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Diseases Resistance and Enzymatic Changes in Pseudomonus aeruginosa Infected Clarias gariepinus treated with Carica Papaya root extracts

Ukwe, I.O.K.[1] and Vopnu, F. B.[2]

1, 2 Department of Fisheries and Aquatic Environment, Rivers State University, Nigeria.

ABSTRACT

One hundred and twenty *Clarias gariepinus* were infected intramuscularly with 1.0ml of *P. aeruginusa (*4.1x10^{4}cfu) using 2ml injection syringe and observed in the laboratory for diseases presence. After diseases presence, they were distributed into four groups in triplicate and treated with various level of *carica papaya* aqueous root extracts (0.0ml, 1.0ml, 2.0ml, and 3.0m), to determine in the disease resistant ability and therapeutic effect on the enzymatic activities of the infected fish using the experimental extract. Blood samples were collected before the infection, after diseases presence and day 2, 5 and 7 of the treatment with experimental extract for biochemical analysis to determine changes in following the enzymes; *aspartatetransaminiase* (AST), *alaninetransaminase* (ALT), *alkalinephosphate* (ATP) and *acidphosphate* (ACP) across the various treatments (0.0ml, 1.0ml, 2.0ml and 3.0ml). And the disease resistance ability of the experimental extract was determined at the end of day 7 of the experiment. At the end of the experiment, it was observed that the activities of; AST, ALT, ATP and ACP were higher ($P>0.05$) after the infection compared to the activities before the infection. After day 2 of the treatment, the activities of all the analyzed enzymes were lower ($P<0.05$) in the treated groups (1.0 – 3.0ml) compared to the values in the untreated group (0.0ml), the same trend was observed at end of day 5. At the end of day 7, the values of the AST and ALT were higher in the infected fish treated with 2.0ml and 3.0ml of the extract compared to the untreated group (0.0ml), but the ALP and ACP values were lower in the treated group compared to be untreated. The disease resistance ability which is a measure of the relative survival percentage was 0% (zero percent) in the untreated group compared to 100% in the treated groups.

Keywords: Disease resistance, enzymatic change, *P. aeruginosu, C. gariepinus, carica papaya root extract*

1 INTRODUCTION

Globally, fisheries production peaked at about 171 million tons in 2016, of which aquaculture production represented 80 million tons and captured production represent 91 million tons (FAO, 2018). 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 (FAO, 2014). 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 between 1992 and 2006 (Hasan and Halwart 2009).

Some of the factors that cause mortality and unproductivity in aquaculture includes: uneaten feeds, fecal and urinary products, chemicals/synthetics drugs, pathogens etc. (Beveridge, 2004;Hargrave, 2005). The release of uneaten food, fecal and urinary wastes may 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 (Ukwe and Gabriel, 2019).

Another factor that affect aquaculture production is stress, because it affects the homeostatic mechanism of the organism(Harper and wolf, 2009) and some of the conditions that causes stress in the fish during culturing according to Rottmann *et al (*1992) includes: increase fish density and poor water quality, injury during handling(i.e., capture, sorting and shipping), poor sanitation and inadequate nutrition.

Chemo-therapeutants, including antimicrobial compounds and pesticides are mainly used in intensive fish and shrimp cultures to control bacterial, fungal and parasitic diseases (Beveridge *et al.,* 2014). But the use of chemicals/synthetic drugs is discouraging worldwide because of their disadvantage of depositing in fish flesh, polluting the environment, causing drug resistance etc. (Adams, 2009; Beveridge *et al.,* 2014). Herbs and herbal products have shown to be reliable replacement for chemical/synthetic drugs in aquaculture because they are eco-friendly, not immospecific and does not deposit in fish flesh (Ukwe and Gabriel, 2019). Plants have been reported to produce various effects such as anti-stress, growth promotion, appetite stimulation, immunostimulation, aphrodisiac and to have antipathogenic properties in fish and shrimp aquaculture due to their varied active phytochemicals such as alkaloids, terpenoids, tannis, saponinsand flavonoids (Eman *et al.,* 2020; Bello *et al.,* 2012; Verma *et al.,* 2013). Some of the plants herbs with medicinal phytochemical properties includes:mango bark (Ukwe and Jamabo, 2020), onion bulb (Bello *et al.,* 2013), aloe vera etc.

