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Enzymes Activities in *Clarias gariepinus* Infected with *Staphlococcus aureus* and Treated with *Chromolaena odorata* Leaves Aqueous Extracts

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Research Article

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Abstract

One hundred and fifty (150) *Clarias gariepinus* table sized of mean length 16.09±2.34cm and mean weight 750.55±23.99g were infected intra muscularly with 1.0ml of 1.4×1010cfu of Staphylococcus aureus using a 2ml syringe and 21 gauge hypodermic needle at day 1,2,3, 4,5 and observed for disease presence. The fish were later exposed to different concentration of aqueous *C. odorata* leave extracts in triplicates for 7 days. The blood samples were taken after 3 and 7 days of treatment through the kidney puncture and were assayed for enzymes: aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP) and alkaline phosphates (ALP). All the analyzed enzymes were higher after infection with the pathogen, when compared to the activities before infection. After seven days of treatment with *C. odorata* leave extract the ALP and ACP were lower in the treated group compared to the untreated group (0.00ml), but the results in the treated groups were not concentration dependent. The AST (33.66±7.57; 30.33±9.71) and ALT (5.0±1.77; 4.33±1.19) were higher in the untreated (0.0ml) group after day 2 and 5 compared to the treated group but when exposed to 2.0ml and 3.0ml, it had higher activities compared to the untreated (0.0ml) at the end of day seven of treatment. At the end of day seven of treatment, the 0.0ml (untreated) recorded 65.01± 2.0% survival rate and 0.00±0.00 RSP compared to the treated groups (2.0ml, 4.0ml and 6.0ml) which record survival percentage and RSP as 100±0.00 in all. The results revealed that *C. odorata* leaves extract is a potential herbal extract for the maintenance and restoration of enzyme activities and enhancement of survival rate and diseases resistance (RSP) against *S. aureus* in *C. gariepinus*.

Keywords: Aquaculture; Fish Disease; Enzymes Herbs; Bacteria

Abbreviations: AST: Aspartate Transaminase; ALT: Alanine Transaminase; ACP: Acid Phosphatase ALP; Alkaline Phosphates; RSP = Relative Percentage Survival; CRD: Complete Randomized Method Design.

Introduction

Aquaculture has become the fastest growing fish producing sector in recent times representing up to 50

percent of global fish supply FAO [1], and developing countries are not behind. In Nigeria, aquaculture has become a big business, serving as a source of animal protein to the populace. Due to significant reduction in captured fisheries as a result of over fishing and pollution due to oil exploration, aquaculture is providing about 70% of fish supply in Nigeria. The increase in population, income, and demography over the years has resulted in the increase in fish consumption due to it nutritional value [2]. The most cultured fish species in Nigeria is *Clarias gariepinus*, hence the species attract significant economic value which makes it a species of debate.

Aquaculture is one of the fastest growing food-producing sectors around the world and high demand for products has led to employment opportunities in both developed and developing sectors [3]. Aquaculture is the means for obtaining more food from our aquatic environments in the future. impact of aquaculture on the biodiversity arise from the consumption of resources, such as land, water, seed, feed and their transformation into products valued by society [4]. Despite Advances been made within the feed industry, resulting in decreased feed conversion ratios and development of suitable alternatives to fish resources, the aquaculture industry use of global fishmeal and fish oil increased three-fold in recent years [5].

Aquacultural products such as fish and shellfish are open to a wide range of bacterial pathogens Rappert S, et al. [6], which have the capacity to cause diseases [7]. Some of the factors that cause mortality and unproductivity in aquaculture includes: uneaten feeds, fecal and urinary products, chemicals/synthetics, drugs, pathogens etc. [8]. The release of uneaten food, fecal and urinary wastes my lead to eutrophication and oxygen depletion, the magnitude of the impact depending on the type and size of operation and the nature of the ecosystem characteristics and assimilative capacity [9]. In aquaculture, veterinary drugs are commonly used to prevent economic losses related to sanitary shortcomings and treat disease outbreaks [10].

Herbal remedies have been used as human therapy for a long time, but there has been relatively little research on the medicinal plants for use against fish bacterial. Herbal drugs can be used not only for human treatment but also for treatment of fish diseases. They are also good growth promoters, stress resistance boosters and preventatives of infections [11]. *Staphylococcus aureus* is a pathogen that is responsible for various diseases causing mortality in fresh water fish. *Chromolaena odorata* leaves have been used for human treatment, but its medicinal values can also be useful in aquaculture. This work is aimed at accessing the medicinal value of *Chromolaena odorata* aqueous leaves extract on the enzymes and disease resistance of *Staphylococcus aureus* infected *Clarias gariepinus*.

Materials and Methods

Experimental Fish

One hundred and 150 healthy *C. gariepinus* of mean length 16.09±2.34cm and mean weight 750.55±23.99g were purchased from the fish farm the Department of Fisheries and Aquatic Environment, Faculty of Agriculture, Nkpolu-Oroworukwo, Port Harcourt. Rivers State. Nigeria.

