

## Effects of Dietary Mango Bark (*Mangniferaindica*) Extract on *Clarias gariepinus* (Burchell, 1822) Infected with *Pseudomonas aeruginosa*

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**Abstract:** Mango bark extract was assessed for its physiological effect on the hematology and organo-somatic indices of *Clarias gariepinus*, as well as its ability to resist *Pseudomonas aeruginosa* infection. Two diets were prepared: a control diet C and a medicated diet (M) that has mango bark extract incorporated to the control diet at 50ml/kg. One hundred and thirty-five (135) *C. gariepinus* of weight  $117 \pm 0.34g$  were distributed into three groups as follows:- group C, fish fed control diet; group M, fish fed mango bark extract incorporated diet continuously and group M/C, fish fed mango bark incorporated diet and control diet intermittently. All groups were in triplicates. Blood samples were collected from the three groups after 3 and 6 weeks of feeding to ascertain the physiological effects of the diets on the hematology and organosomatic indices of *C. gariepinus*. At the end of 6 weeks feeding exercise experimented *C. gariepinus* was injected *P. aeruginosa* and observed for 21 days to ascertain the prophylactic effect of the mango bark extract on the hematology, organosomatic indices and diseases resistance ability of *C. gariepinus*. The results of the hematology at the end of weeks 3 and 6 of the feeding exercise shows that all the values were within a normal range of aquaculture practice and the result of the organosomatic indices (HSI, CSI and SSI) shows that the mango bark extract did not impact any pathological changes in the liver, heart and spleen, which is an indication of non-toxicity. The increased level of lymphocytes in the treated groups is indicative of immune stimulation by the mango bark extract. There was increase in the post-infection values of all the hematological parameters in the treated groups (M and M/C) and a significant reduction in the post-infection values of the control group C. The HSI of the fish in M and M/C were unchanged after the infection period while that of the control group C was significantly high. The unchanged HSI and high hematological parameters after the infection show that the mango bark extract is a strong antibacterial and liver anti-inflammatory agent for *C. gariepinus*.

**Key words:** Mangifera indica extract • *P. aeruginosa* • Hematology • Organosomatic indices • Diseases resistance • *C. gariepinus*

### INTRODUCTION

Aquaculture is one of the fastest-growing sectors in food production worldwide and was observed to increase from 29.9 million tones in 2007 to 41.9 million tones in 2012, FAO [1]. This is a clear attestation to the fact that the demand for fish increase with an increase in population. The world population is expected to be 9 (nine) billion by the year 2050, DESA [2] and this means an increase for the demand of fish and its product from our fresh and marine waters.

The productivity from our natural waters is diminishing each passing day because of massive fishing, organic pollution, contamination from industrial and agricultural waste, climate change, etc. [3]. Aquaculture product like fish contains lipids, vitamins, minerals and some essential fatty acids such as Omega-3 and if consumed at the required quantity eliminates or control some fatal diseases like cancers, cardiovascular diseases, eye defects and the quest to meet up this demand have given birth to series of employment opportunity in the aquaculture sector [4].

Disease presence as a result of bacteria is a bottleneck to a successful aquaculture practice, it is responsible for serious mortalities and severe economic losses. Most of the microorganisms causing these diseases are opportunistic pathogens that attack the fish's immune systems and surrenders the fish to infection [5], they further stated that among the known pathogens that cause bacterial infection, aeromonad, pseudomonad and edwardsiellatarda are the major bacterial fish pathogens. Most of these microorganisms are found in the environment and water and their establishment and propagation can be enhanced by poor water management and improper feeding [4].

According to Khalili *et al.* [6], *Pseudomonas* infection is believed to be the regular bacterial infection in fish, especially the one under culture and they have constituted serious economic losses and reduce productivity in fish farming.

Chemicals have often been used as therapies to treat bacterial diseases or pathogenic attacks in aquaculture, although they encourage antibiotic-resistant pathogens, accumulate in fish flesh, pollute the environment, cause immune suppression, etc. [7]. There is a need to replace the use of chemicals in aquaculture with phytochemicals that are eco-friendly (biodegradable), always available, far less expensive, free from adulteration, will not deposit on fish flesh and treat more than one problem on administration [8]. Plants used in aquaculture as phytochemicals include: *Phyllanthus emblica* [9], *Zingiberofficinale* [10], *Aloe vera* [11], *Azadirachaindica* [12], *Carica papaya* [13], etc.