African sharp tooth catfish is a specie of the catfish family *Claridae,* the air breathing catfishes. *Clarias gariepinus* are readily recognized by their cylindrical body and scaleless skin, flattened bony head, small eyes, elongated spineless dorsal fin and four barbels around the broad mouth. The upper surface of the head is coarsely granulated in adults but smooth in young fish(Van Oijen, 1995). The body is grayish and with the underside of the head and body cavity creamy white. The culture of *Clarias* has grown rapidly because of its high fecundity, flexible phenotype, tolerance to extreme water conditions and the ability to subsist on a wide variety of prey can devastate indigenous fish and aquatic invertebrate population (Bruton, 1986). African catfish (*C. gariepinus*) is the main aquaculture species in Nigeria (Aprodu *et al,* 2015). Catfish has contributed to more than 70% of the inland aquaculture production in Nigeria and it is

considered as the major provider of fish protein through aquaculture (Acosta and Gupta, 2005). The specie is cultured over the world due to its fast growth, omnivorous feeding nature and tolerance to wide water quality and temperature ranges, this fish species can feed in any water part of the water systems (Adebayo *et al.*, 2010).

As many pathogens naturally occur in aquatic environment, all forms of aquaculture are prone to disease outbreaks which are largely determined by host susceptibility (Ringo *et al.,* 1996; Ringo *et al.,* 2001). Also, physiological stress contributes to diseases and increase mortality in aquaculture. It leads to intestinal micro-biota disorder which decrease the level of beneficial microorganism and thereby giving room to invasion from bacterial disease significant cause of mortality in most hatcheries (Ringo*et al.,* 2001;Del Rio Rodriguez *et al.,* 1997). And high mortalities especially during transition from the York sac to the first Feeding stage of development. Bacterial, fungi, protozoa and helminthes are known pathogens of fish. Bacteria genera such as *Aeromonas, Vibro, Staphylococcus, Corynobacteria, Acinettobacter, Enterobacter, Escherichia, Klebsiella,proteus, Serratia* and fungi genus *Cryptococcus*, protozoan (*Ichthyophthiriusmultifilis*)and helminthes (*Huffmanelahuffmani* and *clinostomum*marginatum) have been incriminated in fresh water fish diseases (Rappert and Muller 2005;Ringo*et al.,* 1996; Del Rio Rodrigues *et al.*,1997), and are described as common pathogens in fish farms.

Pseudomonas aeruginosa is an opportunistic Grams negative pathogen, a rod-shaped bacterium which belongs to the *pseudomonadacae* family. It has been established to be the most common bacteria pathogen in marine and fresh water aquaculture (Thomas *et al.,* 2014). It is widely distributed and responsible for the considerable loss of fish production (Thomas *et al.,* 2014). Several species of *Pseudomonas* have been reported to cause disease in a number of fish species

(Bullock, 1965), and are associated with septicemia in aquatic animals (Roberts, 1978). These bacteria have been considered as opportunistic pathogens, causing diseases when the host is subjected to stress. A number of aquatic animals like fish, frogs and soft-shelled turtles were recorded to be susceptible to *Pseudomonas* spp. (Somsiri and Soontornvit, 2002). The symptoms most time manifest as red skin diseases in fish which occurs during stressful conditions, such as inappropriate handling or during transportation (P. Mishra *et al.,* 2014). Some of the disease caused by the bacterium is characterized ulcerative syndrome, hemorrhagicsepticemia and tail, fin, gill rot as well as behavioral alterations linked with impaired locomotion activity (Thomas *et al.,* 2014; Baldissera *et al.,* 2017).

The pawpaw root extract has certain phytochemicals with anti-bacterial and anti-fungi properties such as tannoids, alkaloids, saponins, glycosides and phenols (Doughan *et al.,* 2007; Srivivasan *et al.*,(2001). According to Davi and Patromo (2007), these phytochemicals helps in the thickening of the cell membrane of the fish internal organs and reduces the virulence of the pathogen in the fish organs.

This work is aimed at accessing the usefulness of pawpaw root aqueousextract in the treatment of *P. aeruginosa* attack on fish.

Herbs act as growth promoter, immune stimulant, antimicrobial and anti-fungal agents. (Citarasu, 2010; Ukwe *et al,* 2020a). The use of medicinal plant is an alternative to antibiotics in fish health management (Chakraborty and Chattopadhya, 1998). Many studies have proved that herbal additive enhances fish growth and also protect them from diseases (Sasmal *et al.,* 2005; Ukwe *et al*, 2020b).

