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The Use of *Moringa oleifera* Seed Powder for Treating Recirculatory Aquaculture System (RAS) Discharge

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Abstract:
This study investigated the use of Moringa oleifera seed powder, in water treatment in a Recirculatory Aquaculture System (RAS). RAS is one of the systems of fish rearing in which discharged from the fish pond is treated and then reuse. RAS must effectively remove solids (settle able and suspended), control ammonia and nitrite-nitrogen concentrations, and dissolved gasses. Dried Moringa oleifera drum sticks were harvested, unshelled, dehusked and ground. The oil was removed and dried at room temperature. Four culture tanks were used, in which three were used as experimental tanks and one, as control. A Randomized Complete Block Design (RCBD) in a 3 by 2 factorial was used. The three experimental tanks were treated with 12 g / 90 litres, 25 g / 90 litres and 40 g / 90 litres of Moringa oleifera seed powder. For each quantity of the seed powder, 3 hrs, 4 hrs and 5 hrs retention times were used. The weight of the fish was recorded after each treatment using digital weighing scale. The highest increase in the weight of the fish was recorded when 40 g / 90 litres of the seed powder was used at 3 hrs. Analysis of Variance (ANOVA) was conducted at 5% significance level. Calculated F was greater than Tabulated F for both replicate (5.217 >2.084) and treatment (5.254 > 2.084). This implies that Moringa oleifera has a positive effect on the treatment of aquaculture discharge

Keywords: *Recirculatory Aquaculture System (RAS), Moringa oleifera seeds-powder, coagulants, ammonia, nitrate ion and nitrites ion*

1. Introduction

Naturally occurring coagulants are usually presumed safe for human health. Some studies on natural coagulants have been carried out and various natural coagulants were produced or extracted from microorganisms, animals or plants. One of these alternatives is *Moringa oleifera* seeds. It is a native tree of the sub-Himalayan parts of North-west India, Pakistan and Afghanistan. As cited by Bichi, (2013), the use of natural materials of plant origin to clarify turbid surface waters is not a new idea. Many believe the Biblical book of Exodus(15:23-27) is the earliest written reference to what is most likely Moringa being used to purify water (probably *Moringa peregrina*,): "And the people murmured against Moses, saying, "What shall we drink?" And he cried unto the Lord; and the Lord showed him a tree, which when he had cast into the waters, the waters were made sweet…." (NRC, 2006)

Moringa grows best in the hot, semi-arid tropics. It is drought-tolerant and grows with rainfalls of 250-1500 mm (10-60 in) per year. Altitudes below 600 m (2000 ft) are best for Moringa; however, it grows up to 1200 m (4000 ft) in some tropical areas and has been recorded growing at 2000 m (6000 ft). *M. stenopetala* in Ethiopia is regularly found at altitudes up to 1800 m (6600 ft). At Proyecto Biomasa in Nicaragua, they found the effective altitude limit for growing Moringa to be 500 m (1640 ft), (This might be higher nearer the equator). They also say that excessively windy conditions cause the tree to dry out (Beth, 2005).

Moringa oleifera is a perfect example of a so-called "multipurpose tree". Earlier studies by Grabow *et al*. (1985) have found Moringato be non-toxic, and recommended it to use as a coagulant in developing countries. The use of Moringahas an added advantage over the chemical treatment of water because it is biological and has been reported as edible. According to Muyibi and Evison (1994), hardness removal efficiency of *Moringa oleifera* was found to increase with increasing dosage.

Moringa oleifera seeds act as a natural absorbent and antimicrobial agent as their seeds contain 1% active polyelectrolyte's that neutralize the negatively charged colloid in the dirty water (Eilert *et al.,* 1985). This protein can be nontoxic natural polypeptide for sedimentation of mineral particles and organics in the purification of drinking water. These seeds also act as antimicrobial agent against variety range of bacteria and fungi. The seed contain number of benzyl isothiocyanate and benzyl glucosinolate which act as antibiotic (Eilert *et al.,* 1985). It is believed that the seed is an organic natural polymer. The active ingredients are dimeric proteins. The protein powder is stable and totally soluble in water. The coagulation mechanism of the *Moringa oleifera* coagulant protein has been described as adsorption and charge neutralization and inter-particle bridging (Sutherland *et al.,* 1990). Flocculation by inter-particle bridging is mainly characteristic of high molecular weight polyelectrolytes.