The bark of the Mango Plant (*Mangifera indica*) is highly medicinal and contains protocatechuic acid, catechins, mangiferin, mangiferonic acid, common flavonoids, etc. [14]. Mangiferin is a polyphenolic antioxidant, it has the strong antioxidant ability, anti-lipid peroxidation, immunomodulation, cardiogenic, wound healing, anti-degenerative antibacterial, antimicrobial and antifungal [15]. This experiment was conducted to assess the effect of dietary mango aqueous back extract on the hematology and organosomatic indices of *C. gariepinus* and its anti-pathogenic effect against *P. aeruginosa* infection on the fish.

## MATERIALS AND METHODS

**Experimental Fish:** One hundred and thirty-five (135) healthy *C. gariepinus* of mean weight  $105 \pm 0.13g$  were purchased from Idi-Onyana farms along Abua-Ahoda road in Rivers State, Nigeria and transferred to the research center between 5.30 am - 7.00 am. The fish was

observed for two weeks to evaluate diseases presences or bruises during this period and was fed with Coppens commercial diets twice daily at 5% body weight.

**Source of Pathogen:** *Pseudomonas 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 Herb:** The *Mangifera* bark aqueous extract was prepared using the methods of Oniovosa *et al.* [16]. The harvested *Mangifera* bark was washed clean, dried for four hours and pounded into a semi-paste. The prepared paste was soaked in tap water (25°C) at the concentration of one hundred (100) grams per liter (100g/L) for twenty-four (24) hours. It was filtered and the filtrate was used immediately.

**Preparation of Experimental Diets:** The control diet was prepared using cornmeal (13.36g); wheat offal (9.02g); soybean (43:83); fish meal (24.54g); binder (4.35g); common salt (0.6g); palm oil (3.5g); fish premix, lysine, methionine and vitamin C (0.2g each). The medicated diet was prepared by adding the prepared *Mangifera indica* filtrate to the control diet at the rate of 50ml per one kilogram of diet (50ml/kg). Both diets were mixed properly and pelleted to size.

**Experimental Procedure:** One hundred and thirty-five (135) *C. gariepinus* were divided into three groups (C, M, M/C) in triplicates after the acclimatization. They were distributed into nine (9) fifty-liters plastic tanks at fifteen (15) fish per tank and feeding commenced 24 hours after stocking. Group (C) fish was fed with the control diet (C), the fish in a group (M) was fed with the medicated diet (M) while group (M/C) fish was fed intermittently with diets C and M at weekly interval. Feeding was carried out for six (6) weeks and blood samples were collected at week three (3) and six (6) to assess the effects of the herb in the fish hematology in short and long term feeding. The liver, heart and spleen of the fish from various groups were harvested to assess the effects of the herbs on their indices. The fish were allowed for forty-eight (48) hours to recover from the stress of the blood extraction. They were injected intra-peritoneal with 1.5ml of  $10^5$  cfu/ml of overnight grown *P. aeruginosa* using 2ml injection syringe and observed for 21 days to ascertain the prophylactic ability on the *Mangifera indica* filtrate in the infected fish, the diseases resistance was

calculated as relative percentage survival (RSP) and the liver, heart and spleen were harvested from the infected fish to assess their hist-osomatic index (HSI), cardio-somatic index (CSI) and spleen-osomatic index (SSI).

**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 a 2ml injection syringe.

**Hematological Analysis:** This was done using hamatological analyzer, model MY-BOOZB, Manufactured by MAYA MEDICAL EQUIPMENT LIMITED COMPANY LIMITED in Guangdong, China. The packed cell volume (PCV), White Blood Cells (WBC), Heamoglobin (Hb), Red Blood Cells (RBC) and Thrombocytes (PL) were determined. The blood indices mean corpuscular volume (MCV); mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC) and differential counts Neutrophil (N) and Lymphocytes (Lymp) were determined using the methods of Adeniran *et al.* [17].

