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Phytochemical constituents and anti-microbial effects of *Moringa oleifera* leaf extract on eggs and hatchability in *Clarias gariepinus*

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ABSTRACT

The efficacy of *Moringa oleifera* leaf extract as fish egg surface disinfectant and for improved hatchability and larval survival was evaluated in the *clariid* catfish, *Clarias gariepinus*. The study was conducted at University of Port Harcourt Demonstration Farm for two weeks. The aqueous extract of *M. oleifera* leaves powder was screened for the presence or absence of secondary plant metabolites using standard methods. The result of phytochemical screening showed the presence of flavonoid, tannins, steroid, alkaloid, cardinolide, and carbohydrates in the leaf extract. Fertilized eggs were washed for 5 minutes with three concentrations in three replicates of 1g/L, 2g/L, and 3g/L of aqueous extract of *M. oleifera* leaf. While the total heterotrophic bacteria (THB) and total fungi (TF) of the control were 3.0x106CFU g-1 and 3.0x103CFU g-1, respectively, the TF was significantly reduced ($P<0.05$) to 1.0 x 10^3 CFU g^{-1} in 2g/L and 3g/L concentrations and the THB was also significantly reduced ($P<0.05$) to 1.5 x 10^3 g^{-1} and 1.8 x 10^3 g^{-1}, respectively. The highest mean percentage hatchability (84.84%) was detected at concentration of 2g/l, but the differences between treatments means were not significant ($P>0.05$). The highest mean percentage survival was 83.95% at concentration of 3g/l. *M. oleifera* extract showed inhibitory activity against catfish eggs surface micobes and potential for improving aquaculture production of catfish. In conclusion, *M. oleifera* leaf extract is suitable for fish eggs disinfection; however, further investigations are needed.

Keywords: *Moringa oleifera*, hatchability, *Clarias gariepinus*, anti-microbial, disinfection

INTRODUCTION

Nigeria is currently the seventh largest country in the world and she is projected to be the world's third most populous country by the year 2050 (UN DESA, 2017) by adding no fewer than 189 million people to her current population between 2018 and 2050 (UN DESA, 2018). This projected increase will result in a number of challenges around sustainability, including the need to produce more food. Using 2014 base figures, for example, the total fish demand for Nigeria based on the 2014 population estimate of 180 million is 3.32 million metric tons. By implication, about 6.80 million metric tons of fish will be needed to meet consumption needs in 30 years' time. The supply of locally produced fish is insufficient. Intensive fish farming is expanding in Nigeria. In Nigeria small and large scale intensive commercial farms are developing to meet increasing local demand. As the Nigeria's population increases, so does the demand for protein, including seafood. It is unlikely that wild-caught seafood will be able to meet that demand sustainably, and aquaculture production must therefore increase. The aquaculture industry is becoming highly active in Nigeria, and would play an important role in the production of safe food

and economic growth. However, producing large quantity and high-grade fish with strong nutritional content is a challenge for many farmers. Availability of quality and affordable fish fingerlings is one of the major challenges faced by the sector. To meet the demand of increasing seafood through aquaculture, production of fish seeds of the right quantities and qualities, which represent one of the most critical resources for sustainable fish farming practice, must be stepped up. The increasing rate at which fish fingerlings has been demanded for both commercial and subsistence aquaculture has brought much interest in hatchery propagation of cultivable fish species in Nigeria. One of the most important aspects that must be advanced for sustainable growth of the industry is the management of fish eggs in hatchery.

