ANTIBACTERIAL ACTIVITY OF *MANGIFERA INDICA L.* (MANGO) KERNEL ON *CLARIAS GARIEPINUS* (AFRICAN CATFISH) FINGERLINGS INFECTED WITH *AEROMONAS CAVIAE*

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ABSTRACT
Different approaches are employed to control diseases in fish with particular emphasis on the use of chemotherapeutic agents, however, application of chemicals in aquaculture is expensive and may lead to serious hazards in fish. This study was designed to evaluate the *in vivo* antibacterial activity of Mango kernel on *Clarias gariepinus* experimentally infected with *Aeromonas caviae*. A total of 240 fingerlings randomly divided into four groups of 60 each were used in the study. Fish in groups A, B, C and D were fed diet containing 0, 1, 5 and 10 g/kg of the kernel powder, respectively for 60 days. Sera were collected from 12 fish in each group after the first 20 and 40 days of feeding, and the antibacterial effect of the sera against *A. caviae* was assessed using Agar well diffusion method. Additional 12 fish from each group were challenged with *A. caviae* at 60 days post feeding, and clinical signs, mortalities and gross lesions were observed and recorded over 14 days post-infection. The sera of fish treated with mango kernel at different concentrations produced no antibacterial effect against *A. caviae*, however, less survivability was observed in fish fed control diet (50% survivability). Groups fed 5 and 10 g/kg mango kernel diet had the highest percentage survival (100%). The study showed that mango kernel protects *C. gariepinus* against *A. caviae* infection by enhancing the survivability of the treated fish. More studies to determine the actual dose of the kernel that gives the optimum fish survivability were recommended.

Keywords: *Aeromonas caviae*, Antimicrobial effect, *Clarias gariepinus*, *Magnifera indica* kernel

INTRODUCTION
Aquaculture is one of the fastest growing animal food-producing sectors around the world (FAO, 2005). Among various kinds of cultivated organisms, many marine and freshwater fish species constitute an important industry with their production increasing every year necessitating intensive culture practices (Harikrishnan *et al*., 2011). *Clarias gariepinus*, the most frequently farmed fish in Nigeria, is a fresh water indicator fish species often used as model for ecotoxicological assay (Iheanacho and Odo, 2020). Infectious diseases pose a major problem in the aquaculture industry, and Aeromonas species are arguably the commonest cause of bacterial diseases in cultured warm water fish. The common occurrence of these pathogens relates to stressful conditions such as poor water quality, overcrowding, or rough handling (Majtan *et al*., 2012; Irang and Reyes, 2017). Specifically, *Aeromonas caviae* in fish is known to cause several diseases including, Motile Aeromonas Septicemia (MAS), red sore disease, and ulcerative infections. Mortality rates are high, causing heavy economic losses, thereby necessitating timely measures of control for prevention and treatment (Joseph and Carnahan, 1994; Stratev and Odeyemi, 2017). In aquaculture, disease control using chemotherapeutic

agents is often expensive and undesirable. Moreover, it has been complicated by the misleading advice provided to the farmers by feed and chemical companies (Harikrishnan *et al.*, 2011). Likewise, chemotherapy as prophylactic measures may cause broad negative impacts such as immunosuppression, antibiotic resistance and residue accumulation in tissues (Smith *et al*., 1994; Harikrishnan *et al*., 2009). Conversely, several plant extracts such as *Tamarindus indica* and *Mangifera indica* leaves (Olusola *et al*., 2020), clove basil leaves (Abdel-Tawwab *et al*., 2018) have been reported to possess antimicrobial and antioxidant effects, as well as non-specific immunostimulants that enhance fish performance and modulate the immunity of *C. gariepinus* juvenile against *Aeromonas* species. Similarly, the methanol extract of mango kernel has been established to contains varying secondary metabolites particularly flavonoids, saponins, triterpenes, tannins, and glycosides which have been shown to improve fish growth performance, and exhibit *in vitro* antimicrobial effects at different concentrations (Talba *et al*., 2014; El-Houseiny *et al*., 2017). Therefore, the present study was conducted to assess the antibacterial activity of *M. indica* on *C. gariepinus* fingerlings experimentally infected with *A. caviae*.

MATERIALS AND METHODS

Study location

This study was conducted in the Faculty of Veterinary Medicine, Ahmadu Bello University (ABU) Samaru, Zaria.

Catfish fingerlings and *Aeromonas caviae*

A total of 300 healthy, 4 weeks old catfish fingerlings of average length 10.5 ±1.0 cm, and weigth 10 ± 1.0g were used for the entire study. They were sourced from a supplier in Zaria. The fish were obtained and acclimatized in the laboratory for 12 days before the commencement of the experiment. The *Aeromonas caviae* isolates used in the study were obtained from the Department of Veterinary Microbiology, ABU, Zaria, Nigeria.

Collection, Identification and processing of *Mangifera indica* kernel

Mangifera indica seeds and leaves were collected from mango trees around ABU, Zaria. The mango species were identified based on the collected leaves at Herbarium Department of Biological Sciences, ABU. Zaria, and a voucher specimen no. 1944 was then assigned. The seeds were washed repeatedly under running tap water and the kernels were removed. The kernels were then air-dried at room temperature and ground into powder using mortar and pestle. The powder was preserved in an airtight polythene bag and kept in a cool dry place until used.