2 . MATERIALS AND METHODS

Experimental fish: One hundred and twenty healthy *C. gariepinus* of mean weight 120-13g was purchased from the fish farm the Department of Fisheries and Aquatic Environment, Faculty of Agriculture, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.

The fish was observed for two weeks to evaluate disease presences or bruises during this period there were fed with blue crown commercial diets twice daily to satiation.

Source of pathogen: *Pseudomons aeruginosa* was ordered 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 (Herb Extracts): The *Carica Papaya* aqueous roots extract was prepared using the method of Ukwe and Jamabo (2020). The *C. Papaya*root was washed clean, dried for four hours pounded to paste and soaked in tap water (50^{o}c) at the concentration of One hundred (100/grams per litre) (100g/L) for twenty-four (24) hours. It was filtered and the filtrate was used immediately.

Experimental Design: A complete randomized method (CRD) was used. There were four treatment in triplicates.

Experimental Procedure: One hundred and twenty (120) *Clarias gariepinus* were infected intra muscular with 1.0ml of 4.1 x 10^{4}cfu of overnight grown *P.aeruginosa* using a 2ml injection syringe and 21-guage hypodermic needle at day 1, 2, 4 and 5 and observed in concrete tank for disease presence. After disease presence, the infected fish were distributed into four (4) groups in triplicates and were treated with *C. papaya root* extracts via immersion at 0.00ml/L, 1.00ml/l, 2.00ml/l and 3.00ml for 8hrs. daily.

Blood samples were collected before and after infection, and after day 2,5 and 7 of treatment (exposure) and taken to the laboratory, to ascertain the therapeutic effect of the *C. gariepinus* root 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 six (6) hours.

They were assayed for aspartate *aminotransamanase* (AST), *alanietransamanaase* (ALT), *alkalinephosphate* (ALP) and *acidphosphate* (ACP), using an auto-analyzer, the screen master model, manufactured by Biochemical system. It was used according to manufactures instruction.

Disease Resistance Ability: This was calculated using the formula

$$\text{RSP} = 1 - \frac{\%\ Mortalidy\ in\ treated\ group}{\%\ Mortality\ in\ control} \times 100$$ (Harikrishnan, 2010).

Where RSP = Relative Percentage Survival

Data Analysis: Data will be subjected to a one-way analysis of variables to determine if there was difference in the variables among treatments. Turkey's multiplecomparis test was used to compare the test of the treatment (Wahua, 1999).

3. RESULTS

3.1 Enzymatic Activities in Plasma Biochemistry of *Clarias gariepinus* in the Experimental Fish

The results for the enzymatic changes in *Clarias gariepinus* before and after infection with *P. aeruginsa* is shown in Tables 1, while the enzymatic changes in the infected fish after day 2, 5, and 7 are shown in tables 2-4 respectively, and the comparative values for the enzymes between treatments across the period of the experiment is shown in figures 1-4. Table 5 shows the result of the survival percentage and disease resistance.

3.2 Percentage Survival and Disease Resistance in *C. gariepinus* Exposed to Different Levels of Concentration

The percentage survival was significantly higher in 1.0ml, 2.0ml and 3.0ml (100.00±0.00 %) compared to 0.0ml (65.01±2.02). The relative survival percentage (RSP) which is a measure of the disease resistance was significantly higher in all the *C. papaya* aqueous root extracts treatments (1.0ml, 2.0ml and 3.0ml/l) 100±0.01% each compared to the untreated (0.00±0.00).

Table 1: **Enzyme Activities in the Plasma Biochemistry of *C.gariepinus* before and after Infection (BI and AI) with *P.aeruginosa***

Enzymes (IU/L)	*Before (BI)	Range		*After (AI)	Range	
		Min	Max		Min	Max
AST	22.00±2.00[a]	20.00	24.00	39.67±8.62[b]	32.00	49.00
ALT	3.53±0.51 [a]	3.10	4.10	6.03± 2.16 [b]	4.50	8.50
ALP	6.67±2.60 [a]	4.00	9.20	10.80±0.72 [a]	10.00	11.40
ACP	0.54±0.32 [a]	0.35	1.00	1.08±0.15 [a]	0.94	1.23

***Means within the same row with different superscripts are significantly different (P<0.05)**

Key: ALT -*Alaninetransminase*; AST –*Aspartatetransaminase*; ALP -*Alkalinephosphatase* and ACP-*Acidphosphatase*

Table 2: Enzymes activities in Plasma Biochemistry of *C. gariepinus* Infected with *P. aeruginosa* and exposed to different concentrations of *C.papaya*aqueous root extract for Two (2) days

