratory, Palayamkottai. Subsequently, microbial culture present in the water samples were identified with standard methods. They are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Vibrio cholera* as more dominant in the two ponds. Hence, the identified three pathogenic bacteria were treated with some commercially popular chemicals at various concentrations. The antibacterial activity of three compounds Sodium chloride, Formalin and Copper sulfate was studied against three pathogenic bacteria using agar well diffusion method. The growth suppression rate exhibited by Copper sulfate were higher at 0.08mg/10ml when equivalence to others. When compared to copper sulfate, size of the acquired growth distinct was less when examined with ampicillin. Copper sulfate was identified as the most efficacious against three pathogens.

**Keywords:** Antibacterial activity, ampicillin, commercial chemicals, *Klebsiella pneumoniae*, *pseudomonas aeruginosa*, physio-chemical parameters, *vibrio cholera*.

### Introduction

Water is one of the most important for a system formed by the interaction of a community of organisms with their physical environment. The fact of existing that has the being of water for all the living things on earth. It is necessary for the best adaption to the environment of all living things be it plant or animal life. At most this is the ample valuable in nature but also the most for an improper purpose or abused one. Hence Alwarkurichi village of Tirunelveli district is depending upon the agriculture and aqua culturing products from their pond. Yearly once of rainy monsoon period naturally fill the pond with water run in perennial river Tamiraparani. These ponds or lakes are disturbed by individual human and other main activities like dumping municipality wastages. Although, the freshwater lake or ponds are mainly for the aquaculture has become an important economic activity in many countries. Form the water quality of aquaculture has been accomplished by increasing problem with disease as a substantial source of danger to sustainable production and trade<sup>1</sup>. However, fish are sensitive to various pathogenic microorganisms, for the most part rearing in high densities performance. So the scientists were handling various practices to controlling disease to the aquatic animals. Since the last twenty years, aquaculture has been a growing activity in worldwide and impressive growth has been attended by some patterns potentially damage human and animal health<sup>2</sup>. Fertilizers and human wastes are the most

contaminant in aquaculture pond; it can also cause disease to the aquatic animals. For the purpose of safety risk most of compounds used to ameliorate water quality or soil have limited or no risk safety on pond aquaculture<sup>3</sup>. A forceful consequence of these substances on the resident microflora is not easy to assess because of the quality of being and development of the aquatic environment<sup>4</sup>. The general recommendations of risk associated with chemical usage to the aquaculture and it makes extremely hard to prepare. Due to the farms are settled in waters with dissimilar capability to engage the wastes, including medicinal chemicals, without causing insufferable environmental impacts<sup>5</sup>. From that it will not only be profitable but also be an easier process to fulfill the protein requirement of malnutrition and unprivileged population has the potential to fulfill the nutritive food supply and can also enhance the food security and income generation of fishermen communities of this area<sup>6</sup>. But in the case of this study are to explore the risky and efficacy of certain compounds are in order to cut down the wide use of these chemicals antibacterial activity in aquaculture pathogens, which had caused a series of environmental and food safety problems. For this purpose of the invitro studies on *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Vibrio cholera* were selected to examine the pathogens. Therefore, the investigation of physio-chemical parameter and antibacterial activity with high and low risks of chemicals was used in this study.

## Material and Methods

The sample was collected from aqua culture pond in southern part of Tamilnadu village, Tirunelveli (India). The study sites of Achankulam (Chettikulam) and Therku kurunthudaiyarkulam (Azhagapapuram) at N 8° 47' 08.5" to N 8° 47' 56.9" and E 77° 22' 02.7" to E 77° 19' 51.2". The water samples were collected during 8.00am to 9.00am using sterile 5litre Sunpet plastic jar. These water samples physico-chemical parameters such as pH, conductivity, dissolved oxygen, BOD (Biological oxygen demand), calcium, magnesium, total hardness, chloride, phosphate, nitrate, and total dissolved solid were tested by Tamilnadu water supply and Drainage board, District water testing laboratory, Palayamkottai. Subsequently, the collected water samples were tested for microbial quality and cultures identification were done in Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi. The microbial colonies were isolated using standard serial dilution method<sup>7</sup> and identified by standard biochemical test methods<sup>8</sup>. Then the antibacterial activities of three commercial chemicals were estimated by agar well diffusion method. The effect of different concentration (0.2, 0.4, 0.6 and 0.8 µl/10ml) chemicals of Formalin, Copper sulfate and Sodium chloride were prepared. Hence, the nutrient agar well diffusion plates were incubated at room temperature for 24 – 48 hours. The plates were visually inspected for any zone of inhibition around the wells and the diameter of the zone of inhibition measured in millimeter range from 0.1mm. That the zone of growth inhibition percentage was calculated by the standard growth inhibition formula as,

