



Behavioural and Haematological Changes in *Pseudomonas aeruginosa* Infected *Clarias gariepinus* Exposed to *Carica papaya* Root Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Behavioural and haematological changes were observed in *Pseudomonas aeruginosa* infected *clarias gariepinus* before and after exposure to carica papaya root extracts via immersion. One hundred and twenty (120) *c.garipienus* of mean weight 120g ± 13g were intramuscularly injected with 1.0ml of *P. aeruginosa* (4.1x10⁴cfu), and observed for diseases present/signs. Blood samples were collected before and after disease presence/signs. The infected fish were distributed into four groups in triplicate and exposed to 0.0ml, 1.0ml, 2.0ml and 3.0ml of aqueous *carica papaya* root extracts for a period of seven (7) days, while behavioural changes such as: air gasping, erratic swimming, discoloration, serious wounds, etc were observed from the day of infection to day 7 of treatment. Blood samples were again collected at days 2,5 and 7 of treatment. The haematological analysis of all the blood samples was performed in the laboratory to ascertain the therapeutic effect of the *carica papaya* root extract on the infected fish. The result indicated serious behavioural

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changes in fish exposed to 0.0ml extracts (untreated group) compared to the fish exposed to the various concentrations of the *carica papaya* root extract (treated group). The results from the haematological parameters reveals that Hb, RBC and PLT were lower ($P < 0.05$) in fish after infection compared to the values before infection, but the values increased in the treated groups compared to the untreated group within the 7 days treatment period. The values of the WBC increase ($P > 0.05$) in the fish after infection compared to the value before infection. However the values of WBC in the treated groups were lower compared to the untreated within the period of exposure to the *carica papaya* extract. The values of MCH, MCHC and MCV were fluctuating within the period of treatment. This study has shown that *C.papaya* roots extracts have the potency to improve the health and antimicrobial activities of culture fish during aquaculture operations. Hence, *carica papaya* aqueous root extract is therefore recommended for application in the rearing water at the rate of 1.50 to 2.00mg/l, for both therapeutic and prophylactic use in aquaculture.

Keywords: Aquaculture; fish disease; pathogen; catfish; haematology; behavioural response; *Carica papaya*.

1. INTRODUCTION

Aquaculture is a very important and rapidly developing industry in Nigeria and all over the world [1]. The aquaculture industry has grown considerably in recent years due to the market demand since the wild stocks of most commercially important fish have declined [2]. This rapid growth process is accompanied by an increase in bacterial, viral, fungal and parasitic diseases together with some environmental interactions. This impressive development has been accompanied by the expansion of semi-intensive culture to the intensive culture system. Unfortunately, the intensive culture of fish generates a stressful environment leading to the suppression of the immune system and increased susceptibility of fish to infectious diseases [3].

Conversely, infectious diseases, health problems and economic damages caused by these diseases are the leading ones among the most important problems faced in the fish farming enterprises. Disease can cause significant economic loss through mortality, poor growth rate, low quality of flesh, resulting in reduced profit margins [4]. Various chemotherapeutic agents such as antibiotics and disinfectants have been traditionally used in the treatment and prevention of numerous diseases in farmed fish [5]. However, they cannot be recommended since improper and continuous use of antibiotics may lead to potential development of antibiotic resistant bacteria, environmental pollution and the accumulation of residues in fish [6,5].

Many countries have forbidden the use of certain chemotherapeutics, and also refuse to import aquaculture products treated with antibiotics and chemicals [7]. Therefore, researchers have

intensified efforts to exploit natural products such as herbs and plants in development of alternative dietary supplements that enhance growth performance, health and immune system of cultured fish against chemotherapeutic agents. [8,9,5,10]. Herbs and medicinal plants are promising to be an important source of therapeutics in fish culture since products provide a cheaper source for treatment and greater accuracy without causing toxicity [11].

The hematological characteristics of fishes are an integral part of evaluating their health status. However the diet composition, metabolic adaptation and variation in fish activity are the main factors responsible for the change in hematological parameters of fish [12,13]. The cellular constituents of the fish blood are the red blood cells (erythrocytes), the white blood cells (leucocytes) and thrombocytes [14]. Some fish erythrocytes obtain their characteristic colour from haemoglobin, made-up of the colourless protein globulin and the red-yellow pigment which contains iron. Mature fish erythrocytes are relatively large and oval, with large nuclei [15]. The white blood cells are the major fighters of diseases in both fish and terrestrial animals, and the extracts from some herbs have been reported to have increased the phagocytosis of white blood cells (WBCs) of fish [16].

