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ORIGINAL RESEARCH

Assessment of Ultra-Violet (Uv) light in the Control of Fish Pathogens isolated from the eggs of Catfish (*Clarias Gariepinus*)

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Abstract:
Introduction: Identifying bacterial species from catfish is a vital step in the gene banking of fish bacteria.
Aim: This study assessed the use of ultra-violet (UV) light in the elimination of pathogens isolated from the eggs of the African catfish (*Clarias gariepinus*).
Materials and Methods: Nine broodstocks of *Clarias gariepinus* were used. Their eggs were stripped, collected and taken to the International Institute of Tropical Agriculture (IITA) Nigeria laboratory for culturing, microbial analysis of the eggs and DNA extraction of bacteria from nutrient broth, and the amplicon was taken for molecular characterisation.Ultra-violet (UV) light were used as a form of irradiation on control of bacteria on fish eggs. Bio-edit software was used for importing and mining nucleotide sequences into the gene bank.
Results: The molecular examination of the *Clarias gariepinus* eggs showed the presence of five bacterial species, namely *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas putida,* and *Staphylococcus succinus*. Before UV treatment, *Bacillus* species were the most predominant bacterial species isolated from the fish eggs with 92.95% similarity. After exposure to UV treatment, only *Bacillus* species and *Klebsiella pneumonia* were detected with percentage similarity of 96.87% and 90.33% respectively. *Pseudomonas putida* had the highest nucleic acid concentration (5707.3 ng/µl),while the least nucleic acid was recorded in *Bacillus subtilis* (1299.5 ng/µl).
Conclusion: The ultra-violet (UV) light contributed immensely towards the reduction of microbes and its nucleic acid contents in the fish eggs, however to preclude adverse effects on egg and survival, UV irradiation of (200–280 nm) is advised.

Keywords: Broodstock eggs, Ultraviolet light, bacteria, Molecular method.

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

The aquaculture industry is the fastest-growing food-producing sector, accounting for approximately 50% of global fish production [1]. With the increased demand for Fish, pisciculture has also increased to a great extent. However, due to the rise in fish culture, there has also been a rise in fish diseases [2]. Studies have shown high mortality rates and economic losses in catfish aquaculture due to infectious pathogens such as bacteria [3]. Bush et.al.[4] pointed out that investigating novel non-antibiotic approaches, which can prevent and protect against infectious diseases, should be encouraged as a high priority for research and development projects. Prominent novel non-antibiotic approaches included light-based technologies, like ultraviolet C (UVC) irradiation therapy, photodynamic therapy (PDT), blue light therapy, and other light-based therapies [5]. Ultraviolet light, particularly UVC (200–280nm), is germicidal; it has not been much developed as an anti-infective approach until recently. It was noted that the possible adverse effects on host tissue were relatively minor compared to its high activity in killing pathogens [5]. The ultraviolet radiation (UVR) from the sun that reaches the Earth's surface is divided into three spectral bands: ultraviolet B, which is highly active and moderately harmful (UVB, 280-320 nm); and ultraviolet A, which is mildly energetic and less harmful (UVA, 320-400 nm). Ultraviolet C, which is highly detrimental, is absorbed by stratospheric ozone and oxygen and does not reach the Earth's surface [6-7]. Reports have demonstrated that UVC efficiently inactivated methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), antibiotic-susceptible strains of *E. faecalis* and *S. aureus*, *Enterococci* species, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* in vitro. UVC irradiation disinfected catheters by destroying associated bacterial biofilms, including coagulase-negative *Staphylococcus, Streptococcus*, *E. coli*, *E. faecalis*, *P. aeruginosa*, Coryneforms, and so on [8-9]. Therefore, this study aimed to assess the effects of ultra-violet light as post-treatment of fish bacteria found in eggs of *Clarias gariepinus.*

2. MATERIAL AND METHODS

2.1 Collection of Fish Sample

Nine broodstocks of African catfish *Clarias gariepinus*, were used for this study. They were collected from a private fish farm and transported in a jerry can of water. They were transferred alive to the laboratory in a 20 litre large plastic container filled up to half (10 litres) of its capacity with water and subjected to clinical and bacteriological examinations.

