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Effect of MS (Methylated Spirit) as a Disinfectant and Antisticking Agent on Hatchability of *Clarias gariepinus* Eggs and Survival of the Hatchlings

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Abstract: African catfish *Clarias gariepinus* brood stocks were bred by normal induced breeding method. After fertilization, the eggs were treated with 0.40%, 0.50% and 0.60% concentration levels of MS (methylated spirit) and a control treatment without MS (0%). The objective was to remove the stickiness of the egg outer vitelline membrane to improve hatchability and survival of the hatchlings. Two batches of the treatments were carried out according to treatment duration of 5 s and 10 s. The eggs hatched normally with the highest hatching percentage of 62.31% in eggs treated with 0.40% MS concentration in 5 s treatment and 61.92% in the same concentration for 10 s. The control treatment of 0.00% MS treatment gave the lowest hatchability of 49.69% at 5 s and 45.71% at 10 s exposure time. Growth performance of the hatchlings improved in eggs treated than those not treated. Those treated had higher weight gain and percentage specific growth rates than those not treated. Percentage survival ranged from 75% to 90 % in both treated and untreated groups. MS can therefore be safely used in fish hatchery to prevent egg stickiness to improve hatchability and larval development.

Key words: *Clarias gariepinus*, egg, MS, antisticking.

1. Introduction and Literature Review

Aquaculture is currently one of the fastest growing food production systems in the world with developing countries contributing significantly [1]. With stagnating yields from many capture fisheries and increasing demand for fish and fishery products, there is high expectation for aquaculture to increase its contribution to the world's aquatic food production. It is also hoped that aquaculture will continue to strengthen its role in contributing to food security and poverty alleviation in many developing countries [2]. In view of the significant nutritional, social, economic and environmental benefits associated with aquaculture practices, and the prospects for further development and expansion of the sector, efforts for sustainable development of aquaculture are important [3]. Fish is widely known as one of the cheapest sources of protein for human consumption especially in areas where cattle and beef production is expensive [4]. According to Ref. [5], the demand for fish protein is rising and there is need to increase production of food including fish to feed the population. Fish protein has many advantages over beef, mutton and pork as it supplies essential nutrients such as omega-3 fatty acids and iodine which are lacking in other animal proteins [6].

One major constraint to aquaculture development in developing countries is inadequate supply of good quality fish seed. Successful aquaculture begins with stocking adequate and good quality fingerlings in ponds with optimum conditions that enhance rapid growth and harvest within short time [7]. For maximum production, especially for commercial purpose, the fish farmer has to obtain adequate number of fingerlings to meet the production goals.

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Various techniques have been adopted to control reproduction in cultured fish species with the aim of achieving high fertilization of eggs to produce large number of fingerlings [7]. In artificial fertilization, there is the risk of water touching the eggs before fertilization thereby activating the sticky surface of the eggs (outer vitelline layer) causing them to stick together interfering with fertilization. To prevent this, breeders often use chemical substances such as Cabamide made by mixing 30 g of urea and 40 g of sodium chloride in 10 L of distilled water or saline solution (30 g NaCl + 1 L distilled water) as fertilization solution. After fertilization, the eggs are treated with another solution called antisticking solution such as tannin solution (8-15 g of tannin dissolved in 10 L distilled water) for 3-5 s and then washed thoroughly and transferred to incubators [7, 8]. Some producers use inert materials such as powder milk [9]. Some use alcalase such as talc. A combination of milk and talc gave 80%-90% antisticking while talc alone gave about 70% result [10]. Papain used at 0.2% solution removed the gelatinous matrix of fish egg and improved hatchability and reduced incidence of diseases [11].

MS (methylated spirit) also known as denatured alcohol comprises of ethanol with additives chosen to make it undrinkable. It is often deliberately coloured blue or mauve. The main household use of MS is for camping stoves and burners. It has a number of benefits in that it is inexpensive and can be transported without the need for special containers. It is also not poisonous to the skin. Some types of MS are produced in a jelly form and are dyed. Other domestic uses of MSs are as a cleaning product, to remove stains from clothes or fabrics, windows, electronic equipments and furniture, as a treatment for coldsores, and as a scratch remover. Industrial uses of MS are varied including use as a sanding aid, to control mealy bugs, as a solvent and in the production of biodiesel fuel [12]. Small amount of MSs are used in products such as toothpaste and mouthwash. Medical uses of MS are based on its ability to disinfect skin and to remove fungal infections. Skin can be wiped with MS before injection is given or prior to minor surgery. MS is also used to preserve biological specimens [12]. Thus, it can be used as disinfectant, sterilizer and cleaner of organic substances. It is this property that was applied in this research objective which was to disinfect and clean off sticking substances on *Clarias gariepinus* eggs and improve hatchability and survival of the hatchlings.