This work will supply information on the application of *C.papaya*, root extracts in the rearing of the African catfish (*C. gariepinus*). The mode of application of the herb in this work is such that farmers in the developed and under developed countries can conveniently use it without going through laboratory procedures. Successful trial and findings from this work will go a long way to improve on the growth, survival

and the general health of the fish. It will save the farmer the cost of buying antibiotics and other chemicals, maximize production, lead to pollution free environment and ensure the consumption of disease and contaminants free fish on the side of the consumer. The aim of this project work is to investigate the effect of aqueous extracts *C.papaya* roots on behavior and the diseases resistance of African catfish (*C. gariepinus*), when infected with *P. aeruginosa*.

2. MATERIALS AND METHODS

2.1 Study Location

The project work was carried out in the laboratory of the Fisheries and Aquatic Environment Department, Faculty of Agriculture, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt.

2.2 Experimental Fish and Acclimation

One hundred and Forty (140) healthy *C. gariepinus* of mean weight 120 ± 130 were purchased from Aquaculture Centre of the Department of Fisheries and Aquatic Environment, Faculty of Agriculture, Rivers State University Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria. It was observed for two weeks to evaluate disease presences or bruises during this period and was fed to satisfaction with blue crown commercial diets twice daily.

2.3 Source of Pathogen

The pathogen, *Pseudomonas aeruginosa* 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.

2.4 Experimental Herb Preparation

The *C.papaya* aqueous root extracts used in this study was prepared by using the method of described by [17], *C.papaya* root was washed, clean, dried for four hours, then cut in pieces and pounded to paste and was soaked in tap water (50°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.

2.5 Experimental Design

A complete randomized method 4 x1x 3 (CRD) was used. There were four treatments in triplicates that were infected with *P. aeruginosa*

bacterial concentrations in this experiment with a control. A total of Twelve (12) experimental tanks were used in this experiment.

2.6 Experimental Procedure

One hundred and twenty (120) *C. gariepinus* were infected intra muscularly with 1.0ml of 4.1×10^4 overnight grown of *Pseudomonas aeruginosa* using a 2ml injection syringe and 21 – gauge hypodermic needle at day 1, 2, and 4, 5 and observed for disease presence. While thirty (30) *C. gariepinus* were kept in another tank as control. After disease presence, the infected fish were distributed into four (4) groups of ten (10) fish each in triplicate and was treated via immersion with *C.papaya* aqueous root extracts at 0.00ml/L, 1ml/L, 2.0ml/L, 3.0ml/L.

2.7 Behavioural Changes

Behavioural changes such as:

- Air gasping
- Erratic swimming
- Discoloration
- Serious wounds
- Loss of reflex
- Skin peeling

These were observed manually in the exposed fish in all the experimental periods and recorded.

2.8 Blood Extraction

The fish was blindfolded 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. Blood samples were collected before infection, after disease presence and after day 2, 5 and 7 of treatment, and were taken to the laboratory, to ascertain the therapeutic effect of the *C. papaya* root extracts on the haematological parameters of the fish (*Clarias gariepinus*) as a means of treatment.

2.9 Haematological Analysis

The collected blood samples were transferred into EDTA bottles and sent to the laboratory for analysis. The blood was assayed using automated analyzer for packed cell volume (PVC), hemoglobin (HB), red blood cell count (RBC) total white blood cells count (WBC) Platelets, and red blood cells indices: MCH,

MCHC, MCV were determined using the methods in [17].

2.10 Data Analysis

The data was collated and analyzed using SPSS statistics software 17.0 for windows. A one way analysis of variance (ANOVA) was employed to reveal significant difference in measured variables among control and experimental groups. Tuckey's multiple comparison tests was applied to separate the treatments with significant difference [18].

3. RESULTS

Behavioural observation of the fish showed severe wounds and discolouration on

the fish after infection, and improvement in terms of healing at after day 7 of treatment in treated group (Figs. 1-3). The result for the behavioural response is shown in Table 1, while the result for the haematological analysis before and after the pathogenic infection, and after day 2, 5 and 7 of treatment (exposure) with the *carica papaya* aqueous root extracts are shown in Tables 2-5 respectively. The values of the PCV, Hb, RBC, PLT, MCH, MCHC and MCV were lower after the infection period compared to the values before infection, while the reverse was the case for WBC.

Figs. 4-11 shows the comparative values of the various haematological parameters within the period of the experiment.