Acute Toxicity Test: Limit test

Fish acute toxicity test was carried out as described by Organisation for Economic Cooperation and Development (OECD) testing protocol 203 (1992) with slight modification. Briefly, a total of 60 catfish fingerlings divided randomly into six groups of 10 fingerlings each were used for the study. Mango kernel powder was added into an experimental feed at different doses (0, 1, 4, 20, 40, 60 g/kg of feed). The feeds were given to different fish treatment groups twice daily for 96 hours (4 days). Fish were observed at 24, 48, 72 and 96 hours for any mortality or clinical signs.

Water quality test

The quality of water used was determined using test kit (APITM Aquarium Pharmaceuticals Mars Fish care North America, inc.).

Experimental design

A total of 240 catfish randomly divided into four groups (A, B, C and D) of 60 fingerlings each were used for the study. Each group was maintained in a 500 litre tank. Fish in group A were fed basal diet and served as non-treated control, while fish in groups B, C and D were given feed containing mango kernel at 1, 5 and 10 g/kg of feed for 60 days. After the first 20 and 40 days of feeding, about 0.5 ml of blood for sera were collected (through caudal vein severance) randomly from 12 fish in each group. The collected sera were kept at -20˚C

until used. Agar well diffusion method was used to test for antibacterial activity of the serum samples collected as described by Perez *et al*. (1990). Another set of 12 fish from each group were randomly sampled and challenged at 60 days post feeding through interperitoneal route with 6.7 x 10^{11} cfu/ml of *A. caviae.* Clinical signs and mortality were observed and recorded for the period of 14 days. Gross lesions in the dead fish were also observed and recorded.

Data Analysis

Descriptive statistics was used to measure the percentage survivability between control and treatment groups.

RESULTS AND DISCUSSION

The acute toxicity result of *M. indica* kernel powder incorporated in feed at different doses produced zero mortality in both the treatment and control groups, i.e., the median lethal dose (LD50) is greater than 60 g/kg of feed with 99.9 % confidence. Therefore, mango kernel powder is safe to be incorporated into feed at 60 g/kg of feed and below. The parameters of the water used in this study were found to be within normal range with pH values at 7.0-7.5, temperature (24-27 °C), general hardness (30 ppm mg/l), carbonate hardness (80 ppm mg/l), nitrite ($NO^{-}2$) & nitrates ($NO^{-}3$) being 0 ppm mg/l.

The sera obtained from fish treated with *M. indica* kernel at 0, 1, 5 and 10 g/kg of feed showed no significant antibacterial activity against the test organism as evident by lack of observable inhibition of the *A. caviae* in the media. This finding contradicts the results of Thuy *et al.* (2002) and Sahu *et al.* (2007) who reported significant antibacterial effect of sera obtained from *Labeo rohita* treated with mango kernel against *A. hydrophila.* The discrepancy could be due to the differences in fish species and test organisms.

Mortalities were recorded in fish from untreated group (group A) and group treated with mango kernel at 1 g/kg of feed (group B). Sluggishness, erratic swimming and isolation were noticed in groups A and B. Fish fed both treated and untreated diet (at all doses) had reduced feed intake. A day post infection with *A. caviae*, two and one fish died in groups A and B, respectively. Similarly, two days post-infection, two fish died in group A. finally on the fifth day two and one fish died in groups A and B respectively. Therefore, the total number of fish that died in groups A and B were 6 and 2 respectively. There were no mortalities from day six through day 14 post challenge (Table 1). Post-mortem examination, revealed discolouration of the skin, distended abdomen and discoloured livers in fishes which died from both treated and untreated groups after challenge. In addition, exopthalmus and ulcerations were seen in the untreated group.

Table 1: Effect of *Mangifera indica* kernel on the survival of *Clarias gariefinus* challenged with *Aeromonas caviae* (6.7 x 10^{11} cfu / ml) (12 fish per group)

Groups	**Fish Survival rate (%)**					
	Days post challenge 1	2	3	4	5	6 to 14
A	83.3 (10)	66.6 (8)	66.6 (8)	66.6 (8)	50.0 (6)	50.0 (6)
B	91.6 (11)	91.6 (11)	91.6 (11)	91.6 (11)	83.3 (10)	83.3 (10)
C	100.0 (12)	100.0 (12)	100.0 (12)	100.0 (12)	100.0 (12)	100.0 (12)
D	100.0 (12)	100.0 (12)	100.0 (12)	100.0 (12)	100.0 (12)	100.0 (12)

Values in the parenthesis indicates number of fish survived.

The survivability of fish fed with mango kernel was higher when compared to fish fed with basal diet. Therefore, although negative serum antibacterial activity was recorded, *M. indica* kernel enhanced the survivability of *C. gariepinus* fingerlings against *A. caviae* infection possibly through improved fish immune status, as well as antioxidant action. This assertion correlate well with the findings of

Schaperclaus, (1992) and Suhu *et al*. 2007 who independently reported resistance to *A. hydrophila* infection with increased superoxide anion and its reactive derivatives production in *Labeo rohita* fingerlings fed with *M. indica* kernel.

CONCLUSIONS AND RECOMMENDATIONS

Mangifera indica kernel protects *C. gariepinus* against *A. caviae* infection as evident by higher survival rates in fish fed with the kernel-enriched diets (83 to 100%) as compared to those fed with the control diet (50 to 83%) post challenge. The enhanced survivability of the treated fish might be due to augmented immune status, and antioxidant effects rather than direct inhibitory effect of the kernel on the test organism. Mango kernel should be added into fish feed at 5-10g/kg as it has some beneficial effects, however, more elaborate studies to determine the dose of the kernel that gives the optimum fish survivability should be carried out. In addition, the actual component of the mango kernel exerting the desired effect should be identified.

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