**Organosomatic Indices (OSI):** It was calculated as:

$$OSI = \frac{\text{Weight of organ}}{\text{Weight of fish}} \times 100 \quad [18]$$

**Diseases Resistance:** This was determined as the relative survival percentage (RSP) using the formula:

$$RSP = \frac{\% \text{Mortality in treated group}}{\% \text{Mortality in control}} \times 100 \quad [19]$$

**Data Analysis:** Data were subjected to a one-way analysis of variance to determine if there were difference in the variables among treatments. Duncan test was used to compare the means of the treatments.

## RESULTS

**Behavioural Observations:** There was a restlessness in the fish across the groups after the infection with *P. aeruginosa* but the restlessness lasted much longer (over 24 hours) in the fish fed the control diet. Signs and symptoms of infections such as skin ulcer and hemorrhage started appearing in the fish fed the control diet C after two (2) weeks of infection, but became severe at end of twenty-one (21) days post-infection (Figures 1 and 2).



Fig. 1: *C. gariepinus* After two (2) weeks of infections with *P. aeruginosa*



Fig. 2: *C. gariepinus* After three (3) weeks of infections with *P. aeruginosa*

**Hematological Parameters:** The hematological parameters of *C. gariepinus* at the end of weeks 3, 6 and after twenty (21) days infection with *P. aeruginosa* are shown in Tables 1-3 respectively. At the end of week 3, the fish in the (M) group had higher values ( $P < 0.05$ ), with fish in the M/C group having the least compared to the fish fed control diet (C) group. The values of Lymphocytes, MCV, MCH, MCHC and PL were significantly the same across the various groups, while the higher value for WBC was recorded in (C) and Neutrophils recorded higher ( $P < 0.05$ ) values in (M) and (M/C). At the end of week 6, there was a significant difference in all the tested hematological parameters except the PL. The PCV was higher ( $P < 0.05$ ) in the C and M groups, HB was higher ( $P < 0.05$ ) in C and lower in M/C groups, RBC was higher ( $P < 0.05$ ) in C and M and lower in group M/C. WBC and N were higher in C and lower in M, L was higher in groups M and M/C and lower in C. MCV was higher ( $P < 0.05$ ) in C and M and lower in

Table 1: Hematology of *C. gariepinus* fed dietary mango back extract for 3 weeks

| Diets | PCV (%)                 | HB (g/dl)              | RBC (Cellsx10 <sup>12</sup> ) | WBC (Cellsx10 <sup>9</sup> /L) | Neutrophils (%)         | Lymphocytes (%)         | MCV (FL)                | MCH (Pg)                | MCHC (g/dl)             | PL (%)                   |
|-------|-------------------------|------------------------|-------------------------------|--------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|
| C     | 37±1.00 <sup>b</sup>    | 12.4±0.10 <sup>b</sup> | 5.3±0.20 <sup>b</sup>         | 15.53±0.11 <sup>a</sup>        | 29±2.00 <sup>b</sup>    | 60±2.00 <sup>a</sup>    | 69.83±0.75 <sup>a</sup> | 23.42±0.81 <sup>a</sup> | 33.52±0.81 <sup>a</sup> | 208.33±8.51 <sup>a</sup> |
| M     | 41.33±1.53 <sup>a</sup> | 13.4±0.10 <sup>a</sup> | 6.0±0.15 <sup>a</sup>         | 7.6±0.10 <sup>c</sup>          | 35±2.00 <sup>b</sup>    | 63.33±4.51 <sup>a</sup> | 68.17±2.17 <sup>a</sup> | 22.10±0.69 <sup>a</sup> | 33.78±1.21 <sup>a</sup> | 196.67±4.16 <sup>a</sup> |
| M/C   | 32±1.00 <sup>c</sup>    | 11.5±0.50 <sup>c</sup> | 4.97±0.15 <sup>b</sup>        | 13.47±0.06 <sup>b</sup>        | 34.67±0.58 <sup>a</sup> | 64.67±0.58 <sup>a</sup> | 64.51±4.01 <sup>a</sup> | 23.16±0.75 <sup>a</sup> | 35.98±2.30 <sup>a</sup> | 202.33±6.65 <sup>a</sup> |

Means within the same roll with different superscript are significantly different (p<0.05)