Fig. 1. *C. gariepinus* before it is infected with *P. aeruginosa*



Fig. 2. *C. gariepinus* after it is infected with *P. aeruginosa*



Fig. 3. Infected *C. gariepinus* after seven (7) days exposure/treatment with 2.0ml/l of *P. americana* aqueous leaves extracts

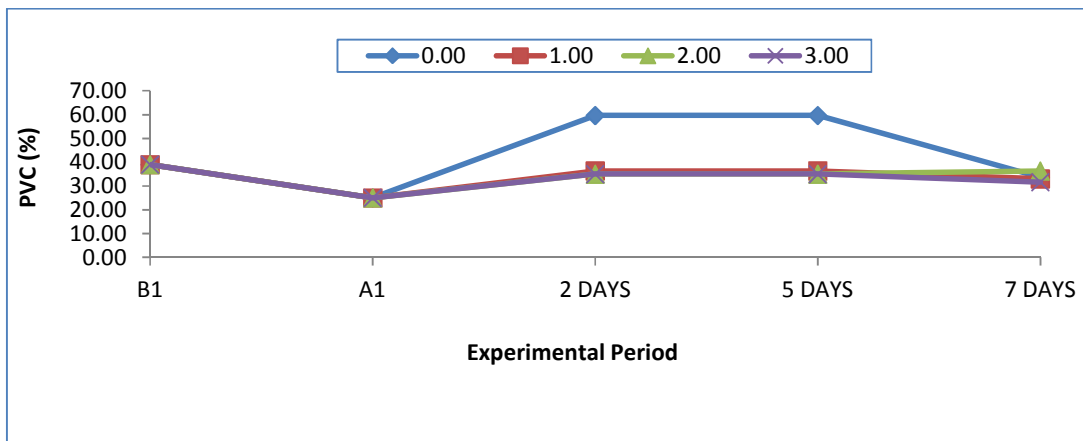


Fig. 4. Comparative values of PCV in the plasma of *C.gariepinus* infected with *P.aeruginosa* and exposed to *C.papaya* extracts at different experimental period

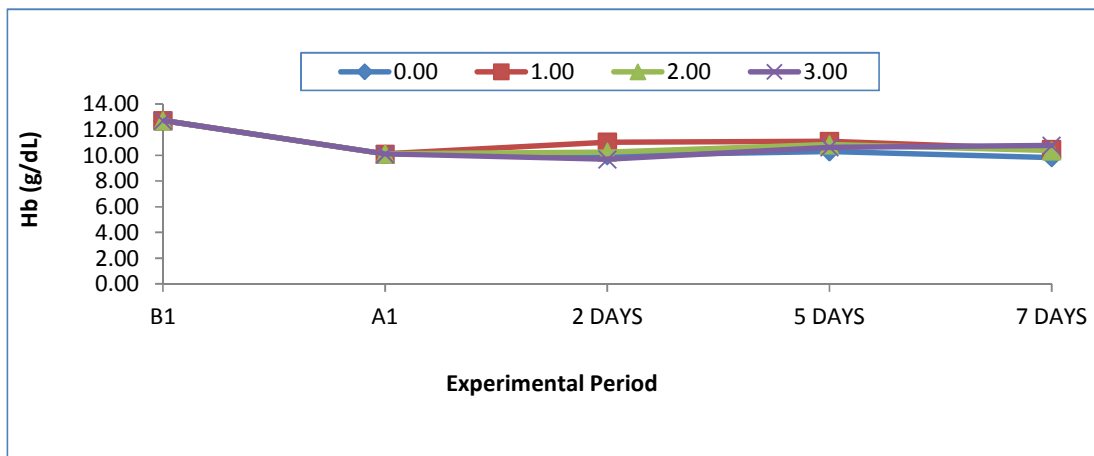


Fig. 5. Comparative values of Hb in the Plasma of *C.gariepinus* Infected with *P.aeruginosa* and exposed to *C.papaya* Extracts at different experimental periods

Table 1. Behavioural response of *Clarias gariepinus* infected with *P. aeruginosa* and exposed to different concentrations of *C.papaya* leaf aqueous extract for seven (7) days

Behaviour/ Concentrations	A1	After two (2) days				After five (5) days				After seven (7) days			
	0.0ml	1.0ml	1.5ml	2.0ml	0.0ml	1.0ml	1.5ml	2.0ml	0.0ml	1.0ml	1.5ml	2.0ml	
Air gasping	+	-	-	-	-	-	-	-	-	-	-	-	
Erratic swimming	†	-	-	-	-	-	-	-	-	-	-	-	
Discoloration	++	++	+	+	+	+	-	-	-	-	-	-	
Serious wounds	++	++	+	+	+	+	+	+	+	+	+	-	
Loss of reflex	++	-	-	-	-	-	-	-	-	-	-	-	
Skin peeling	++	+	+	+	+	+	-	-	-	+	-	-	

Key : ++ = highly present; + = present; - = absent

Table 2. Haematological parameters of *Clarias gariepinus* before and after infection (B1 and A1) with *P. aeruginosa*

