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Short Communication

The use of aqueous extracts of *Vernonia amygdalina* in the control of saprolegniasis in *Clarias gariepinus*, a freshwater fish

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Saprolegniasis infection causes severe damage to fish integument. To control this fungal infection in the fish environment, Asteraceous plant (*Vernonia amygdalina*) extract at varying concentrations (50, 40, 30, 20%, control) was used to test its potency in controlling the infection and to ascertain its inhibitory capacity. The axenic culture of the test fungus was maintained on PDA at 28°C. The rate of the colony forming units was determined and the test inocula adjusted to 1.5 X 10^5 spores per ml. The Asteraceous plant extract generally depressed the growth of the fungi and cleared the spores earlier noticed on the fish integument. The potency of inhibition of the growth of *Saprolegnia* increased with increase in concentration. The control fish without the plant extract showed fluffy tufts growing on their body after 28 days and they appeared lethargic. This research hold great promise in elucidating the potency of the plant extract (*V. amygdalina*) in suppressing fungal growth in the integument of *Clarias gariepinus,* a freshwater fish.

Key words: *Saprolegnia* spp.*, Vernonia amygdalina,* fish integument, fish environment, fungi bait.

INTRODUCTION

Saprolegniasis, primarily caused by *Saprolegnia* spp. is a very common fungal infection in freshwater. Saprolegniasis is mainly a secondary infection seen after damage to the fish integument caused by parasites such as viruses and bacteria (Rai et al., 2002; Khulbe et al., 1995). Even though synthetic fungicides are available and are used to control the disease, their indiscriminate use causes environmental hazards. Although the use of these chemicals are effective in controlling fungal diseases, there are however some major set backs which tend to limit the usage. Some fungi have developed resistance to these chemicals. This necessitates higher dosage or the development of new chemicals to replace those to which fungi are resistant. Secondly, some fungicides are not readily biodegradable and tend to persist in the environment. Hence, it becomes necessary to develop ecologically safe, effective and economically feasible method of disease management (Khoo, 2000). Aqueous extract of many allelopathic plants are known to exhibit antifungal properties. Allelochemicals reduce the germination of spores and mycelial growth of pathogenic fungi (Begum et al., 2008; Haikal, 2007; Sahayaraj et al., 2006; Bajwa et al., 2003). The use of plant extract to control fungal growth in freshwater fish is scarce in the literature (Rai et al., 2002). The aim of this study therefore was to evaluate the potential of aqueous extract of aerial parts of the asteraceous plant extract (*V. amygdalina*) to control the pathogenic fungus (*Saprolegnia* spp) of the freshwater fish, *Clarias gariepinus*.

MATERIALS AND METHODS

Preparation of the asteraceous plant extract

Fresh healthy aerial parts (leaf, flower and stem) of *V. amygdalina* (family: Asteraceae) growing as wild in the premises of Delta State University, Abraka, Nigeria, were collected. These were washed

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Table 1 Effect of different concentrations of aqueous extracts of *V. amygdalina* on the mycelial production of *Saprolegnia* on the fish integument (CFU/ mL).

Day	Tank 1 (control)	Tank 2 (10%)	Tank 3 (20%)	Tank 4 (30%)	Tank 5 (40%)	Tank 6 (50%)
1	1.5×10^5	1.5×10^5	1.5×10^5	1.5×10^5	1.5×10^5	1.5×10^5
8	1.5×10^{10}	1.5×10^{10}	1.5×10^6	1.5×10^5	1.5×10^4	1.5×10^3
15	3.0×10^{18}	1.5×10^8	1.5×10^5	1.5×10^4	1.5×10^3	1.5×10^2
21	3.0×10^{28}	1.5×10^6	1.5×10^4	1.5×10^3	1.5×10^2	1.5×10^1
28	3.0×10^{36}	1.5×10^4	1.5×10^3	1.5×10^2	1.5×10^1	1.5×10^0

thoroughly under running tap water and were cut into pieces and sun dried. A 100% w/v stock solution of *V. amygdalina* extract was obtained by soaking the crushed plant materials in sterilized water for 48 h at room temperature, sieved through muslin cloth and finally through Whatman filter paper.

