

EFFICACY OF GARLIC *Allium sativum* L. AQUEOUS EXTRACT IN CONTROLLING SAPROLEGNIA HATCHERY-LINKED INFECTION IN *Clarias gariepinus* (BURCHELL, 1822)

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ABSTRACT

The efficacy of Garlic, *Allium sativum* L. aqueous extract in controlling *Saprolegnia* hatchery-linked infection in African Catfish *Clarias gariepinus* (Burchell, 1822) were investigated. Fresh *Allium sativum* was procured from Maiduguri Monday Market, peeled, washed, and ground to obtain the garlic juice. The prevalence of *saprolegnia* on eggs, fry, and fingerlings was determined by submerging fertilized eggs into different concentrations of the garlic extract for 5 minutes, smear sample from each treatment were collected at different developmental stages of the fish, and samples were cultured on Sabour dextrose Agar (SDA). The experiment was carried out using a completely randomized experimental design with six (6) treatments and five (5) replicates. The data obtained were subjected to one-way analysis of variance, and the differences between the means were separated using Duncan Multiple Range test. The results obtained at egg stage showed total elimination of *saprolegnia* in T5 (2.0 mL/L) and T6 (2.5 mL/L), with 40.8% as the highest, which was recorded in T1. Similarly, total elimination of *saprolegnia* was observed at fry stage in T4, T5, and T6, while highest prevalence of 48.6% was recorded in T1 (control). At fingerling stage, total elimination of *saprolegnia* was observed in T3, T4, T5, and T6 and the highest prevalence of 10.2% was recorded in T1. Generally, there was a decrease in the occurrence of *saprolegnia* with increase in concentration of garlic extract and developmental stage of the fish.

Keywords –*Allium sativum*, *Saprolegnia*, Elimination, Fingerlings, Extract

INTRODUCTION

Saprolegniasis is one of the most aggressive and problematic diseases for several wild and farmed species of fish in different life stages, especially eggs (Songe *et al.*, 2016). The pathogen hyphae break the chorionic membrane that regulates the osmosis process of the embryo (Liu *et al.*, 2014), causing its mortality and consequent production losses.

The available drugs are neither safe for the fish nor for human consumption of the treated fish (Sudava, 2007). The use of chemicals such as malachite green and formalin is effective in treating *Saprolegnia* fungal diseases at very low concentrations; however, they are hazardous to humans; hence, malachite green and formalin have been banned worldwide (Robertson *et al.*, 2009).

Garlic *Allium sativum* L. can help in the control of pathogens, especially bacteria and fungi, and increase the welfare of fish (Corzo-Martinez, 2007). The plant is a bulb growing to 25–70 cm with hermaphrodite flowers. The use of plant extract (*Allium sativum*) to control Saprolegniasis in freshwater fish is limited in the literature (Rai *et al.*, 2002). Garlic has been reported to control pathogens, combat stress, increase the welfare of fish (Megboon 2013) and enhance the immune response (Corzo-Martinez *et al.*, 2007).

The extensive use of synthetic antifungal chemicals such as formalin and malachite green has resulted in drug residues in treated fish, could result in to teratogenic, mutagenic, and carcinogenic effects and malformation of embryo and fetus in fish and human, respectively. The aim of the study is to minimize the use of synthetic chemicals by evaluating the potential of aqueous extract of garlic in prevention of the pathogenic fungus *Saprolegnia* species on *Clarias gariepinus* eggs.

MATERIALS AND METHODS

The study was conducted in the teaching and research fish farm of the Department of Fisheries, University of Maiduguri, situated between latitude 11° 48' 16" North and longitude 13° 12' 12" East. The Mean monthly temperature is (40.2°C) prior to the onset of the rain in June and the lowest (31.3°C) during the peak of the rainy period in August. The average annual rainfall is about 550 mm³.