Due to the small size of the *Moringa oleifera* coagulant protein, a bridging effect may not be considered as the likely coagulation mechanism (Muyibi and Evison, 1994). Moringaseeds possess antimicrobial properties therefore recombinant protein in the seed is able to flocculate Gram-positive and Gram-negative bacteria cells. In this case, microorganisms can be removed by settling in the same manner as the removal of colloids in properly coagulated and flocculated water. On the other hand, the seeds may also act directly upon microorganisms and result in growth inhibition. Antimicrobial peptides are thought to act by disrupting the cell membrane or by inhibiting essential enzymes. Folkard and Sutherland (2001) reported that *Moringa oleifera* seeds could inhibit the replication of bacteriophages.

Amagloh and Benang (2009) in the study of comparison of effectiveness of *Moringa oleifera* and alum, at 95.0% confidence level, showed that there was significant difference among all the treatments at the varying loading dose concentrations on the pH. Increase in alum dosage leads to increase in acidity of water but the reverse was observed with the Moringa treatment. The use of natural materials of plant origin to clarify turbid water is not a new idea cited by Sani (1990), Ndabigengesere *et al*., (1995), Folkard and Sutherland (2001). Among all the plant materials that have been tested over the years, powder processed from the seeds from *Moringa oleifera* has been shown to be one of the most effective as a primary coagulant for water treatment and can be compared to that of Alum (conventional chemical coagulant). It was inferred from their reports that the powder has antimicrobial properties. Also, Oria-Usifo (2014) in a comparative coagulation studies between alum and *Moringa oleifera* concluded that compared to the commonly used coagulant chemicals, *Moringa oleifera* has a number of advantages: it is of low cost, it produces biodegradable sludge,it produces lower sludge volume and it does not affect the pH of the water.

The above listed advantages make *Moringa oleifera* consumer and environmentally friendly low cost alternative with significant potential both in developing and developed countries. One major advantage that *Moringa oleifera* seed has over all other coagulants is that it's readily available at cheaper amount. It can be propagated from seed or from cutting of the stem. Within 3 years of planting one tree will produce 300 to 400 pods every year and a mature tree can produce up to 1000 pods. Frequent pruning of the growth tips will maintain and increase leaf growth and the height can be controlled to make harvesting easier (HDRA, 2002).

The cheapest source of good protein in the diet of a greater population of Nigerians remains fish, and the nutritional value of fish is impressive because of its rich display of amino acids (protein/body builder). Fish has a greater impact on the masses because of its affordability as a good protein source, as it is cheaper than beef, pork and other meat sources. According to Fabian (2016), in developing countries including Nigeria, about 60 per cent of the protein requirement comes from fish. Though, Nigeria's per capita fish consumption of 11kg against a global average of 21kg is still low, there is yet not enough supply to meet national demand.

According to the Federal Department of Fisheries (FDF), available statistics on fish production and supply in Nigeria have shown a consistent shortfall in the supply of fish, either farmed fish via aquaculture or capture from the wild, in spite of the effort in the past few years to increase production. National demand in 2012 was 2,000,000 tonnes, with supply of 690,000 tonnes and a deficit of 1,329,000 tonnes; for 2014, there was demand of 2,175,000 tonnes, supply of 730,000 tonnes, leaving a deficit of 1,404,000 tonnes (Fabian, 2016).

He further reported that, though there was an increase in fish supply over the succeeding years, the growing population seemed to have paled the effort, especially from aquaculture. The development of the fish industry will increase local production of fish and save much of the foreign exchange being used for fish importation. Specifically, it has a special role of ensuring food security, alleviating poverty and provision of animal protein. Nigeria can substitute fish importation with domestic production to create jobs, reduce poverty in rural areas where 70% of the population lives and ease the balance of payments.