$$GI (\%) = \left( \frac{Z_t - Z_c}{Z_c} \right) \times 100$$

Where, GI is the growth inhibition percentage;  $Z_t$  is the zone inhibition of treatment;  $Z_c$  is the zone inhibition of control. The measured growth inhibition values were estimated for F-value significant by one way ANOVA calculation using SPSS software version 20.0.

## Results and Discussion

The physico-chemical parameters of aquaculture pond varying results indicates that the highest value of Electrical conductivity (612 Micro mho/cm), calcium (29 mg/L), magnesium (17 mg/L), total hardness (144 mg/L), chloride (40 mg/L), nitrate (3 mg/L), and total dissolved solid (416 mg/L) in the water samples collected in Achankulam (Chettikulam). However, highest value of pH (7.47), Biological oxygen demand (27.45 mg/L), phosphate (0.10 mg/L) and dissolved oxygen (2.6 mg/L) were in Therku kurunthudaiyarkulam (Azhagapapuram) ponds are represented in figure-1. Simultaneously the water samples were tested for the microbial colonies were observed and the identified colonies were treated with commercial chemicals. The antibacterial activity studied with various compounds which are frequently used to control the disease causing agent of

aquaculture pathogens. To control *Pseudomonas aeruginosa* pathogenic bacteria, among the tested chemicals, formalin showed 4.48 mm zone formation, sodium chloride produced zone size of 3.31mm, copper sulfate showed 4.63mm zone. The same experiment was carried out for the bacteria *K.pneumoniae* and *Vibrio cholera* formalin exhibited a zone size of 4.42mm and 4.38, sodium chloride showed 2.76mm and 3.54mm and Copper sulfate induced 6.82mm and 4.85mm (Table 1) respectively. The different concentrations of growth inhibition of all the three chemicals against three pathogens were shown in (figure-2A, B, and C). All the three chemicals showed better activity over ampicillin treated as control. Minimal inhibitory concentrations of sodium chloride against all the three pathogens were 0.08mg/10ml and Copper sulfate under maximal concentration of 0.08mg/10ml showed maximum activity over other chemicals.

Aquaculture farming now a day's getting lots of problem due to disease causing agent create damaging the economic trade of production. So the invitro study of physio-chemical parameter and pathogen identification from the aquaculture pond water has been done. Also the water quality monitoring and management with the objective of make better the quality of water with keep better capable of being sustained management. In that comparative analysis suggests the distinct nature of different river water and it depends on geographical location, time zone and geological foundation. Water quality admits all physio-chemical parameters, to life and living things that promoting use of water<sup>9</sup>. This case occur in the rivers also the total coliform count, except at the upstream station of Gokak town, were found to exceed the limits of standard of class 'C' (< 5000 MPN/100 ml) of the river. This increase in the values could be mainly attributed by the discharge of untreated domestic waste at the upstream of all the monitoring stations and urban runoff<sup>10</sup>. Also the increase in number of zooplanktons was in accordance with temperature of its habitat. The study also showed that zooplankton species survive in the neutral condition. Thus the condition of the River could suppose to be eutrophic as pointed by the noticeable heterogeneity of zooplankton. Physico-chemical conditions of River Kapila can be changed because of industrial effluents which release to the water. Therefore, conducting further studies in this area is essential to measure the diversity of zooplanktons<sup>11</sup>. Such as the earlier work was done to the physico and chemical characteristics of water and soil in Aquaculture pond are investigated in season before seedling with a view to optimize the fish productivity and to incorporate the scientific way of fish rearing in the ill effects caused by imbalance in the quality of water and soil of fish ponds for survival of aquatic life<sup>12</sup>. However, the aquaculture is concerned the aquatic performance in any event of a water quality liable and any characteristic of water that affects the survival, reproduction, growth or management of fish<sup>13</sup>. Although, the previously reported as physio-chemical parameters as water quality in ground water and pond water, from that pond water indicated that the community ponds are highly polluted and unsafe for human use<sup>14</sup>. Thus the pathogenic bacteria were

