SAFETY ASSESSMENT OF CRUDE AND AQUEOUS EXTRACTS OF *Syzygium aromaticum* in *Oreochromis niloticus* (Linnaeus, 1758) JUVENILES

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ABSTRACT

The application of plant extracts in aquaculture is growing due to their various biological benefits; however, some are toxic. This study was carried out to evaluate the safety doses of S. aromaticum (clove buds) in O. niloticus juvenile. Air dried buds were pulverized to obtain crude powder while aqueous extraction was done following standard method to obtain aqueous extract. Median lethal dose (LD_{50}) was determined using Lorke's method. Behavioural responses were monitored and recorded. When crude powder was administered, there was no adverse reaction observed in the first 6 hours at doses below 1000mg/kg while reactions like weak swimming, loss of scale and fin erosion were noted at increased doses. In the group administered aqueous extract, no adverse responses were recorded in the first 6 hours. Beyond this period, ragged fins, slow opercular movement and loss of scale were observed even at the lowest dose of 10mg/kg. The calculated LD_{50} for crude powder was 223.61mg/kg and 538.52mg/kg for aqueous extract. Conclusively, the LD_{50} values recorded in this study indicate moderate toxicity. It is recommended that S. aromatica be used with caution and the calculated LD_{50} should not be exceeded to avoid toxic responses from fish.

Keywords: Clove, Safety, Lethal dose, Toxicity, Fish health

INTRODUCTION

Medicinal plants have been used as a source of herbal remedies in human traditional medicine (Mishra *et al*., 2013; Murugan *et al*., 2021) and many have been reported to possess pharmacological activities that are attributable to the presence of bioactive compounds such are glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes (Batiha *et al*., 2018; Beshbishy *et al.,* 2019). In traditional use, the extent of application of an herb ensures that a corresponding herbal drug is safe. However, traditional or folk medicine comprises practices, approaches, knowledge and beliefs not based on scientific evidence that are applied to treat, diagnose and prevent illness. Hence, there is need for cautious use as some herbal remedies can be toxic (Mounanga *et al*., 2015; Schultz *et al*., 2020; Olaniyi *et al.,* 2020).

In recent decades, medicinal plants have been gaining wider acceptance in aquaculture due to the perception that these plants being natural products, have lesser side effects and improved efficacy than their synthetic counterparts (Ody, 2017; Ruddaraju *et al*., 2020). *Syzygium aromatica* is commonly known as clove. In Nigeria, it is commonly called "kanufari" and "kanafuru" by the Hausas' and Yorubas' respectively, and used as a health spice in food and drinks such as 'yaji' (grilled meat sauce), 'kunu zaki' (millet drink), and 'jedijedi' (herbal concoction). Agbaje *et al*. (2009) reported that the flower bud of the plant has spermicidal effect in male rats, hence, can be a potential anti-fertility feed additive in controlling prolific breeding in *Oreochromis niloticus.* However, there are some medicinal plants that are toxic (Antache *et al.,* 2013), hence, there is need to ascertain the phyto-toxicity of *Syzygium aromatica* in fish.

Ascertaining the toxicity of medicinal plants in fish production can be challenging, whereas, several strategies including genotoxicity and carcinogenicity assessments have been employed to mitigate this problem in mammalian cells (Kirkland *et al*., 2016; Li *et al*., 2019; Allemang *et al*., 2021). However, another strategy is the use of LD_{50}, also called the median lethal dose. The value of LD_{50} for a substance is the dose required to kill half the members of a tested population after a specified test duration. LD_{50} is usually determined by tests on animal models or cells. LD_{50} tests on laboratory mice (Kouadio *et al*., 2014; Olatunbosun *et al*., 2018) and fish (Sreedevi and Vijayalakshmi, 2018; Jobi and Kshetrimayum, 2020; Ojetayo *et al*., 2022) have been conducted.

To the best knowledge of the authors, there are no studies presently that have examined LD_{50} of

S. aromatica extracts (crude and aqueous) in *O. niloticus.* To this end, the focus of this study is to examine LD_{50} of *S. aromatica* extracts in *O. niloticus.* Findings from the study will serve as a guide on safe inclusion levels of *S. aromatica* extract in further applications in fish nutrition.

MATERIALS AND METHODS

Procurement and identification of clove buds

The study was carried out at the Department of Forestry, Wildlife and Fisheries, College of Agricultural Sciences, Olabisi Onabanjo University, Ayetoro Campus. *S. aromatica* was procured from a reputable market in Ibadan, Oyo State, Nigeria. The plant (bud) was identified and authenticated in the Forestry unit in the Department of Forestry Wildlife and Fisheries.

