

Application of *Moringa oleifera* for Development of Sustainable and Biosecure Aquaculture

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Abstract

Manoj T. Kamble, Balu R. Chavan, Ataguba Gabriel, Txomin Azpeitia, Seema V. Medhe, Surangna Jain, and Rakesh R. Jadhav. 2014. Application of *Moringa oleifera* for Development of Sustainable and Biosecure Aquaculture. *Aquacultura Indonesiana*, 15 (2) : 64-73. As the global scenario is now changing towards the development of sustainable and bio-secured aquaculture by using of safe and eco-friendly products. This paper reviews the properties and applications of *Moringa* plant in aquaculture. The *Moringa* products have been shown to possess many properties that can apply in aquaculture. These properties include antimicrobial activity, larvicidal activity, piscicide, and, antifertility properties. The plant can also be used as a food supplement and as seafood preservative. The use of *Moringa* seed coat and pod husks as antimicrobial substances or as a medicinal plant have reported in shrimp where *Penaeus indicus* were found to protect from pathogenic *Vibrio harveyi*. There was also an evidence for cyanobactericidal activity of filtrate from crushed *Moringa* seeds that a protease inhibitor isolated. In conclusion, this review suggests that *Moringa oleifera* is a valuable plant that could be used for development of sustainable aquaculture

Keywords: Activity; Antimicrobial; Aquaculture; *Moringa*; Sustainable

Introduction

Sustainable aquaculture developments focus on increasing aquaculture production and productivity to alleviate poverty and hunger without compromising the environment. Aquaculture is the fastest-growing food production sector in the world and now supplies almost half of the global fish consumption. Trends in many of the drivers of demand indicate that aquaculture continue to expand. In particular, Asian and African aquaculture must grow significantly to meet domestic and regional demand for fish by increasing production by using underutilized water resources.

In the aquaculture, fish farmers always encounter predatory problem during the stocking of new fish species, and most often, they tend to use synthetic chemicals, which are non-biodegradable, as an effective solution. The bio-safety of products from aquaculture is paramount to consumers of aquatic foods from the aquaculture source. *M. oleifera* is biodegradable, environmentally friendly and cost-effective, and could use as supplemental protein source for fishmeal, and as aquaculture pond management tool to wipe predators in the fish pond prior to

stocking with new fish species (Adesina and Omitoyin, 2011). Focus on plant research has increased all over the world and a large amount of data has collected which shows enormous potential of medicinal plants used in various traditional systems. Many herbs have developed into herbal supplements which are claimed to assist in healthy lifestyle (Sirimongkolvorakul *et al.*, 2011).

M. oleifera (Lam) belongs to the family Moringaceae and is commonly called drumstick tree, horseradish West India Bern tree or "Miracle tree". The *Moringa* plant has been the object of much research due to its multiple uses and well-known bactericidal activity. It is rich in nutrients and, apart from a range of industrial and medicinal applications, is used to purify water for human consumption (Vieira *et al.*, 2010). As the global scenario is now changing towards the use of safe and eco-friendly products, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human and animal diseases. *M. oleifera* is one such plant which has been reported to possess many medicinal properties (Dewangan *et al.*, 2010). *M. oleifera* is a natural nutrition plant in tropic countries. The leaves, fruits, flowers and immature pods used as a highly nutritive

vegetable. This plant contains a profile of essential minerals, good source of protein, vitamins, amino acids, β -carotene and various phenolics. In addition, all parts of the tree are considered to possess medicinal properties, such as cardiac and circulatory stimulant, possess antitumor, antipyretic, anti-inflammatory, antiulcer, antispasmodic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities (Anwar *et al.*, 2007).

High nutrient of the *M. oleifera* leaves is promoted as "Trees for life", by an NGO based in the United States. Gram for gram fresh leaves of *M. oleifera* have 4 times the vitamin A of carrots, 7 times the vitamin C of oranges, 4 times the calcium of milk, 3 times the potassium of bananas, $\frac{3}{4}$ the iron of spinach and 2 times the protein of yogurt (Lotaka and Piyatiratitivorakul, 2012). Antimicrobial resistance has become a global public health problem impacted both by human and non-human microbial usage (WHO, 2001 and 2003 and Henning, 2006). There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicines, especially in developing countries. Synthetic chemicals and alum has for years been used, as fish disinfectant, and therapeutant. However recently, attention has been shifted to plant based chemical, for water treatments and possibly as therapeutic. Recently, *M. oleifera* seed powder has been tested and proved to have better performance in purifying turbid water, but little work has done on the toxicity of *M. oleifera* seed powder to some important cultivable fish species. *Moringa* plants are widely available in the tropic. Their leaves, fruits, bark and roots have economic importance for industrial and medicinal uses (Ayotunde *et al.*, 2011^a). In this paper, published data on *Moringa* plant extract and any of its use in aquaculture were extensively reviewed and analyzed.