Concentration (ml)	Enzymes (IU/L)			
	AST	ALT	ALP	ACP
0.0	33.66±7.57[b]	5.0±1.77[b]	9.03±1.70[b]	1.12±0.11[b]
1.0	27.33±3.51[a]	3.43±0.85[a]	7.06±2.73[a]	0.69±0.06[a]
2.0	27.33±7.57[a]	3.70±0.91[a]	7.03±0.92[a]	0.66±0.08[a]
3.0	26.0±2.64[a]	3.00±0.91[a]	7.30±3.04[a]	0.65±0.44[a]

Means within the same column with different superscripts are significantly different (P<0.05)

Key: ALT -*Alaninetransminase* ; AST - *Aspartatetransaminase* ; ALP -*Alkalinephosphatase* and ACP-*Acidphosphatase*

Table 3: Enzymes activities in Plasma Biochemistry of *Clariasgariepinus* Infected with *P. aeruginosa* and exposed to different concentrations of *C.papaya*aqueous root extract for Five (5) days

Concentration (ml)	Enzymes (IU/L)			
	AST	ALT	ALP	ACP
0.0	30.33±9.71[b]	4.33±1.19[b]	8.33±2.25[b]	0.73±0.36[a]
1.0	22.66±6.42[a]	3.46±0.65[a]	6.63±3.32	0.73±0.21[a]
2.0	25.66±11.01[a]	3.33±0.87[a]	5.36±1.23[a]	0.64 ±0.23[a]
3.0	29.66±17.38[a]	3.60±2.27[a]	7.13±3.42[a]	0.71±0.47[a]

Means within the same column with different superscripts are significantly different (P<0.05)

Key: ALT -*Alaninetransminase* ; AST - *Aspartatetransaminase* ; ALP -*Alkalinephosphatase* and ACP-*Acidphosphatase*

Table 4: Enzymes activities in Plasma Biochemistry of *Clariasgariepinus* Infected with *P. aeruginosa* and exposed to different concentrations of *C.papaya*aqueous root extract for seven (7) days

Concentration (ml)	Enzymes (IU/L)			
	AST	ALT	ALP	ACP
0.0	26.66±13.01 [a]	4.03±1.70 [a]	7.43±2.70 [b]	0.69±0.30 [a]
1.0	25.33±20.79 [a]	3.76±3.21 [a]	7.13±4.56 [b]	0.65 ±0.33 [a]
2.0	32.33±5.85 [b]	5.00±2.51 [b]	7.30±2.93 [b]	0.58 ±0.28 [a]
3.0	32.00±12.16 [b]	5.73±0.11 [b]	6.90±2.76 [a]	0.65 ±0.46 [a]

Means within the same column with different superscripts are significantly different (P<0.05)

Key: ALT -*Alaninetransaminase*; AST - *Aspartatetransaminase*; ALP -*Alkalinephosphatase* and ACP-*Acidphosphatase*.