Recirculating aquaculture systems (RAS) is also known as water reuse systems. RAS are commonly found in aquaculture production facilities, public aquaria, tropical fish wholesale operations, and retail pet stores (Yanong, 2003). RAS have the advantages of reducing overall water usage, as compared to flow-through systems, and improving control of many aspects of nutrition and water quality if properly managed. However, recirculating systems have their own unique set of problems. They are not a substitute for good management and often require more time and care than flow-through systems. They certainly are not a "silver-bullet" for eliminating fish diseases (Yanong, 2003). In addition, defining the primary intended use of a system will help optimize efficiency and utility in the design process. The major problem of aquaculture in Nigeria is lack of adequate quality water. This project will help the fish farmers to treat and reuse the water thereby eliminating shortage of adequate water.

Recirculating aquaculture systems are designed to provide excellent culture conditions, supporting high densities of the species being cultured, providing adequate feed, and maintaining good water quality. Poor water quality, while not necessarily lethal to the crop, results in reduced growth and stress related diseases. Critical water characteristics include concentrations of dissolved oxygen, un-ionized ammonia-nitrogen, nitrite/nitrate-nitrogen, carbon dioxide, pH, alkalinity, and chloride levels. The by-products of metabolisms include carbon dioxide, ammonia-nitrogen, particulate and dissolved fecal solids, and uneaten food (Akegbejo-Samsons *et al.,* 2013).

The major objective of this research was to investigate *Moringa oleifera* seed powder in treating Aquacultural waste

2. Review of Literature

2.1. Basic Stages in an Aquaculture System

2.1.1. Culture Tank

About the only thing that Aquacultural Engineers can unanimously agree upon is that a body of water is required to raise fish. How this body of water should be contained is open to considerable debate? Should the fish be reared in ponds, raceways, or tanks? If tanks are chosen, to what geometry should the tank conform? How deep should a tank be or more descriptively, what should the tank diameter to depth ratio be? A system that does not effectively and quickly remove manure from the culture water will never produce fish economically. All individual components of the system will fail to perform efficiently. Thus, the first question that one should ask is whether or not the tank will effectively clean, because of this, most farmers prefer circular tanks

Secondly, the capability to effectively manage the fish within the tank vessel is the next question that must be addressed.

Intensive tank culture offers several advantages over the use of ponds. The high density of fish in tanks disrupts breeding behavior and allows male and female tilapia to be grown together. If cultured together, females will be half the size of the males (340g vs. 680g) (DeLong *et al*., 2009). Females will not reach marketable size at the same time as the males unless there is a market for the smaller fish. In ponds, mixed-sex populations breed so prolifically that parents and offspring compete for food, individual fish growth is reduced, and the population becomes stunted.

A well-managed pond not fertilized nor aerated can support 335 – 561 kg of fish per hectare while fertilized and aerated can support 896 – 1,121 kg per hectare (Malone, 2011). Dunn (2014) also stated that 635 fingerlings per hectare is advisable, thereafter sorting is needed as the fishes grow. Using tanks allows the fish culturist to manage stocks and have a good deal of control over environmental parameters (e.g., water temperature, dissolved oxygen concentration, pH, and waste) that can be adjusted to promote maximum production. In addition, feeding and harvesting operations require less time and labor than in ponds. In small tanks it is practical and economical to treat diseases with therapeutants applied to the culture water. Intensive tank culture can produce high yields, year-round, on small parcels of land.

Tank culture also has disadvantages, because fish have little natural food in tanks, they must be fed a complete diet containing the protein, vitamins and minerals necessary for good growth. Finishing (often referred to as on-growing) feed for tank culture operations generally has more protein than that used for pond culture, usually 32 to 40 percent protein (DeLong *et al*., 2009).

2.1.2. Sedimentation Tank

Sedimentation is the process by which particles in suspension in liquid form sediment. Also, sedimentation in formation of rocks is the process by which rocks are formed by the accumulation of sediment (Encarta, 2009). One of the stages of water treatment is sedimentation. Raw water in this case is allowed to flow at the silting velocity to allow for sedimentation. Most times, a coagulant must have been added to the raw water to allow for flocculation and coagulation thereafter leaving the water for hours to settle. When a river flows, it carries some particles with it which are been deposited along the path of flow.

