Research Article

Control of *Ichthyophthirius multifiliis* Infecting African Sharptooth Catfish *Clarias gariepinus* Using *Ocimum gratissimum*

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Abstract | Ichthyophthiriasis, caused by ciliate protozoa, *Ichthyophthirius multifiliis*, persists in freshwater fish and affects aquaculture production globally. An experiential study on *Clarias gariepinus* to investigate the potential of graded concentrations of ethanol leaf extract of *O. gratissimum* (ELEOG) for control of *I. multifiliis* (Ich) in a 96 h short bath treatment. A total of 120 ich-infected catfish, *Clarias gariepinus* were infected with 10,875 theronts and were randomly distributed in triplicates into five groups (A-E). Groups A, B and C (500mg/L chloramphenicol) served as the normal, negative control, and positive control, while groups D and E were treated with 500mg/L and 1,500mg/L ELEOG, respectively. ELEOG controlled the parasitaemia after the seventh-day post-inoculation but persisted after treatment relative to the standard drug, chloramphenicol's significant (P=0.05) performance. However, between 72 h and 96 h post-treatment, both the intensity of theronts and trophonts declined to a non-detectable level at a concentration of 500mg/L ELEOG with linear similarity with the standard drug. A positive and strong relationship was observed between pH and Ich intensity, although no Ich was detected at 5.5. There was no relationship between the temperature (25° C and 29° C) and Ich intensity. *O. gratissimum* is a good anti-ich plant material favouring 500mg/l and 1500mg/L ELEOG in bath treatment against *I. multifilis*.

Keywords | *Ichthyophthirius multifilis*, *Ocimum gratissimum*, *Clarias gariepinus*, Aquaculture

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INTRODUCTION

Overall, 47% of global fish production is in aquaculture (FAO, 2018). Nigeria is the largest aquaculture producer in sub-Saharan Africa, from 21700 tonnes in 1999 to 316700 tonnes in 2015 (FAO, 2017). It has created over 13,627 (2% women) employment in Nigeria. Small-scale fishers generate over 80% of Nigeria's total domestic production. The culture of clariids species in Nigeria has attracted the interest of private sectors to support the propagation of the most commonly farmed catfishes such as *Clarias gariepinus*, heteroclarias (*Heterobranchus bidorsalis*), and *Clarias nigrodigitatus* (Adewumi and Olaleye, 2011). The expansion of freshwater aquaculture worldwide and the intensification of the production system have facilitated the propagation of pathogens, of which *Ichthyophthirius multifillis* infection under favourable water temperature conditions (particularly, the trophont stage development between 20 days at 7°C to five to seven days between 20°C-24°C) multiplies speedily and mostly generate significant economic losses every year. One of the major parasitic infections affecting fish both in the ponds and wild is the *Ichthyophthirius multifilis* which causes ichthyophthiriasis. It is characterized mainly by the presence of white spots