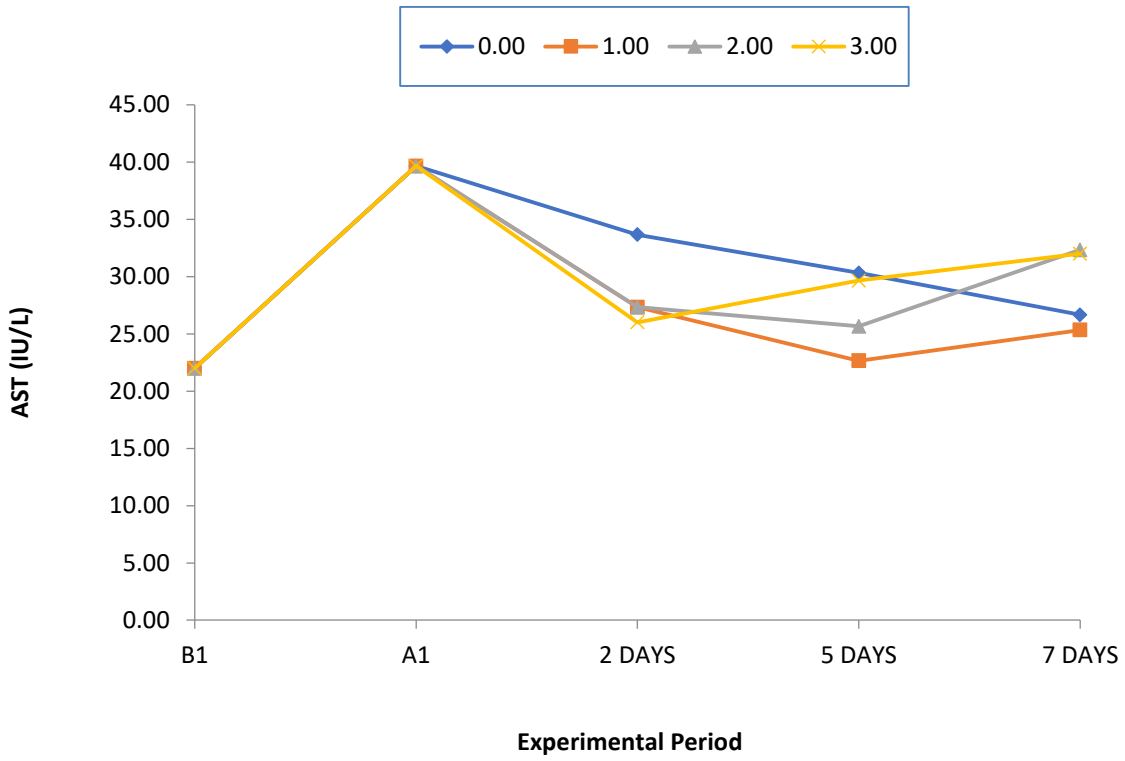

Figure 1: Comparative Values of AST in the Plasma of *C.gariepinus* Infected with *P..aeruginosa* and exposed to *C.papaya* Extracts at Different Experimental Period

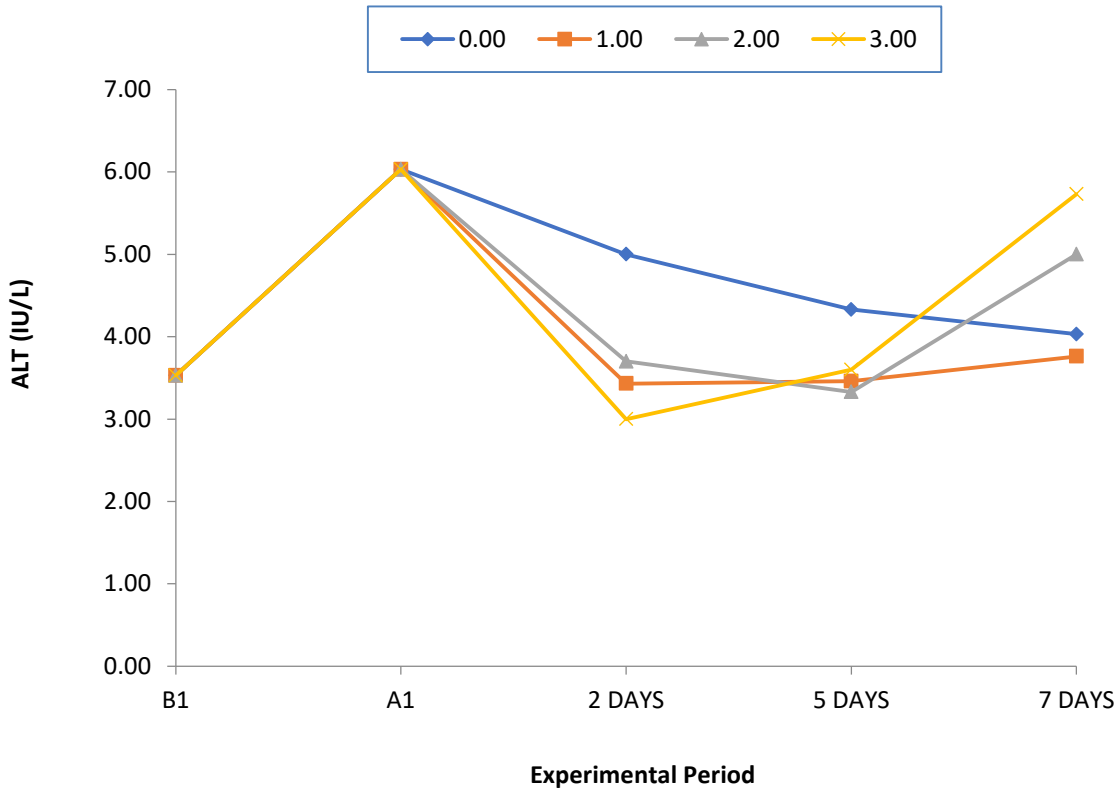

Figure 2: Comparative Values of ALT in the Plasma of *C.gariepinus* Infected with *P.aeruginosa* and exposed to *C.papaya* Extracts at different Experimental Period