Clarias gariepinus is the most common catfish cultured in Nigeria. It is the most suitable species for aquaculture due to its hardiness, resistance to handling stress, disease and high stoking density. It also has high and fast growth rate and good feed conversion ability. They are highly adaptable to artificial environment and feed [13]. This research was therefore conducted to test the effect of MS as antisticking agent on incubation and hatching of *Clarias geriepinus* eggs to improve fingerling production.

2. Materials and Methods

Five ripe brooders (two males and three females) ranging from 1.30-1.80 kg were purchased from a fish farm and transported in 50 L open plastic cans to the hatchery. On arrival, they were disinfected with 5% salt bath for 5 min [1] and then allowed to acclimatize for 5 days. They were fed with 40% crude protein commercial feed. Water quality parameters were maintained at optimum required limit for fresh water fish culture. After acclimatization, one male and one female of sizes 1.68 kg and 1.80 kg TBW (total body weight), respectively, and fully ripe were selected and bred by normal induced breeding methods [7]. The female was injected with ova prim hormone and after the latency period of 8 h, eggs were stripped into a plastic bowl. Fecundity was determined by multiplying the number of eggs in a subsample of 5 g by the total weight of eggs stripped. The male was dissected to remove the testis. A saline solution was prepared to serve as fertilization solution. Milt was mixed with the

saline solution in a petri dish and poured onto the eggs and stirred gently with a plastic spoon to fertilize the eggs. After fertilization, the eggs were divided into eight batches, each with about 1,200 eggs and used for the three MS treatments and a control (T1: 0%; T2: 0.40% MS; T3: 0.50% MS; and T4: 0.60% MS), each replicated three times. Each treatment was subjected to two exposure times of 5 s and 10 s, to determine the most effective exposure time. This gave a total of eight treatments replicated three times or 4 × 2 × 3 experimental designs.

Methylated spirit containing 95% alcohol and 5% wood naphtha was used at 0.40%, 0.50% and 0.60% concentrations, respectively. The concentrations were obtained by diluting 4, 5 and 6 mL of MS in 96, 95 and 94 mL of distilled water, respectively. Twenty four (24) plastic aquaria with size 53 cm × 36 cm × 42 cm were used for incubation. After fertilization, with the aid of two assistants in the lab, 18 glass beakers were set, three for each treatment replicated three times for 5 s exposure and repeated for 10 s exposure time. Based on the fecundity determined which gave about 400 eggs per 0.5 g of egg weight, 0.5 g of fertilized eggs were quickly measured and poured into the beakers according to treatments exposure times, respectively. The eggs were mixed thoroughly by gently agitating the glass beaker containing the eggs and the chemical for 5 s and 10 s, respectively and then rinsed with pure water and transferred to the incubators. Incubation was monitored for 24 h with gentle water flow through in addition to aeration with aquarium electric pump. Water quality parameters particularly DO (dissolved oxygen), pH, temperature, conductivity and ammonia were measured during incubation and rearing of the hatchlings. Percentage hatchability was calculated as the number of hatched larvae against the estimated number of eggs incubated using the formula:

$$\text{Percentage hatchability} = \frac{\text{Number of hatched larvae}}{\text{Total eggs incubated}} \times 100$$

This was used to determine cumulative mean percentage hatching for each treatment.

The fry were reared in the incubation tanks for 4 weeks after which 60 hatchlings were stocked in each rearing tank and replicated three times giving 180 hatchlings for each treatment. They were then reared for another 8 weeks. Water flow through in addition to aeration with aquarium electric pump was also maintained in the nursery tanks. Growth parameters in terms of weight and length increase, *SGR* (specific growth rate), percentage mortality and survival of the fish were recorded fortnightly. Water quality parameters were also measured fortnightly.

SGR was determined by the formula:

$$SGR = \frac{WF - WI}{T} \times 100$$

WF = final body weight of fish;

WI = initial body weight of fish;

T = time in days.

The percentage survival rate for each treatment was determined by the formula:

$$\text{Percentage survival} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Results of hatchability, length and weight gain, survival rates and water quality parameters for all the treatments were analyzed by one way analysis of variance using SAS (statistical analysis system), while means were compared for significant differences at 0.05 significant levels using Duncan's multiple range tests using SPSS version 15.0.