observed on both the skin and fins of the infected fish. The disease is highly epizootic without the need for an additional host. Periodically, it causes respiratory distress, ulcers, bacterial infection due to ulcers and 100% mortality if it spreads in an enclosed pond (Stoskopf, 2015). Ulcers and lesions observed on the infected fish's body impede the fish's ability to control water movement relative to its body and intolerance to low dissolved oxygen levels. The life cycle of *I. multifiliis* involves 4 distinct forms of the parasite (mature trophont, encysted, rapidly dividing tomites and ciliated infective theronts). Mathias (2014) reported the outbreaks of ichthyophthiriasis on *Clarias gariepinus* reared in the Tella area of River Taraba, Taraba State, Nigeria. Omeji *et al*. 2011 reported 37.08% *of I. multifiliis* infections in *Clarias gariepinus* reared in homestead ponds against 42.51% of fishes in the wild. While it is true that *I. multifilis* is unlikely to be transmitted to man, It is, however, a threat to our food source, leading to a whopping loss of fish yearly, rendering them unmarketable due to the skin lesion, and drains resources invested in treatment and eradication (Omeji *et al*., 2011). Most chemotherapeutics such as chlorine T (Rintamaki-kinnunen *et al*., 2005; Balta *et al*., 2008), metronidazole (Noga, 2010), potassium permanganate (Straus and Griffin, 2002; Noga, 2010; Tieman and Godwin, 2001; Balta *et al*., 2008), citrocide (Shin *et al*., 2005), hydrogen peroxide (Landstainer and Weismann, 2007), copper sulphate (Straus *et al*., 2009), ornidazole (Toksen and Nemli, 2010), acetic acid (Balta *et al*., 2008), sodium chloride (Shin *et al*., 2005; Lahnstainer and Weismann, 2007), formaldehyde (Shinn *et al*., 2005), tannic acid (Alavinia *et al*., 2018) have been adopted in controlling the Ich parasite. Notwithstanding their efficacy in controlling the parasites, most of the chemicals are harmful to both the fish and man following bioaccumulation, off-flavour to the fish reducing consumers' preference and an unsustainable practice in aquaculture, which has created serious public concern (Lusiastuti *et al*., 2017). Chemicals, especially malachite green, formaldehyde are also one of the banned chemicals due to their toxic impact on humans. Most chemicals can deplete dissolved oxygen levels in water and its toxicity to humans. However, considering the commercial fish production setting that houses fingerlings and adult fish, how ideal it would be to move these fish and house them appropriately in the interim pending theront clearing, is up for debate. Positive alternative measures have been put in place to explore natural herbs and environmentally friendly alternatives to develop an effective and efficient alternative treatment towards enhancement of growth performance, health, and immune system. Some previously used medicinal plants which are bio-degradable and cost-effective utilized in eradicating *I. multifillis* adult parasites include *Cynachum paniculatum* (Ji-Hong *et al*., 2017), *Polygonum cuspidatum* (Zhou *et al*., 2018), *Allium sativum* (Garlic) (Nya and Austin, 2009; Gholipour-kenani *et al*., 2012; Williams *et al*., 2016), *Carica papaya* (Ekanem *et al*., 2004), *Matricaria chamomilla* (Ghalipour-kenani *et al*., 2012), *Macleaya microcarpa* (Yao *et al*., 2011), *Sophora alopecuroidis* (Yi *et al*., 2012), *Toddalia asiatica* (Xiao-Feng *et al*., 2014), *Galla chinensis* (Zhang *et al*., 2013), and also encouraged organic production adopted in food fish farms. The herbaceous plant, *Ocimum gratissimum*, which belongs to the Labiatae family, is popularly used in most countries, especially in Nigeria, to treat various ailments such as diarrhoea and skin diseases, conjunctivitis, and pneumonia (Prabhu *et al*., 2009). Less information exists on its efficacy in controlling *I. multifillis* parasite infection in freshwater fish. The study aims to ascertain the potential of ethanol extract of *Ocimum gratissimum* against *I. multifillis*.

MATERIALS AND METHODS

Plant materials

Five kilograms of the fresh leaf of *Ocimum gratissimum* were collected from a nearby farm at the University of Nigeria, Nsukka and were identified in the Department of Plant Science and Biotechnology Laboratory University of Nigeria, Nsukka. The healthy, fresh leaf was removed from the stalk and washed to remove debris and dust particles without squeezing. The leaf was dried under room temperature for two weeks, ground into a fine powder using a commercial blender, and weighed. A weight of 1342g of the powder was concentrated in 1000ml of ethanol, shaken vigorously and left for 24 hours. The mixture was filtered using a muslin cloth and evaporated to dryness. The percentage yield was calculated by dividing the weight of concentrated extract by the weight of dried-ground leaf, multiplied by 100, and a 23.5% yield was obtained. The extract was stored in an airtight container until ready for use.

Phytochemicals

Alkaloids, flavonoids, saponins, tannins, terpenoids, and phenol were done using Dahiru *et al*. (2006) (Table 1).

Table 1: Phytochemical constituents of ethanol leave extract of *O. gratissimum*.

Parameters	Bioavailability	Amount (%)
Terpenoid	+	0.187±0.03
Phenol	+	0.252±0.02
Saponin	++	1.095±0.00
Flavonoid	+	0.265±0.00
Tannins	+	0.214±0.01
Alkaloid	+	0.425±0.01

+: Present; ++: moderately present.

Experimental fish

One hundred and twenty (120) juvenile *Clarias gariepinus*

with an average weight of 97.24±13.26g and standard length of 13.2±0.87 cm were obtained from Freedom fisheries, Nsukka, Enugu State, Nigeria. They were disinfected using 1% potassium permanganate, certified free from ectoparasitic infestations, acclimatized for two weeks, and fed twice daily at 5% body weight with commercial fish pellet feed; faecal matters were daily removed. The water temperature was 23.3–24.4°C, pH 6.8–7.3 and dissolved oxygen 5.8–6.7mg/L, and aeration was optimum. The fish were fed 5% body weight per day with commercial feed (Coppens, Coppens International, Netherland) containing 42 % crude protein, 19.8 MJ/kg gross energy and 17.3MJ/kg digestible energy. Faecal matters were siphoned off daily.

The fish was handled following the approved regulatory guideline of the University of Nigeria, Nsukka committee on animal and research ethics (UNN-ACC, PROTOCOL/NO 0709/2018). No discomfort was observed in the experimental fish. A complete randomized design was adopted such that each of the experiments was in triplicates. The experiment consisted of 5 groups (A-E); where A-C were the normal, negative and positive control (500mg/L chloramphenicol), respectively and groups D and E were exposed to 500mg/L and 1,500mg/L ELEOG, respectively, were maintained daily via water exchange. Meanwhile, water pH, temperature (°C) and dissolved oxygen (mg/L) were monitored throughout the experiment. The concentrations selected were obtained from the probit of acute toxicity tests of ELEOG.