Properties and Applications of *Moringa* Plant in Aquaculture

Antimicrobial Activity

Medicinal herbs are moving from the fringe to mainstream use with a greater number of people seeking for remedies and health approaches from side effects caused by synthetic chemicals. It has aggravated the search for antimicrobials from plant sources. Rajamanickam and Sudha, (2013) conducted a study to obtain the preliminary information on the phytonutrient profile, toxicity, and antimicrobial property of

Allamanda cathartica and *M. oleifera* against clinical pathogens. The effects of antibiotic sensitivity test showed that petroleum ether extract of *A. cathartica* and ethanolic extract of *M. oleifera* showed better antimicrobial property against many pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Cornebacterium* sp., *Klebsiella pneumonia*, and *Acinetobacter* sp. than other extracts. However, methanol extracts of *M. oleifera* inhibit bacteria better than ethanol extracts (Oluduro 2012), but Pal *et al.* (1995) reported the effectiveness of ethanolic extracts of *M. oleifera* against both gram positive and negative bacteria. Bichi *et al.* (2012) on the other hand, reported that a combination of hexane oil extraction and aqueous extraction followed by freeze drying produced an extract with greater antimicrobial activity compared to standard aqueous extraction, salt extraction and a combination of hexane extraction and these methods. In general, natural drugs have much less side effects when compared to that of synthetic and other chemical drugs (Table 2). Efficacy of some extracts is better than others primarily due to the method of extraction. Methanol extracted products present better results for antimicrobial activity than other methods.

Onsare *et al.* (2013) reported the use of aqueous extracts of *M. oleifera* samples from different areas of India against two yeast strains, three Gram positive and seven Gram negative bacteria by agar well diffusion assay. In that report, the extracts from seed coat, stem bark and pod husks were found to be organism specific with minimal inhibition concentration (MIC) ranged from 0.5 – 1.25 mg/mL, 3.0 – 4.2 mg/mL and 4.0 - 5.6 mg/mL, respectively (Table 1^A). Both seed's coat and pod's husks extracts exhibited microbicidal properties which were totally inhibited at 10 and 12 hours respectively. The seed coat extract was the most effective against *E. coli* at zero hours, while some pathogens, treated with stem bark extract, exhibited regrowth again after 24 hours apparently. Antimicrobial activity of the seed coat and pod husks reported for the first time. The active constituents may be considered potential candidates for the development of broad spectrum antimicrobial drugs. It also is in agreement with Oluduro (2012) who found that aqueous and methanolic extracts of *M. oleifera* showed visible inhibitory effects against bacteria but was not effective against *Aspergillus flavus* whereas ethanolic extract was effective against

many fungi. Pal *et al.* (1995), used ethanolic extracts from the leaves of *M. oleifera* and found that efficacy also was specific and decreased in the order: *E. coli* > *Bacillus subtilis* > *Mycobacterium phlei* > *Bacillus cereus* > *Micrococcus luteus* > *S. aureus*. This point to the fact that leaves, seed coat and pod husk of *M. oleifera* possess antimicrobial constituents that can be extracted using either methanol, ethanol, or a combination of hexane and aqueous extraction which showed high potency against bacteria as demonstrated by Bichi *et al.* (2012).