The fish was observed for two weeks to evaluate disease presences or bruises during this period they were fed to satisfaction with blue crown commercial diets twice daily at 5% body weight per day.

Source of Pathogen

Staphylococcus aureus was procured from the National Veterinary Institute, Vom in Jos, Plateau State, Nigeria and was transferred to the Microbiology department of the Rivers State University for preservation.

Preparation of Experimental Treatments

The *Chromolaena odorata* aqueous leave extract was prepared using the method described by Ukwe OIK, et al. [11], *Chromolaena odorata* leaves were harvested, washed and pounded to paste, soaked in tap water at the concentration of 100g/L for twenty four(24) hours. It was filtered and the filtrate used immediately.

Experimental Design

A complete randomized method design (CRD) was used. There were four treatments in triplicates.

Experimental Procedure

One hundred and fifty (150) *Clarias gariepinus* were infected intra muscular with 1.5ml of 1.4 x 10^10 cfu of overnight grown *Staphylococcus aureus* using a 2ml injection syringe and 21-guage hypodemic needle and observed in plastic tank for disease presence. After disease presence, the infected fish were distributed into four (4) groups in triplicates and were treated with *Chromolaena odorata* leave extract via immersion at 0.00ml/l, 2.00ml/l. 4.00ml/l and 6.00ml/l. Blood samples were collected before and after infection, and after day 3 and 7 of treatment (exposure) and taken to the laboratory, to ascertain the therapeutic effect of the *Chromolaena odorata* leave extracts on the enzyme activities of the infected fish (*Clarias gariepinus*).

Blood Extraction

The fish was blindfold by covering the head with a thick cloth, to attain calmness, and blood was extracted via kidney puncture through the genital opening using 5ml injection syringe.

Enzymes Analysis

The collected blood sample were transferred into LITHUM HEPARIN tube and sent to the laboratory for biochemical analysis within twelve Wahua TAT (12) hours. They were assayed for aspartate amino transferase (AST), alaineamoni transferase (ALT), alkaline Phosphate (ALP) and Acid Phosphate (ACP), using an auto-analyzer, the screen master model, manufactured by Biochemical system. It was used according to manufactures instruction.

Disease Resistance Calculation

This was calculated using the formula

$$RSP = \frac{1 - \%\, mortality\ in\ treated\ group}{\%\, mortality\ in\ control} \times 100$$

Where RSP = Relative Percentage Survival

Data Analysis

Data were subjected to a one-way analysis of variables to determine if there was difference in the variables among treatments. Turkey's multiplied with comparis test was used to compare the means of the treatment [12].

Results

The enzymes activities in the experimental after day 3 with *S. aureus* are shown in Table 1, in the AST (Aspartate transaminase) there was significant difference with the treated group and the untreated group with the control (42 ± 14.73). In the ALT (Alanine Transaminase) there was significant difference (6.76±2.29). The ALP (Alkaline phosphatase) injection with *S.aureus* (61± 26.66). The ACP (Acid Phosphatase) records in day 3 exposure with *S.aureus* (0.63 ± 0.32). The variables varies between the control, the untreated, 2ml, 4ml and 6ml. the enzyme activities are higher in the untreated as compared to the treated and control (Table 1). After days 7 of exposure to aqueous *C. odorata* leave extract, Table 2 the value of AST was significantly higher at 0.0ml, which is the control (43.33±15.88), the value of AST in 2ml increased at day 7, AST decreased in untreated and reduced significant at 4ml and 6ml after exposure to *chromolaena odorata* (Table 2). The percentage survival rate in Table 3 were significantly higher ($p<0.03$) in 2.0ml, 4.0ml and 6.0ml (96.67±5.77, 100.00±0.00, 90±10.0 %) compared to infected (10±10.00). The relative survival percentage resistance (RSP) were significantly higher ($p<0.03$) in all the *C. odorata* aqueous leave extracts treatments (2.0ml, 4.0ml and 6.0ml/l 100±0.01% each compared to the untreated (0.00±0.00).

Treatment	Plasma enzymes			
	AST (U/L)	ALT (U/L)	ACP (U/L)	ALP (U/L)
Control	42.00±4.73[a]	6.76±2.29[a]	0.63±0.32[a]	61.09±6.66[a]
Untreated	57.67±5.96[b]	8.33±2.08[a]	0.79±0.22[a]	72.09±2.93[a]
2ml	45.00±9.16[a]	6.73±1.10[a]	0.64±0.14[a]	64.67±9.23[a]
4ml	45.67±3.78[a]	6.46±1.51[a]	3.63±5.19[a]	67.33±7.63[a]
6ml	54.98±3.07[a]	6.70±1.96[a]	0.72±0.01[a]	71.33±2.66[a]

Means within column with the same superscripts are not significant different at $p>0.05$.