In designing for bigger sedimentation tank, Stokes' law for terminal settling velocity can be used.Stokes' law for terminal settling velocity states that net gravitational force is equal to drag force. This is shown in equations 1 and 3

$$\pi D^3 g(\rho_s - \rho_l) / 6 = 3\pi D\eta U \quad (1)$$

Stokes drag force:

1/3 due to pressure, 2/3 due to shear stress

$$U = \frac{H}{t}\text{To} \quad (2)$$

obtain diameter from height and settling time:

$$D^2 = ((18\eta H) / (\rho_s - \rho_l)) \quad gt \quad (3)$$

D = diameter (m)

g = gravitational constant (m/s^2)

ρ_s = effective solid density (kg/m^3)

ρ_l = liquid density (kg/m^3)

η = liquid viscosity (kg/ms)

U = settling velocity (height/time) (m/s)

Ommen (2010),

2.1.3. Sand bed filter tank or Biofilter

A sand bed filter is a kind of depth filter. Broadly, there are two types of filter for separating particulate solids from fluids: Surface filters, where particulates are captured on a permeable surface and Depth filters, where particulates are captured within a porous body of material. In addition, there are passive and active devices for causing solid-liquid separation such as settling tanks, self-cleaning screen filters, hydrocyclones and centrifuges. There are several kinds of depth filter, some employing fibrous material and others employing granular materials. Sand bed filters are an example of a granular loose media depth filter. They are usually used to separate small amounts (<10 parts per million or <10 g per cubic metre) of fine solids (<100 micrometres) from aqueous solutions. In addition, they are usually used to purify the fluid rather than capture the solids as a valuable material. Therefore, they find most of their uses in liquid effluent (wastewater) treatment.

Slow sand filtration is a simple technology used for pathogen and particle removal in drinking water purification (Langenbach *et al.,* 2009). The slow sand filter was also tried in biological denitrification of drinking water (Aslan & Cakici, 2007). The physical, chemical and biological means of removing bacteria and suspended particles in raw water can be done using slow sand filter (Bauer et al., 2011; Ijadunola et al., 2011). Formation of *schmutzdecke* or colmation layer on the surface of the sand bed as filtration progresses is considered as the important process of purification mechanism of slow sand filters (Farooq, 1994).

Conventional fluidized-sand biofilters (FSBs) have been widely adopted in North America, especially in recirculating systems that must reliably maintain excellent water quality to produce species such as salmon smolt (Summerfelt *et al*., 2004), FSBs can typically remove 50–90% of the ammonia each pass and thus maintain total ammonia-nitrogen and nitrite-nitrogen concentrations in their discharge of 0.1–0.5 mg/L and <0.1–0.3, respectively, in cold- and cool-water aquaculture systems (Heinen *et al*., 1996; Summerfelt *et al.,* 2004).

3. Methodology

Four (4) culture tanks of 0.8 by 0.8 by 0.8m were constructed according to Malone (2011) and Dunn (2014). Thirty – five juveniles were introduced into each of the culture tanks. A picture of the culture tank is displayed in Figure 3.8. One of the tanks serves as control in which the water is not being treated but changed at the required time. Ninety litres of water from borehole was introduced and the fishes were fed twice daily. Due to change in environment of the fish, feeding rate was low for the first 18 days. Therefore, water used 7days before been changed but on the 19^{th} day, feeding rate increased.

The numbers of days the water uses before change depends on the turbidity level required and this result from the feeding habit of the fishes. The water was stirred rapidly for 2minutes and slowly for 10mins to allow even distribution of the powder. The water was allowed to sit undisturbed (2 to 5 hours). It was noticed after the addition of the powder that the dissolved solids increased. This resulted into coagulation of the particles in which formed floccs which later settled down. Figure 1 shows the de – oiled Moringa seed powder used and Figure 2 shows two of the culture tanks

Figure 1: De-oiled Moringa powder

Figure 2: Culture Tank

The sand filter tank was arranged from the smallest particle diameter starting from fine sand to gravel and granite. Using a pump of 1hp, the settled water was pumped into the sand filter. The sand filter helps to filter the suspended solids in the water and there by remove any suspended particles. The treated water was then allowed to flow under gravity into the culture tank. Accurate quantity of water loss along the process was substituted with clean water.

A randomized complete block design in a 2 factors 3 levels (3 by 2) factorial experiment was used to carry out the experiment and the result is subjected to ANOVA. Variation in the quantity of Moringa powder and sedimentation hours were the levels while the main factors were the Moringa powder and sedimentation hours.