3. Results

The female brood fish used weighed 1.80 kg with egg weight of 428.12 g and had fecundity of 85,6241 eggs. Results of hatchability and percentage hatchability of eggs treated with MS as antisticking agent before incubation are presented in Table 1.

The highest hatching of 747.75 ± 3.25 (62.31%) was obtained from eggs treated with 0.4% MS concentration at 5 s exposure and 743.01 ± 3.14 (61.92%) hatching

Table 1 Hatchability of *Clarias gariepinus* eggs exposed to varying concentrations of MS for 5 s and 10 s.

Exposure time (s)	Treatment	Percentage MS concentration (%)	Mean total egg incubated	Mean hatching	Percentage hatching (%)
5	1	0.00	1,200	596.34 ± 2.31	49.69
	2	0.40	1,200	747.75 ± 3.25	62.31
	3	0.50	1,200	679.32 ± 2.41	56.61
	4	0.60	1,200	620.37 ± 2.02	51.69
10	1	0.00	1,200	548.49 ± 2.23	45.71
	2	0.40	1,200	743.01 ± 3.14	61.92
	3	0.50	1,200	660.66 ± 2.36	55.06
	4	0.60	1,200	652.32 ± 2.21	54.36

at 10 s treatment. The control, 0.00% MS concentration, gave the lowest hatching of 548.49 ± 2.23 (45.71%) at 10 s and 596.34 ± 2.31 (49.69%) at 5 s treatment. Results of comparison of hatchability within and between treatments in terms of duration of exposure (5 s and 10 s) are presented in Table 2.

Comparison within treatments indicated significant difference ($P < 0.05$) between all the treatments, while comparison between treatments, that is between 5 s and 10 s treatments, indicated no significant difference ($P > 0.05$) between 0.4% and 0.5% MS treatments, but the control treatment (0.00%) MS and 0.6% MS were significantly different from each other ($P < 0.05$). Table 3 shows the growth performance of the fingerlings hatched from eggs treated with MS for 5 s. Weight gains in fingerlings of 5 s treatment were not significantly different from each other ($P > 0.05$) though 0.6% MS treatment had the highest weight gain.

Table 4 shows the growth performance of the fingerlings hatched from eggs treated with MS for 10 s. In 10 s treatment, the control (0.00%) had the lowest weight gain showing significant difference from other treatments. Weight gains from 0.4%, 0.5% and 0.6% were not significantly different ($P > 0.05$).

Results of cumulative mortality and survival of the fingerlings during experiment are presented in Table 5.

Table 2 Comparison of hatchability within and between treatments in terms of exposure time and MS concentration.

Treatment	Percentage MS concentration	Within treatments		Between treatments	
		5 s	10 s	5 s	10 s
1	0.00	596.34 ± 2.32^{d}	548.49 ± 2.23^{d}	596.34 ± 2.32^{a}	548.49 ± 2.23^{b}
2	0.40	747.75 ± 3.25^{a}	743.01 ± 3.14^{a}	747.75 ± 3.25^{a}	743.01 ± 3.14^{a}
3	0.50	679.32 ± 2.41^{b}	660.66 ± 2.36^{b}	679.32 ± 2.41^{a}	660.66 ± 2.36^{a}
4	0.60	620.37 ± 2.02^{c}	652.32 ± 2.21^{c}	620.37 ± 2.02^{b}	652.32 ± 2.21^{b}

[a], [b], [c] and [d]: level of significance from ANOVA (analysis of variance) a > b > c > d;
Values in column with same superscripts are not significantly different ($P > 0.05$);
Values in row with same superscripts are not significantly different ($P > 0.05$).

Table 3 Growth performance of *Clarias gariepinus* fingerlings hatched from eggs treated with MS for 5 s and reared for 12 weeks.

Treatment	MS concentration (%)	Mean initial total length (cm)	Mean final total length (cm)	Mean initial total body weight (g)	Mean final total body weight (g)	Mean length gain (cm)	Mean weight gain (g)	Specific growth rate (%)
1	0.00	1.50 ± 0.10	8.20 ± 1.02	2.65 ± 0.05	28.50 ± 0.65	6.70	25.85	2.15
2	0.40	1.80 ± 0.12	8.56 ± 1.21	2.78 ± 0.08	30.22 ± 0.85	6.76	27.44	2.29
3	0.50	1.50 ± 0.10	7.85 ± 0.65	2.66 ± 0.06	25.30 ± 0.50	6.35	22.64	1.89
4	0.60	1.75 ± 0.11	8.44 ± 1.20	2.80 ± 0.08	32.45 ± 1.01	6.69	29.65	2.47

Table 4 Growth performance of *Clarias gariepinus* fingerlings hatched from eggs treated with MS for 10 s and reared for 12 weeks.