Parasite

The native strain parasite source (infected *Clarias gariepinus*) was identified by visible white spots, and mature trophonts (Figure 14) were procured from Anambra River, Nigeria. The infected fish was anaesthetized with 150 mg/l tricaine methanesulfonate (MS-222, Sigma), and the skins were gently scraped to dislodge the trophonts. Isolated parasites were transferred into beakers containing 500ml of dechlorinated tap water to remove organic matter for four hours and were incubated in the dark chamber (slight modification) to allow mitotic division, which finally produced free-swimming theronts within 48 h. Furthermore, groups C-E excluding the control (A, normal control and B, negative control), were challenged with approximately 10,875 isolated infective theronts and quantified under a binocular microscope (Olympus-CH, Japan) using a Sedgewick-Rafter cell to detect the number of viable theronts produced. As a modification, the fish were kept in darkness for 5-10 days at 24°C. Active white spots observed on the fish's body were an indication of Ich infection. Before parasite examination, the fish (6 fish per group) were immobilized using clove oil, the gill was excised and cut through the operculum and the gill arch to obtain a three-gill arch containing the filaments of the lamellae. The excised gill and skin smears were placed on a clean glass slide; three drops of water were added and covered with a coverslip to check for the presence of parasites under 40X magnification. Parasites were further stained with Lugol iodine for a clear morphological view.

Data analysis

Data were analyzed using R package version 3.6.1 (R core team, 2019) and statistical package for service solution version 23.0 (IBM cooperation, Armonk, USA). Generalized linear model univariate analysis of variance was estimated between and within treatment effects of the treatment and duration of exposure. Parasite intensity was not normally distributed and was therefore compared between groups of interests using the Kruskal wallis H-test, the ggpubr package (Kassambara, 2019), coupled to ggplot2 (Wickham, 2016), used to for this purpose. The level of significance was set at P= 0.05.

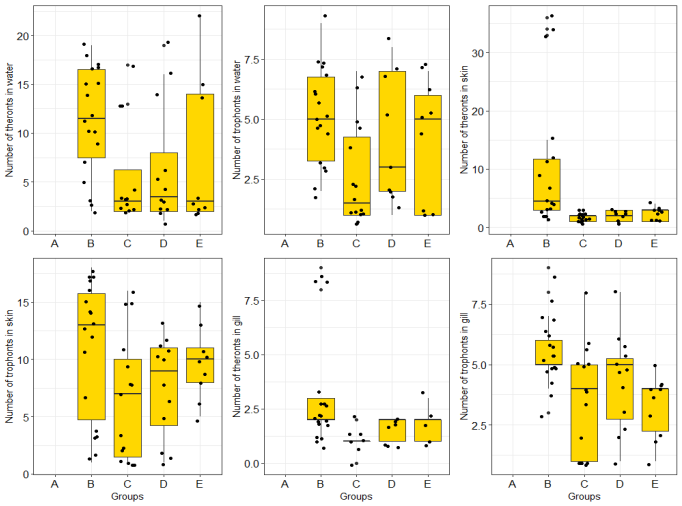

Figure 1: Intensity of *Ichthyopthirius multifilis* infection on *Clarias gariepinus* juvenile 48h pre-treatment with *Ocimum gratissimum*. Key: A: Normal control (not infected and not treated); B: Negative control (Infected, not treated); C: Standard control (infected and treated with 150mg/L chlomphenicol); D: 500mg/L Ethanolic leaves extract of *O. gratissimum* (ELEOG); E: 1500mg/L ELEOG.

RESULTS AND DISCUSSION

In the present study, *O. gratissimum* controlled *I. multifilis* infection, irrespective of its asynchronous life cycle and the continuous release into the water column of different stages, considering the water temperature and applications of the environmentally friendly plant. Infective theront inoculation into the experimental media B, C, D and E resulted in a parasitic infection in all the groups, which became apparent from the seventh-day post-inoculation (Figure 1). The application of low and high concentrations of ethanol extract of *O. gratissimum* leaf eliminated *I. multifiliis* infection within 96 h. More efforts have caused positive alternative, sustainable strategies for the eradication

(Yao *et al*., 2010; Gholipour *et al*., 2012) and management of *I. multifiliis* infection, as reported by Zhang *et al*. (2013).

