Behavior Changes and LC$_{50}$ of Dried Garlic (*Allium sativum*) Acute Toxicity in Nile Tilapia (*Oreochromis niloticus*) Juvenile

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Plant-based compounds are emerging substitutes for chemical treatments in aquaculture since they are known to cause less harm to both the environment and its organisms. Garlic (*Allium sativum*) is a popular herb that has a broad range of uses, especially in the control and treatment of bacterial and parasitic diseases. However, its toxicity to tilapia is not well-studied. Hence, this study was conducted to test the toxicity levels of garlic powder in juvenile Nile tilapia (*Oreochromis niloticus*) by determining the cumulative mortality and median lethal concentration (LC$_{50}$). Test fish were exposed through immersion in 6 concentrations (150, 200, 250, 300, 350, and 400 mg L$^{-1}$) of garlic powder with 25% allicin and a control in 96 h static bioassay. Within 24 h, test fish exposed to 250 mg L$^{-1}$ garlic and above, exhibited abnormal behaviors such as lethargy, gasping for air, and weak responses to environmental stimuli. Cumulative mortality was highest at 100% in 400 mg L$^{-1}$ garlic at 48 h. Mortalities were dependent on the concentration and duration of exposure. The 96 h LC$_{50}$ of garlic powder to *O. niloticus* was 225.86 mg L$^{-1}$ with lower and upper confidence limits of 210.37 and 242.50 mg L$^{-1}$, respectively at 27°C.

Keywords: behavioral response, cumulative mortality, garlic, tilapia, median lethal concentration (LC$_{50}$), 96 h static bioassay

INTRODUCTION

Tilapia is one of the most important fish commodities in the Philippines, contributing to almost 11% of the country’s total aquaculture production in 2020 (PSA 2021). Due to its higher growth rates and high tolerance to environmental stress, tilapia is highly preferred by fish farmers as a species for culture. Tilapia can be cultured in a high stocking density; however, this setup allows parasites to proliferate rapidly, leading to outbreaks of parasitic diseases (Michel 1989; Nowak 2007).

Garlic can be a potential alternative treatment for various parasitic infections of finfishes. It is rich in sulphur-containing compounds such as allicin, diallyl sulfide (DAS), diallyl trisulphide (DATS), diallyl disulfide (DADS), ajoene, and 2-vinylthiins, which play active roles in immunostimulation and have antioxidant properties (Subroto et al. 2021). Garlic is also found to reduce the prevalence and intensity of ectoparasites including *Gyrodactylus turnbulli* (Fridman et al. 2014), *Trichodina heterodentata* (Abd El-Galil and Aboelhadid 2012), and *Ichthyophthirius multifiliis* (Gholipour-Kanani et al. 2012). The incorporation of garlic in the diet of fish may also stimulate the immune system and enhance growth rate (Shakya and Labh 2014). Additionally, active compounds of garlic such as allicin have anti-microbial properties against Gram-positive and Gram-negative bacteria (Nakamoto et al. 2020). Allicin solubility to water reaches 0.8% and has a half-life of only 2.5 d from freshly cut garlic (Jiang et al. 2020). Although chemical treatments are effective in preventing and controlling parasite infestation, they can be detrimental to the environment and may impact the overall health of fish (Subasinghe et al. 2000). Therefore, studies have been focusing more on the application of plant-derived treatments because they are organic and environmentally...
safe. Limited studies have been conducted on the toxicity of garlic powder to tilapia. Hence, this study was conducted to determine the 96 h median lethal concentration (LC50) of garlic powder for juvenile tilapia.

MATERIALS AND METHODS

Preparation of Garlic Solution

Garlic powder containing 25% allicin was obtained from Hebei Kangdali Pharmaceutical Co., Ltd. Garlic aqueous stock solution at 9,000 mg L\(^{-1}\) was prepared by adding 9 g powdered garlic in 1 L distilled water and stored in a glass bottle at room temperature.

