USE OF PLANT EXTRACT AND PROBIOTICS IN PREVENTING *PSEUDOMONAS AERUGINOSA* INFECTION IN *CLARIAS GARIEPINUS*

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ABSTRACT
This study was aimed at investigating the use of plant extract and probiotics (Bacillus subtilis) in preventing bacterial infection in Clarias gariepinus. 120 post-juvenile C. gariepinus were challenged with Pseudomonas aeruginosa and neem leaves extract and B. subtilis were administered concurrently with the infected fish for fourteen days. Clinical signs and mortality were the parameters measured to observe if fresh aqueous neem leaves extract and Bacillus subtilis can prevent P. aeruginosa infection in C. gariepinus. Lesions were more in group infected with P. aeruginosa bacterial only, while lesions were absent to minimal in groups treated with neem extract and Bacillus subtilis. Mortality rate of P. aeruginosa infected group was 30%, while those treated with neem extract and Bacillus subtilis groups were 10 and 5% respectively. Both neem leave extract and B. subtilis were able to prevent P. aeruginosa infection in C. gariepinus base on the clinical sign observed, but mortality rate was higher in group administered with neem than group administered with Bacillus.
Keywords: Administered, Lesion, Mortality, Probiotics, Plant extract

INTRODUCTION

Gram-negative bacteria cause most diseases in tropical fish (Efuntoye *et al.,* 2012). In aquaculture, a wide range of antimicrobials are being used to control these diseases, (Rodgers and Furones, 2009). Unfortunately, the use of antibiotics for the control of bacterial diseases in aquaculture has proven unsustainable and ineffective due to development of multidrug antibiotic resistance in pathogens and retention of high levels of residues in animals (Cabello, 2006; Winkaler *et al*, 2007). In recent studies, some of the bacteria flora in fish intestine have been isolated and cultured and used as probiotics (Verschuere *et al.,* 2000; Vines *et al.,* 2006; Marzouk *et al.,* 2008). This innovative method has substituted for the use of chemotherapy in many fish diseases. However, another most promising natural compound is Azadirachtin (AZA), which is an active compound in neem tree (*Azadirachta indica*). Its antiviral, antibacterial and antifungal properties has been known for more than 2000 years (Isman *et al.,* 1990; Hariskrishnan *et al.,* 2003). Therefore, the objective of this study is to prevent *Pseudomonas* infection in *Clarias gariepinus* using aqueous extract of neem leaves (*Azadirachta indica*) and probiotic (*Bacillus subtilis*).

MATERIALS AND METHODOLOGY

120 post-juveniles *Clarias gariepinus* used for this study were allotted into six groups and two replicates each. First three groups were the uninfected groups. Group one (control) were given normal saline, group two were bath in neem leaf extract (60 g of freshly harvested neem leaves was rinsed, blended in 1 litre of water, sieved and mixed with water 15 litres of water before introducing the fish for 15 minutes, group three were bath in 1g/litre of *Bacillus subtilis* (packaged by Animal Care as MaxCare®). Group four to six were administered with 1.6 x 10^{11} CFU/100µl of *P. aeruginosa* (sourced from Nigerian Institute for Oceanography and Marine Research, Lagos) orally, while group five and six were administered fresh aqueous neem leaf extract and *B. subtilis* respectively for fourteen days. Clinical signs and mortality rate were measured during the course of the experimentation.

RESULTS

Table 1: Clinical signs of exhibited by different groups

Groups	Replicate	Clinical signs
Control	1	Nil
Control	2	Nil
Neem	1	Nil
Neem	2	fin ulcer on the dorsal fin with skin lightening
Bacillus	1	Nil
Bacillus	2	Change in skin colour of fish in the group
Infected	1	Several fish had different skin ulcers
Infected	2	Patches of haemorrhage on the skin of fish
Neem treated	1	Nil
Neem treated	2	Injury and slow movement
Bacillus treated	1	Change in skin colour
Bacillus treated	2	Change in skin colour, ulcer on the dorsal fin, wound, haemorrhage on the pectoral fin

Table 2: Mortality rate for different groups

Groups	Mortality rate (%)
Control	$0.00\pm.00^{b}$
Neem	$0.00\pm.00^{b}$
Bacillus	5.00 ± 5.00^{ab}
Infected	$30.00\pm.00^{a}$
Neem treated	10.00 ± 10.00^{ab}
Bacillus treated	5.00 ± 5.00^{ab}
M±SEM	3.445
P -Value	.037