Parameters	*Before (B1)	Range		*After (A1)	Range	
		Minimum	Maximum		Minimum	Maximum
PCV	39.00±5.56 ^b	34.00	45.00	25.00±7.05 ^a	20.10	33.20
Hb	12.70±1.35 ^a	11.40	14.03	10.00± 2.47 ^b	7.30	12.00
RBC	5.53±0.96 ^a	4.50	6.40	4.38±1.14 ^a	3.00	5.20
WBC	8.80±1.49 ^a	7.70	10.50	11.77±1.76 ^b	10.70	13.80
PLT	340.39±90.13 ^b	247.25	455.52	276.56±53.11 ^a	215.00	315.00
MCH	23.15±1.72 ^a	11.30	14.00	23.60±0.65 ^a	23.00	24.33
MCHC	32.84±4.21 ^a	28.44	36.84	42.17±5.88 ^b	33.18	60.00
MCV	71.29±10.45 ^b	59.38	78.05	60.66±19.29 ^a	38.46	73.33

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

Key: PCV- Packed Cell Volume (%); Hb- Haemoglobin (g/dL); RBC- Red Blood Cell (Cells X10¹⁰/L); WBC- White Blood Cell (Cells x 10⁸/L); PLT- Platelets (Cells x10⁹/L); MCH- Mean Corpuscular Haemoglobin (pg); MCHC- Mean Corpuscular Haemoglobin Concentration (g/dL); MCV- Mean Corpuscular Haemoglobin (fL)

Table 3. Haemtological parameters of *Clarias gariepinus* Infected with *P.aeruginosa* and exposed to different concentrations of *C.Papaya* root aqueous extract after (2) days

CONC (ml)	PCV	HB	RBC	WBC	PLT	MCH	MCHC	MCV
0.0	28.00±8.18 ^a	10.00±2.93 ^b	4.06±1.32 ^a	10.73±2.81 ^b	260.66±61.49 ^a	24.80±3.61 ^a	32.88±9.60 ^b	69.36±2.69 ^a
1.0	34.66±8.32 ^b	11.03±2.57 ^b	4.66±1.15 ^a	8.53±1.66 ^a	318.33±96.50 ^b	24.86±10.89 ^a	31.85±0.30 ^b	80.26±38.21 ^b
2.0	34.00±7.93 ^b	10.26±2.37 ^b	4.67±1.15 ^a	8.37±2.65 ^b	314.33±50.01 ^b	23.51±10.72 ^a	30.68±5.85 ^b	75.37±24.50 ^b
3.0	33.33±4.93 ^b	9.70±0.79 ^a	4.33±0.40 ^a	8.93±3.23 ^a	324.33±87.52 ^b	22.59±3.53 ^a	29.70±6.18 ^a	76.79±6.24 ^b

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

Key: PCV- Packed Cell Volume (%); Hb- Haemoglobin (g/dL); RBC- Red Blood Cell (Cells X10¹⁰/L); WBC- White Blood Cell (Cells x 10⁸/L); PLT- Platelets (Cells x10⁹/L); MCH- Mean Corpuscular Haemoglobin (pg); MCHC- Mean Corpuscular Haemoglobin Concentration (g/dL); MCV- Mean Corpuscular Haemoglobin (fL)

Table 4. Haemtological parameters of *Clarias gariepinus* infected with *P.aeruginosa* and exposed to different concentrations of *C.papaya* root aqueous extract after five (5) days

CONC (ml)	PCV	HB	RBC	WBC	PLT	MCH	MCHC	MCV
0.0	59.67±9.14 ^b	10.30±2.40 ^a	3.56±1.36 ^a	10.13±2.41 ^b	285.00±15.32 ^a	32.84±16.82 ^b	42.31±15.83 ^b	75.52±15.92 ^a
1.0	36.33±1.15 ^a	11.10±1.55 ^a	4.80±0.76 ^b	8.86±1.85 ^a	338.33±55.07 ^b	23.19±0.61 ^a	31.33±3.45 ^a	74.77±10.76 ^a
2.0	35.00±2.00 ^a	10.83±2.04 ^a	5.00±0.43 ^b	9.33±3.08 ^a	308.00±99.32 ^b	21.65±3.64 ^a	31.22±7.40 ^a	70.51±9.20 ^a
3.0	35.00±5.00 ^a	10.63±2.37 ^a	4.63±1.40 ^b	9.53±3.45 ^a	331.67±68.25 ^b	25.50±12.64 ^a	30.17±3.581 ^a	83.15±38.24 ^b