The quality of eggs and larvae produced in hatcheries is considered an important limiting factor in the larvae production and, consequently, in the development of the aquaculture industry (Kjørsvik *et al.*, 1990; Peck *et al.*, 2004). The surface of fish eggs provides excellent medium for the growth of microbial organisms (Grotmol and Totland, 2000; Miguez and Combarro, 2003). Fish eggs can be subjected to contamination by bacteria and fungi through several ways including contact with bacteria and fungi in the hatchery environment, transmission from broodstock and during the process leading to fertilization. The quality of water determines to a great extent how these organisms will build-up. Water of poor quality promotes microbial contamination of eggs incubated in it (Trust, 1972; Cahill, 1990; Okpokwasili and Ogbulie, 1998; Kubilay *et al.*, 2009). The presence of these organisms affects fertilization, subsequent development of eggs, hatchability and larval viability (Jung, 2004). The damage by fungi alone on fish eggs is estimated to cause an annual loss in production of about 20 %, which may rise to above 40 % (Forneris *et al.*, 2003). The elimination of loss of eggs and mortality at the larval stage caused by bacterial and fungal contamination in the hatchery will ensure dependable and predictable supply of quality fry. In this regards, disinfection of eggs has been widely used to reduce egg mortality and to improve rearing success during the yolk sac and first feeding stages (Morehead and Hart, 2003; Madsen *et al.*, 2005; Stuart *et al.*, 2010). The prevalence of these pathogens in hatcheries makes fish egg disinfection one of the essential practices among hatchery operators. The practice helps to avert the danger of transmission of external pathogen from brood stock to larvae (Stuart *et al*., 2010) which will help in the reduction of mortality rate of larvae.

Several researches have been conducted to improve the hatchability and survival of fish larvae under culture condition by treating eggs with chemicals or exposing them to Ultraviolet radiation. Chemicals such as formalin, malachite green, methylene blue and salt have been applied by many researchers (Rahman *et al.*, 2017; Akpoilih and Adebayo, 2010). Malachite green and formalin are the most widely disinfectants for fish eggs. However, because of the awareness of the hazards to fish eggs as well as potential carcinogenic and mutagenic properties of these methods, their applications have been banned in many countries. This has informed the continuous search of organic alternatives to chemicals substances.

Plants, having antimicrobial properties including *Moringa oleifera*, are suggested as alternatives. Therefore, in order to attain more economical, sustainable, environmental-friendly and viable production, research interest has been directed towards the evaluation and use of nonconventional sources of antimicrobial

agents from plants. Recently, researchers have increasingly been paying attention to *M. oleifera* (Lam.), which is a widespread drought tolerant tree, as antimicrobial agent. Its effectiveness has not been tested on fish eggs. Research on fish disease, particularly those affecting fish eggs and preventive measures, are scarce in Nigeria. . The goal of this study was to evaluate the efficacy of *M. oleifera* leaf extracts as anti-microbial agent in the treatment of *C. gariepinus* eggs to improve hatchability and larval survival.

MATERIALS AND METHODS

Collection of plant material

Leaves of *M. oleifera* plant were collected from a garden in Asaba, Delta state. The leaves were washed with water to eliminate dust and other foreign particles. The fresh leaves of *M. oleifera* were then shade-dried at room temperature (32-35°) to a constant weight over a period of four days. The dried leaves were ground into powder with blender and stored in polythene bags for later use.

Phytochemical Screening of extract

Sample of powdered *M. oleifera* leaves were taken to the Department of Phamacognosy Laboratory, University of Port Harcourt to screen for phytochemical or bioactive components. The obtained extract was screened for the presence or absence of secondary plant metabolites such as saponins, alkaloids, steroids, phenolics compounds and tannins using standard methods as described by Trease and Evans (1989), Harborne (1998) and Sofowora (1983).

(I)Tannins

$FeCl_3$ test: 0.5g of sample was weighed into a test tube and 5ml of distilled water was added to the test tube, shaken vigorously and filtered. To the filtrate, 5% ferric chloride was added. A blue-black colouration indicates the presence of tannin in the extract.
Test for phlobatannins: (HCl test). 2 ml of the aqueous solution of the extract were added into dilute HCl and observed for red precipitate that was indicative the presence of phlobatannins.

(II) Alkaloids

About 0.5g of dried sample was extracted with 10ml of 10% hydrochloric acid. After warming for about 5minutes on a water bath and filtering, the filtrate was allowed to cool and was divided into two equal portions. The presence of alkaloid was detected using the following tests:
Mayer's test
To the portion was added two drops of Mayer's reagent. A cream coloured precipitate is a positive test for alkaloids.
Dragendorff's test
To the second portion was added two drops of Dragendorff's reagent. A reddish orange precipitate shows a positive test for alkaloids.
Hager's test
To the third portion was added two drops of Hagers reagent. Ayellow coloured precipitate is a positive test for alkaloids.