Onyuka *et al.* (2013) reported the effectiveness of the *M. oleifera* plant extracts against bacteria in fish samples, which was never described before (Table 2). It means that *M. oleifera* plants that easily grow in most arid and sandy conditions, as found near the Lake Victoria basin in Kenya, can be exploited to provide extracts for preserving fish for long term storage and safety for human populations. Ekanem *et al.* (2011) evaluated the antibacterial effectiveness of seven plant extracts against *Aeromonas* and *Pseudomonas* bacteria of farmed catfish (*Heterobranchus longifilis*). The results from this study have demonstrated that preparations from

medicinal plants have potentials for the control of *A. hydrophila* and *P. fluorescens* in cultured catfish. *M. oleifera* showed antibacterial activities against *A. hydrophila* and *P. fluorescens* of *H. longifilis* with MIC of 50 mg/mL (Table 2). The high host tolerance to the plant extracts is also an indication that a reliable therapeutic regime could develop from herbal products for the treatment of aquaculture diseases. This discovery from various plants involved the use of leaf and bulb or fruit extracts using ethanol as solvent. Although efficacy was recorded, for some plants, others were not as effective. It is quite clear that parts of the plant used for extraction of the antimicrobial agent affects its effectiveness. *Allium cepa* bulb extract did not inhibit *A. hydrophila* and *P. fluorescens* of *H. longifilis* (Ekanem *et al.*, 2011) but Muniruzzaman and Chowdhury (2004) reported the effectiveness of ethanol extracted *A. cepa* leaf extract against *A. hydrophila*, *P. fluorescens* and *Edwardsiella tarda*. The difference in part of the plant used is responsible for the disparity of results coupled with the fact that Ekanem *et al.* (2011) used other solvents apart from ethanol that was, however, the significant solvent (70%).

Table 1^A: Efficacy of Moringa with aqueous extracts in controlling/reducing the population of pathogenic bacteria

Parts used	Target species	Activity/Effect	Concentration/ Biological Activity	Reference
Seed	<i>Anopheles gambiae</i>	Larvicidal	72 hour- LC50 and LC90 of 1885.4 µg/mL, 3902.4 µg/mL respectively, ($P < 0.05$), causing 99.33 ± 1.15% mortality at 8700 µg/mL.	Ohia <i>et al.</i> , 2013
Seed	<i>Poecilia reticulata</i>	Toxicity	Toxic reactions (erratic movements, air gulping with prolonged exposure resulted in dullness and discoloration in higher concentrations)	Ohia <i>et al.</i> , 2013
Seed	<i>Staphylococcus aureus</i> , <i>Vibrio cholerae</i> <i>Escherichia coli</i>	Antibacterial	1:5 and 1:10 in volumes 50, 100, 150 and 200 µL were efficiently inhibited (halo > 13 mm) the growth of bacteria isolated from shrimp samples, especially at 150 and 200 µL/dish.	VIEIRA <i>et al.</i> , 2010
Seeds coat, bark, pods husks	<i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Shigella flexneri</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumonia</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhimurium</i> , <i>Candida albicans</i>	Antimicrobial	totally inhibited at 10 and 12 h respectively	Onsare <i>et al.</i> , 2013
Seed Coat	<i>Escherichia coli</i>	Antimicrobial	Seeds' coat extract was the most effective against <i>E. coli</i> at 0 hours	Onsare <i>et al.</i> , 2013

Vieira *et al.* (2010) found antibacterial activity (inhibition halo >13 mm) against *S. Aureus* (Gram positive), *Vibrio cholerae* and *E. coli* (Gram negative) isolated from the whiteleg shrimp (*Litopenaeus vannamei*) using aqueous and ethanolic seed extracts of *Moringa*. While *E. coli* isolated from tilapia, *Oreochromis niloticus*, was sensitive to the ethanolic extract of *Moringa*. However, LC50 of 242 mg/L of seed extract has been reported by Ayotunde *et al.* (2011^a). While histological changes such as gill degeneration, gill arch hypertrophy, as well as liver and kidney hyperplasia, necrosis, and vacuolation have been seen with severity increasing with concentration of seed extracts (Ayotunde *et al.*, 2011^b). Dewangan *et al.* (2010) conducted an experiment to determine the antibacterial activity of different extracts of *M. oleifera* root bark against *S. aureus*, *E. coli*, *Salmonella gallinarum* and *P. aeruginosa* *in vitro* (Table 2). Both the gram-positive and gram-negative organisms showed variable sensitivity to different extracts of *M. oleifera* root bark in organic solvents as methanol, acetone, ethyl acetate and chloroform and in the inorganic solvent, water. In general, ethyl acetate and acetone extracts showed maximum antibacterial activity. The aqueous extract had minimum antibacterial activity against the test organisms.