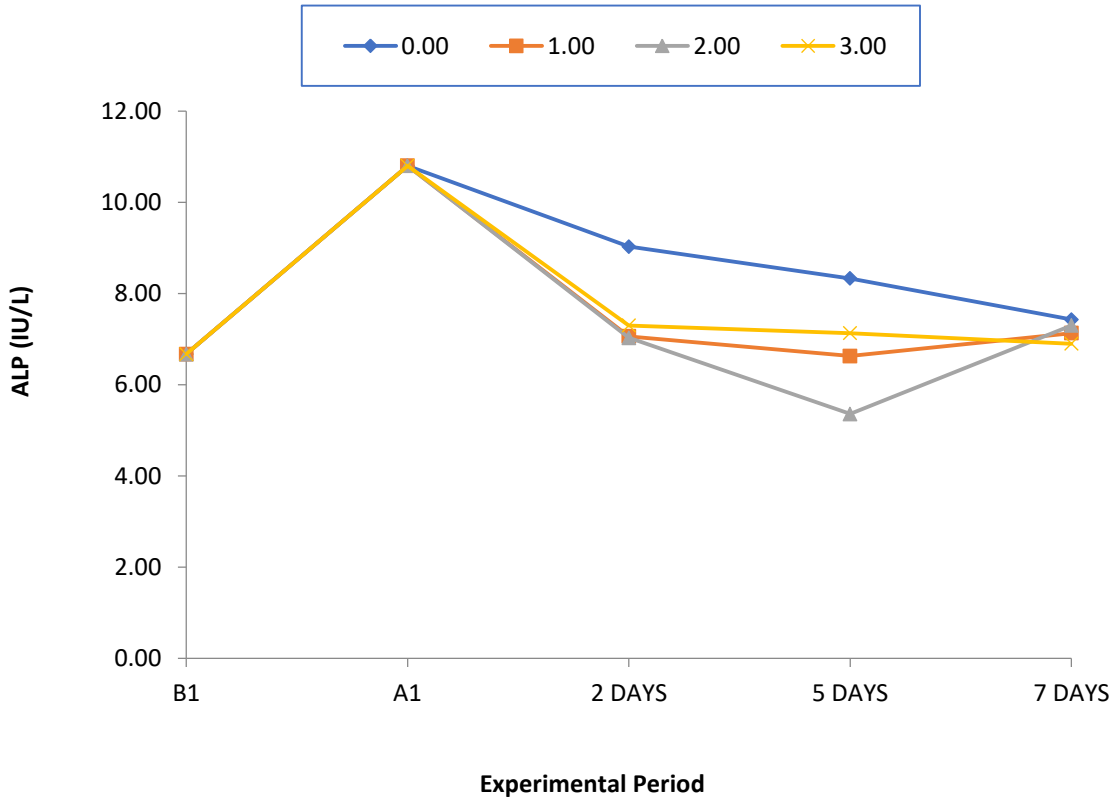

Figure 3: Comparative Values of ALP in the Plasma of *C.gariepinus* Infected with *P.aerigunosa* and exposed to *C.papaya* Extracts at different Experimental Period