2.2 Morphometric Measurements

Morphometric differentiation,such as body weight, total length, standard length, and head length, among the selected broodstock were measured and recorded. All the lengths were measured in cm with the meter rule, while body weight was determined using an electric meter balance model PM400 to the nearest whole number in grams.

2.3 Collection of Eggs

The nine broodstocks tagged A, B and C containing three each in A, B, C were injected with a synthetic ovaprim hormone of 1.4 ml, 0.6 ml and 0.4 ml respectively. After that, they were allowed to stay for 9-15 hours to complete the latency period before stripping. Stripping of the female spawners was done by gently pressing their abdomen with a thumb from the pectoral fin towards the genital papilla. Ovulated eggs flowed out effortlessly. The ovulated eggs were more or less transparent and large, and a gram of egg contains approximately 600 eggs. The average weight of eggs extracted for broodstock A, B and C were 332 g, 177 g and 116 g, respectively.

All procedure was carried out under aseptic conditions using sterile materials such as conical flask (250ml), Sterile Petri dishes, Measuring cylinder, sterile syringes, Beakers, Mac Cartney bottles, and Cover slip.

2.4 Isolation of bacteria and purification

Isolation of bacteria was carried out aseptically from eggs collected from female broodstocks. The isolation involved streaking of eggs and were cultured on different media for bacteria growth on tryptic soya broth at 25°C and 37°C for (18-24) hours, then poured onto tryptic soya agar, blood agar, Rimler-Shoots agar,

Thiosulfate Citrate Bilesalt Sucrose agar (TCBS), and incubate at the same time and temperature.The isolated bacterial strains were purified according to Austin [10].

2.5 Molecular analysis and characterisation of bacterial isolates

Bacterial isolates were characterised using the molecular method.

2.5.1 RAPD- Polymerase Chain Reaction (PCR) Analysis DNA Extraction

The Murray and Thompson (1980) procedure was adapted to extract DNA from bacterial broth cultures in the log phase. A 2 ml bacterial culture produced quality DNA amounting to about 20 g using the modified protocol without using proteinase K.

2.5.2 Primers and PCR Amplification and resolution of RAPD markers

A panel of 2 numbers of decamer random primers were used for PCR amplification of bacterial DNA template. The PCR cocktail mix consists of 2.5 µl of 10x PCR buffer, 1µl of 25mM $MgCl_2$, 1µl each of forward primer and reverse primer, 1 µl of DMSO, 2 µl of 2.5 mMDNTPs, 0.1µl of 5U/µl Taq DNA polymerase, and 3µl of 10ng/ul DNA.Utilising 13.4 µl of nuclease-free water, a 25 µl total reaction volume was created.

2.5.3 Primer Sequence for Bacterial Identification

27 F: AGAGTTTGATCCTGGCTCAG

1429R: GGTTACCTTGTTACGACTT

2.5.4 PCR cycling parameters

Initial denaturation took place at 94 ^oC for 5 minutes, followed by 36 cycles of denaturation at 94 ^oC for 30 seconds, annealing at 56 ^oC for 30 seconds and elongation at 72^oC for 45 seconds. The temperature is maintained at 10 °C indefinitely after a final elongation stage that lasts 7 minutes at 72°C.Amplified fragments were visualised on ethidium bromide-stained 1.5% agarose electrophoresis gels. The size of the amplicon is about 1500bp and the DNA ladder used is 50bp from New England Biolabs (NEB). The sequencing was done using the genetic analyser ABI 3500 from Thermo Fisher.