Clarias gariepinus, a mostly consumed freshwater fish, is generally considered one of the most susceptible and vulnerable hosts to *I. multifilis* infections. The *I. multifilis* susceptibility may vary considerably, including commercially important species observed in channel catfish (*Ictalurus punctatus*, Rafinesque, 1818) (Jorgensen *et al*., 2001). The most common form of treatment to control this ciliate in farm systems is trough-based bath treatments over a short exposure time, e.g. 30 min – 96 h (considering marketability and sustainability), which target the free-swimming stages of the parasite (i.e. tomonts and theronts). Meanwhile, parasitaemia persisted after treatment, except in group A which did not receive the inoculums, although a general fluctuation in parasitaemia was observed. The parasitic stages included the theronts and trophonts (Figures 12 and 13). Parasitaemia was affected by the treatment, while the number of theronts and trophonts in water, on the skin and gills of fish was affected strongly (p=0.05) by *O. gratissimum*. Parasitaemia of theronts and trophonts in the water culture medium, skin and gill of fish were significantly different between the groups at most of the points of comparison ($p < 0.05$).

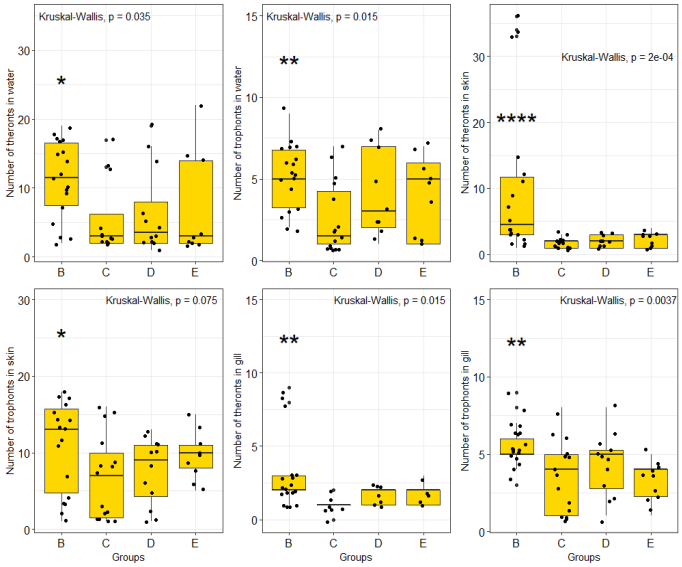

Figure 2: Intensity of *Ichthyopthirius multifilis* infection in *Clarias gariepinus* juvenile 24h pre-treatment with *Ocimum gratissimum*. Key: B: Negative control (Infected, not treated); C: Standard control (infected and treated with 150mg/L chloromphenicol); D: 500mg/L Ethanolic leaves extract of *O. gratissimum* (ELEOG); E: 1500mg/L ELEOG.

Notwithstanding, the variation noticed in the number of trophonts present in both the skin and gill was due to coverage of the body by a mucous layer, a defence mechanism against the *I. multifiliis* parasite. In addition, water movement over the gills increased the possibility of parasite contact with the host Erkin *et al*. (2012), and the gills appeared to be more susceptible to the infection. The extract performed impressively relative to the standard drug, but parasitaemia was not different between the extract groups and the standard control. These findings coincided with Chika *et al*. (2020) report, which opined that standard control and 4500mg/L aqueous leaves of *Moringa oleifera* eliminated 83.33% trophonts present in the gill of ich-infested fish. However, parasitaemia in the infected and untreated (negative control) was significantly higher P=0.05 than standard control for theront and trophont in water, fish skin and gill.

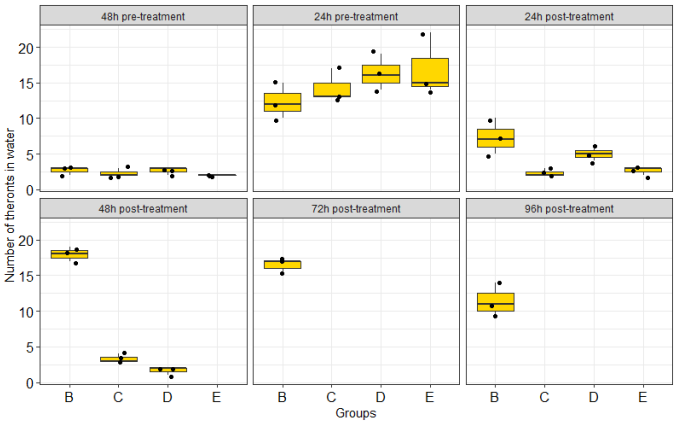

Figure 3: Theront intensity pre- and post-treatment with *O. gratissimum* extract in water. Key: A: Normal control (not infected and not treated); B: Negative control (Infected, not treated); C: Standard control (infected and treated with 150mg/L chlomphenicol); D: 500mg/L Ethanolic leaves extract of *O. gratissimum* (ELEOG); E: 1500mg/L ELEOG.