Table 1: Enzymes Activities in plasma Biochemistry of *Clarias garieinus* after day three of treatment infected with *S aureus*.

Treatment	Plasma enzymes			
	AST (U/L)	ALT (U/L)	ACP (U/L)	ALP
Control	43.33±5.88[a]	6.80±2.59[a]	0.63±0.14[a]	61.09±4.58[a]
Untreated	56.09±6.24[a]	7.70±2.76[a]	0.54±0.11[a]	72.88±4.63[a]
2ml	47.33±8.50[a]	7.10±2.30[a]	0.66±0.12[a]	61.67±2.34[a]
4ml	40.00±8.19[a]	7.23±2.50[a]	0.54±0.14[a]	61.33±3.00[a]
6ml	51.33±2.58[a]	7.06±2.43[a]	0.61±0.28[a]	75.99±9.53[a]

Means within column with the same superscripts are not significant different at $p>0.05$.

Table 2: Enzymes Activities in plasma Biochemistry of *Clarias garieinus* after day seven of treatment infected with *S aureus*.

Treatment	Relative survival			
	stocking density	% Mortality	% survival	RSV
Control	10±0.00[a]	0±0.00[b]	100±0.00[a]	100.00±0.00[a]
Infected	10±0.00[a]	30±5.77[a]	10±10.00[b]	0.00±0.00[b]
2ml	10±0.00[a]	3.33±5.77[b]	96.67±5.77[a]	100.00±0.00[a]
4ml	10±0.00[a]	0±0.00[b]	100±0.00[a]	83.33±28.86[a]
6ml	10±0.00[a]	6.67±5.77[b]	90±10.0[a]	66.67±28.86[a]

Means within column with different superscripts are significant different at $p<0.05$.

Table 3: Percentage and relative survival of *S.aureus* infected *C.gariepinus* after seven days of exposure to *C. odorta* aqueous leaves extract.

Discussion

The enzymes activities were higher in the fish after infection compared to the activities before infection. The increase in the enzyme activities in the infected fish was evident to the fact that the function of some internal organs of the fish such as the liver and kidney are malfunctioning [13]. Similar result was obtained by Khalil RH, et al. [14] who reported increase in this enzyme when *Anguilla Anguilla* was exposed to *vibro anguillarium* and Rashannasan Ramasamy H, et al. [15] reported an increase in enzyme as problems associated with haemapoetic organs.

However, during the period of treatment the analyzed enzymes reduce in activity up to day 3 and increased gradually at the end of day 7 in the treated group (2.0ml-6.0ml) but they were not higher than the enzyme activities in the untreated group (0.00ml) except the AST and ALT at the end of day 7. The decrease in the enzyme activities in the treated fish could be as a result of the antibacterial activities of the *Chromolaena odorata* leave aqueos extract which tends to boast the immune system of the fish Sales CH, et al. [16] or it could be as a result of essential amino acid present in the leaf. This result is similar to Favero MS, et al, [17] reported a reduction in the percentage increase the enzymes in Nile tilapia fed anabaena and infected with *A. hydrophila* when compared to the untreated. But Liu B, et al. [18] who reported a reduction in AST and ALT activities compared to the control.

At the end of the 7 days experiment the survival rate and disease resistance were higher in the treated group (2.0ml-6.0ml) of aqueous *Chromolaena odorata* leaf aqueous extract compared to the untreated group (0.00ml) of aqueous *Chromolaena odorata* leaf extract. Similar results were reported by Yao J, et al. [19] who observed higher survival percentage and RSP in the bath treatment of grass carp infected with *Icthymoltiphilis* (white spot disease) in different concentration of *Macleayacordata* species leave extract and Olusola ES, et al. [20] who observed an improvement in RSP and percentage when C. gariepinus fed with diet of various inclusion of bitter leaves (*Veronica amygdalina*) and pawpaw (C. papaya) leaves extract which were infected with *A. hydrophila*. The increase in survival rate and disease resistance could be as a result of the presence of some biotic substances that are antibacterial found in plant extract. Some phytochemicals found in *Chromolaena odorata* includes; tannis, saponins, phytates.

Conclusion and Recommendations

Staphylococcus aureus has been observed to be infectious bacteria causing diseases such as eye protrution and mort in fresh and marine water fish. These bacteria increase the enzymatic activities of some plasma enzymes, which is an indication of organ damage in the fish. This study shows that *Chromolaena odorata* aqueous leave extract has therapeutic effect on the AST, ALT, ATP and ACP of *C. gariepinus* infected with *S. aureus* as well as antibacterial activities against *S. aureus* as it enhances the survival rate and disease resistance of the infected fish.

The following recommendations should be considered:

- Fish farmers should be encouraged to plant awolowo tree in their farms for more availability of the leaves.
- The experiment should be carried out in other aquatic animals to ascertain the efficiency of the *C. odorata* leaves in aquaculture.
- *Chromolaena odorata* leaves aqueous extract should be prepared in pellet form for easy usage by farmers.

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