2.5.5 UV treatment of eggs inoculated with bacterial cells

Fresh eggs stripped out of female *Clarias gariepinus* were left at room temperature for approximately 20 minutes before UV light treatment. In the ongoing UV-C light studies, samples were processed in a specially built aluminium chamber (1.0 x 0.5 x 0.6 m^3) with two UV-C lamps (2 3 95 W; UV-C Kompaktleuchte, B aro GmbH, Leichlingen, Germany) mounted in the ceiling. The UV-C light was emitted essentially at 253.7 nm, and the intensity was measured using a UVX Radiometer (Ultra-Violet Products Ltd., Cambridge, UK) with a UV-C sensor (model UVX- 25, Ultra-Violet Products Ltd.). Samples were subjected for exposure durations of 5, 10, 30, 60, and 300 seconds, giving fluences of 0.05, 0.1, 0.3, 0.6, and 3.0 J/cm^2, respectively, at a power intensity of 10 mW/cm^2, which is near to the maximum when using commercial lamps.

The instrument was equipped with a xenon flash lamp (19 cm), which was water-cooled, with an aluminium reflector (with an opening of 10 by 20 cm), it emitted light of 200 to 1,100 nm with up to 45% of the energy being in the UV-light region with maximal emission at 260 nm for high-energy pulses (SteriBeam Systems GmbH). Nearly beneath the reflector's entrance, at a distance of 6.5 cm, eggs were exposed to light. By dividing the total discharge energy of the lamp by the reflector's opening area at this distance, the fluence can be computed following the manufacturer's instruction. The input voltage was changed to alter the fluence of each pulse to either 1.25 J/cm^2 (low) or 3.6 J/cm^2 (high). The samples were exposed to single pulses, once to the low pulse and three or five times to the high pulse, respectively (3.6, 10.8, or 18.0 J/cm^2). The treated areas were then illuminated, and any remaining germs were collected by swabbing them. Temperatures were recorded using a Raynger MX infrared thermometer (Raytek Corporation, Santa Cruz, CA).

2.5.6 Analysis of cells

After exposure to UV light, the eggs were swabbed, and the surviving bacteria were collected. The bacteria were then plated onto tryptic soy agar (TSA; Oxoid) with 200 lg/mL Rif using an automated plate spreader

(Whitley Automatic Spiral Plater, Don Whitley Scientific Ltd., West Yorkshire, U.K.), eggs placed in incubator were then incubated for an overnight period at 37 ^{o}C. The number of colonies was determined using an automatic plate reader (The Neogen® Petrifilm® Plate Reader Advanced). Because RifR strains were used, the background flora on the eggs was negligible.

UVdose = (UVintensity) x (exposure time) 10-30 sec [11].

2.6 Statistical Analysis

Bio Edit software was used for importing and mining nucleotide sequences into Gene Bank, while BLAST was carried out on the NCBI website. Data were presented using table.

3. RESULTS

3.1 Morphometric Features of the Sampled Broodstocks

The morphometric characters (body weight, total length, standard length and head length) dosage of ovaprim and weight of eggs extracted from the sampled broodstocks of *Clarias gariepinus* were presented in Table 1. Broodstock 1 had the highest values for all the parameters listed in **Table 1** while broodstock 3 had the lowest values. However, the mean body weight and total length were 1.69±0.65 kg and 59.17±3.12 cm, respectively. The mean standard length was 51.67±2.09 cm, while the mean head length was 11.67±1.32 cm. While the average weight of egg extracted was 208.33±5.83 g, the mean ovaprim dosage applied to the broodstock was 0.8±0.08 ml.

3.2 Molecular Characterisation of Bacteria Isolates

The molecular examination of the eggs of brood stocks of *Clarias gariepinus* showed the presence of five different bacterial species (**Table 2**). Bacterial species identified were Bacillus species, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas putida*, and Staphylococcus succinus. Prior to ultra-violet (UV) treatment, based on percentage similarity, Bacillus species was the most probable bacterial species isolated from the eggs of the fish sample with 92.95%. *Escherichia coli* with 91.83% followed closely, while the least was recorded in *Pseudomonas putida* with 84.89%. After exposure to UV treatment, only Bacillus species and *Klebsiella pneumonia* with % similarity of 96.87% and 90.33%, respectively could be the species present.