In contrast, the significant difference between standard and negative controls was more evident for dermal theronts ($p<0.0001$). Changes in theront and trophonts intensities are presented in Figures 3 to 8. Post-treatment parasitaemia of theronts in water decreased as the duration of treatment increased. It dropped drastically 24 h post-exposure treatment compared to 24h pre-treatment (Figure 2). By 72 h post-treatment, theront intensity in the water had decreased to a non-detectable level (Figure 3). Ekanem *et al*. (2004) reported that 200 mg/L of *Carica papaya* wiped out 100% of Ich parasite within 6 h, while 200 mg/L *Mucuna prurens* reduced the adult parasites by 90% after 72 h and 96 h, exposure in Goldfish. A similar pattern as theront in water was observed for trophonts in water. However, from 48 h to 96 h, parasitaemia was zero in the groups administered at 500mg/L and 1500mg/L ELEOG (Figure 4). The same pattern re-occurred for theront and trophont in the skin

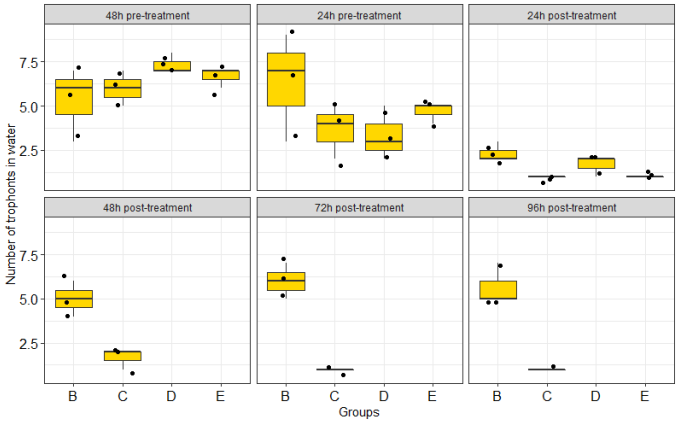

Figure 4: Trophont intensity pre- and post-treatment with *O. gratissimum* extract in water. Key: A: Normal control (not infected and not treated); B: Negative control (Infected, not treated); C: Standard control (infected and treated with 150mg/L chlomphenicol); D: 500mg/L Ethanolic leaves extract of *O. gratissimum* (ELEOG); E: 1500mg/L ELEOG.

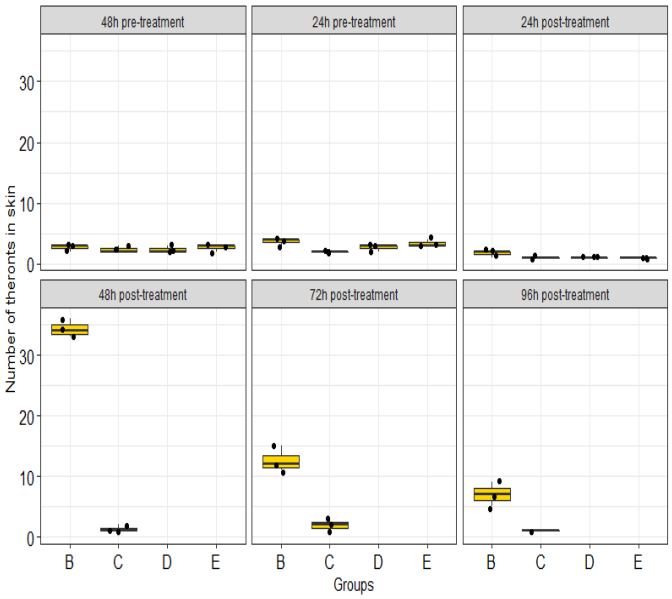

Figure 5: Theront intensity pre- and post-treatment with *O. gratissimum* extract on the skin. Key: A: Normal control (not infected and not treated); B: Negative control (Infected, not treated); C: Standard control (infected and treated with 150mg/L chlomphenicol); D: 500mg/L Ethanolic leaves extract of *O. gratissimum* (ELEOG); E: 1500mg/L ELEOG.

(Figures 5 and 6). Post-treatment, the intensity of parasitaemia was consistently higher in the negative control at all time intervals. Chika *et al.* (2020) reported similar intensity of parasitaemia in the negative control within 24 hours. The performance of the extract against water and skin Ich parasites was impressive when considered against the standard conventional drug used in this experiment. The efficacy of *O. gratissimum* ethanol leaf extract depended on the duration of treatments for the total eradication of the parasites. Though the standard drug was active against the Ich parasite, the infection persisted in the standard control in some cases where the extract reduced the infection to a below detectable level. In the gills, *I. multifiliis* clearance was not as impressive as was detected on the skin. Ich persisted 48 h post-treatment, except for 1500mg/L ELEOG. Complete clearance of infection at a lower extract concentration occurred at 500mg/L ELEOG (Figures 7 and 8). Yi (2012) reported eliminating theronts within 4h methanol extract of *Magnolia officinalis* and *Sophora alopecuroides* (pea flowered tree) eradicated *I. multifiliis* within 4hours by 2.45 mg/L and 3.43 mg/L, respectively.