Procurement of experimental Fish

One hundred and fifty *O. niloticus* juveniles were purchased from a reputable farm in Ikorodu and transported in oxygen bags to the laboratory. They were acclimatized for two weeks under laboratory condition while being fed twice daily with commercial fish feed. The fish were kept in aerated 1000L tanks containing freshwater and the water was drained partially and refilled every alternate day.

Preparation of *S. aromatica* (powder and aqueous)

The collected clove buds were rinsed in clean water and air dried at room temperature for 14 days (Obaroh and Nzeh, 2013). The air dried buds were pulverized to powder using an electric blender machine. The powder obtained was weighed (550g) and half of this was used to prepare the aqueous extract. For aqueous extraction, powdered buds sample obtained was soaked at ratio 1:12 clove to water at room temperature for 72 hours with constant mixing within this period (Ojetayo *et al*., 2022). The resulting extract was filtered with sterile muslin cloth and concentrated using water bath.

Determination of LD_{50}

LD_{50} was determined following Lorke's method (Lorke, 1983). This method involved two phases. In Phase 1, nine fish samples were required. The nine fish samples were divided into three groups and each group had three fish. Each group of fish were administered 10mg/kg, 100mg/kg and 1000 mg/kg of test substance (powder and aqueous extract). The fish were placed under observation for 24 hours to monitor their behaviour and mortality. For Phase 2, three fish samples were distributed into three groups of one fish each. Each group were administered 1600mg/kg, 2900mg/kg and 5000 mg/kg of clove powder and aqueous extract and then observed for 24 hours for behaviour as well as mortality. Then the LD_{50} was calculated by the formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where:

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality

RESULTS

Behavioural responses of *O. niloticus* administered crude clove buds

Behavioural responses of fish administered crude clove are presented in Table 1. In the first 6 hours of Phase 1, fish administered 10mg/kg of crude clove showed no adverse reaction; in the 12th hour, normal opercular movement and ragged fins were recorded and in the 24th hour, weak swimming movement and ragged fin with mortality was recorded. In fish administered 100mg/kg of the test ingredient, in the first 6th hour, no adverse reaction was observed; in the 12th hour, normal opercular movement and mild fin erosion were observed and in the 24th hour, weak opercular movement, pronounced fin erosion with mortality were recorded. In fish administered 1000mg/kg of test ingredient, no adverse reaction was observed in the first 6 hours; in the 12th hour, mild fin erosion was noted and in the 24th hour, weak swimming, mild fin erosion with mortality were recorded. In Phase 2, the group administered 1600mg/kg of test ingredient exhibited upside down swimming in some fishes and laboured breathing in the first 6 hours; in the 12th hour, scale loss was recorded and in the 24th hour, scale loss, and mortality were recorded. In group administered 1600mg/kg of the test ingredient, no adverse reaction was observed in the first 6 hours; in the 12th hour, pronounced fin erosion and slow opercular movement were observed and in the 24th hour, pronounced fin erosion, slow opercular movement and no mortality were recorded. In the group administered 5000mg/kg of crude clove, in the first 6 hours, weak swimming movement was observed; in the 12th hour, pronounced scale loss, abnormal swimming and pronounced fin erosion

was recorded and in the 24th hour, pronounced scale loss, weak swimming, pronounced fin erosion but no mortality was observed.

Behavioural responses of *O. niloticus* administered clove buds aqueous extract

The result of behavioural responses of *O. niloticus* administered clove buds aqueous extract is presented in Table 2. In fish fed with 10mg/kg of test ingredient, in the first 6 hours, no adverse reaction was observed; in the 12th and 24th hour, ragged fins and slow opercular movement were observed. In fish fed with 100mg/kg of test ingredient, no adverse reaction was observed in the first 6 hours post administration; in the 12th and 24th hour post administration, ragged fins with slow opercular movement were observed and mortality was recorded. In fish fed with 1000mg/kg of test ingredient, no adverse reaction was observed until the 12th hour when loss of scale and ragged fins were noted with weak opercular movement and mortality recorded by the 24th hour.