Isolation of the fungus, *Saprolegnia* sp.

Maize seeds (*Zea mays*) were used as baits for trapping *Saprolegnia* and some other fungi from River Ethiope, Delta state, Nigeria employing the method previously described by Ilondu et al. (2001). *Saprolegnia* was the dominant fungi and was isolated in the labora-tory. The axenic culture of the test fungus was maintained on PDA at 28°C. To avoid bacterial contamination, Chloromycetin capsules were used as antibacterial agent. One week old culture was washed with sterile saline and the spore suspension was prepared by using glass wool filtration. The rate of the colony forming units was determined and the test inocula adjusted to 1.5 X 10^5 spores per ml.

Live fish samples of *Clarias gariepinus* juveniles of mean length and weight of 20.0 ± 2.0 cm and 40 ± 2.0 g respectively were obtained from a fish farm in Delta State, Nigeria and used for this research. They were left for 14 days in the laboratory to acclamatize in glass aquaria of 30 liters capacity before the commencement of the research.

The fungi isolates were then introduced to the fish environment (1.5 X 10^5 spores per ml) and left in the aquarium tanks for 21 days. This was done so that the fungi would have enough time to attack the integument of the fish. When signs of fungal growth were evident on the fish, varying concentrations of the plant extract (50, 40, 30, 20, 10 and 00%) were introduced into the various aquaria while monitoring the growth of the fungus in the fish integument. This was monitored for another 28 days. All test tanks were replicated twice and the entire experiment was repeated two times. Some water quality parameters such as pH, dissolved oxygen, alkalinity and free CO_2 were also monitored regularly in the test tanks using APHA (1992) methods.

For the second experiment, Petri dishes were filled with 10 ml of PDA in aseptic conditions and 1 ml of the spore suspension (recovered from the infected fish) was added per plate after the medium solidified. Sterile discs (5 mm diameter, Whatman filter paper no. 42) were soaked in different concentrations of the plant extract (10, 20, 30, 40 and 50%) up to saturation. These saturated discs were placed in the centre of the Petri dishes, which were then incubated at 28°C for 48 h. For each concentration of the plant extract, triplicates were maintained. The clear inhibition zones were measured and noted.

Statistical analysis

Analysis of variance (ANOVA) was employed in determining the statistical significance of the different concentrations of the plant extract on the fungal species and regression analysis was used to determine the relationship between the inhibition zone and the different plant extract concentrations. The SPSS 12 programme was used in all cases.

RESULTS AND DISCUSSION

Seven days after introducing the isolated fungi into the fish tanks, the skin colour of the fish appeared whitish brown. After 14 days, fluffy tufts were observed on the integuments of the fish with heavy growth of mycelia of the fungus. The mycelial production response of *Saprolegnia* sp. on the fish integument was variable when grown in the presence of different concentration of aqueous extracts of *Vernonia amygdalina* (Table 1). Concentrations and the exposure period were statistically significant ($P < 0.05$) using a two-way ANOVA indicating that fungi inhibition increases with a corresponding increase in concentration. All concentrations of the tested plant were found to inhibit the growth of fungal spores and the rate of inhibition increased gradually by increasing the concentrations (Figure 1). After 28 days, the fish in tanks without the plant extract developed more fluffy tufts growth on their body and they appeared lethargic. The fish in the highest concentration (50%) showed more healthy conditions although water quality conditions were slightly altered.