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

Key: PCV- Packed Cell Volume (%); Hb- Haemoglobin (g/dL); RBC- Red Blood Cell (Cells X10¹⁰/L); WBC- White Blood Cell (Cells x 10⁸/L); PLT- Platelets (Cells x10⁹/L); MCH- Mean Corpuscular Haemoglobin (pg); MCHC- Mean Corpuscular Haemoglobin Concentration (g/dL); MCV- Mean Corpuscular Haemoglobin (fL)

Table 5. Haemtological parameters of *Clarias gariepinus* infected with *P.aeruginosa* and exposed to different concentrations of *C.Papaya* root aqueous extract for seven (7) days

CONC (ml)	PCV	HB	RBC	WBC	PLT	MCH	MCHC	MCV
0.0	33.66±3.51 ^a	9.83±3.35 ^a	4.33±1.05 ^a	9.60±1.91 ^b	268.33±50.33 ^a	24.85±13.40 ^a	20.04±13.00 ^a	79.70±12.49 ^b
1.0	33.00±6.08 ^a	10.46±2.56 ^b	4.63±1.11 ^a	8.33±2.17 ^a	289.00±45.53 ^a	22.58±0.39 ^a	32.82±11.94 ^b	74.44±23.37 ^b
2.0	36.33±5.68 ^a	10.37±0.63 ^b	4.60±0.60 ^a	8.80±3.56 ^a	274.00±38.03 ^a	22.85±3.79 ^a	28.97±4.47 ^a	79.20±10.47 ^b
3.0	31.66±7.37 ^a	10.76±2.10 ^b	4.60±0.70 ^a	8.530±1.45 ^a	309.00±71.92 ^b	23.40±2.71 ^a	34.89±8.95 ^b	68.67±9.62 ^a

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

Key: PCV- Packed Cell Volume (%); Hb- Haemoglobin (g/dL); RBC- Red Blood Cell (Cells X10¹⁰/L); WBC- White Blood Cell (Cells x 10⁸/L); PLT- Platelets (Cells x10⁹/L); MCH- Mean Corpuscular Haemoglobin (pg); MCHC- Mean Corpuscular Haemoglobin Concentration (g/dL); MCV- Mean Corpuscular Haemoglobin (fL)

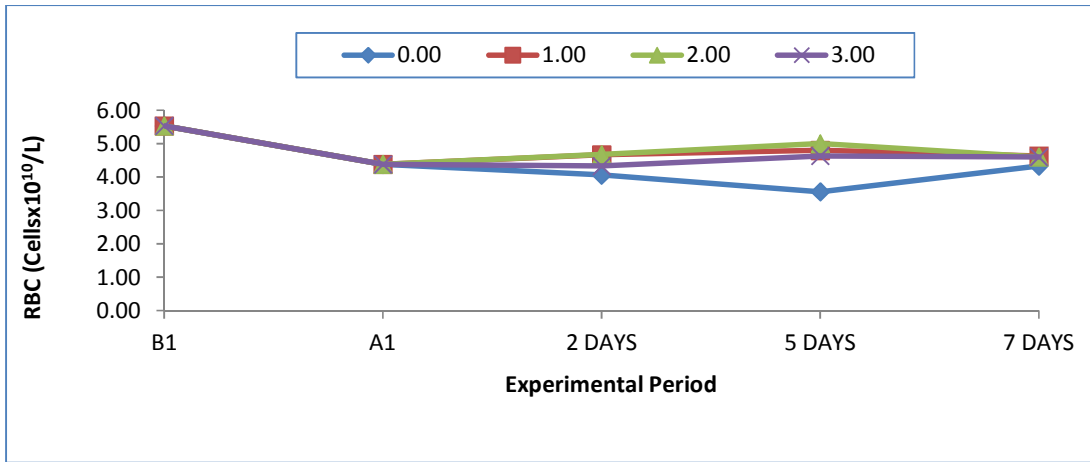


Fig. 6. Comparative values of RBC in the plasma of *C.gariepinus* infected with *P.aeruginosa* and exposed to *C.papaya* extracts at different experimental period