Larvicidal Activity

Plant-based insecticides have been long proposed as alternatives to synthetic chemical insecticides for vector and pest control because they pose little threat to human and environmental health (Choochote *et al.*, 1999 and Murray, 2006). Several plants around the world have been identified with insecticidal (larvicidal, pupicidal and adulticidal) properties against mosquitoes either in the seeds, fruits, barks, roots or their root exude. The diversity of plant species is huge and phytochemicals obtained from them are important sources of safe and biodegradable chemicals which can be screened for mosquito and insecticidal activities and tested for safety in mammals (Mittal and Subbarao, 2003).

Ohia *et al.* (2013) evaluated the aqueous extract of the *Moringa oleifera* seed as a control agent against third instar larvae of *Anopheles gambiae* in the laboratory. It showed that it was larvicidal to the mosquito species and with very minimal toxic effects seen in the non-target animal, *Poecilia reticulata* (Table 1^A). These results suggest that *M. oleifera* may be

a potentially valuable source of insecticide and may well play a more prominent role in mosquito control programs in the future. Similarly, Njom *et al.* (2011) assessed the toxicity and growth regulatory effects of *M. oleifera* seed extracts on third larval instars of *Anopheles gambiae*, under laboratory conditions. At highest and lowest concentrations of 5% w/v and 1% w/v, larval mortality in a day was 80% and 10%, respectively. No adult emerged in higher concentrations though the few adults that emerged in lower concentrations were too weak to fly. This study clearly showed that *M. oleifera* seed extract has larvicidal and growth inhibitory effects on larvae of *Anopheles gambiae*.

Prabhu *et al.* (2011) conducted a study on larvicidal and repellent potential of *M. oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). This study indicates that the phytochemicals derived from *M. oleifera* seeds extracts are effective anti-mosquito agents and the plant extracts may be used for further integrated pest management programs. Low toxicity of *M. oleifera* has been reported in mice and daphnia (Ferreira *et al.*, 2009) but 100% mortality of simuliid larvae has been reported with the use of petroleum extract of *M. oleifera* (Etusim *et al.*, 2013). The use of oil extracted from *M. oleifera* as a method of controlling the armyworm (*Spodoptera frugiperda*) was demonstrated by Kamel (2010). It indicates that the extract from *M. oleifera* is also effective against other insect larvae. The effectiveness of the extract against both human disease vectors and plant pests shows its versatility and importance in ensuring food security and social wellbeing.

Piscicide

Elimination of unwanted fish is a common practice among fish farmers prior to stocking with desirable fish fingerlings. However, the synthetic piscicides used to eliminate unwanted fish are persistent and could get into the food chain. Thus, the use of environmentally safe organic piscicides as an alternative is being embraced in aquaculture because they are biodegradable over short periods, and there is a possibility that the fish killed by them are edible with no health hazards (Chiavareesajja *et al.*, 1997; Agbon *et al.*, 2004; Akinbulumo *et al.*, 2004; Akinwande *et al.*, 2007). *M. oleifera* is suggested as an alternative to synthetic piscicides because it is commonly

available, is less expensive for fish farmers and has a lower toxicity against non-target species. Adesina and Omitoyin (2011) conducted a study on the potential of *M. oleifera* fresh root-bark extract as an organic piscicide in aquaculture pond management. The results of the study revealed that the fish exposed to fresh root-bark extract of *M. oleifera* at different concentrations exhibited toxic responses which eventually lead to death. This extract could use as an organic piscicide in aquaculture pond management, and baseline information on its toxicity to fish could serve as a fisheries management tool to wipe out predatory fishes in ponds prior to stocking.

Adesina *et al.* (2013) reported that biosafety of food products from aquaculture is now considered as a public health concern in Nigeria. It is due to the nature and component of materials used as rearing facilities on the fish farm. Elimination of unwanted fishes and other predators is a common practice among fish farmers in Nigeria prior to stocking of desirable fish fingerlings (Table 1^B). The use of synthetic pesticides to eliminate unwanted fishes and other predators in the pond is persistent and could get into the food chain. Thus, the use of environmentally safe, natural pesticides as an alternative is being embraced in aquaculture because they have a short period of toxicity disappearance and biodegradable. It enhances the principles of sustainable aquaculture production and its management in Nigeria.