Collection and preparation of garlic extract

Six hundred grams (600g) of garlic bulbs were purchased from the local market in Maiduguri. Fifty (50) garlic were peeled and washed with tap water. The peeled garlic were weighed using a digital electronic scale (Gallenkomp, EB-4300 England). It was then ground using a wooden pestle and mortar to obtain the garlic juice. The garlic was filtered with muslin cloth as described by Suleria *et al.* (2013).

Experimental fish

To obtain the fry for the experiment, one-year-old gravid female and male fish of *C. gariepinus* the female weigh (1500g) and the male weigh (1000g) were procured from a reputable fish farm for induced breeding. The gravid fish were being transported in a twenty-five (25)-liter capacity horizontally cut Jeri can half filled with water to the fish hatchery complex of the Department of Fisheries, University of Maiduguri. The gravid fish were conditioned for 15 days in a polyethylene-lined fish pond (7x5x1.2m) outdoor. During the acclimatization, the fish were being fed commercial feed with 35% crude protein before the commencement of the inducement.

The breeding was done using one female and one male. The female brood fish was induced with ovaprim at 0.5 ml/kg of fish and kept in a separate tank (2m²) before the latency period. At the end of the latency period, milt from the male was obtained using the method described by (Diyaware *et al.*, 2010). The milt sacs were washed to remove blood and fat using physiological saline solution and placed in a 100-ml beaker containing 0.9% saline solution. The milt was squeezed into a 10 ml beaker container with 1-2ml of physiological solution (0.9%) saline. Then eggs from the female was stripped into a clean receptacle. The milt in the beaker was used to fertilized the stripped eggs.

Experimental design

A total of 1g of fertilized eggs was placed on a nylon net (2cm) as an egg substrate. The fertilized eggs on the nylon egg substrates were placed in six (6) different concentrations of garlic aqueous extracts of 0, 0.5, 1.0, 1.5, 2.0, or 2.4 mL/L of water for five (5) minutes. The treatment was replicated five times in a complete Randomized Design (CRD) manner. The aqueous garlic-treated eggs were incubated in a two (2)-liter capacity trough (10cm diameter x 5cm depth) filled with one (1) liter of water using a flow-through system.

Preparation of sabouraud dextrose agar (SDA) medium

The Sabouraud Dextrose Media (SDA) was prepared in adherence to the manufacturer's instructions. Firstly, 32.8g of the media was weighed and placed into a conical flask. Then, exactly 500 ml of distilled water was poured into the flask, and the mixture was stirred with a stirring rod and shaken thoroughly to create a uniform solution. Next, the mouth of the flask was covered with non-absorbent cotton wool and aluminum foil. Afterwards, the conical flask and its contents were autoclaved at 121°C for 15 min. Finally, the SDA

was placed into sterile Petri dishes and left to solidify (Atlas 2010)

Isolation of fungi

A swap stick was used to collect smear samples from eggs, fry, and fingerlings. Smears were cultured in SDA. The agar plates were incubated in an autoclaving machine at 26°C, and *Saprolegnia* growth was observed after 5 days under the microscope using lacto phenol cotton blue stain (APHL, 2017).

Monitoring of water quality parameters

Dissolved Oxygen (DO) (mg/L) and water temperature (°C) were recorded daily using digital DO/temperature analyzer (Model: JPB-608 DO made in Japan) also pH was recorded daily using digital pen pH meter (Model: ATC pHmade in China).