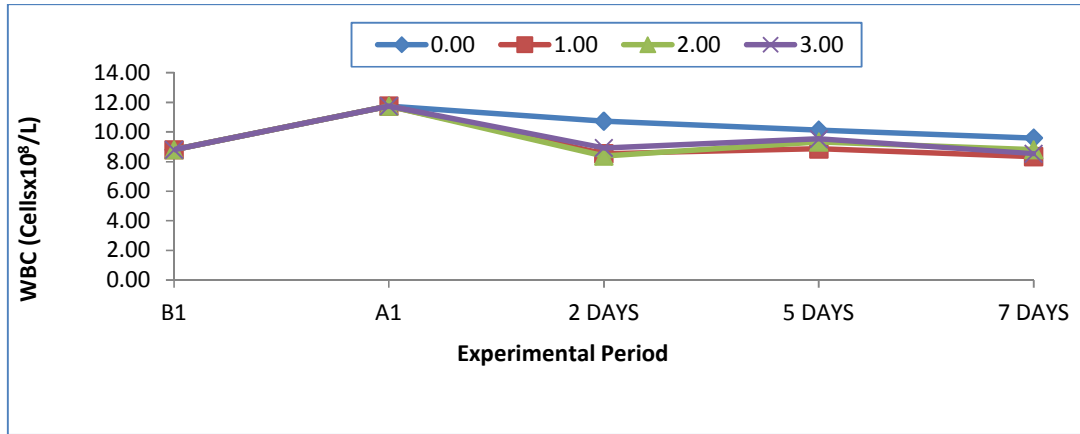


Fig. 7. Comparative values of WBC in the plasma of *C.gariepinus* infected with *P.aeruginosa* and exposed to *C.papaya* extracts at different experimental period

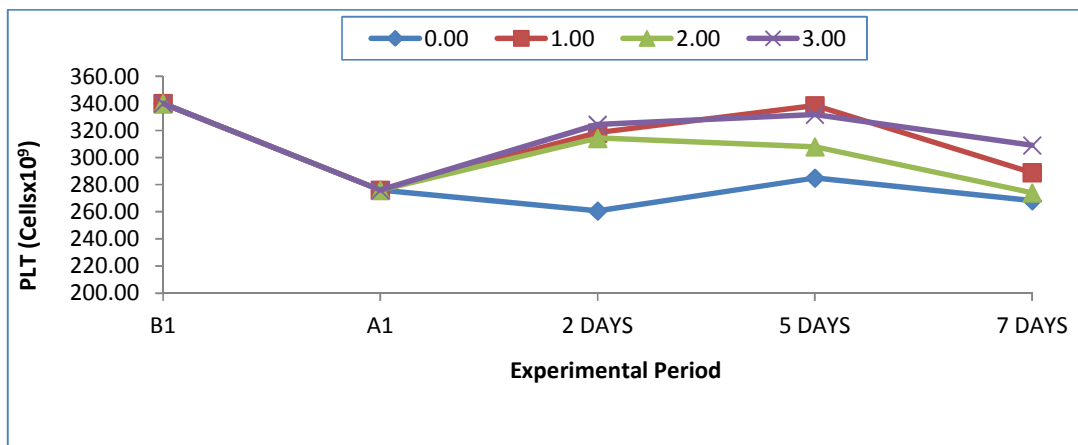


Fig. 8. Comparative values of PLT in the plasma of *C.gariepinus* infected with *P.aeruginosa* and exposed to *C.papaya* extracts at different experimental period

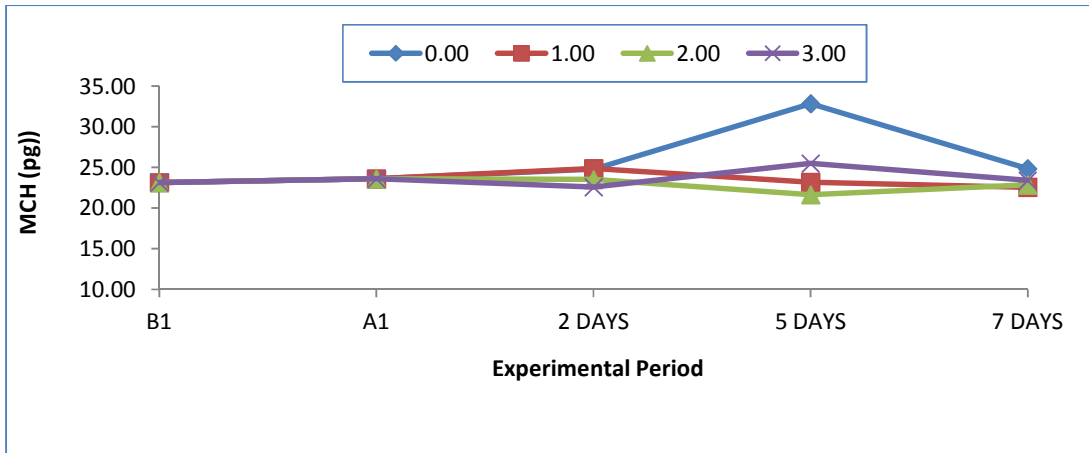


Fig. 9. Comparative values of MCH in the plasma of *C.gariepinus* infected with *P.aeruginosa* and exposed to *C.papaya* extracts at different experimental period