Table 2: Hematology of *C. gariepinus* fed dietary mango back extract for 6 weeks

| Diets | PCV (%)              | HB (g/dl)               | RBC (Cellsx10 <sup>12</sup> ) | WBC (Cellsx10 <sup>9</sup> /L) | Neutrophil s (%)        | Lymphocy tes (%)        | MCV (FL)                | MCH (Pg)                | MCHC (g/dl)             | PL (%)                   |
|-------|----------------------|-------------------------|-------------------------------|--------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|
| C     | 44±1.00 <sup>a</sup> | 14.4±0.110 <sup>a</sup> | 6.13±0.15 <sup>a</sup>        | 15.83±0.75 <sup>a</sup>        | 42±1.00 <sup>a</sup>    | 51.33±1.53 <sup>b</sup> | 71.78±2.81 <sup>a</sup> | 23.49±0.66 <sup>a</sup> | 32.74±0.52 <sup>b</sup> | 208.33±4.93 <sup>a</sup> |
| M     | 45±1.00 <sup>a</sup> | 13.3±0.2 <sup>b</sup>   | 5.9±0.44 <sup>a</sup>         | 7.4±0.20 <sup>c</sup>          | 32.67±1.53 <sup>c</sup> | 64±5.57 <sup>a</sup>    | 74.19±1.46 <sup>a</sup> | 21.92±0.24 <sup>b</sup> | 29.56±0.57 <sup>a</sup> | 206.00±7.81 <sup>a</sup> |
| M/C   | 35±1.00 <sup>b</sup> | 12.33±0.32 <sup>c</sup> | 5.37±0.15 <sup>b</sup>        | 14.23±0.15 <sup>b</sup>        | 37.67±0.58 <sup>b</sup> | 58.67±1.53 <sup>a</sup> | 65.26±3.42 <sup>b</sup> | 22.99±0.53 <sup>a</sup> | 35.26±1.23 <sup>a</sup> | 198.00±1.00 <sup>a</sup> |

Means within the same roll with different superscript are significantly different (p<0.05)

Table 3: Hematology of fish fed various levels of mango back extract inclusion feeds after 3 weeks of *P. aeruginosa* infection

| Diets | PCV (%)                 | HB (g/dl)               | RBC (Cellsx10 <sup>12</sup> ) | WBC (Cellsx10 <sup>9</sup> /L) | Neutrohils (%)       | Lymphocy tes (%)        | MCV (FL)                | MCH (Pg)                | MCHC (g/dl)             | PL (%)                   |
|-------|-------------------------|-------------------------|-------------------------------|--------------------------------|----------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|
| C     | 41±1.00 <sup>b</sup>    | 13.37±0.89 <sup>b</sup> | 5.8±0.20 <sup>c</sup>         | 10.2±0.20 <sup>c</sup>         | 29±1.00 <sup>c</sup> | 41.33±1.53 <sup>a</sup> | 70.73±2.21 <sup>a</sup> | 23.07±0.98 <sup>a</sup> | 32.62±0.84 <sup>b</sup> | 178.00±0.00 <sup>b</sup> |
| M     | 47±1.00 <sup>a</sup>    | 15±1.00 <sup>a</sup>    | 6.73±0.15 <sup>a</sup>        | 8.2±0.10 <sup>b</sup>          | 48±1.00 <sup>a</sup> | 60.67±2.08 <sup>b</sup> | 69.82±1.76 <sup>a</sup> | 22.28±1.41 <sup>a</sup> | 31.86±1.50 <sup>b</sup> | 194.10±1.00 <sup>a</sup> |
| M/C   | 41.33±1.53 <sup>b</sup> | 14.5±0.2 <sup>a</sup>   | 6.13±0.06 <sup>b</sup>        | 9.3±0.10 <sup>b</sup>          | 31±1.00 <sup>b</sup> | 62±1.00 <sup>a</sup>    | 67.38±1.90 <sup>a</sup> | 23.64±0.40 <sup>a</sup> | 35.11±1.20 <sup>a</sup> | 204.00±0.98 <sup>a</sup> |

Means within the same roll with different superscript are significantly different (p<0.05)