(III) Saponins

Frothing test: 3ml of each extract and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously and allowed to stand for 30 minutes. A persistent honey comb froth indicates the presence of saponins.
Emulsion Test Procedure: 3mls of the extract was pipette out into a test tube and 5 drops of olive oil was also added and it was shook vigorously. Emulsification was observed

(IV) Steroids

Liebermann-Burchard's test: A 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled well in ice; followed by addition of 1ml of concentrated sulphuric

acid down the wall of the test tube to form a layer. A pink-red colour indicates triterpenoid nucleus.
Salkowski's Test: A 2ml of concentrated sulphuric acid was carefully added down the wall of the test tube containing the chloroform filtrate, after which concentrated sulphuric acid was added to form a lower layer. A reddish brown colour at the interface between the two liquids indicates the presence of steroidal nucleus.

(V) Cardinolide
Kedde test for lactone ring: 1ml of an 8% solution of the extract in methanol was mixed with 1ml of kedde's reagent. An immediate violet to reddish brown colour with the precipitate of a whitish crystalline solid indicates the presence of cardenolides.
Keller-killani test for deoxy sugars: a 0.5g of the extract was dissolved in 2ml glacial acetic acid containing one drop of ferric chloride solution 1ml of concentrated sulphuric acid was poured down the side of the test tube. A brown ring obtained at the interphase indicates the presence of de-oxy sugar characteristics of cardenolides

(VI) Carbohydrates
A 0.5g of sample was boiled in 5ml distilled water using a water bath for 3minutes. The mixture was filtered while hot and the resulting filtrate cooled and used for the test described below:

Fehling test for reducing sugars
To 2ml volume of the filtrate was added a mixture containing equal volume of Fehling solutions A and B; and boiled in water bath for 5minutes. A brick red coloured precipitate indicates the presence of reducing sugars

Molisch's test
A few drops of molisch's reagent was added to 2ml of each of the aqueous filtrate obtained in section 2.5.5 above, followed by a small quantity of concentrated sulphuric acid and allowed to form a lower layer. A purple ring at the interface of the liquids indicates the presence of carbohydrates.

(VII) Flavonoids
The following test were carried to detect the presence of flavonoids according to Trease and Evans, (1989):

Shinoda's test for flavonoids: About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids (Trease and Evans, 1989). Lead ethanoate test for flavonoids: Few quantity of the each portion was dissolved in water and filtered. To 5 ml of each of the filtrate, 3 ml of lead ethanoate solution was then added. Appearance of a buff-coloured precipitate indicates the presence of flavonoids (Trease and Evans, 1989).

Preparation of *Moringa oleifera* leaf extracts
1 g, 2 g and 3 g, respectively of finely ground *M. oleifera* were dissolved in 1 litre of water heated to 100^0 C and allowed to stand for 24 hours before filtering and use.

Egg collection and treatment
Broodstock of male and female *Clarias gariepinus* were obtained from a commercial fish farm at Rukpokwu in Obio-Akpo LGA of Rivers State. Each fish weighed above 2kg. The brood fish were screened for external wounds or injury and presence of parasites. They were acclimatised for four days in holding indoor concrete tanks. They were maintained under prevailing temperature and fed with Coppens. The brood fish were examined for gonaal

development according to the method of Blythe *et al*., (1994) and reported by Yisa *et al*., (2010). Males were examined for rigid and reddish infusion of genital orifices and the females for reddish infusion of genital orifices, distension of the belly and release of egg when gentle pressure was applied on the abdomen. The selected samples were properly maintained separately before being used for breeding.

Spawning and fertilization

The female brood fish was injected with the hormone Ovaprim according to the method of Goudie *et al.*, (1992) and hand-stripped for eggs into dry clean container after a latency period of about eleven hours at a temperature of between 25-29 ^{0}C. The milt was obtained by killing the male and the testis were removed, cleaned with cotton wool to remove the blood stains and kept in a clean bowl. The testis was dissected to squeeze out the milt. The stripped eggs were fertilised with milt by mixing them together gently with plastic spoon for about 2-3 minutes.