Treatment	MS concentration (%)	Mean initial total length (cm)	Mean final total length (cm)	Mean initial total body weight (g)	Mean final total body weight (g)	Mean length gain (cm)	Mean weight gain (g)	Specific growth rate (%)
1	0.00	1.52 ± 0.10	7.85 ± 1.55	2.70 ± 0.06	25.05 ± 0.40	6.33	22.35	1.86
2	0.40	1.85 ± 0.13	8.25 ± 1.56	3.08 ± 0.13	32.00 ± 0.50	6.40	28.92	2.41
3	0.50	1.77 ± 0.11	8.75 ± 1.58	3.09 ± 0.15	36.10 ± 0.65	6.98	33.01	2.75
4	0.60	1.75 ± 0.11	8.65 ± 1.45	2.85 ± 0.11	34.17 ± 0.70	6.90	31.32	2.61

Table 5 Cumulative mortality and survival of *Clarias gariepinus* fingerlings hatched from eggs treated with MS for 5 s and 10 s and reared for 12 weeks.

Exposure time (s)	Treatment	MS concentration (%)	Total initial stocking	Total mortality	Mortality (%)	Total survival	Survival (%)
5	1	0.00	180	18	10	162	90
	2	0.40	180	27	15	150	85
	3	0.50	180	18	10	162	90
	4	0.60	180	36	20	144	80
10	1	0.00	180	27	15	150	85
	2	0.40	180	36	20	144	80
	3	0.50	180	45	25	135	75
	4	0.60	180	36	20	144	80

Mortality was very minimal during the rearing period. The highest cumulative mortality was 45 recorded in 0.5% MS treatment under the 10 s exposure time. The lowest cumulative mortality was 18 recorded in the control (0.00%) and 0.5% MS treatments respectively under the 5 s exposure time.

Results of water quality parameters measured in all the treatments were very similar. Their means were therefore calculated. Mean pH was 7.87 ± 1.02; mean DO was 6.24 ± 0.23 $mg \cdot L^{-1}$; mean temperature was 27.92 ± 2.12 °C; mean conductivity was 354.36 ± 52.03 μS and mean NH_3 (ammonia) was 0.038 ± 0.008 $mg \cdot L^{-1}$.

4. Discussion

Treatment of fertilized fish egg with MS did not have any negative effect on egg quality and viability. The treated eggs had higher percentage hatching than those not treated (control). Though no significant difference ($P > 0.05$) was observed between hatchability of eggs treated with 0.4% and 0.5% MS for 5 s and 10 s, respectively, treatment with 0.4% MS gave the highest hatchability both at 5 s and 10 s exposure (Table 1). The eggs became discrete and non sticky after treatment and this allowed easy spread of eggs in the incubator. It also facilitated easy separation of hatchlings from egg cases. According to Ref. [14], the cortical envelope of fish egg is rich in protein and acid phosphates which are responsible for stickiness of eggs when in contact with water. Elimination of stickiness is therefore critical to reduce adhesive effect, increase embryonic development, hatching and easy management of the incubator. According to Ref. [15], egg stickiness does not occur when in contact with saline solution. The use of powdered milk as organic dissolvent to remove egg stickiness has been reported by Soin [9]. Linhart et al. [16] reported the use of enzymes. According to Ref. [10], a combination of whole milk and talc gave 80%-90% anti stickiness and hatching while whole milk only gave 70% result.

Treatment of eggs with MS had positive effect on the growth performance of the hatchlings. Specific growth rate and weight gain were normal and were observed to be even higher in treatments 2, 3, and 4 than the control (treatment 1) under the 10 s exposure

time (Table 4). Survival of the hatchlings was also not affected as 75%-90% survival was recorded from all the treatments (Table 5).

5. Conclusion

Results of the experiment showed that MS at 0.40% concentration removed stickiness of fish eggs most effectively at both 5 s and 10 s exposure. The conclusion therefore was that MS can be used in fish hatchery to remove egg stickiness and enhance fingerling production.

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