Anti-cyanobacterial Activity

Climate change is expected to aggravate hazardous blooms of Cyanobacteria (Paerl and Huisman, 2008) while safe and aesthetically acceptable water is a growing need in a modern society (Steffensen, 2008). Hence, water resource management is challenged worldwide to reduce the vulnerability to the threats of harmful cyanobacterial blooms. Particularly in developing countries where the high costs of chemicals for water and wastewater treatment might limit their application, the development of cost-effective and environmentally acceptable mitigating measures is beneficial. In that context, water clarification and disinfection with natural products, such as the seeds of the pan-tropical tree, *M. oleifera*, is of particular interest. Lurling and Beekman, (2010) stated that presumably, in the higher doses of 20- to 160-mg crushed seeds per liter, the cyanobacteria would die. At the highest dosage of 160 mg/L, the efficiency of photosystem II (ΦPSII) dropped to zero rapidly and remained nil during the experiment that lasted 14 days (Table 1^B). Hence, under laboratory conditions, a complete wipeout of the bloom could be achieved. It is the first study that yielded evidence for cyanobactericidal activity of filtrate from crushed *Moringa* seeds, suggesting that *Moringa* seed extracts might have a potential as an effect-oriented measure lessening cyanobacterial nuisance.

Table 1^B: Efficacy of Moringa with aqueous extracts in controlling/reducing the population of pathogenic bacteria.

Parts used	Target species	Activity/Effect	Concentration/ Biological Activity	Reference
Seeds	<i>Microcystis aeruginosa</i>	Anti-Cynobacterial	Good growth in controls and treatments with 4- and 8-mg crushed Moringa seeds per liter, having similar growth rates of 0.50 (±0.01) per day. In exposures of 20- to 160-mg crushed Moringa seeds L ⁻¹ , growth rates were negative and on average -0.23 (±0.05) .day ⁻¹ .	Lurling and Beekman, 2010
Seeds	Nile tilapia	Toxicity	24-h, 48-h, 72-h and 96-h LC50 of M includes erratic movement, air gulping, loss of reflex, discoloration, molting, loss of scale, and hemorrhage.	Ayotunde <i>et al.</i> , 2004
Root Bark	Nile tilapia	organic piscicide	Acute-lethal toxicity (LC) of Moringa oleifera extract for 96-h exposure for Tilapia fingerlings was determined at 26.45 mg l ⁻¹	Adesina and Omitoyin 2011
seed	Nile tilapia	Toxicity	The pack cell volume increased from 17.5±0.7 in the control to 23.0±4.2 in concentration of 17.5±0.7 in the control to 23.0±4.2 in concentration of 250 mg/L Moringa/ liter of water.	Ayotunde <i>et al.</i> , 2011
seed	Nile tilapia	Histological Changes	Pathologic lesions observed : levels of cells degeneration in the gill, lamellar hyperemia; hypertrophy of gill arch occurred, while hyperplasia, disarrangement of hepatic cell, necrosis, vacuolation occurred in fish liver and kidney	Ayotunde <i>et al.</i> , 2011

Antifertility Effect

Ampofo-Yeboah *et al.* (2013) conducted a study on the effect of Moringa seed powder on the gonadal integrity of Mozambique Tilapia, *Oreochromis mossambicus*. The result of the study indicated that including *Moringa* seed powder at 5.0 g/kg basal diet and higher inclusion levels have an antifertility effect, significantly affecting gonad integrity and sperm production in sexually mature *O. mossambicus* males.

Food Supplement

Kumalaningsih *et al.* (2011) conducted a study on encapsulation of *Lactobacillus* sp. with *M. oleifera* leaves extract for food supplement. He concluded that the addition of *Moringa* leaf extract increased the growth of lactic acid bacteria. The use of 8% *M. oleifera* leaves extract is the best treatment for making encapsulated food supplement. The feeding trial showed that the food supplement increased the weight of rats approximate by 30%. Rayes (2013) investigated the effect of dietary *M. oleifera* leaf for the first time in aquaculture as a medicinal plant for protection of *P. indicus* from pathogenic *V. harveyi*. The study found that survival of shrimp juvenile in *M. oleifera* leaves was 86.4%. The study also indicated that *M. oleifera* leaves contained appreciable amounts of carbohydrate, protein and minerals, which are the nutritional requirements of all livestock animal. Possibly, the leaves from this plant could be useful as feed supplement and as a medicine in poultry to improve health and increase performance. Lotaka and Piyatiratitivorakul (2012) concluded that the supplementation of *M. oleifera* leaf powder in the diet after 63 days had no adverse effect on the shrimp survival. The results also indicated that *M. oleifera* leaves could be an alternative herb for improvement of marine shrimp culture by conducting an experiment on effects of supplemented diets on growth, survival and

immune response of pacific white shrimp (*Litopenaeus vannamei*).