Table 4: Relative survival percentage (RSP) of *C. gariepinus* fed Dietary mango back extract infected with *P. aeruginosa*

| Diets | Survival (%)            | RSP (%)                  |
|-------|-------------------------|--------------------------|
| C     | 54±5.58 <sup>b</sup>    | 0 <sup>b</sup>           |
| M     | 96±5.29 <sup>a</sup>    | 91.54±14.32 <sup>a</sup> |
| M/N   | 88.33±2.89 <sup>a</sup> | 74.77±4.07 <sup>a</sup>  |

Means within the same roll with different superscript are significantly different (p<0.05)

Table 5: Organosomatic indices of *C. gariepinus* fed dietary mango back extract before and after infection with *P. aeruginosa* infection

| DIETS | HIS                    | CSI                    | SSI                    |
|-------|------------------------|------------------------|------------------------|
| C     | 0.96±0.06 <sup>a</sup> | 0.08±0.01 <sup>a</sup> | 0.05±0.02 <sup>a</sup> |
| M     | 0.99±0.01 <sup>a</sup> | 0.08±0.01 <sup>a</sup> | 0.06±0.02 <sup>a</sup> |
| M/C   | 0.89±0.08 <sup>a</sup> | 0.07±0.01 <sup>a</sup> | 0.05±0.03 <sup>a</sup> |

Means within the same roll with different superscript are significantly different (p<0.05)

Table 6: Organosomatic indices of *C. gariepinus* fed dietary mango back extract after infection with *P. aeruginosa*

| DIETS | HIS                    | CSI                    | SSI                    |
|-------|------------------------|------------------------|------------------------|
| C     | 1.25±0.41 <sup>a</sup> | 0.08±0.01 <sup>a</sup> | 0.05±0.02 <sup>a</sup> |
| M     | 0.88±0.09 <sup>b</sup> | 0.09±0.01 <sup>a</sup> | 0.06±0.02 <sup>a</sup> |
| M/C   | 0.93±0.06 <sup>b</sup> | 0.08±0.00 <sup>a</sup> | 0.10±0.13 <sup>a</sup> |

Means within the same roll with different superscript are significantly different (p<0.05)

**KEY**

HSI - Histosomatic index

CSI - Cistomatic index

SSI - Splenosomatic index

C - Control

M - Mango back inclusion diets fed constantly

M/C - Mango back inclusion diets fed intermittently

M/C. MCH was higher in C and M/C, but lower in M, while MCHC was higher (P<0.05) and lower in M. After the three weeks infection with *P. aeruginosa* the PCV, HB,

RBC, N, L, MCHC and PL had significantly lower values in the fish fed the control diets (C) compared to the treated groups M and M/C. The WBC had high value in fish fed (C) while the MCV and MCH recorded no significant difference across the treatments.

**Relative Survival Percentage:** The result of the relative survival percentage is shown in Table 4. At the end of the infection period, the rate of survival was significantly higher in groups M and M/C (96±5.29 and 88.33±2.89 respectively) and lower (P<0.05) in group C (54±5.58). The relative survival percentage for M and M/C was 91.54±14.32 and 74.77±4.07 respectively.

**Organosomatic Indices:** The organosomatic indices before and after the infection with *P. aeruginosa* is shown in tables 5 and 6 respectively. At the end of six (6) weeks feeding trials, there was no significant difference in the tested organosomatic indices (HSI, CSI and SSI) across the various treatments. After the three weeks infection period, the HSI was significantly higher in the fish fed the control diet (C) compared to the fish in the treated groups which were significantly the same. The CSI and SSI were significantly the same across the various groups.

**DISCUSSION**

**Behavioural Observations:** The behavioral observations after infecting the fish (*C. gariepinus*) with *P. aeruginosa* were similar to the observations in Amrevuawho *et al.* [20] and Ukwe *et al.* [21] when *P. aeruginosa* was introduced to *C. gariepinus* orally and intramuscularly respectively. They observed ulceration and hemorrhage in the skin of the fish. There were no signs of hemorrhage or ulcers on

the skin of the fish in the treated groups infected with the pathogen, this could be as a result of the presence of mangiferonic acid in the mango bark extract that has been reported to be antimicrobial and wound healing [33, 34].

