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Use of *Euphorbia Kamerunica* (Spurge) Extract in the Control of *Saprolegnia Species* Growth in Incubated Eggs of *Clarias Gariepinus*

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Abstract - *Saprolenia* is a major aquatic fungus affecting the smooth successful operation of fish hatcheries because it causes mortality during incubation. A number of cheap synthetic antifungal products have been prohibited because of human health risks associated with them. A study was conducted to investigate the antifungal potential of *Euphorbia kamerunica* (Spurge) using latex from fresh extract. Fertilized catfish (*Clarias gariepinus*) eggs were incubated in three different treatment concentrations of 100ml, 50ml and 25ml extract with a control.This study on the effectiveness of *Euphorbia kamerunica* (Spurge), a perennial woody shrubs whose caustic milky sap (Latex) is readily soluble in water was extracted as anti fungi agent on the growth of *Saprolegnia species* on incubated eggs of *Clarias gariepinus species*.

Keywords : *spurge, saprolegnia, incubation, fungi extract, concentration.*

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Abstract - *Saprolenia* is a major aquatic fungus affecting the smooth successful operation of fish hatcheries because it causes mortality during incubation. A number of cheap synthetic antifungal products have been prohibited because of human health risks associated with them. A study was conducted to investigate the antifungal potential of *Euphorbia kamerunica* (Spurge) using latex from fresh extract. Fertilized catfish (*Clarias gariepinus*) eggs were incubated in three different treatment concentrations of 100ml, 50ml and 25ml extract with a control.This study on the effectiveness of *Euphorbia kamerunica* (Spurge), a perennial woody shrubs whose caustic milky sap (Latex) is readily soluble in water was extracted as anti fungi agent on the growth of *Saprolegnia species* on incubated eggs of *Clarias gariepinus* species.The study revealed that was a significant difference ($P<0.05$) in the number of eggs infected at different concentration of 100ml (38 ± 10), 50ml (109 ± 8), 25ml (140 ± 14) and 0ml (197 ± 2.186) and the number of eggs hatched 100ml (648 ± 45.863), 50ml (942 ± 21.032), 25ml (1088 ± 52), and 0ml (1950 ± 63). Some of the physico-chemical parameters of the water monitored are pH, Electrical conductivity, Total dissolved solids and Temperature which were within the tolerable range/limit of the fish species. From the results, it was inferred that 25ml concentration of the spurge extract could be used to control the growth of the fungi *Saprolegnia species* in fish hatchery operations as a result of high hatchability rate (1088 ± 52.699) and reduced level of infection.

Keywords : spurge, saprolegnia, incubation, fungi extract, concentration.

I. Introduction

Saprolegnia is ubiquitous in freshwater ecosystem and is the main genus of water moulds responsible for fungi infection of freshwater fish and eggs. *Saprolegnia* has a fairly wide range of temperature tolerance from 3°C to 33°C, this appears to reflect the thermal preferences of the host (Pickering and Willoughby, 1982). Almost every freshwater fish is exposed to at least one species of fungi during its lifetime (Neish, 1991; Noga, 1996), especially from the egg stage through smoltification (Bruno and Wood, 1994). *Saprolegnia* is characterized by an external cotton-like appearance that radiates out in a circular crescent shaped or whorled pattern. It infects eggs by adhesion to and penetration of the egg membrane (Willoughby, 1994) and can spread from dead eggs to live eggs via positive chemotaxis (Bruno and Wood, 1994). The physiological state of fish generally determines if fungi will be successfully established (Neish, 1977). *Saprolegnia* generally invades dead eggs in hatchery condition and fish that have been stressed or otherwise have a weakened immune system (Pickering, 1994). Sudden changes in temperature can also make fish vulnerable to *Saprolegniasis* due to increased physiological stress.

Euphorbia *kamerunica* (Family: *Euphorbiaceae*) is found primarily in the tropical and subtropical region of Africa and America as well as in the temperate zones of the world. Succulent species originate mostly from Africa, the Americas and Madagascar (Bruyns, 2006). Several spurges are grown as garden plants, and the succulent species are used in traditional medicine in China. The latex (milky sap) of *E. kamerunica* is usually white, but in rare cases yellow. The latex exudates congeal within a few minutes on contact with air. Partially or completely congealed latex is often not soluble in water. Several *Euphorbia species* serve as food for the larvae of some Lepidoptera (butterflies and moths) like the spurge Hawk-moth and the Giant Leopard Moth (Carter and Smith, 1988).

The use of some commonly available chemicals such as Hydrogen Peroxide, Formalin and Malachite green etc. in the control of *Saprolegnia species* has been reported to have teratogenic and carcinogenic effects on both fish and man (Doerge *et al*., 1998) and their use has been prohibited. The search for alternative anti-fungal agents for use in fish hatchery has intensified in recent years. The search has been extended to plants that possess fungicidal properties. Mori *et al*. (2002) reported that some plant extracts possesses anti-fungal properties which inhibits the growth of aquatic fungi such as *Saprolegnia species*.