The results obtained in the present study revealed that aqueous extracts of *V. amygdalina* reduced the pathogenic fungal growth in the skin of the test fish. In general, the higher the concentration of the plant extract the greater the inhibition potential. Studies of plant extract against fungal growth in fish are scarce in the literature. However, Rai et al. (2002) reported the efficacy of essential oils of five plants from Asteraceae family against the fungus, *Saprolegnia ferax*. There are numerous reports to show that certain plant extracts are highly effective in suppressing the growth of fungal infections on crops. For example, Haikal (2007) reported marked suppression of growth of *Fusarium solanmi* by the aqueous leaves extract of *Azadirachta indica, Ziziphus spina-christi* and *Zygophylum coccineum*. Paul and Sharma (2002), Devanath et al. (2002), Pavlou and Vakalounakis (2005) and Sahayaraj et al. (2006) also reported similar inhibi-

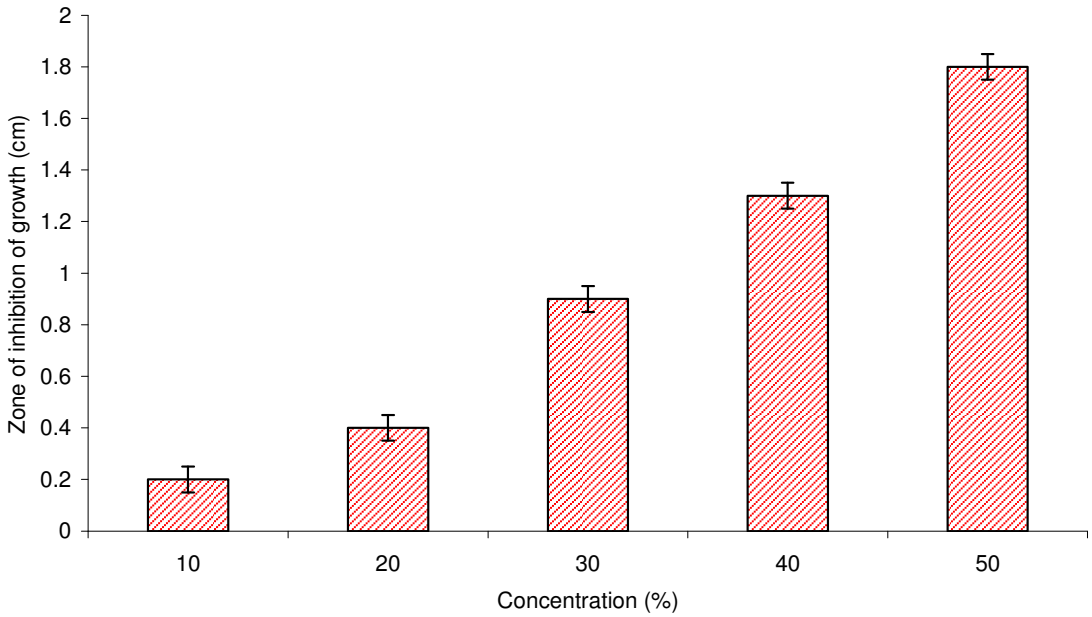

Figure 1. Antifungal activity of *V. amygdalina* extract on *S. ferax,* showing the zone of inhibition (cm).

tory properties of different plant extract against fungal species. Bajwa et al. (2003) reported antifungal activity of aqueous extract of allelopathic weed *Parthenium hysterophorus* against three pathogenic fungi *Drechsler* sp., *Aspergillus niger* and *Phoma hysterophorus.* The author however cautioned on the use of very high concentrations of this plant extract as it may likely enhance the fungal biomass production.

The use of plant extract for prevention and control of fungal infection in fish ponds holds great prospect especially for the peasant local fish farmers who can not afford the high cost of chemicals/fungicides and will also reduce the hazard caused by chemical fungicide in the environment. Normally it is expected that fish treated with malachite green would not be sold until after six months when the effect of the chemical substance must have been degraded. It is not the same as the use of plant extract which is relatively non toxic and the fish can be sold at any time after treatment.

Conclusion

This research has elucidated the potency of the plant extract (*V. amygdalina*) in controlling fungal infection caused by *Saprolegnia* spp in the freshwater fish. Fish pond could therefore be treated regularly with extracts from this plant which have proved effective for combating fungal growth in fish integument. Furthermore, research in the area of extracting the active ingredients of this plant is recommended.

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