Seafood Preservative

Bijina *et al.* (2011) conducted a study on protease inhibitor from *M. oleifera* with potential for use as therapeutic drug and as seafood preservative. Among the different parts of *M. oleifera* tested, the crude extract isolated from the mature leaves and seeds showed the highest level of inhibition against trypsin. Among the various extraction media evaluated, the crude extract prepared in phosphate buffer showed maximum recovery of the protease inhibitor. The protease inhibitor recorded high inhibitory activity toward the serine proteases thrombin, elastase, chymotrypsin and the cysteine proteases cathepsin B and papain which have more importance in the pharmaceutical industry. The protease inhibitor also showed complete inhibition of the activities of the commercially available proteases of *Bacillus licheniformis* and *Aspergillus oryzae*. However, inhibitory activities toward subtilisin, esperase, pronase E and proteinase K were negligible. Further, it was found that the protease inhibitor could prevent proteolysis in a commercially valuable shrimp *Penaeus monodon* during storage indicating the scope for its application as a seafood preservative. It is the first report on the isolation of a protease inhibitor from *M. oleifera*. Onyuka *et al.* (2013) conducted a study on the comparison of the antimicrobial activities of brine salting, chlorinated solution and *M. oleifera* plant extracts in fish from the Lake Victoria basin of Kenya. The results of this study show that for the two fish species *Raillardella argentea* and *O. niloticus*, 12% sodium chloride solution was the most effective in reducing bacterial load, compared to chlorinated solution even at the highest concentration of 200 mg/L (Table 2).

Table 2: Efficacy of Moringa with different solvent extracts in controlling/reducing the population of pathogenic bacteria

Solvent Extract	parts used	Target species	Activity/Effect	Concentration/ Biological Activity	Reference
Ethanol	dry seed	Enteric bacteria in <i>Rastrineobola argentea</i> , <i>Oreochromis niloticus</i>	Antimicrobial	At 8 hours, microbial load was 536.6 MPN/g at control 0 µg and this decreased to 144.3 MPN/g at M. oleifera ethanol extract concentration at 80 µg/mL at 8 hours, P= 0.00001.	Onyuka <i>et al.</i> , 2013
	Leaf	<i>Aeromonas</i> , <i>Pseudomonas</i> bacteria	Antibacterial	less sensitive to <i>A. hydrophila</i> and effective against <i>P. fluorescens</i>	Ekanem <i>et al.</i> , 2011
	leaf	<i>S. aureus</i> , <i>E. coli</i> , <i>Bacillus</i> sp., <i>P. aeruginosa</i> , <i>Cornebacterium</i> s, <i>Klebsiella pneumonia</i> , <i>Acinetobacter</i> sp.	Antimicrobial	Significant inhibition (<i>S. aureus</i> (20 mm), <i>E. coli</i> (20 mm), <i>Bacillus</i> sp. (18 mm), <i>P. aeruginosa</i> (14 mm), <i>Cornebacterium</i> sp. (12 mm), <i>Klebsiella pneumonia</i> (12 mm), <i>Acinetobacter</i> sp. (14 mm)).	Rajamanickam and Sudha, 2013
	leaf	red-tail tin foil barb	Lead Toxicity	24 hours LC50 lead exposure, hepatocytes were showed cytoplasmatic vacuolation with hypertrophy and pycnotic nuclei.	Sirimongkolvorakul <i>et al.</i> , 2011
Methanol, acetone, ethyl acetate, chloroform	Root bark	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Salmonella gallinarum</i> , <i>Pseudomonas aeruginosa</i>	Antibacterial	variable sensitivity to different extracts	Dewangan <i>et al.</i> , 2010
n-hexane	Seed	Enteric bacteria in <i>Rastrineobola argentea</i> , <i>Oreochromis niloticus</i>	Antimicrobial	At 8h the control tilapia (0 µg/mL) had a microbial load of 326.2 MPN/g and at 80 µg/mL it decreased to 87 MPN/g, P=0.00001	Onyuka <i>et al.</i> , 2013