The colony counting of the species isolated, as revealed by microscopic examination of these microbes during pre-UV treatment, showed that Klebsiella spp was 12, *E.coli* (28), *Staphylococcus spp* (20), *Bacillus subtilis*(30), and *Pseudomonas aeruginosa* (6); while in post-UV treatment, identified microbes were *Bacillus subtilis*(2) and Klebsiella spp(8).

3.3 DNA amplicon from the isolated bacteria strains separated on the agarose gel

Plate 1 showed the amplification products of two RAPD products in the isolates and the types of amplified DNA bands generated by these primers. The molecular size of the PCR products generated by these primers ranged from 1500 to 300 bp. The two primers generated fifty-one polymorphic bands. The primer 1429R was found to be more potent, generating 07 unique bands while the latter primer produced 06 unique bands. The primer 27F revealed clear variations in RAPD products between the studied bacterial isolates.

Table 1: Average values of Measurements of Broodstocks Used

Category of Broodstocks	Weight of Fish(kg	Total Length (cm)	Standard Length(cm)	Head Length cm)	Quantity of Ovaprim(ml)	Weight of Eggs Stripped(g)
Broodstock 1	2.90	76.5	67.0	15.0	1.4	332.0
Broodstock 2	1.20	53.0	45.0	11.0	0.6	177.0
Broodstock 3	0.96	48.0	43.0	9.0	0.4	116.0
Mean±STD	**1.69±0.65**	**59.17±3.12**	**51.67±2.09**	**11.67±1.32**	**0.8±0.08**	**208.33±5.83**

Table 2: Molecular Characterisation of Organisms found in the Eggs of *Clarias gariepinus*

Fish Specimen	Biotypes	Similarity (%)	Accession number	Strain Characterisation
1	*Bacillus* species	92.95	KP183926.1	*Bacillus* sp. BAB-4642
2	*Escherichia* coli	91.83	MG566068.1	*Escherichia coli* strain HPCAQ7CR13
3	*Klebsiella* species	85.94	MH141479.1	*Klebsiellapneumoniae* strain YCTCP01
4	*Pseudomonas* species	84.89	KX343950.1	*Pseudomonas putida* strain AR3
5	*Staphylococcus* species	85.79	KJ534522.1	*Staphylococcus succinus* strain F5
6	*Bacillus* species	96.87	KP183926.1	*Bacillus* sp. BAB-4642
7	*Klebsiella*	90.33	AY838355.1	*Klebsiellapneumoniae*