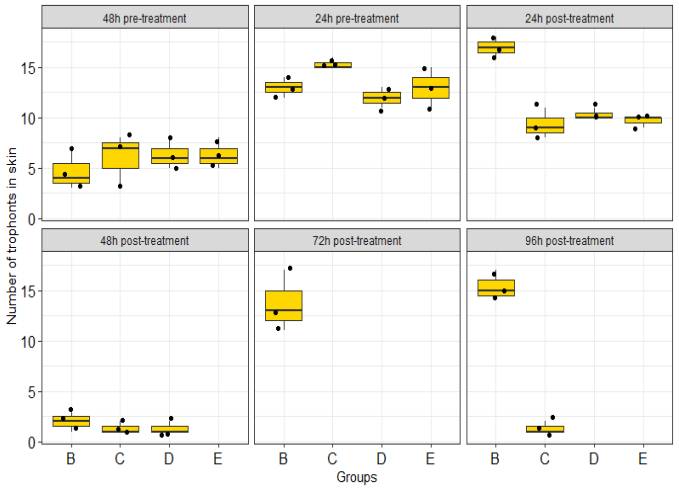

Figure 6: Trophont intensity pre- and post-treatment with *O. gratissimum* extract on the skin. Key: A: Normal control (not infected and not treated); B: Negative control (Infected, not treated); C: Standard control (infected and treated with 150mg/L chlomphenicol); D: 500mg/L Ethanolic leaves extract of *O. gratissimum* (ELEOG); E: 1500mg/L ELEOG.

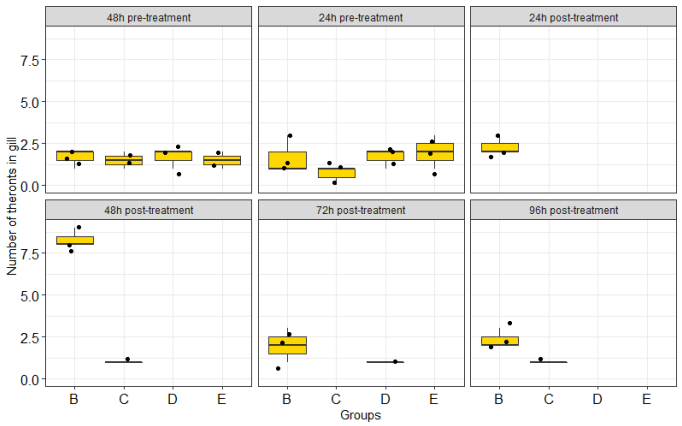

Figure 7: Theront intensity pre- and post-treatment with *O. gratissimum* extract on the gill. Key: A: Normal control (not infected and not treated); B: Negative control (Infected, not treated); C: Standard control (infected and treated with 150mg/L chloromphenicol); D: 500mg/L Ethanolic leaves extract of *O. gratissimum* (ELEOG); E: 1500mg/L ELEOG.

Nevertheless, there is still a paucity of literature on the efficacy of *O. gratissium* in the treatment of *I. multifiliis* infected fish. *O. gratissimum* served as a better alternative to chemical drugs in controlling *I. multifiliis* in fishes which can improve consumer preference and acceptability in utilizing *Clarias gariepinus* as a good source of protein due to zero off-flavour caused by the plant material.

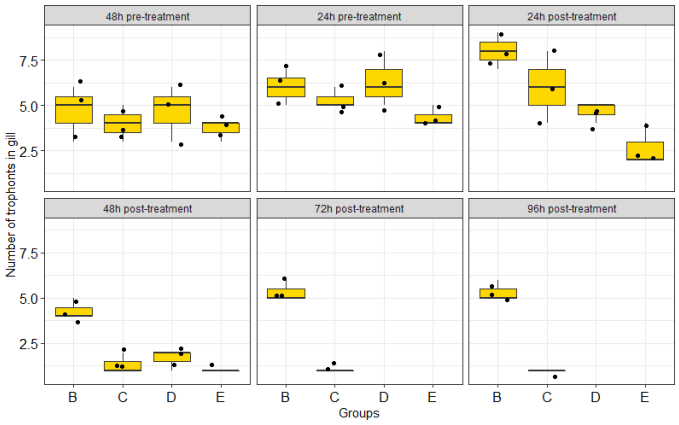

Figure 8: Trophont intensity pre- and post-treatment with *O. gratissimum* extract in gill. Key: A: Normal control (not infected and not treated); B: Negative control (Infected, not treated); C: Standard control (infected and treated with 150mg/L chloromphenicol); D: 500mg/L Ethanolic leaves extract of *O. gratissimum* (ELEOG); E: 1500mg/L ELEOG.