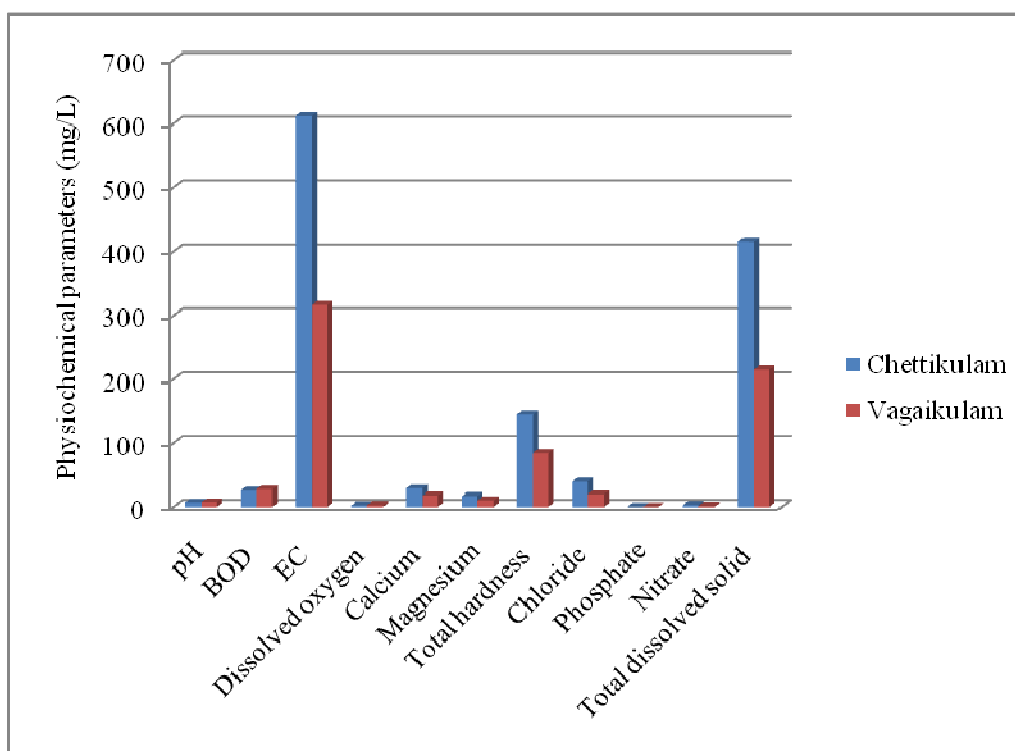
treated with certain chemical compound. The spreading of disease led to a large and growing trade in live aquatic animals in the aquaculture<sup>15-17</sup>, Disease is now considered to be as one of the most important task for efficient and sustainable aquaculture production and trade<sup>18</sup>. However, some other compounds using for the aquaculture as the Phospholipids compound shows higher activity towards *Staphylococcus aureus* this compound can be used against infection caused by *Staphylococcus aureus*<sup>19</sup>. But in this study of investigating is to assign a chemical drug that shows broad range of susceptibility of dread full disease causing pathogenic bacteria species. We investigation is considered to be one of the case study of using Copper sulfate, Formalin and Sodium Chloride for effective development of aquaculture, Copper sulfate is selected to use as

a good chemical algacide and drug against aquaculture pathogens in controlling diseases has been decided after reviewed the work, use of copper in aqua culture and pond<sup>20</sup>, a complete guide of Copper usage in field application, it shall may beneficial if Copper sulfate may be used in small indoor system of aquaculture. Although the Copper may used as an antifouling paint and also component of the food fed to farmed various fleshy fishes in particular concentration<sup>5</sup> in we study it was well known that the drug using against aquaculture pathogen and algacide than commercial drug, is easily available and less toxic when used in minimal concentration, finally it was conclude that the usage of this chemical is affordable against disease causing pathogen, copper sulfate is recommended to use in all aquatic system.