Test Fish

Tilapia juveniles with a mean body weight of 21.9 ± 5.1 g and a total length of 10.6 ± 1.34 cm were obtained from a commercial fish farm in Zarraga, Iloilo, Philippines. Fish were then transported to the Southeast Asian Fisheries Development Center/Aquaculture Department in Tigbauan, Iloilo using oxygenated polyethylene bags and acclimatized for 2 wk under laboratory conditions. Fish were held through a static system in 500 L circular fiberglass tanks filled with filtered freshwater and continuous aeration. During acclimatization, fish were fed with commercial feeds at 3% of their body weight. Debris and waste were siphoned out daily.

Water Quality Monitoring

Water quality parameters were monitored daily and recorded using Milwaukee MW 101 pH meter for pH, standard mercury thermometer for temperature, Milwaukee MW 600 DO meter for dissolved oxygen (DO), and AtagoS/Mill hand-held optical refractometer for salinity. For total ammonia nitrogen (TAN), water samples were collected and analyzed using Skalar Methods Catnr. 155-006 w/r(+P3) using San++ continuous flow analyzer.

Acute Toxicity Test

The experimental procedure for the acute toxicity tests to determine the 96 h LC50 of garlic on O. niloticus via bath immersion treatment was based on the outlined toxicity tests by Bridgewater et al. (2012). Ten randomly collected fish were distributed in each test treatment and control group, fish showed normal swimming and behavioral patterns, and no mortalities were recorded from the start to the end of the experiment. Fish were observed every 24 h for 4 d (96 h) for behavioral changes and, likewise, mortality and water parameters were recorded. Behavioral changes included slow swimming behavior, decreased response to stimuli (gentle prodding of water), and gasping for air. Dead fish were removed, recorded, and properly disposed.

Statistical Analysis

Probit analysis program by Srinivasan (2004) based on Probit Analysis by Finney (1971) was used to calculate the median lethal concentration (LC50) values and corresponding 95% confidence limits.

RESULTS

The water quality parameters during the toxicity test were shown in Table 1. The temperature was consistent at 27.8°C ± 0.9 for the entire duration of the experiment whereas pH and dissolved oxygen values slightly increased towards the last day of monitoring.

The acute toxicity effect of garlic powder on O. niloticus was studied in terms of behavioral patterns. In the control group, fish showed normal swimming and behavioral patterns, and no mortalities were recorded (Table 2). In contrast, fish exposed to 150 and 200 mg L\(^{-1}\) garlic powder at the first 24 h exhibited slow swimming behavior and decreased response to stimuli, while these behaviors persisted only for 2 h in fish exposed to 250 mg L\(^{-1}\) and 300 mg L\(^{-1}\) garlic powder with occasional gasping for air. At higher concentrations (350 and 400 mg L\(^{-1}\)), similar behavior was observed, except that the gasping for air lasted for 1 h, and fish recovered from the changes after 24 h.

Median lethal concentration (LC50) test was conducted to determine the concentration of garlic powder in the water which cause 50% mortality of the test population of O. niloticus. The LC50 value of garlic powder at 96 h was 225.86 mg L\(^{-1}\) with 95% confidence limits ranging from 210.37 to 242.5 mg L\(^{-1}\) (Table 3). The cumulative mortality of O. niloticus during the 96 h exposure to garlic powder was shown in Fig. 1. The cumulative mortality