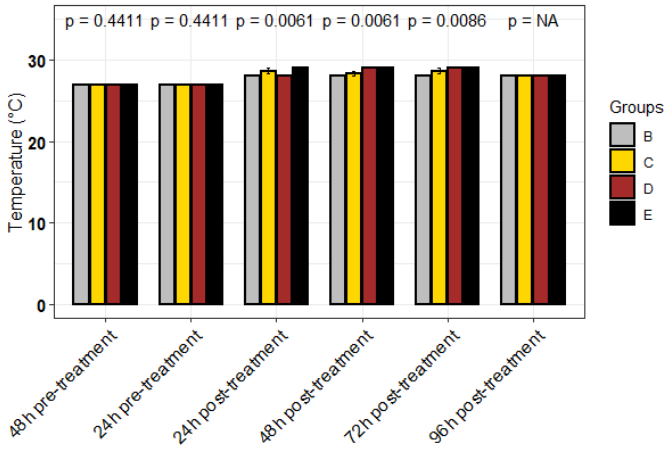

Figure 9: Changes in temperature in the experimental media.

There were few variations in the water temperature for the duration of the study. The temperature of the media varied significantly 24-, 48- and 72 h post-treatment. On further analysis, there was no relationship between the temperature of 24°C and Ich intensity for a temperature range of between 25°C and 29°C (Figure 9). However, the pH was different between medium, and 24h pre-treatment of Ich infection was significantly different between the setups (p=0.00046). At the same time, the standard control group had the most petite pH. At this time, however, no drug had been introduced. Upon introducing the extract and control drug, there were variations in pH believed to be extract induced. All the groups that received the extract had a marked drop in their pH relative to the negative control (Figure 10). An exciting relationship was noticed on further analysis to assess the implication of pH change on Ich intensity. There was a positive and robust relationship between pH and Ich intensity (Figure 11).

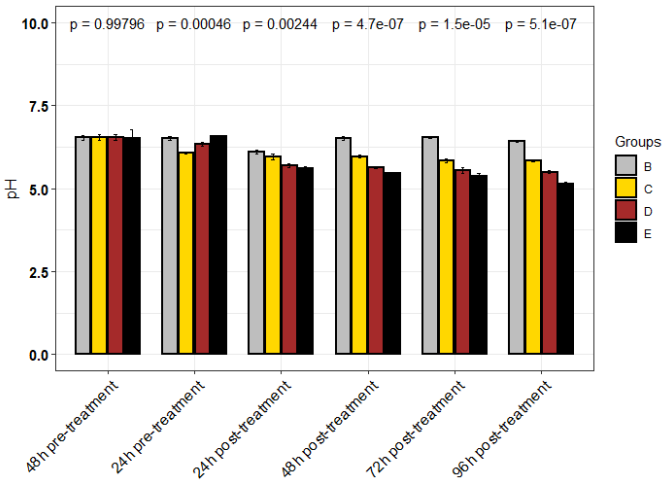

Figure 10: pH of experimental medium.

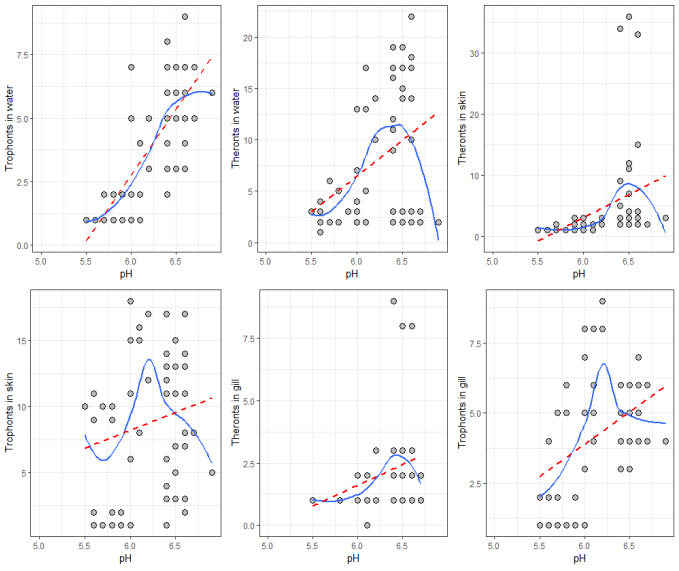

Figure 11: Relationship between culture water pH and intensity of *I. multifiliis*.