Statistical analyses

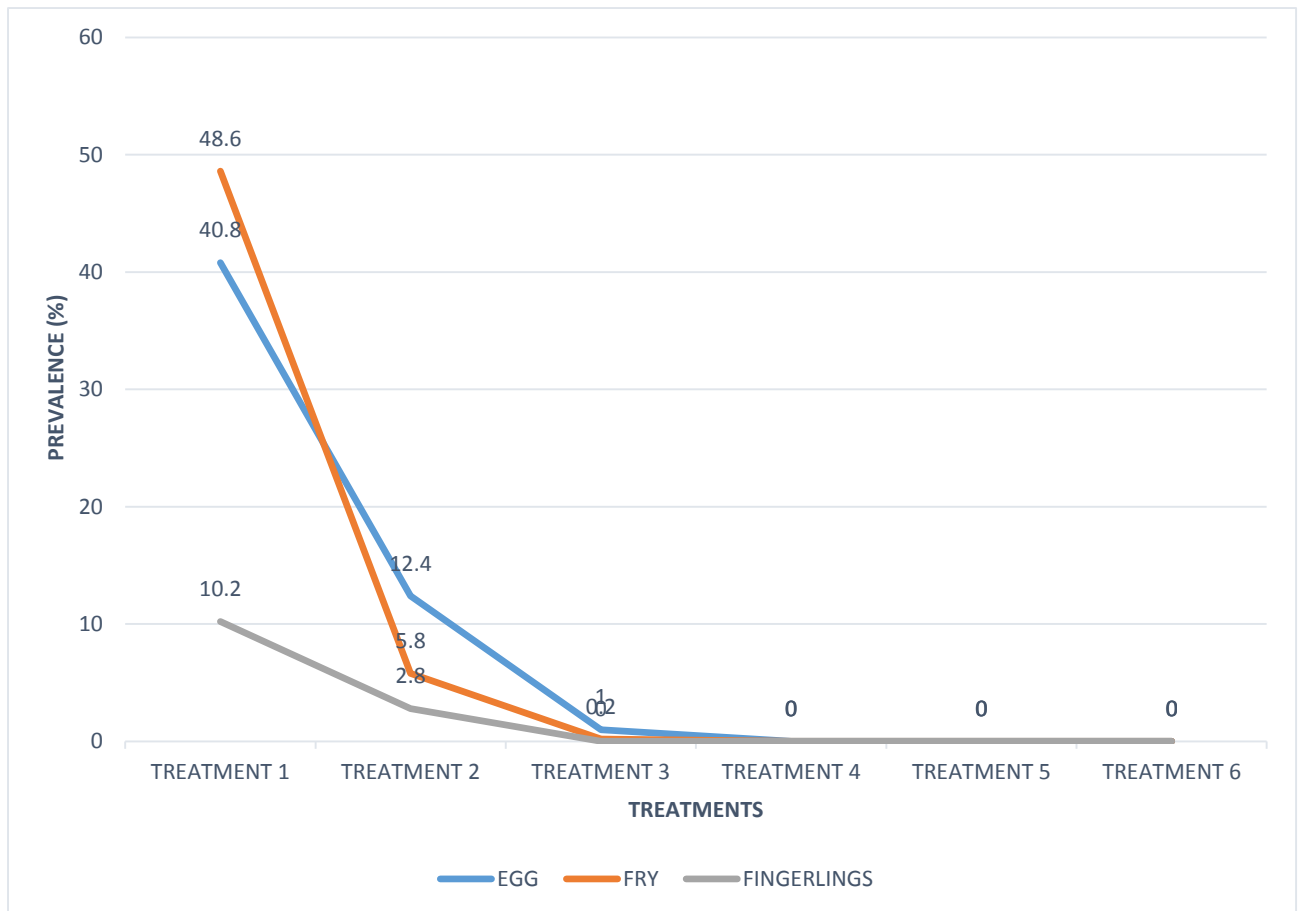
Data obtained from the study were subjected to one-way Analysis of variance (ANOVA), to test for significant difference in treatment means. Differences between the means were separated using Duncan Multiple Range Test (DMRT) at 95% level of confidence with aid of statistic 8.0.

RESULTS

The percentage prevalence *Saprolegnia* on eggs, fry, and fingerlings of *Clarias gariepinus* is presented in Figure 4.1. The highest prevalence of *Saprolegnia* (40.8%) on fertilized eggs was recorded in T1 of *A. sativum* extract, followed by treatment 2 (12.4%) of *A. sativum* aqueous extract, total elimination (0.0%) of *Saprolegnia* was observed in T5 and T6, respectively. There was no significant difference ($P>0.05$) between prevalence obtained in treatments 3,4, 5 and 6 of *A. sativum* aqueous extract, while significant differences ($p<0.05$) existed between prevalence obtained in T1 and T2 of *A. sativum* extract.