4. Results and Discussion

Table 1 shows the result of the fish and table 2

M (g/90 l)	HOS	Tank 1 (g)			Tank 2 (g)			Tank 3 (g)			Control Tank (g)		
12	3	36.9	35.4	36.1	35.3	35.5	35.6	29.2	29.0	30.4	32.6	32.0	31.1
	4	36.0	37.6	39.5	35.7	36.1	38.8	29.4	29.0	30.1	31.3	35.4	37.6
	5	42.6	44.8	49.6	42.0	45.2	48.5	36.1	37.2	37.1	40.5	40.9	42.6
25	3	52.9	53.4	55.7	51.9	52.5	55.7	42.0	45.2	48.5	45.6	46.8	50.5
	4	58.8	59.4	62.4	55.7	58.4	60.1	52.9	53.4	55.7	52.9	54.5	55.7
	5	66.4	69.4	72.3	65.5	67.9	73.0	60.1	62.4	69.5	56.5	61.6	72.4
40	3	77.3	80.2	84.5	81.4	85.4	90.5	75.8	76.3	81.2	81.2	82.5	84.9
	4	91.4	95.2	101.5	90.4	99.4	95.4	85.5	88.9	89.8	90.5	91.3	92.6
	5	107.5	107.8	108.2	100.2	104.5	105.2	93.5	95.4	101.5	94.7	102.3	104.5

M - Moringa

HOS - Hours of Sedimentation

Table 1: Weight of the Fish

SOV	DF	SS	MS	Fcal	Ftab
Rep	8	101830.248	12728.78	5.216852	2.084
Trt	8	102550.849	12818.86	5.253769	2.084
Error	64	156155.841	2439.935		
Total	80	360536.938			

Table 2: ANOVA table

From the results, it was noticed that all the fish in the tanks treated with *Moringa oleifera* seed-powder had a steady growth but in the control tank, the growth was fluctuating and at the end of the experiment, the total weight of the fish in the treated tank were higher than the fish in the control tank. Tank 1 has the highest weight of 108.2g whereas in the control tank, 104.5g was the highest weight of fish. The highest increase of fishes within the experimental unit was recorded in tank 2 which has the value of 8.4g; this was possible because the environment in the pond was conducive and comfortable.

Also, in tank 1, throughout the period of treatment, 2.86% mortality was recorded and it was when 25g of Moringa powder at 3hrs of sedimentation was used as treatment whereas 8.57% mortality was recorded in the second tank, 0% mortality in the third tank and also 8.57% in the control tank. Tank 2 has the highest increase in weight when 40g of Moringa powder was used at 3hrs retention time. The increases in the weight of the fish in the experimental plots were possible because of comfortable and conducive environment for the fish which was possible because of the *Moringa oleifera* seed-powder.

At the first week of the treatment, fishes in tank 1, tank 2, tank 3 and culture tank consumed an average of 24.0g, 19g, 22.4g and 20.6g per day respectively. These further increases as the fishes grew in sizes and at the last week of treatment, the consumption increased to: Tank 1 = 84.10g, Tank 2 = 79.9g, Tank 3 = 83.4g, and control tank = 82.2g. From the ANOVA table, it was shown that the *Moringa oleifera* has a positive effect on the treatment of aquaculture effluents since Calculated F (5.217) is greater than Tabulated F (2.084) for replicate and also greater in treatment (5.254 > 2.084).

The pHs of all the culture tanks were all within range when different quantity of *Moringa oleifera* seed powder was used. This support the findings of Amagloh and Benag (2009) when *Moringa oleifera* powder was used as a coagulant in comparison with alum, their results showed that alum increases the acidity of water while this was not so for *Moringa oleifera* seed powder.

5. Conclusion

According to Schwarz (2000), the seed kernels of *M. oleifera* contain lower molecular weight water-soluble proteins which carry a positive charge. When the seeds are crashed and added to water, the protein produces positive charges acting like magnets and attracting predominately negatively charged particles such as clay, silk, and other toxic particles. Under proper agitation, these bound particles then grow in size to form the flocculates which are left to settle by gravity. This accounted for the effectiveness of *Moringa* as a coagulant for aquaculture water purification.

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