CONCLUSIONS AND RECOMMENDATIONS

Utilization and proper regulation of ethanol leaf extract of *O. gratissimum* within 96 h bath treatment to control protozoan parasite, *I. multifilis* was effective and showed a positive influence over the adult parasite (trophonts) at both high and low concentrations. It also demonstrated efficacy of novel non-chemical treatment against the adult and infective parasite.

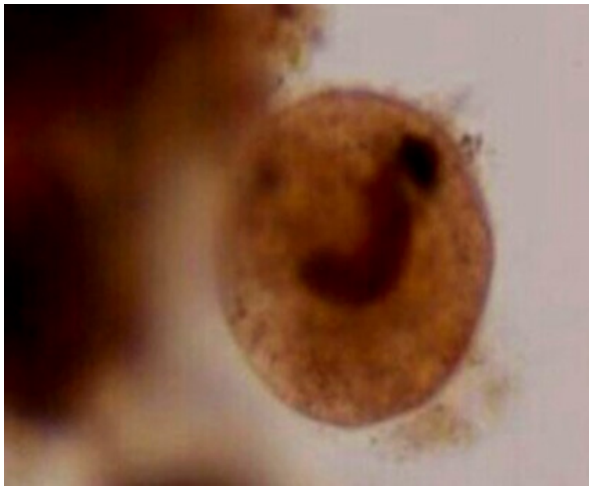

Figure 12: Trophont (Adult stage).

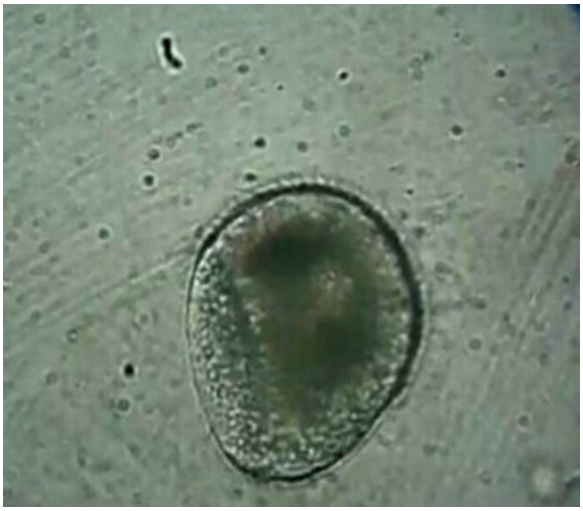

Figure 13: Theront (Infective stage).

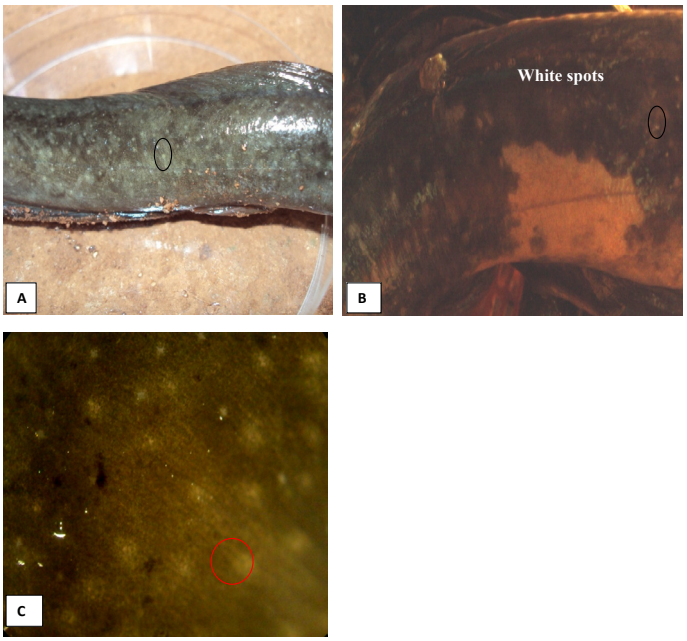

Figure 14: Gross morphology of infected Fish, *Clarias gariepinus* showing white spots (circles); A and B: Through visual observations; C: White spots under stereo microscope. Mag. 20X.

ACKNOWLEDGEMENTS

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NOVELTY STATEMENT

The research work currently evaluated potential of *Ocimum gratissimum* against *I. multifillis. O. gratissimum* has seldomly be used to control protozoa ich parasite

AUTHOR'S CONTRIBUTION

ICB designed the experiment, isolated and identified the parasite and supervised the research. OCMG proof read the manuscript. ECC conducted and monitored the experiment, including trophonts and theronts counts in the water, skin and gill of the ich-infected fish.

Ethical approval

All the fish used in the research work followed the guidelines of animal ethics and was approved by the appropriate ethical committee (UNN-ACC, protocol No. 07/09/2018) of the University of Nigeria, Nsukka and does not contain clinical studies or patient data.

Conflict of interest

The authors have declared no conflict of interest.

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