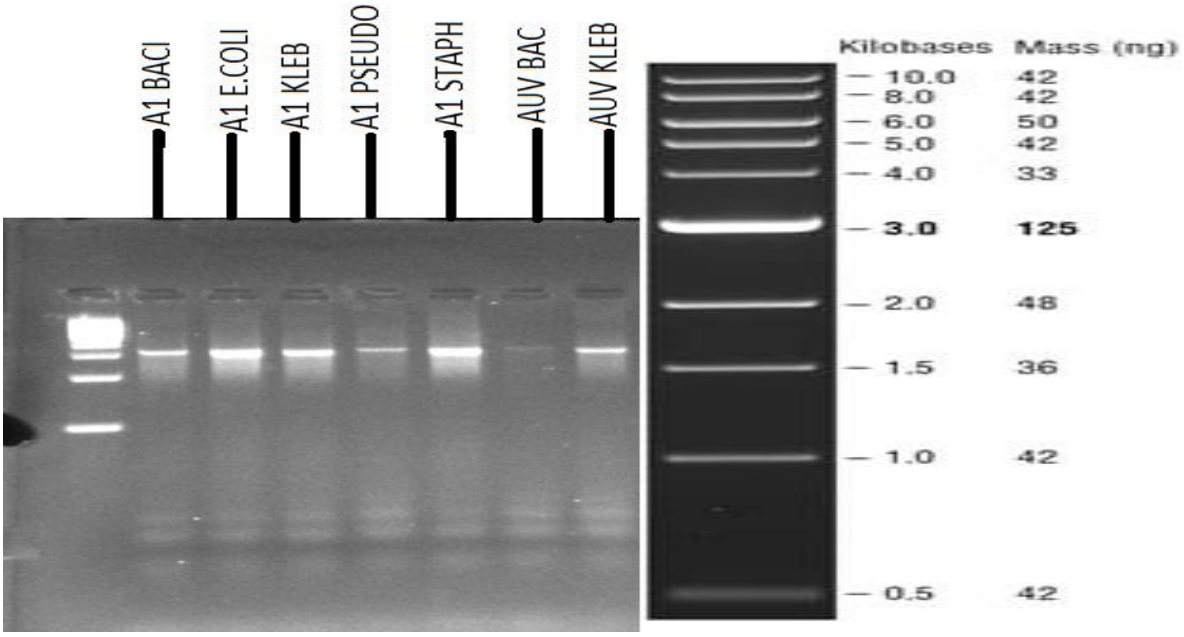

Plate 1: DNA amplicon from isolated bacteria strains separated on the agrose gel (M-maker).

3.4 Nucleotide concentration found in egg samples of *Clarias gariepinus* from Spectrophotometry

Table 3 showed that AI PSEUDO had the highest nucleic acid (5707.3 ng/µl), followed by A1 KLEB (4770.1 ng/µl), while the least nucleic acid was recorded in AUV BACILLUS (1299.5 ng/µl). Three kinds of absorbent (A), namely A230, A260 and A280, were recorded for all the sampled bacterial organisms, and their proportionality among the organisms follows the same trend as the proportion of the nucleic acids.

Sample ID	Nucleic acid (ng/µl)	A230(Abs)	A260 (Abs)	A280 (Abs)	A260/A280	A260/A230
AI PSEUDO	5707.3	50.507	114.145	53.984	2.11	2.26
A1 BACILLUS	3169	28.550	63.381	31.846	1.99	2.22
AUV BACILLUS	1299.5	11.707	25.989	12.367	2.1	2.22
A1 E COLI	1612.7	15.072	32.254	15.12	2.13	2.14
A1 KLEB	4770.1	42.590	95.402	46.105	2.07	2.24

A1 STAPH	4225.3	38.238	84.505	40.846	2.07	2.21
AUV KLEB	4223.2	37.540	84.465	41.133	2.05	2.25

Abs= Absorbent, A1 = Eggs before exposure to ultraviolet light (UV), AUV=Eggs after exposure to UV, AI PSEUDO= *Pseudomonas putida* in eggs A1, A1 BACILLUS = *Bacillus* species in eggs A1, AUVBACILLUS = *Bacillus* species in eggs AUV, A1 E COLI= *Escherichia coli* in eggs A1, A1 KLEB=*Klebsiella pneumonia* in eggs A1, A1 STAPH= *Staphylococcus succinus* in egg A1, AUV KLEB=*Klebsiella pneumonia* in eggs AUV.

4. DISCUSSION

The mean total length and body weight of broodstocks of *Clarias gariepinus* used for this study indicated that they had appropriate growth from where they were cultured. Similarly, the colours of the extracted eggs did not differ from conventional colours,as reported for *C. gariepinus*[12]. However, the average weight of the egg extracted (208.33±5.83g) could imply that the broodstocks were less fecund compared to some smaller sizes of *C.gariepinus* whose mean extracted eggs was about 600g[13]. This result indicates that weights do not necessarily imply high fecundity but may be dependent on other variables.