Exposure to *M. oleifera* extract/ Microbial load analysis

Samples of normal unfertilised eggs, fertilised eggs and hatchery water samples were collected using aseptic techniques and kept in sterile bottles, which were taken to Microbiology laboratory of the University of Port Harcourt within 30 minutes of collection and fertilisation. For each of the leaf extracts comprising 1g/l, 2g/l and 3g/l, 550 eggs weighing 1g of fertilized egg was washed by immersion for 5 minutes only. The control (0g/l) had no extract of the leaf. Each treatment was replicated three times. Then the fertilised eggs were taken to the incubator for incubation. Aeration was maintained by flow-through system. The hatchlings for each treatment were raised for 2 weeks. After absorption of yolk sac, the hatchlings were fed with artemia. Water quality parameters including temperature, pH, and Dissolve oxygen were monitored during the period of study. Microbial analysis of the fertilized eggs and hatching medium (water) in the incubating tanks were carried out immediately at the Microbiology Laboratory at the University of Port Harcourt to determine total heterotropic bacteria (THB) and total fungi (TF).

Untreated eggs and disinfected eggs and hatchery water sample for microbial analyses were sampled soon after stripping and after fertilization and immersion, respectively. Eggs were disinfected externally by immersion for 5 minutes in aqueous extract of *M. oleifera* leaf.

Determination of Hatchability

The following indices were determined:

$$\text{Percentage Hatchability} = \frac{\text{No of hatched egg}}{\text{No of egg incubated}} \text{ x } 100$$

Determination of survival

$$\text{Percentage Survival} = \frac{\text{Total no of larvae} - \text{No of dead larvae}}{\text{Total no of larvae}} \text{x } 100$$

Data analysis

One way Analysis of variance (ANOVA) was used as a statistical tool for analysis. Data obtained for each treatment was the mean value of the replicates. The results are expressed as total colony number or the number of colony forming units (CFU) per gram of sample. All differences in mean values were determined at P=0.05 level of significance

RESULTS

Phytochemical Constituents of Aqueous Leaf Extract of *M. oleifera*

The result of phytochemical test (Table 1) showed the presence of the flavonoid, tannins and steroids in the extract. Cardinolide, alkaloids and carbohydrates were also found in the leaf extract of the plant. However,

Cyanogenic glycosides and saponins were absent from the leaf extracts.

Table1: Phytochemical components of aqueous extract of *M. oleifera* leaf

Phytochemical components	Methods/Test	Result
Alkaloid	Drandedorff	Present
	Mayer	Absent
	Hager	Absent
Flavonoid	Shinoda	Present
	Lead acetate	Present
Tannins	$FeCL_3$	Present
	Phlobatannis	Present
Steroid	Leibemann-buchard	Present
	Salwoski	Present
Carbohydrates	Molisch	Present
	Fehlings	Present
Cardinolide	Keller killani	Present
	Kedde	Present
Cyanogenic glycosides		Absent
Saponin	Frothing	Absent
	Emulsion	Absent

Preliminary observations

There was an unanticipated reaction resulting in colour change of the fertilized eggs from somewhat greenish yellow to golden colour. Secondly, the eggs were aggregated together, sticking to the bottom of the plastic container, leaving a clear liquid. However, the eggs were freed from each after adding saline water.

Temperature, pH and Dissolved oxygen were measured during the period. The pH ranged from 7.2 to 8.1 while the temperature was within the range of 22 ^{0}C to 26 ^{0}C. There was sharp drop in temperature on the 12th day to 22°C. The mortality of larvae was highest on the 12th day, representing 9, 37, 14 and 20 individuals in T-I, T-II, T-III and T-O, respectively. The dissolved oxygen varied between 5.0 and 7.5mgL^{-1}.

Effect of *Moringa oleifera* leaf extract on microbial load of catfish eggs

The effects of aqueous *M. oleifera* leaf extract on total heterotrophic bacteria (THB) and total fungal (TF) load in water, unfertilised eggs and fertilised eggs exposed to different concentrations of the extract are presented in Table 2.0. THB and TF of untreated fertilised eggs (0g/L) were 3.0x10^{6}CFU g-1 and 3.0x10^{3}CFU g-1, respectively; lower values of THB and TF were, however, observed in the incubation water, T-H20. TF was not found in T-I extract but considerably reduced (1.0 x 10^3 CFU g^{-1}) in T-II and T-III. The leaf extracts reduced significantly the activities of THB in 2g/L and 3g/L.