Higher prevalence of *Saprolegnia* on fry (48.6%) was observed in T1 of *A. sativum* aqueous extract, with total elimination (0.0%) of *Saprolegnia* recorded in T4, T5 and T6 of *A. sativum* aqueous extract, respectively. There was no significant difference ($P>0.05$) between treatment group T3, T4, T5 and T6 of *A. sativum* extract.

The highest prevalence of *Saprolegnia* recorded on fingerlings was (10.2%) observed in T1 of *A. sativum* aqueous extract, with total elimination of *Saprolegniasis* in T3, T4, T5, and T6, respectively. Significant difference ($P<0.05$) was observed between T1 and T2 of *A. sativum* aqueous extract.



DISCUSSION

Saprolegnia growths on the surface of eggs, fry, and fingerlings of fish can cause extensive direct mortalities. In this study, total elimination was recorded on eggs of *Clarias gariepinus* using garlic extract as a fungicide. The findings in this are in line with those obtained in the study Faranak (2016) who reported that an ethanolic extract of the garlic with a higher concentration showed the highest inhibitory effect on the *Saprolegnia* fungus growth. Dilute solutions of garlic completely inhibit the growth of *S. aureus* at higher concentrations (Deresse, 2010). Ozcelik *et al.* (2020) reported that the aqueous methanolic extracts of garlic stem and garlic skin were effective in preventing *Saprolegnia infection* in rainbow trout eggs. The elimination recorded at the study may be due to the concentrations used, as reported by Daniel *et al.* (2018), garlic in higher concentrations showed more antifungal effects, also, lower concentration of garlic had no antimicrobial effect (Shokrzadeh and Ebadi, 2006).

The sulphur compound present in fresh garlic plays a crucial role in inhibiting the growth of *Saprolegnia* fry and fingerlings. (Gomaa, 2003) demonstrated antifungal potency of garlic, where inhibition of *Trichophyton* and *Microsporium* species using fresh

garlic juice was shown due to stronger activity of ajoene. Buchmann *et al.* (2021) also, investigated the effects of *A. sativum* extract on *I. multifiliis* theronts and tomocysts in vitro and found that it killed theronts within 15 hrs at a higher concentration. Garlic in saline solution inhibited the growth of *Aeromonas hydrophila* at higher concentration (Blanco, 2013). Also, the method of application plays an important role in eliminating the saprolegnia. As reported by Harikrishnan *et al.* (2010), injection and immersion methods may be effective in delivering herbal extracts.

CONCLUSION

Garlic extract at a concentration of 2.5 mL/L was able to eliminate the growth of saprolegnia at the egg fry and fingerling stage of *Clarias gariepinus*. These means garlic extract at a concentration of 2.5 mL can be used in hatcheries in the control of saprolegniasis.