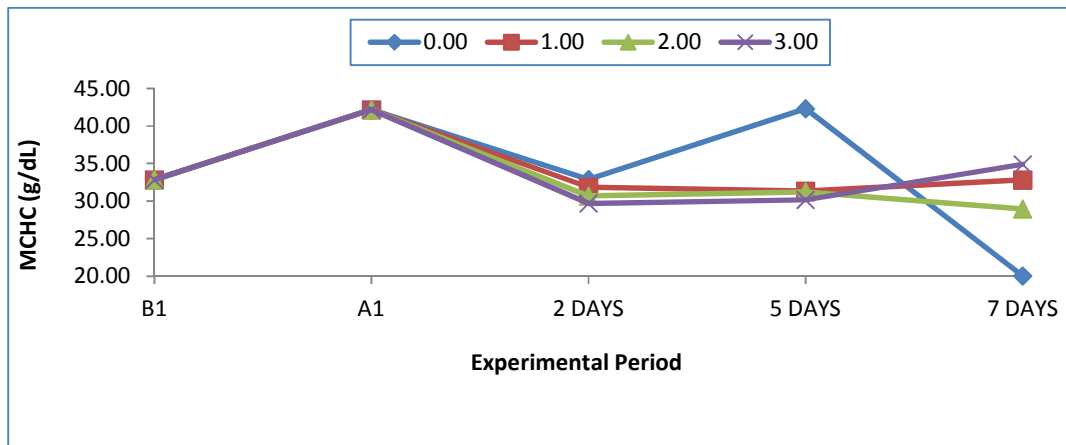


Fig. 10. Comparative values of MCHC in the plasma of *C.gariepinus* infected with *P.aeruginosa* and exposed to *C.papaya* extracts at different experimental period

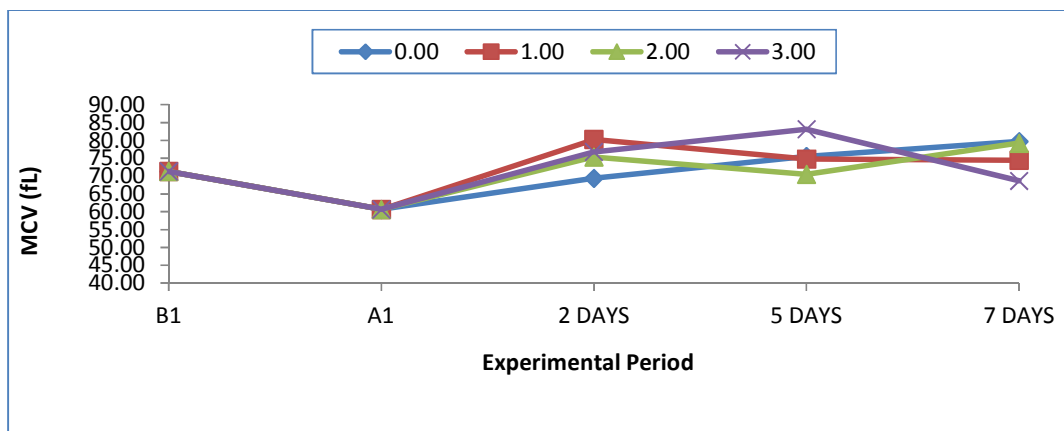


Fig. 11. Comparative values of MCV in the plasma of *C.gariepinus* infected with *P.aeruginosa* and exposed to *C.papaya* extracts at different experimental period

4. DISCUSSION

Microorganisms are significant component of the aquatic ecosystem. The interaction between these pathogens and the aquatic environment increase the rate of contagious diseases during aquaculture operations. After infection with the *P.aeruginosa*, the infected fish showed similar behaviours such as restlessness, incessant jumping, irregular swimming pattern, wounds and skin discolourations. These abnormal behaviours were more noticeable in the fish after infection and the ones exposed to 0.00ml extracts. Similar observations were reported by [19] and [20]. The ability of the fish exposed to various concentrations of the extracts to prevent ulceration could be as a result of the presence of alkaloids, resins, saponins, glycoside, tannins, flavonoids, steroid, terpenes and anthraquinones in pawpaw root extract [21]. The presence of these bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the demonstration of antibacterial activity by the plant extracts used in this study [22]

The immune system of fish is often compromised as a result of bacterial infection, and haematological parameters have proven to be very useful in the assessment of the health status of infected fish [23]. Haematological indices are essential health indicators that reveal the health status of fish before and after an experiment [24]. In this study, the results obtained indicated that PCV, Hb and RBC in the blood of infected fish reduced after infection. This result agrees with the findings of [25] who reported similar result in *C. gariepinus* experimentally challenged with *Escherichia coli* and *Vibrio fischeri*. This is an indication that the productions of red blood cells have been impaired by the pathogen, which result in anemic condition of the fish a situation that depicts low oxygen in circulation [26]. There were slight increases in PCV, Hb and RBC of the infected fish exposed to *C. papaya* roots extracts during the experimental period. The increase in the PCV, Hb and RBC in the infected fish exposed to different concentrations of *C.papaya* root extracts is an indication that there were enhanced antibody production which promotes survival and recovery, and the fish had better oxygen circulation [27].