The molecular examination of the eggs of brood stocks of *C. gariepinus* examined in this study showed the presence of five different bacterial species:*Bacillus* species, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas putida*, and *Staphylococcus succinus*. Two Species (*E. coli and S. succinus*)observed in this study were also reported in the eggs of *C. gariepinus* isolated by Akinyemi [14]using the Biochemical method. However, *Salmonella sp, Proteus sp, Vibrio sp, Shigellasp, and Providencia rettgeri* found by Akinyemi were not present in this study. Also, microorganisms(*Pseudomonas stutzeri, Acinetobacter generic, Enterobacter caviae, Acinetobacter haemolyticus,*and *Aeromonas caviae)*isolated by Awe *et al*.[15]were not similar to those microbes found in this study. Therefore, the variation in the microbial load may be due to different sources of the broodstocks, the health status of their culturing media, and the kind of feed they were fed.

One of the several species discovered in this investigation was *Klebsiella spp.*, which is widely distributed in the natural world. The mucosal surfaces of animals, such as humans, horses, or swine, are likely their second common home after the environment, where they can be found in surface water, sewage, soil, and plants. This is where the genus *Klebsiella* differs from *Shigella spp.* and *E. coli*, which are prevalent in humans but not in the environment. Instead, it is similar to *Enterobacter* and *Citrobacter.*

Furthermore, Awe *et al*.[16] isolated the following bacterial organism from three different parts (skin, intestine, and gills) of African catfish (*Clarias gariepinus)* (Burchell, 1822) collected from private fish farmers along Uren river in Odogbolu, Odogbolu Local Government of Ogun state, Nigeria. These include *Aeromonas veronii, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus agalactiae and Enterococcus faecium*. The authors [16] also identified a new strain of *Aeronomonas* that is *Aeromonas veronii,* and has added to the strain of bacteria in Nigeria found on African catfish *Clarias gariepinus* in the gills and skin from the earthen fish ponds.

In this study, before the ultra-violet (UV) treatment, *Bacillus* species was the most predominant bacterial species isolated from the eggs of the fish sample with 92.95%, followed by *Escherichia coli* with 91.83%. However, after exposure of the eggs to UV treatment, only *Bacillus* species and *Klebsiella pneumonia* were detected with a % similarity of 96.87% and 90.33%, respectively. This observation indicated that *Bacillus species and Klebsiella pneumonia* are not irradiation susceptible. Therefore, the need to invent other methods of reducing their population in fish eggs cannot be over-emphasised. However, UVtreatment effectively eliminated *Escherichia coli*, *Pseudomonas putida*, and *Staphylococcus succinus* from the egg samples. Furthermore, reports have demonstrated that UVefficiently inactivated methicillin-resistant *Staphylococcus aureus*

(MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), antibiotic-susceptible strains of *E. faecalis* and *S. aureus, Enterococci* species, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* in vitro.Additionally, UV irradiation effectively cleaned catheters by eliminating bacterial biofilms such as those made up of coagulase-negative *Staphylococcus, Streptococcus*, *E. coli*, *E. faecalis, P. aeruginosa*, *Coryneforms,* and others [8-9]. When the inactivation efficacies of UVC were evaluated in vitro on harmful microorganisms such as bacteria (*P. aeruginosa* and *Mycobacterium abscessus*) and fungi (*Candida albicans, Aspergillus fumigatus*), it was discovered that fungi required considerably greater fluences to kill than bacteria [17]. Christian Gottfried Ehrenberg published the first account of *Bacillus* in 1835[18]. Since then, *Bacillus* has been discovered in many creatures and environments, including pigs, ponds, and dirt [19-20]. *Bacillus* is a common bacterium that is gram-positive, rod-shaped and ranges in size from 0.3 to 22 millimeters to 1.2 to 7 millimeters. It also forms spores and is aerobic to facultative. According to the morphology of the spore, *Bacillus* can be divided into three groups: (1) seven species, including *Bacillus subtilis* in the first genus, have ovular- or pillar-shaped spores and sporocytes that are not significantly expanded; (2) nine species, including *Bacillus circulans* in the second genus, have an oval spore and an enlarged sporocyte; and (3) *Bacillus sphaericus* in the third genus [21].Industrial enzymes, bioinsecticides, antibiotics, and other goods are frequently produced using *Bacillus* strains [22]. One of the most popular strains that is functioning is *B. subtilis*. Several studies have shown that *B. subtilis* has a lot of potential uses in animal husbandry right now [23].