**Effect of Dietary Mango Bark on Hematology:** The PCV, Hb and RBC values were significantly higher in the group fed continuously with the mango bark extract diet (M), this finding is in agreement with the reports of Baba *et al.* [22] who fed common carp with dietary *Avena sativa* extract and Gabriel *et al.* [23] who reported the effect of dietary *Aloe vera* polysaccharides on *C. gariepinus* fingerlings. However, Zaid *et al.* [24] reported reductions in the PCV, Hb and RBC when *C. gariepinus* was treated with herbal mixture. The increase in these parameters in the experiment is an indication that the dietary mango bark extract is medicinal [14]. The report of the WBC in this work is in agreement with the result obtained by Gabriel *et al.* [23]. Though the WBC count was low in the treated groups, the increase in L and N in circulation is an indication of the readiness of the immune system within this period (six weeks) and it's suggestive of the immune-stimulating ability of the mango bark extract [16]. There was no significant difference in the MCV, MCH, MCHC and PL in the fish across the various treatments, this is an indication that the fish within the three weeks of feeding was not anaemic [25]. The pre and post-infection values of PCV, Hb and RBC in the fish fed the control diet (C) were lower ( $P < 0.05$ ) compared to the values in the treated diets. This result is in agreement with Sivagurunathan *et al.* [10] who assess the potency of *zingiberofficinale* and curcuma lung on haematology of *cirrhoumagrigala* exposed to *P. aeruginosa*. This reduction could be as a result of destruction of some haematopoietic organs such as the kidney, liver or spleen that may have affected their production [35], while the increase in the treated group could be as a result of immunostimulatory and prophylactic effect of the mango bark extract that may have increased the phagocytic and bactericidal activities of the fish against the *P. aeruginosa*. [27, 36].

**Effect of the Dietary Mango Bark on Relative Survival Percentage:** The disease resistance which was calculated as relative survival percentage (RSP) and percentage survival was high in the (M) and (M/C) groups fed fish compared to the control in this experiment. This result is in agreement with the reports of Verma *et al.* [27] in common carp fingerlings fed *Azadirachaindica* powdered

leaf at 0.5% (W/V) and challenged with *A. hydrophila*, Award and Austin [28] in dietary mango leaf extract fed Rainbow trout challenged with *A. hydrophila*. The high RSP in the fish fed dietary mango bark extract could be as a result of many phytochemicals including flavonoids contained in mango bark extracts that are bactericidal, or may have activated the innate defense mechanism to improve the antimicrobial activities of the fish [29, 30].

**Effect of the Dietary Mango Bark on Organosomatic Indices:** The organosomatic indices (HSI, CSI and SSI) were significantly the same across the various treatments, this findings is in agreement with Gabriel *et al.* [23] when *C. gariepinus* was treated with aqueous leaf extract of *Lepidagathisalopecuroides*, while the result of SSI is in agreement with Kareem *et al.* [31] who fed rainbow trout with dietary yarrow extract. Though [32] identified feed or supplemented diets as factors that alter the organosomatic indices of fish, the results of this experiment shows that dietary mango bark extract at the used concentration has no adverse effect on the liver, heart and spleen of the *C. gariepinus*. The presence of the pathogen led to an increase in the HSI of the *C. gariepinus* fed the control diet (C) compared to the treated diets. This results is in agreement with the result of Gupta *et al.* [37] who reported increase in HSI when *rattusrattus* was infected with *cysticercusfasciolaris*. The increase HSI could be as a result of loss of glycogea [38], which may have cause weight increase in the liver [39].

## CONCLUSION

The dietary mango bark extract has proven to be highly medicinal for fish since it was able to improve the hematological parameters in *C. gariepinus* even with the infected *P. aeruginosa* and noticeably improve the survival and diseases resistance after the period of infection. The mango bark aqueous extract is a good liver anti-inflammatory and prophylactic agent which can be used in fish farming against pathogenic bacteria such as *P. aeruginosa*.

**Recommendation:** We recommend that mango bark extracts be used at varying concentrations to assess its potentials in other aspects of fish farming such as growth, survival, improve reproduction, flesh quality among others. Other means of application of the mango bark should be tried.

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