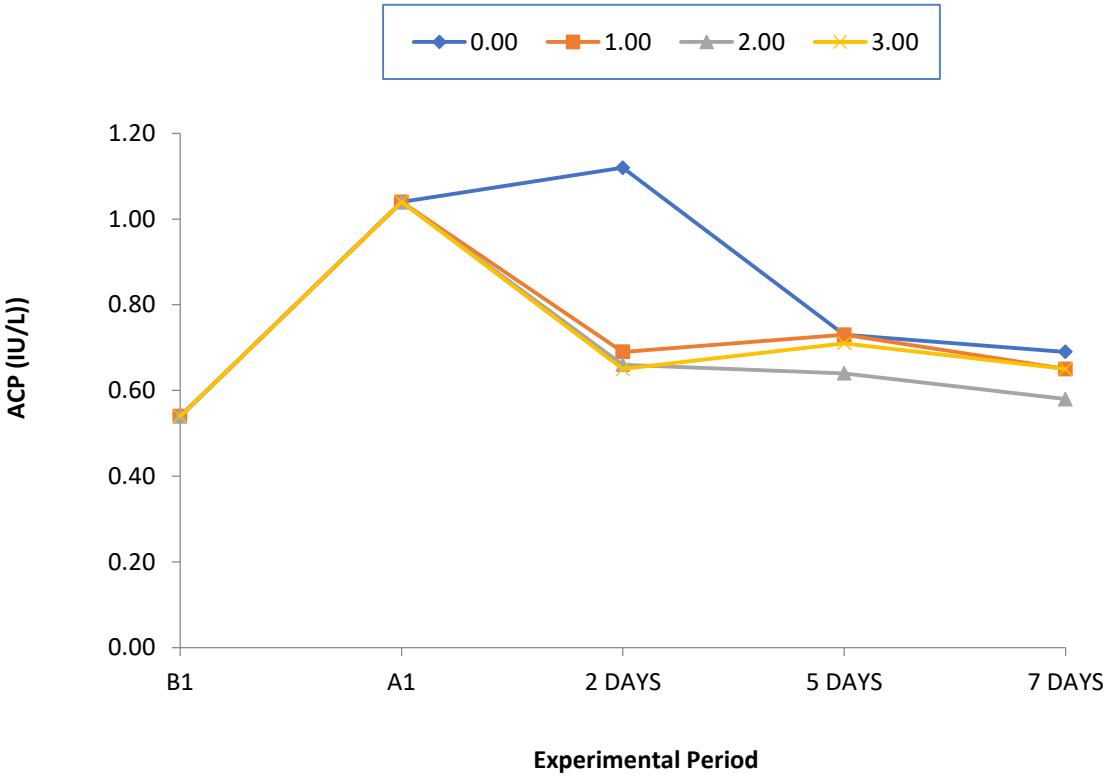

Figure 5: Comparative Values of ACP in the Plasma of *C.gariepinus* Infected with *P.aeruginosa* and exposed to *C.papaya* Extracts at different Experimental Period

Table 5: Percentage Survival and Diseases Resistance (RSP) of *C. gariepinus*Infected with *P. aeruginosa* and exposed different concentration of Pawpaw roots extracts

Concentrations (ml)	Stocking Density	% Mortality	% Survival	**RSP (Disease Resistance)**
0.00	10.00 ± 0.02^{a}	25.00 ± 1.02^{b}	65.01 ± 2.02^{a}	0.00 ± 0.00^{a}
1.00	10.00 ± 0.01^{a}	0.00 ± 0.00^{a}	100.00 ± 0.01^{b}	100.00 ± 0.01^{b}
2.00	10.00 ± 0.02^{a}	0.00 ± 0.00^{a}	100.00 ± 0.01^{b}	100.00 ± 0.01^{b}
3.00	10.00 ± 0.01^{a}	0.00 ± 0.00^{a}	100.00 ± 0.01^{b}	100.00 ± 0.01^{b}

Means within the same column with different superscripts are significantly different (P<0.05)

4. DISCUSSION

4.1 Enzyme activities in *C. gariepinus* exposed to different concentration of aqueous *C. papaya* root extract.

Aspartate transaminiase (AST), *Alanin transaminase* (ALT), Acid phosphare (ACP) and *Alkaline phosphare* (ATP) are recognized biomarkers in determining the functionality of the body organs such heart, liver, kidney and other haematopoetic organs (Zaki *et al,* 2007; Yang *et al,* 2003). Ukwe and Oladapo (2019) also reported the increase in these enzymes as diseases associated, when *C. gariepinus* were infected with *P. aeruginosa* and *A. hydrophila,* Dacet *et al* (2004) also reported increase in these enzymes when Indian major carp was exposed to nitrate. Rashannasab *et al* (2016) stated that elevation of ALT is an indication of medical problems such as heart failure, diabetes, myopathy among others, while Chi *et al* (2019) reported increase in ACP and ALP as defence mechanism to poisoning toxin produced by Pseudo-nitzclina and Nitzchina against bay scallop.

This study shows that there were elevations the AST, ALT ACP, and ALP of the fish infected with the pathogen (*P. aeruginosa*), and the elevation could be as a result of an infringement in some hematopoeticorgans of the fish (Rashannasa *et al*. 2016; Najeeb and Azize, 2013). Ukwe and Oladapo(2019)stated that the increase in the enzyme activity in the infected fish is evident to the fact that the function of some internal organs of the fish such as the liver and kidney are malfunctioning. Similar result was obtained by Khalil *et al.,* (2011)who reported increase in this enzymeswhen *Anguilla anguilla*was exposed to *vibroanguillarium*, and Rashannasan *et al.* (2016)who reported an increase in these enzyme activities as problems associated with hemapoetic organs. The increase in these enzymes could also be a means of self defence by the experimental fish (Chi *et al*, 2019).