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Dissolved Oxygen (ppm)</th>
<th>Ammonia (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>27.7 ± 0.8</td>
<td>8.49 ± 0.03</td>
<td>6.2 ± 0.54</td>
<td>0.01 ± 0.1</td>
</tr>
<tr>
<td>24</td>
<td>27.9 ± 0.6</td>
<td>8.47 ± 0.04</td>
<td>6.6 ± 0.45</td>
<td>0.36 ± 0.4</td>
</tr>
<tr>
<td>48</td>
<td>27.3 ± 0.8</td>
<td>8.58 ± 0.10</td>
<td>6.6 ± 0.48</td>
<td>0.91 ± 0.32</td>
</tr>
<tr>
<td>72</td>
<td>28.1 ± 0.7</td>
<td>8.53 ± 0.06</td>
<td>6.7 ± 0.28</td>
<td>1.20 ± 0.25</td>
</tr>
<tr>
<td>96</td>
<td>27.8 ± 1.0</td>
<td>8.59 ± 0.13</td>
<td>6.9 ± 0.48</td>
<td>1.87 ± 0.31</td>
</tr>
</tbody>
</table>
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Table 2. Behavioral changes of Oreochromis niloticus exposed to various concentrations of Allium sativum powder containing 25% allicin.

<table>
<thead>
<tr>
<th>Exposure Time</th>
<th>Control</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
<th>350</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>30 min</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>45 min</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>1 hr</td>
<td>+</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>2 hr</td>
<td>+</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>3 hr</td>
<td>+</td>
<td>+</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>6 hr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>9 hr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>12 hr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>24 hr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
<td>a,b,c</td>
</tr>
<tr>
<td>48 hr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 hr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>96 hr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1 - no behavioral changes, 2 – slow swimming behavior, 3 – decreased response to stimuli, 4 – gulping for air
5 – recovered from altered changes

Table 3. Computed lethal concentration (LC) values and confidence limits after 96 h exposure of Oreochromis niloticus juveniles (TL = 10.6 ± 1.34 cm) to garlic powder containing 25% allicin with three replicates per treatment and ten fish per replicate using probit analysis.

<table>
<thead>
<tr>
<th>Point</th>
<th>Probit</th>
<th>Concentration (mg L⁻¹)</th>
<th>Log Concentration</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC 1.0</td>
<td>2.67</td>
<td>104.7</td>
<td>2.02</td>
<td>85.94 – 127.54</td>
</tr>
<tr>
<td>LC 10.0</td>
<td>3.72</td>
<td>147.88</td>
<td>2.17</td>
<td>129.85 – 168.40</td>
</tr>
<tr>
<td>LC 50.0</td>
<td>5.00</td>
<td>225.86</td>
<td>2.35</td>
<td>210.37 – 242.50</td>
</tr>
<tr>
<td>LC 95.0</td>
<td>6.64</td>
<td>388.99</td>
<td>2.59</td>
<td>344.93 – 438.69</td>
</tr>
<tr>
<td>LC 99.0</td>
<td>7.33</td>
<td>487.26</td>
<td>2.69</td>
<td>414.13 – 573.31</td>
</tr>
</tbody>
</table>

percentages at 96 h were 0, 46.7%, 63.3%, 76.7%, 86.7%, and 100% at concentrations of 150, 200, 250, 300, 350, and 400 mg L⁻¹, respectively. No mortality was recorded in the control group for the whole duration of the experiment.

The regression equation of the relationship was calculated to be probit $y = -26.945 + 13.117 \log \text{Conc.} \times x$ and on R square value ($R^2$) of 0.7606 (Fig. 2). The regression equation ($R^2$) value showed a positive correlation between the mortality rate of the test fish and the concentration of garlic powder. This means that the mortality rate of the fish increased with the increasing concentration of A. sativum.

DISCUSSION

The addition of garlic in the test water may alter the water quality parameters and affect the survival of fish. The temperature was constant at 27.8°C ± 0.9 from start to end which is far from the upper (42°C) and lower limit temperature (12°C) of O. niloticus according to Albaba and Barroso (2007). Based on the study of Makori et al. (2017) on the physio-chemical parameters during culture of O. niloticus in earthen ponds, pH values of 8.5 to 8.6 and DO levels of 6.2 to 6.9 are considered to be optimal for the culture of tilapia.