This study was designed to provide baseline information on the potential of *Euphorbia kamerunica* in the control of *Saprolegnia* in catfish hatchery by determining the effect of various concentrations of the extract on the growth of *Saprolegnia* during incubation of the catfish eggs with a view to ascertain the most convenient concentration of the extract that will be most effective in controlling the growth of *Saprolenia*.

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II. Materials and Methods

Gravid brooders of *Clarias gariepinus* were selected and kept in plastic bowls prior to inducement for breeding. Single doses of 0.5ml/kg body weight of ovaprim (Syndel) was injected into the female broodfish intramuscularly below the dorsal fin above the lateral line and were monitored during latency period of that lasted from 10-12 hours before stripping. Thirty minutes prior to stripping, the testes of male brooder fish were surgically excised and upon stripping of the females of their eggs into a bowl, the testes were lacerated and the milt squeezed on to the eggs. The eggs were fertilized by gently mixing them with the milt and freshwater added and the bowl swirled for about 3 minutes at 26^{0}C to allow for proper fertilization. Fertilized eggs were incubated in standard hatching jars.

a) Preparation of Euphorbia kamerunica extract

100g of fresh spurge *Euphorbia kamerunica* was crushed in a porcelain mortar and dissolved in one litre of distilled water. The mixture was sieved through a whatman filter paper and the filtrate stored in a refrigerator at 4°C inside a clean sterile plastic contain and labeled as the stock solution until ready for use. The following volumes: 100ml, 50ml and 25ml were measured out with the aid of a glass measuring cylinder into glass aquaria (hatching trough) containing 10 litres of water each for incubation of the eggs. There was also a control (0ml extract).

There were three replicates for the treatments (100ml, 50ml, 25ml and 0ml) which were distributed in twelve hatching trough of 0.49m×0.49m×0.2m (L×B×D:) size containing 5g of fertilized eggs each for incubation using static renewal method with aerators. They were monitored for three days to observe the growth of *Saprolegnia species* in the different treatment concentrations and their respective replicates.

b) Determination of physico-chemical parameters

The following physico-chemical parameters: pH, Temperature, Electrical conductivity and the Total Dissolved Solid (TDS), of the water in the incubation troughs were monitored with the aid of Hanna HI 9810 meter and their values were recorded.

c) Statistical analysis

The results obtained were subjected to subjected to Analysis of Variance while Duncan Multiple Range test was used to separate means that showed significant level of variance at 5% with the aid of SPSS version 15.

III. Results

The mean number of eggs infected with *Saprolegnia* at different concentration level of plant extract, number of eggs hatched and number of eggs not hatched are shown in Table1.

Table 1 : Mean number of eggs infected, hatched eggs and not hatched eggs.

Parameters	100ml	50ml	25ml	0ml (Control)
Number of eggs infected	38 ± 10^{a}	109 ± 8^{b}	140 ± 14^{b}	197 ± 2^{c}
Number of eggs hatched	648 ± 45^{a}	942 ± 21^{b}	1088 ± 52^{b}	1950 ± 63^{c}
Number eggs not hatched	2352 ± 45^{a}	2058 ± 21^{b}	1912 ± 52^{b}	1050 ± 63^{c}

Values with the same super-script on the same row are not significantly different at P<0.05.

The control (0ml) had the highest mean number of eggs infected (197±2) followed by 25ml (140±14) with 100ml (38±10) being the least infected with *Saprolegnia*. The number of hatched eggs ranged from 648±45 (in 100ml) to 1950±63 (in 0ml). The highest treatment with eggs not hatched was recorded in 100ml concentration (2352±45) followed by 50ml (2058±21 and the least in 0ml (1050±63). Anova revealed that there was significant difference (P<0.05) in the number of eggs infected, number of eggs hatched, and number of unhatched eggs.

The highest mean number of percentage hatchability was recorded in 0ml (In Table 2) concentration (65±2) followed by 25ml concentration (36±1) and the least in 100ml concentration (21.60±1.525). The highest value of percentage unhatched eggs was (78±1) in 100ml concentration followed by 50ml and the least in the control 0ml concentration (34±2). Percentage infected within unhatched eggs ranges from (18±1) to (1 ±0.41).

Table 2 : Percentage hatchability, percentage infection within unhatched eggs and percentage unhatched eggs.