Toxicity Effect

Despite widespread use of *Moringa*, its toxicity and effectiveness to aquatic organisms, particularly fishes, have not been examined. Acute and chronic toxicity test was conducted using aqueous extract of *M. oleifera* seeds on the most widely cultivated African freshwater fishes. Sirimongkolvorakul *et al.* (2011) presented a study on the influence of *M. oleifera* on histopathological changes due to lead toxicity in red-tail tin foil barb, *Puntius altus*. The present study revealed that the crude leaves powder of *M. oleifera* has a potential to reduce lead toxicity. The greater potency of *M. oleifera* leaves against lead could be due to the antioxidant property or chelating properties. Thus, they recommend that a suitable selection and manipulation of their diet might be useful for a significant extent in

reducing toxicity risk in populations exposed to lead. Ayotunde *et al.* (2004) conducted acute and chronic toxicity tests using aqueous extracts of *M. oleifera* seeds on *O. niloticus* fingerlings and adults in order to provide baseline information and establish a limit of using aqueous extracts of *M. oleifera* seeds in freshwater fish ponds. Hematological examination of the fingerlings showed (Table 1^B) an increase in red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values. While there was a decrease in the erythrocyte sedimentation rate (ESR), MCH, and mean corpuscular volume (MCV) values. Whereas in the adults, the RBC, PCV, Hb and MCH values increased and there was a decrease in the ESR, WBC and MCV, while there was no

change ($P>0.05$) in MCHC values. Mortality increased with increase in concentration of *M. oleifera* and time of exposure in both *O. niloticus* fingerlings and adults.

Ayotunde *et al.* (2011^a) determined the toxicity of *M. oleifera* seed powder to the most cultivable fish species in Africa, tilapia, *Oreochromis niloticus*, fingerlings (Table 1^B). *M. oleifera* seed powder is a good water purifier; it contains polyelectrolytes, which constitute active ingredients in water treatment. The histological changes in the gill, skin, liver and kidney include different level of degeneration of cells, lamellar hyperemia; hypertrophy of gill arch, shrinkage and dermal erosion and necrosis of the skin. While hyperplasia, disarrangement of hepatic cell, necrosis, and vacuolation occurred in liver and kidney of tilapia fingerling. The damages became severe with increasing concentration of *M. oleifera* to fish and time of exposure. There was no significant difference in water quality in the test, the result obtained before the test, during the test and after the test. It found close to the water quality parameters of the control. The results of the test provided baseline information and established safe limit of using an aqueous extract of *M. oleifera* seed powder in fresh water pond. It ascertained that aqueous extract of *M. oleifera* seed powder did not have adverse effects on water quality. Ayotunde *et al.* (2011^b) defined the pathological effects of *M. oleifera* seed powder, natural alternative to alum, used as a water purifier in a fish culture enclosure in Nigeria (Table 1^B). The histological changes in gill, liver, skin and kidney of fingerling and adult of Nile tilapia exposed to aqueous extract of *M. oleifera* seed at different sub-lethal concentration under static bioassay procedure described. There were pathologic lesions observed, which included different levels of cell degeneration in the gill, lamellar hyperemia; hypertrophy of gill arch occurred while hyperplasia, disarrangement of hepatic cell, necrosis, and vacuolation occurred in fish liver and kidney. Damages of different organs became severe with increasing concentration of aqueous extract of *M. oleifera* seeds and time of exposure.

Conclusion

After analysis of available information on *Moringa* plant regarding its potentials to contribute multipurpose use in sustainable aquaculture found that *Moringa* exhibited multipurpose activities. These activities include

antimicrobial activity, larvicidal activity, organic piscicide, anti-cyanobacterial activity, antifertility effect, food supplement and seafood preservative. Reliable therapeutic regime could develop from *Moringa* products for the treatment of aquaculture diseases. These results suggest that *M. oleifera* may be a potentially valuable novel plant for sustainable aquaculture in the future.

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