The WBC is an important components of the immune system, and they are concerned with defending the cell against invasion by foreign

bodies. A high count of WBC is usually an indication of stress response in animals [28]. A high count of leukocytes is usually an indication of response to stress in animals [29,30,31]. In this study, infected *C. gariepinus* produced a higher level of WBC in contrast to the levels before infection. This observation is consistent with the findings in similar studies in which WBC were elevated during bacteria challenge [32,33,34]. [35] also reported that diseased *C. gariepinus* exposed to neem leaves extract for 12 – weeks reveals higher levels of WBC, phagocytic activation and diseases resistance.

Also, the significant increase in the values of WBC in infected fish could be attributed to the increase in WBC synthesis as a defense mechanism against the destruction of erythrocytes. Lymphocytes are the most numerous cells comprising the leucocytes, which function in the production of antibodies and chemical substances serving as a defense against infection [34]. In this study, the values of WBC in infected *C.gariepinus* exposed to 1.0 – 3.0ml concentrations of *C.papaya* roots extracts are lesser than that of untreated (0.0ml) fish. This result align with the reports of [35] who observed the same trend in *C.gariepinus* challenged with *E. coli* and *V. fisher* .

The values of platelets reduced after infection. This agrees with the findings of [36], who obtained similar results in *Oreochromis mossambicus* infected with *Aeromonas hydrophila*. Regarding fish, the role of the platelets in the inflammatory process is still unknown. According to [37], these cells participate, together with the lymphocytes, directly in the granulomatous reaction. According to [38] platelets are present in recent fish lesions, more as a secondary cell type rather than one playing an active role. In the present study, platelets counts were altered, decreasing after fish infection.

However, the values of platelets increased in the infected fish exposed to *C. papaya* root extracts compared to the fish exposed to 0.0ml in all the experimental period. This could be as a result of the phytochemicals present in their blood consequent of their exposure to *C. papaya* root extracts [39,40] which enhanced the production of platelets and lead to the protection of the exposed fish against external wounds [41]. This result agrees with the postulation that increase in platelets prevents injuries or leads to quick recovery from injuries [42,17].

The values for blood indices (MCH, MCHC and MCV) in the fish after infection with the pathogen varied with respect to the various concentrations of *C. papaya* root extracts. In the fish exposed to *P. aeruginosa*, there were fluctuating values of MCH, MCV and MCHC across the various experimental treatments within the period of the experiment. This result is in line with the report of [43] who reported the antibacterial activities of mango leaf extracts on *C. gariepinus* exposed to *P. aeruginosa*.

In prophylactic studies on fish, it has been reported that immunostimulants such as some plants extracts can enhanced the general defence system, decreased mortality against pathogens and increase the viability rate [43,44]. The results in our study on the effects *C.papaya* extracts on the haematology of *C. gariepinus*, were compatible with those obtained in various studies [45,46,47].

5. CONCLUSION AND RECOMMENDATIONS

The abnormal behaviour in *C.gariepinus* exposed to *P.aeruginosa* pathogen were minimized as the inclusion level of *C.papaya* roots extracts increased. The disease resistance ability of the exposed fish as shown in the blood parameters increased with increase concentrations of *C.papaya* roots extracts. The haematological parameters of *C. gariepinus* at the end of the experimental period indicates that the used concentrations of aqueous extracts of *C. papaya* roots did not impair, but rather improved. The formation of RBC, Hb and PCV in the infected fish. Conversely, the result of the thrombocytes showed that the aqueous extracts of *C. papaya* roots have wound healing abilities. Though the values of WBC fluctuated in the exposed fish, during the experimental period this could be attributed to handling and other experimental procedures. This study has shown that *C.papaya* roots extracts have the potency to improve the health and antimicrobial activities in culture fish during aquaculture operations. Also, *C.papaya* roots are readily available, cheap, accessible, eco-friendly, non-toxic to the fish and consumers. Hence, it therefore recommended to be applied in the rearing water at the rate of 1.50 to 2.00mg/l, for both therapeutic or prophylactic use in aquaculture:

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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