M±SEM - Mean ± Standard Error of Mean

Means with different superscripts along the column indicate significance ($p < 0.05$) according to Duncan's Multiple Range Test

The result of clinical signs of *Clarias gariepinus* subjected to *Pseudomonas aeruginosa* and concurrently treated with aqueous neem leave extract and *Bacillus subtilis* is represented in Table 1: control, neem (replicate [rep.] 1), bacillus (rep. 1) and neem treated (rep. 1) groups had no lesion. Fin ulcer was observed at the dorsal fin with skin lightening in neem (rep. 2), there was change in skin colour of fish in bacillus (rep. 2) group (grp.), skin ulcerations and haemorrhage were observed on the skin of fish in infected grp. Injury and slow movement were observed in neem treated (rep. 2) grp, bacillus treated grp had change in skin colour, ulcer on the dorsal fin, wound, haemorrhage on the pectoral fin of a fish. Mortality rate of *Clarias gariepinus* subjected to *P. aeruginosa* and concurrently treated with aqueous neem leave extract and *B. subtilis* is shown in Table 2. Mortality rate (%) for this study are as follows: control and neem group had $0.00\pm.00^{b}$, bacillus grp and bacillus treated grp had 5.00 ± 5.00^{ab}, infected grp had $30.00\pm.00^{a}$, neem treated grp had 10.00 ± 10.00^{ab}, M±SEM (standard error of mean) is 3.445, P -Value is.037.

DISCUSSION

In this experiment, *Clarias gariepinus* were subjected to *Pseudomonas aeruginosa* and concurrently treated with aqueous neem leave extract and *Bacillus subtilis.* Clinical signs were observed in some groups and absence in some groups. Control group (CG) had no lesion at all, while the in-neem grp

(NG) and bacillus grp (BG.) lesions were absent in the first replicates but present in the second replicates. Both replicates in infected grp (IG) and Bacillus treated grp (BTG) had lesions. The lesions documented were fin and tail ulcer with skin lightening, skin dis-colouration, patches of haemorrhage on the skin, injury and slow movement. External lesions found in second replicates of NG and BG. had not been documented in any article for neem and *Bacillus subtilis*. Lesions observed in IG is in accordance with what was observed by Magdy *et al.* (2014) and Amrevuawho *et al.* (2014) the two authors experimentally infect *P. aeruginosa* in heteroclarias and *C. gariepinus* and similar clinical signs were reported. Abd Allah *et al*. (2019) reported lesions in *C. gariepinus* that was infected with *Aeromonas hydrophila* and given *Bacillus subtilis* as was observed in BTG of this study. Lesion was not found in first replicate of neem treated grp (NTG) but was observed in the second replicate. Absence of lesion was reported by Abd Allah *et al*. (2019) when *Aeromonas hydrophila* and oil extract of *Origanum vulgare* was included as feed additive in that study, therapeutic effect of aqueous *A. indica* leaf extract had been documented by Harishrishnan (2002) against *A. hydrophila*. Therefore, absence of lesion could be the effect of the neem extract. Significant difference p-value (< 0.05) was observed in mortality rate (MR) across the groups. Zero MR was recorded in CG and NG, while BG. and BTG had 5% MR, NTG had 10% and IG had 30%. Result obtained in CG and IG were supported by Obisesan *et al.* (2022); Amrevuawho *et al.* (2014); Hossain *et al.* (2006) and Magdy *et al.* (2014) were heteroclarias, *C. gariepinus* and *Oreochromis niloticus* were infected with *P. aeruginosa*. The CG had no mortality while the IG had different ranges of mortality. Reduction in mortality observed in NTG corroborates with Mona *et al*. (2015) findings where different concentration of neem leave extract were administered to Chinese carp and exposed to *A. hydrophila*, the groups with different concentration of neem leaf extract had reduction in mortality rate than the grp without neem leaf extract. Cha *et al*. (2013) got similar result with BTG in this study, he evaluated the effect of dietary and water additive of *Bacillus* spp. on olive flounder (*Paralinchthys olivaceus*) against *Streptococcus iniae,* and recorded lesser mortality compared to grp exposed to only *S. iniae*.

CONCLUSION

It was observed that *Azadirachta indica* and *Bacillus subtilis* has the capacity to prevent infection of *Pseudomonas aeruginosa* in *Clarias gariepinus*.

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