Table 2: Effect of different concentrations of aqueous extract of *Moringa oleifera leaf* on microbial load on catfish eggs

SAMPLE	THB (CFU g^{-1})	TF (CFU g^{-1})
0mg/l	$3.0x10^6$	$3.0 x10^3$
1mg/L	$2.8x10^3$	Nil
2mg/L	$1.5x10^3$	$1.0 x10^3$
3mg/L	$1.8x10^3$	$1.0 x10^3$
T-H20	$1.8x10^6$ CFU gm^{-1}	$2.0 x10^3$CFU gm^{-1}

CFU = Colony forming unit

Hatchability and larval survival of *C. gariepinus* exposed to leaf extracts of *M. oleifera*.

The result of the effect of leaf extract of *M. oleifera* on hatchability of *C. gariepinus* eggs and survivability of larvae are presented in Table 3.0. It showed that differences in hatchability or hatching rate which were not significant among the concentrations including the control (P>0.05). However, the highest hatching rate was obtained at 2g/l. The results further showed that *M. oleifera* leaf extract significantly enhanced larval survival. The mean percent survival was between the range of 72.5±9.39 for 0g/L and 83.95±11.61 for 3g/L. The mean percent survival for 1g/L and 2g/L concentrations were 75.37±12.14 and 78.91±10.0, respectively. The differences in survival rate between concentrations were significant (P<0.05). Eggs treated with 3g/l had significantly highest (P<0.05) mean survival (83.95%) compared to control (72.5%), 1g/L (75.37% and 2g/L (78.91%).

Table 3: Mean hatching rate and survival of *Clarias gariepinus* exposed to aqueous leaf extract *of M. oleifera*

Concentration of leaf extract	0gm/l	1gm/l	2gm/l	3gm/l
% Hatchability	84.75^a	81.81^a	84.84^a	83.33^a
% Survival	$72.5±9.39^a$	$75.37±12.14^b$	$78.91±10.0^c$	$83.95±11.61^d$

*Letters with the same superscript in the same row are not significant (p > 0.05).

The highest percentage survival was recorded at temperature of 26°C and the lowest at 22°C.The temperature was between 25 and 26°C from the first day up to the 11th day when it dropped to 22oC on the 12th day, coinciding with high mortality of the larval fish.

DISCUSSION

Phytochemical constituents are important factors that will determine the antimicrobial properties of the moringa leaf extract. The phytochemical screening indicated presence of secondary metabolites such as steroids, alkaloid, flavonoids and tannins in the leaf extract of *M. oleifera*, which confers on it the antimicrobial potentials (Sofowora, 1983; Abdallah, 2011; Nepolean *et al.*, 2009; Abubakar and Usman, 2016). Abdulkadir *et al* (2015) found similar bioactive components except saponin in *M. oleifera* leaf extracts. However, Okah and Walter (2019) and Effiong *et al.* (2016) detected the saponins in the leaves of *M. oleifera* in contrast to this study. Bukar *et al*. (2010) work showed lack of alkaloid and tannin in the leaf extract of the same plant. The method of extraction of bioactive ingredient and genetic differences among cultivars may be responsible for the differences. Ndhlala *et al*. (2014) reported

differences in bioactive components among cultivars of the plant. Therefore, cultivars in Nigeria need to be characterized for their optimum utilization. In general, the bioactive constituents in plants can enhance the innate immune system, possess antimicrobial and antioxidant characteristics that may help to improve the general physiological condition of fish (Chakraborty et al., 2014).

From preliminary observations, the *C. gariepinus* eggs' aggregation and sticking to the bottom of the fertilising container could have affected hatchability. Late addition of saline water helped to free the aggregated egg mass. Yisa *et al.,* (2014) used saline solution to prevent the eggs of the catfish, *Clarias gariepinus* from sticking together. The aggregation of the egg mass could have some undesirable effects on hatchability. It is important to observe what happens to development during the aggregation and gumming of eggs. El-Gamal and El-Greisy (2008) used 4 gm urea in addition to 4 gm NaCl/L of water to free aggregated eggs of the common Carp. The utilisation of *M. oleifera* extract as a natural adsorbent for removal of pollutant has also been documented (Kumari *et al*., 2006 and Araújo *et al.,* 2010). The seeds and leaves of Moringa are proteins rich that act like cationic polyelectrolytes once they are added to water (Ghebremichael *et al*., 2005; Zayed, 2012; Mune Mune *et al.,* 2016). Extracts from different parts of the plant have been used in water purification. Ali *et al.* (2009) and Vieira *et al*. (2010) suggested their use in removing turbidity and providing safe drinking water devoid of microbes. In view of our observations, it appears that this plant exhibit properties similar to those of alum and could be useful in solving turbidity problems associated with aquaculture pond water. It can also be used in small-holder fish ponds to treat waste water for reuse especially in areas where water for aquaculture is scarce. We hereby suggest that saline water should be added to the extract in a defined proportion in future experiments to determine optimum combination.