Changes in the behavioral patterns of fish were indications of the potentially toxic effects of the substance that was added to the water. The observations were similar to the study of Syngai et al. (2016) who exposed juvenile common carp (Cyprinus carpio) to different concentrations of aqueous garlic extract; the tested fish exhibited slow-moving swimming behavior and were unresponsive to sudden changes in the environment for 24 h. The work of Aqel et al. (1991) involved testing the effects of garlic juice in vitro on the isolated segments of the aorta, trachea, intestine, and heart of a rabbit; the study showed that acetylcholine, a neurotransmitter that plays a role in muscle movement, was inhibited by garlic. This may be associated with the sedated behavior of...
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CONCLUSION

This study was conducted to evaluate the toxicity of garlic to tilapia via bath immersion method. Toxicity of garlic was measured through LC₅₀ and the behavioral changes of tilapia for 96 h. In the results, exposing the tilapia to test concentrations caused behavioral changes, such as slow swimming behavior, decreased response to stimuli and gulping for air, but at 300 ppm garlic and below fish were able to recover through time. The behavioral changes also persisted at increasing concentration. The 96 h acute toxicity concentration of garlic to tilapia juvenile was 225.86 mg L⁻¹ at 27.8 ± 0.4 °C. This value can be a baseline concentration for future studies that involve testing the efficacy of garlic in *in vivo* and *in vitro* trials for controlling parasitic and bacterial diseases. It also gives information to allow comparison between the toxicity of garlic and other herbal products of the same species of fish or to other species of fish.

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REFERENCES CITED


It was also found that the 96 h LC₅₀ of garlic to tilapia was 225.86 mg L⁻¹ at 27.8 ± 0.4 °C, which was comparable to the reported LC₅₀ value of garlic on common carp (Syngai et al. 2016). The closeness of the 96 h LC₅₀ values of garlic between tilapia in this study and carp in the study of Syngai et al. (2016) may be attributed to the similarities in size and ontogeny of investigated fish that both studies used juveniles. Generally, the tolerance of fish to xenobiotics or synthetic chemicals increases as the size of the fish increases (Alam and Maughan 1995). Moreover, tilapia and common carp are both considered hardy species that can tolerate a wide range of environmental conditions e.g. poor oxygen levels and can be easily bred in various culture systems (Hoseini et al. 2018; Prabu et al. 2019). Both have similar stress responses in hypoxic environments wherein they undergo a profound, rapid, and reversible metabolic rate depression as shown by large decreases in oxygen consumption rate (Speers-Roesch et al. 2010).

The LC₅₀ test gives the assumption that exposure time associated with the specified LC₅₀ is sufficient to allow almost complete chemical equilibrium between the fish and the test water (Syngai et al. 2016). LC₅₀ test was necessary to evaluate the nature and degree of adverse effects of chemicals and plant-derived compounds on the test organisms which will serve as a basis for choosing concentrations for aquaculture treatments. The LC₅₀ value of a therapeutant to fish varies depending on the fish species and size, weight, sex, and behavior of the same species (Tiwar et al. 2011). In this study, the garlic powder toxicity in tilapia was concentration- and time-dependent—mortalities were higher at increasing concentration and time of exposure. This observation was in agreement with the previous report on the toxicity of garlic on guppies (*Pocelia reticulate*) with a size range of 0.4 – 0.06 g (Fridman et al. 2014). Moreover, the dose- and time-dependent response was observed in other species of fish subjected to other herbal extracts such as neem (*Azadirachta indica*) leaf extract on 9 g streaked prochilod juveniles (*Prochilodus lineatus*) (Winkaler et al. 2007) and *Terminalia catappa* leaf aqueous extract on guppies (Claudiano et al. 2012).

The LD₅₀ value of garlic to tilapia in this study. Moreover, gasping for air may indicate deficiency in the oxygen intake of fish due to hypoxic conditions (Tiwari et al. 2011). However, DO levels in this were within the acceptable limits for tilapia. High concentrations of garlic powder may interfere with the gaseous and ionic exchange since it has low solubility in polar solvents such as water (Fujisawa et al. 2008; Claudiano et al. 2012).

TERMINALIA catappa

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