Parameter	100ml	50ml	25ml	0ml
% Hatchability	21 ± 1^{a}	31.40 ± 0.685^{b}	36.27 ± 1.752^{b}	65.00 ± 2.868^{c}
%infection unhatched eggs	1 ± 0.41^{a}	5.31 ± 0.469^{b}	7.30 ± 0.525^{b}	18.98 ± 1.019^{c}
% unhatched eggs	78.41 ± 1.528^{a}	68.60 ± 0.699^{a}	63.72 ± 1.756^{a}	34.99 ± 2.132^{a}

Values with the same super-script on the same row are not significantly different at P<0-05.

There was a significant difference ($P<0.05$) in the percentage hatchability and percentage infection of unhatched eggs but there is no significant difference in the percentage of unhatched eggs.

In Table 3, the value of temperature in all the concentration is the same (31.3±0.000) and the electrical conductivity increase as the concentration level increases with value ranging from 225±0.471 to 274±0.471. Also the Total dissolved solids value range from 113±0.816 to 141±0.471 (0ml to 100ml) while the highest pH value (7.11±.0.005) was associated with 0ml and least value (6.83±0.005) with 100ml.

Table 3 : Mean values of physicochemical parameters of the culture media.

Parameter	100ml	50ml	25ml	0ml
Temperature (°C)	31.3	31.3	31.3	31.3
Electrical conductivity (us/cm)	274±0.471[a]	236±0.471[b]	226±0.471[c]	225±0.471[c]
Total Dissolved Solids (ppm)	141±0.471[a]	119±0.943[b]	114±0.471[b]	113±0.816[b]
pH	6.83±0.005	6.87±0.009	7.02±0.005	7.11±0.005

Values with the same super-script on the same row are not significantly different at P<0.05.

There were significant differences ($P<0.05$) only in the Electrical conductivity and Total Dissolved Solids. Temperature and pH had no significant difference ($P>0.05$).

IV. Discussion

The physico-chemical parameters of the incubation media were adequate. Boyd (1990) and Adeniji (1986) reported that to maintain a good population of Fish, it was necessary to keep the pH between 6.5 and 9.0. The pH values obtained during this study (6.83-7.11) were suitable for fish eggs to develop. The conductivity of the water was observed to decrease as the concentration decreased. The conductivity values in all the treatments were high than that of the control (0ml). Boyd (1990) stated that natural water conductivity ranges from 20 to 1500us/cm. The Electrical Conductivity value obtained ranged between 225-274μs/cm, which was within the range for development and survival of the eggs. The mean temperature obtained from the result showed that it was constant (31.30°C) in all the treatments during the study. Boyd (1990) reported that eggs hatched at temperature between 25°C to 32°C and between 24 to 48 hours. The values of the Total Dissolved Solids obtained were within the range 113-141 ppm. These values agrees with that of Boyd (1990).

The study revealed that there was an inverse relationship between the plant extract and eggs hatchability. There was an increase in the number of infected eggs as the concentration of plant extract decreased. The study further showed that there was a significant difference ($P<0.05$) between the concentrations of the *E. kamerunica extract* used in the experiment. On the infection with *Saproglenia*, the mean number of infected eggs in the control (0ml) was greater when compare to the treatment with the 100ml concentration treatment. Similarly the infection was visibly higher in 0ml concentration. This may probably due to the absence of spurge. This agrees with the report by Khomvilai *et al* (2006) who used Horseradish Extract on *Saprolegnia parasitica.* Moreso, there was a significant difference ($P<0.05$) in the number of eggs hatched at different concentration level. The result indicated that the number of eggs hatched increases as the concentration level decreases. The control (0ml) recorded highest mean value of hatchlings compare with the 100ml treatment concentration.

There was a significant difference ($P<0.05$) in the number of eggs unhatched at different treatment concentration levels. The highest mean number of unhatched eggs was recorded in 100ml treatment concentration while the lowest was in control thus indicating that the extract of *E. kamerunica* had aborticidal effect on the fertilized eggs of *C. gariepinus.* There was a direct relationship for as the concentration level the extract of *E. kamerunica* increased, the percentage of unhatched eggs also increased.

On the percentage infection of unhatched eggs, there was a significant difference in the percentage infection of unhatched eggs ($P<0.05$). The highest treatment concentration of the extract of *E. kamerunica* had the lowest mean number of infected unhatched eggs while the control had the highest mean number of infected unhatched eggs. This shows that the spurge (*E. kamerunica*) inhibited the development of *Saprolegnia species* on the eggs of catfish. This finding agrees with Isshiki *et al* (1992) and Mori *et al* (2002) who reported that allyl isothiocyanate (AIT) present in *E. kamerunica* had strong antifungal activity against aquatic fungi including *S. parasitica.*

V. Conclusion

From the result obtained in the present study, it can be concluded that 25ml and 50ml concentration of spurge (*Euphorbia kamerunica*) could be used to control the growth of *Saprolegnia species* in fish

hatchery operations since the level of hatchability was high and degree of infection reduced thus making it a good candidate to be considered as an antifungal agent.

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