**Table-1**  
**Effects of the commercial chemicals against on three pathogens**

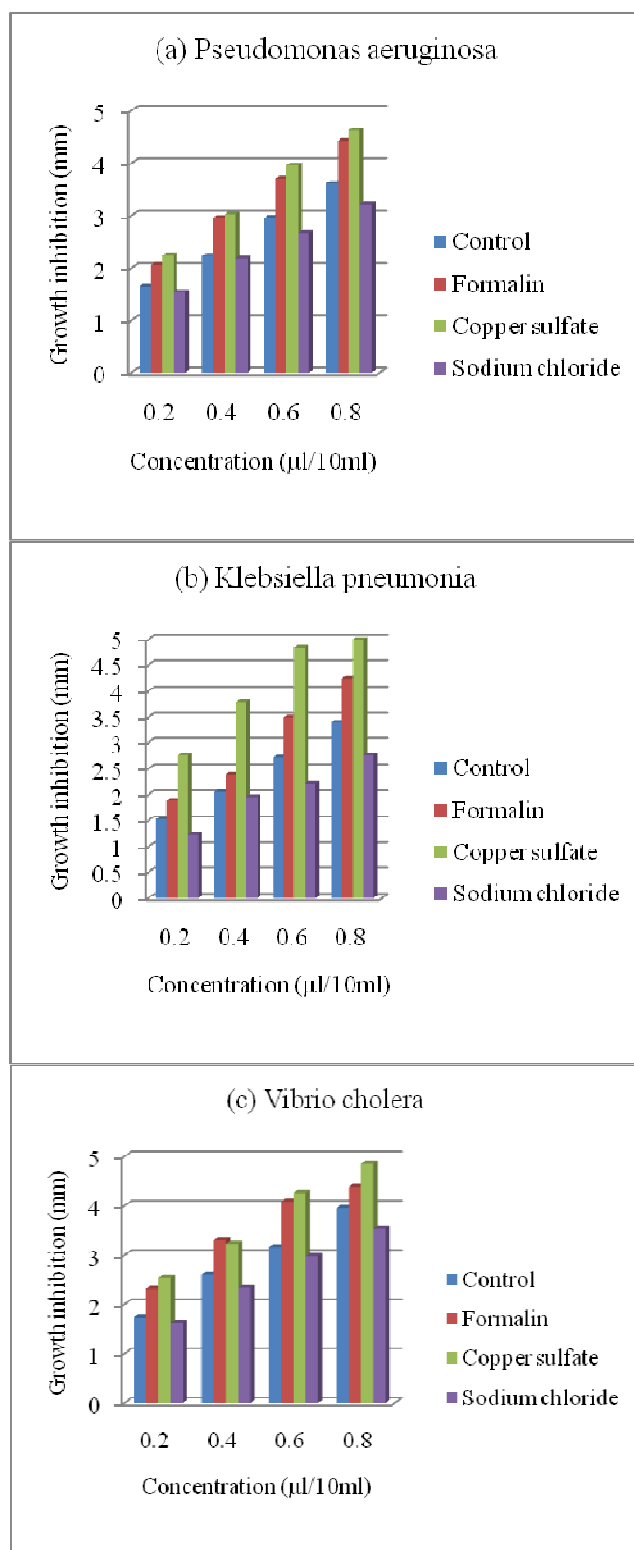
Chemicals	Microbial species						F value
	Pseudomonas aeruginosa		Klebsiella pneumonia		Vibrio cholera		
	Mean±SD	%growth inhibition	Mean±SD	%growth inhibition	Mean±SD	%growth inhibition	
Control (Ampicilin µl/10ml)	2.6 ± 0.85	8.54	2.4 ± 0.81	8.6	2.9 ± 0.93	9.3	0.257
Formalin (µl/10ml)	3.3 ± 1.02	26.29	3.0 ± 1.06	22.87	3.5 ± 0.92	25.02	0.267
Copper sulfate (mg/10ml)	3.5 ± 1.05	33.41	4.6 ± 1.73	80.89	3.7 ± 1.03	31.83	0.755*
Sodium chloride (mg/10ml)	2.4 ± 0.71	7.44	2.0 ± 0.64	15.56	2.6 ± 0.82	8.18	0.641*

SD= Standard deviation; “\*” is significant level at 0.5%



**Figure-1**

Graphical diagram showing the physio-chemical parameters of aquaculture ponds at Chettikulam and Vagaikulam, Tirunelveli district, Tamil Nadu



**Figure-2**

A, B, C shown as different concentration of growth inhibition on *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Vibrio cholera*

## Conclusion

Hence it was conclude that copper sulfate was a good drug in controlling disease causing pathogenic bacteria in the aquaculture and its administration in disease control efficacy should be used in field purpose reduce the expense of antibiotic drugs.

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