Abdallah (2016) found *M. oleifera* leaf to be a good candidate in the search for new antibacterial agents from natural products against different pathogens. According to Pietta (2000) and Patel *et al*. (2014) alkaloids and tannins as constituents of *M. oleifera* leaf extract have antibacterial and antioxidant properties. This view is supported by the current study, which revealed significant antimicrobial activities from *M. oleifera* leaf extract. All the concentrations of the leaf extract showed some degrees of antibacterial activity against THB and TF. Accordingly, Zaffer *et al*. (2014) found that bark extracts from Moringa under different concentrations inhibited growth of bacterial pathogens in pure culture tests to varying degrees, with activity decreasing with decreasing concentration of the extract. From our result, it can be deduced that aqueous extract of *M. oleifera* leaf reduced the microbial activity but no trend could be discerned. This calls for further research exploring more concentrations using different parts of the plants. Looking at these results it may be concluded that *M. oleifera* may be a potential source for the disinfection of eggs of different species against different kinds of microbes. Patel *et al.* (2014) demonstrated high antifungal activity of ethanolic and aqueous extract of *M. oleifera* against *Saccharomyces cerevisiae*, *Candida albicans* and *Candida tropicalis*. The present study on the antifungal activity of *M. oleifera* leaf aqueous extract show that fungi activity was completely eliminated at the lowest concentration but significantly reduced at higher concentration. Antifungal activity of *M. oleifera* was studied against several concentrations of *M. oleifera* extract and aqueous leaf extract showed maximum

activity on fungi. This result is similar to the work of Patel *et al.* (2014).

One of the most important factors affecting early development of all poikilotherms is temperature. It has been demonstrated to affect almost every aspect of fish early development: hatching, emergence and initial feeding times. The highest mortality recorded during this experiment was on the 12th day when temperature dropped from 26 to 22^{o}C. In terms of survival, the zone of thermal tolerance for early life history of *C. gariepinus* ranged from 18.9 to 33.2°C, with the thermal optimum from 22.9 to 30.3°C. Though, within the suitable range for larval rearing, the sudden change or rapid decrease in temperature was or could be responsible for high mortalities of eggs observed during the experiment. It is important that temperature be maintained at a fairly stable level during larval rearing to minimize early deaths. This is in agreement with Blaxter (1992) and Brett (1979) who observed that temperature directly affects metabolism and can influence growth, survival, and development of larval fishes.

The aqueous extract from the leaves of *M. oleifera* contains secondary metabolites with antimicrobial properties. The aqueous extract is a promising disinfectant to improve the hatching of *C. gariepinus* egg and larval survival. The procedure can be applied by local catfish farmers without any technical training or skills. Besides, its flocculating action could be technically applied in the control of undesirable physicochemical properties of water in aquaculture facilities including removal of turbidity and management of waste.

In conclusion, this research strongly suggests that *M. oleifera* is a valuable plant that could be used for development of sustainable aquaculture in Nigeria. The discovery of safe and economical antimicrobial substances for catfish farmers is essential for the continued success of the aquaculture industry in Nigeria.

Since the procedure does not require any special equipment or training, it can be easily adopted by local farmers to improve productivity. However, there is need for further studies to determine the optimum levels for applications. Future studies should focus on undertaking a toxicity test with more levels of the phytochemical disinfectants to identify optimum doses. This should be coupled with improved microbiological assays to validate the apparent effectiveness found in this study and identify the key microbial species that are responsible for mortalities during incubation eggs of *C. gariepinus*. The plant promises as a future low cost disinfectant for routine use to deal with microbial infestation of eggs in hatcheries.

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