In Phase 2, in fish administered 1600mg/kg of test ingredient, no adverse reaction was observed; by the 12th hour, fin erosion and normal opercular movement was observed while in the 24th hour, fin erosion and weak opercular movement were recorded. In fish administered 2900mg/kg of test ingredient, in the first 6th hour and the 12th hour, no adverse reaction was observed and in the 24th hour, mild fin erosion and weak swimming was recorded. In fish administered 5000mg/kg of test ingredient, in the first 6th hour, no adverse reaction was observed; in the 12th hour, mild tail fin erosion and slow opercular movement was recorded and in the 24th hour, mild tail fin erosion, slow opercular movement and mortality were recorded.

Table 1: Behavioural responses of *O. niloticus* administered crude clove buds

Phase 1	6th hour	12th hour	24th hour
10 mg/kg	A1	B1, C1	A2, C1, M
100 mg/kg	A1	B1, C1	B2, C2, M
1000 mg/kg	A1	C1	A2, C1, M
Phase 2			
1600 mg/kg	A2, B2	D1	D1
2900 mg/kg	A1	C2, B2	C2, B2
5000 mg/kg	A2	D2, A2, C1	D2, A2, C2

Table 2: Behavioural responses of *O. niloticus* administered clove buds aqueous extract

Phase 1	6th hour	12th hour	24th hour
10 mg/kg	A1	C1, B2	C1, B2
100 mg/kg	A1	C1, B2	C1, B2, M
1000 mg/kg	A1	D1, C1	D1, C1, B2, M
Phase 2			
1600 mg/kg	A1	C1	C1, B2
2900 mg/kg	A1	A1	C1, A2
5000 mg/kg	A1	C1, B2	C1, B2, M

A1: no adverse reaction/swimming

A2: weak swimming/ abnormal swimming

B1: normal opercular movement

B2: laboured breathing/opercular movement

C1: mild fin erosion/ragged fins

C2: pronounced fin erosion

D1: mild scale loss

D2: pronounced scale loss

M: mortality

Calculated LD_{50} value for crude and aqueous extract of clove buds

The calculated LD_{50} for crude clove buds was 223.61mg/kg while 538.52mg/kg was the calculated LD_{50} for clove aqueous extract.

DISCUSSION

Behavioural responses such as gasping for air, erratic or lethargic swimming, and even death have been reported in fish exposed to certain plant extracts (Zannatul *et al*., 2018; Ali, 2013). Fin erosion and loss of scale have also been reported. Orji *et al*. (2014) reported distress responses such as gulping of air, rapid opercula movement and erratic swimming within 15 minutes post exposure to aqueous extract of *Psychotria microphylla*. It was also noted that these responses were more pronounced with increasing concentrations. Ajani and Ayoola (2010) and Sultana *et al*. (2021) also reported dose dependent behavioural responses in fish administered herbal products. These reports are similar to what was observed in this study when crude and aqueous extract of clove were administered to *O. niloticus*. However, in a study by Afanyibo *et al*. (2019), clove did not exert any toxicity or stress responses in shrimp larvae when administered in vitro. The reason for this variation could be due to the type of extract used, concentration of extract and species used for the studies.

In the present study, doses above 10mg/kg caused mortality in the first 24 hours when crude clove was administered to *O. niloticus*. However, mortality was observed when doses of clove aqueous extract administered were above 10mg/kg. This report is not in agreement with the findings of Isaac *et al*. (2015) and Saeed *et al*. (2017) who reported that doses between 0.5-2.5g/kg of clovinol, a compound extracted from clove, did not induce mortality or abnormal responses. Humbal *et al*. (2019) in another study also noted no mortality in rats fed clove oil at doses between 50-200mg.kg for 28 days.

Although clove is considered safe for consumption by Food and Drug Administration, however, there are emerging reports on its possible toxicity. Tanko *et al*. (2008) reported LD_{50} of 567.7mg/kg in rats administered ethanolic extract of clove. In another study by Agbaje *et al*. (2009), LD_{50} values of 263mg/kg and 2500mg/kg were recorded in rats administered aqueous extract of clove intraperitoneally and orally, respectively. In the present study, LD_{50} values of 223.6mg/kg and 538.52mg/kg were obtained for clove crude and aqueous extract respectively. These values were within the values reported in previous studies. According to Hodge and Sterner (2005), these values are considered moderately toxic.

CONCLUSION

The study revealed that low doses (below 100mg/kg) of clove caused behavioural responses that can be attributed to toxic effect of the plant. Although the calculated median lethal doses for the crude and aqueous extract fall within moderate toxicity range, it should however be used cautiously. In addition, sub-lethal assessments of clove should be determined to ascertain its safety on long term administration in *O. niloticus*.

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