For instance, it was discovered that the *B. subtilis* stress-responsive alternative sigma factor (SigB) increased the production of the lipopeptide surfactin, which improved antifungal effectiveness. Additionally, bacteria producing fengycin could prevent mice from becoming colonised by *Staphylococcus aureus* [24-25]. Also, *B. subtilis* was identified to inhibit the growth of pathogenic *E. coli in vitro* and *in vivo*, which implied that the isolate of *B. subtilis*WS^{-1} could have potential usage in the future.

The presence of *Pseudomonas putida* in the egg sampled in this study buttressed the report of Sugita *et al*.[26] that *Flavobacterium sp., Ahydrophila, Pseudomonas spp., Micrococcus spp., and A. punetata* were predominant on goldfish(*C. auratus)* eggs*.* The highest nucleic acid (5707.3 ng/µl) found in the *Pseudomonas putida*, followed by *Klebsiella pneumonia*(4770.1 ng/µl), indicated that these species, if not eliminated from fish eggs, could have great nucleic acid potential to thrive even in isolation of the yolk of the fish eggs. However, the least nucleic acid recorded in *Bacillus spp.* (1299.5ng/µl) exposed to UV indicates that UV can suppress the growth of this microbe in fish eggs. However, the duration of exposure of fish eggs to ultraviolet light (UV) is essential. For instance, it has been observed in bluegill larvae (*Lepomis macrochirus*) in a UV-transparent lake wherein 19% of nests estimated, UV-induced mortality of larvae exceeds 25%. Thus, specific measures are introduced in fish aquaculture, such as installing UV sunscreens to avoid UV damage to larval fish in the usually shallow habitats [27]. According to research, whether exposed to an acute dose or for a brief time, UVR (mostly UVB) radiation has some of the most obvious impacts, including a decrease in survival rates and an increase in the number and types of developmental abnormalities in both eggs and larvae. Following UVR exposure, several developmental defects have been linked to high mortality. For instance, Nunez *et al*.[28] divulged that four (4) hour post fertilisation (hpf) zebrafish (*Danio rerio*) embryos exposed for 2.4 h (UVB, 295 nm cutoff) showed a reduction of more than 50% in their survival rate after six days, with a high incidence of developmental abnormalities, including caudal (posterior) notochord torsion and bending. Similarly, studies have shown that levels of UVA and UVB radiation in aquatic ecosystems can cause damage at different levels to a broad range of organisms, from bacteria to higher vertebrates [24]. According to Sucre *et al*. and Vehniainen *et al*.[18] fish species exhibit a high degree of variation in their ability to tolerate UVB during the larval stage,

5. CONCLUSION

The ultraviolet light contributed immensely towards reducing the microbes and their nucleic acid contents in the fish eggs. However, to preclude the adverse effects of UV irradiation, UVC (200–280 nm) has the best potential ability to inactivate microorganisms because the wavelength of 250–270nm is strongly and mainly absorbed by the nucleic acids of microbial cells and, therefore is the most lethal range of wavelengths.

COMPETING INTERESTS

We declare that there are no competing interests

AUTHORS' CONTRIBUTIONS

Folalu Adekunle AWE, Ebere Lilian CHIDI-EZEH and Gabriel Olarinde MEKULEYI designed the study. Ebere Lilian CHIDI-EZEH and Folalu Adekunle AWE performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Folalu Adekunle AWE and Gabriel Olarinde MEKULEYI reviewed the first draft before submission.

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