However, during the period of treatment the analyzed enzymes reduce in activities up to day 5 and increased gradually at the end of day 7 in the treated groups (1.0ml-3.0ml) but they were not higher than the enzyme activities in the untreated group (0.00ml) except the AST and ALT in 2.0 and 3.0ml aqueous *carica papaya* root extracts 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 *Carica papaya* root extract which tends to boast the immune system of the fish (Sales *et al.,* 2013) or it could be as a result of the phytochemicals such as; tannins, alkaloids, saponinsetc. found in pawpaw root extract (Doughan *et al.,* 2007; Srivivasan *et al,* 2001) which may have thickened the cells membrane of the fish internal organs and reduce the virulence of the pathogen on the fish organs (Davi and Patromo, 2007), and these phytochemicals are also known to be antipathogenic (Verma e*t al,* 2013; Bello *et al,* 2012).Thisresult is similar to Fadi *et al* (2013) who reported a reduction in the percentage increase of some enzymes in Nile tilapia fed anabaena and infected with *A. hydrophila* when compared to the untreated, and Liu *et al*(2012)who reported a reduction in AST and ALT activities compared to the control, when *Megalobramaamblycephala* treated with anthraquinine was infected with *A. hydrophila*. The increase of AST and ALT in 2.0 and 3.0ml of the extracts compared to the 0.0ml indicates that exposing the fish at these concentrations for more than 5 days is detrimental to the fish, due to prolong phytochemicals exposure (Palanisamy *et al,* 2011), and may have caused distortions on the hematopetic organs (Oyeyemi and Oyeyemi, 2015).

4.2 Percentage Survival and Disease Resistance

At the end of the 7 days experiment the survival rate and disease resistance were higher in the treated group (1.0ml, 2.0ml and 3.0ml of aqueous *carica papaya*root extract) compared to the untreated group (0.00ml). Similar results were reported by Yao *et al.*,(2010) 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* leave extract and Olusola and Nwokike (2018) who observed an improvement in RSP and percentage survival when *C. gariepinus* fed with diet of various inclusion of bitter leave (*Veronica amygdalina*) and pawpaw (*C. papaya*) leaves extracts were infected with *A. hydrophila*.

There was no mortality observed at the end of day 2, 5 & 7 of the exposure/treatment with different levels of exposure (1.0ml – 3.0ml) of aqueous *c. papaya*root extract. Similar result was reported by Rattanachaikunsopon and Phumkhachom (2009) who observed no mortality in the Nile tilapia fed diet supplemented with garlic chives (*Alliumtuberosum*) oil after infection with *F. columnare* and Ekanem *et al* (2004) reported a 90% reduction in the number of parasites and zero mortality when Gold fish infected in the ciliate *Ichthyophthririusmultifilis* were immersed for 72hrs in baths with crude methanoic extract of leaves of *mucunapruriens* and *C. papaya* seeds containing similar bioactive substances found in *C. papaya* root. 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 which are also found in pawpaw root extract (Srivivasan *et al.,* 2001). Some medicinal phytochemicals also found in pawpaw root includes: saponins, alkaloids, tannins, glycosides and phenol (Doughan *et al.*, 2007) and they have been reported to be antibacterial, antipathogenic and antimicrobial (Sales *et al.*, 2013; Verma *et al.*, 2013).

5. CONCLUSION AND RECOMMENDATION

P. aeruginosa have been observed to be an infectious bacteria causing diseases such as ulcers, and hemorrhage, and mortality in fresh water fish. These bacteria increase the activities of some plasma enzymes such as AST, ALT, ALP, and ACP which is an indication of organ damage in the fish. This study shows that *Carica papaya* aqueous root extract has therapeutic effect on the hematopoetic organs of *P. aeruginosa* infected *C. gariepinus* as well as antibacterial activities against *P.aeruginosa* as it enhances the survival rate and disease resistance of the infected fish. It is therefore recommended that pawpaw plant should be planted within fish farms to enhance the availability of the root, to be used as therapeautic agents against pathogenic attacks on fresh water fish. More works should be carried out with other aquatic animals to ascertain the efficacy of the C. papaya aqueous root extract in aquaculture.

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