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REFERENCE

- Associatin of Public Hhealth Laboratories (2017). Swap Collection and Handling.
- Atlas, R.M., and Snyder, J. (2010). Handbook of media for Clinical microbiology. CRC Press.
- Blanco Silva S.A. (2013). Effect of the inclusion of garlic extract (*Allium sativum*) in a balance ration, on the growth and survival of goldfish (*Carassius auratus*) and levistes (*Poecillareticulata*) in culture *Biblioteca digital Veterinaria*, 1688-6909.
- Buchmann, K. Mathiessen, H., Jaafar, R. M., Al-Jubury, A., Gersdorff Jørgensen, L. and Kania, P. W., (2021). Comparative in Vitro and in Vivo Effects of Feed Additives on Rainbow Trout Response to *Ichthyophthirius multifiliis*. *North American Journal of Aquaculture*, 83 (2), 67–77.
- Corzo-Martinez, M., Corzo, N. and Mar V. (2007). Biological properties of onions and garlic, *Trends in Food Science and Technology*, 18,609-625.
- Deresse. D. (2010). Antibacterial Effect of Garlic (*Allium sativum*) on *Staphylococcus aureus*: An in vitro Study *Asian Journal of Medical Sciences*, 2 (2), 62-65.
- Daniel, D., Aisha, H. and, A. B. K. M (2018). Antifungal Activity of Garlic (*Allium sativum*) Extract on Some Selected Fungi. *Journal of Medicinal Herbs and Ethnomedicine*, 4, 12–14.
- Faranak Shafiei, Davoodi R, Bagheri D, Jamali F, and Nooryazdan H. (2016). Effect of the Garlic extract (*Allium sativum*), on *Saprolegnia* isolated from Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) eggs compared with Malachite green and Bronopol. *Journal of Artificial Intelligence Research*, 4 (3), 93-105.
- Gomaa, N.F., and Hashish, M.H, (2003). The inhibitory effect of garlic (*Allium sativum*) on the growth of some microorganisms. *Egypt Journal of Public Health*, 78:361-372.
- Harikrishnan, R., Balasundaram, C., and Heo, M. S. (2010). Effect of chemotherapy, vaccines and immunostimulants on innate immunity of goldfish infected with *Aeromonas hydrophila*. *Diseases of Aquatic Organisms*, 88 (1), 45–54.
- Liu, Y., De Bruijn, I., Jack, A. L., Drynan, K., Van Berg Den, A. H., Thoen, E., Sandoval-Sierra, V., Skaar, I., Van West, P., Dieguez-Uribeondo, J., Van Voort Der, M., Mendes, R., Mazzola, M., and Raaijmakers, J. M. (2014). Deciphering microbial landscapes of fish eggs to mitigate emerging diseases. *ISME Journal*, 8 (10), 2002–2014.
- Megbowon, A., Adeyemi Adejonwo, O. A., Y.B., Kolade, O.y., Adetoye, A.A.A., C.A, Edah., B, Okunade, O.A., and Adedeji, A.K. (2013). Effect of Garlic on Growth Performance, Nutrient Utilization and Survival of an Ecotype Cichlid, ‘Wesafu. *Journal of Agriculture and Veterinary Science*, 6 (3) 10-13.
- Ozcelik, H.Y., Tastan, E. and Adam (2020). Use of Onion (*Allium cepa*) and garlic (*Allium sativum*) waste for the prevention of fungal disease (*Saprolegnia parasitica*) on Eggs of Rainbow Trout (*Oncorhynchus mykiss*) *Journal of Fish Disease*, 43 (10),1325-1330.
- Rai, M.K., Kaushal, S.K, and Acharya, D (2002). In vitro effect of five Asteraceous essential oils against *Saprolegnia ferax*, a pathogenic fungus isolated from fish. *The Antiseptic* 99 (4), 136-137.
- Robertson E.J., Anderson V.L., Phillips A.J., Secombes C.J., Dieguez-Uribeondo J. and van West P. (2009) *Saprolegnia* – fish *Oomycete Genetics and Genomics*, p407-424.
- Shokradeh M. and Ebadi A.G. (2006). Antibacterial effect of Garlic (*Allium sativum*) on staphylococcus *Pakistan Journal of Biological Sciences*. 9 (8), 1577-1579.
- Songe, M., Willems, A., Wiik-Nielsen, J., Thoen, E., Evensen, Ø., Van West, P., and Skaar, I. (2016). *Saprolegnia diclina* IIIA and *S. parasitica* employ different infection strategies when colonizing eggs of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases*, 39 (3), 343-352.
- Sudova. E., Machova. J., Svobodova. Z. and Vesely T. (2007) Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: a review. *Veterinarni Medicina*, 52, (12): 527–539.
- Suleria H.R, Butt M.S, Anjum F.M, and Khalid N, (2013). aqueous garlic extract; natural remedy to improve haematological, renal and liver status. *Journal of nutrition and food science*, 63, 431-439.