

***Moringa oleifera* as a sustainable fish feed ingredient: nutritional benefits and applications; a comprehensive review**

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Abstract *Moringa oleifera* exists as a wonder plant because of its multiple applications across both the food industry and healthcare field. Its cultivation is widespread in tropical and subtropical regions, particularly in South Asia, due to its nutritional and medicinal properties. Its various parts offer significant food value, addressing nutrition-related issues, vitamin deficiencies, and mineral shortages. Its roots together with leaves, bark, pods and various other parts contain substantial phytochemical levels that demonstrate active biological properties including anticancer, antioxidant, anti-inflammatory and hepato-protective and cardio-protective actions etc. In recent years, there has been a fast-growing trend to use *M. oleifera* as a dietary supplement for fishes. The nutrient composition highlights its high protein, vitamins and minerals content as well as the presence of bioactive compounds like glucosinolates, phenolic acids and flavonoids. These bioactive compounds have potential to improve fish growth, health and disease resistance. Besides having great nutritional potential, it also contains many anti-nutritional components including saponins, phytates, oxalates and tannins, etc. Many studies reported that the effect of these anti-nutritional components can be lowered by using dehydration, soaking, fermentation and other strategies. This review article gives an overview of the potential benefits and some drawbacks of incorporating *M. oleifera* in fish diet and highlights its use as potential supplement in fish feed.

Keywords Miracle plant . Fish feed supplement . Dietary uses . Fish growth . Fish immunity

Introduction

For both humans and animals, plants are unique source of energy, food, shelter and medicinal substances. Numerous beneficial plant products acquired directly or indirectly, attest the importance of plants to humans and other living beings (Kawo 2007). *M. oleifera* belongs to Moringaceae family is a 2.5-10m tall, leguminous shrubby plant (Vlahof et al. 2002; Teixeira et al. 2014) that is native to South Asia. It is mostly growing from North-Eastern Pakistan to North-Western Bengal, India and in the Himalayan foothills (Sharma et al. 2011). It is grown in Pakistan's irrigated plains, dry regions, and Sindh province (Iqbal and Bhanger 2006). *M. oleifera* regional name is Sohanjana in Pakistan (David et al. 2015). *M. oleifera* is termed as the "Tree of Life" due to its advantages for the environment, in water bodies cleaning, health, and nutrition. The root, leaves, bark, flower, seeds, seed oil and gum, almost all of its parts have been used for a variety of advantageous purposes (Anwar et al. 2007).

M. oleifera is also regarded as "the miracle tree" due to its special helpful medical characteristics like

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its antioxidant (Rani et al. 2018), antimicrobial, anti-inflammatory, and hepato-protective effects (Cheenpracha et al. 2010) and wide range of phytochemicals present in it. Every component of this plant is edible by both fish and animals (Kou et al. 2018). A plant contains few significant nutrients as a basic component. *M. oleifera* Studies indicate that its leaves contain four types of powerful antioxidants, namely flavonoids, carotenoids, phenolics with antibacterial properties, and ascorbic acid (Das et al. 2012). The use of *M. oleifera* leaves extends the shelf life of ghee according to Siddhuraju and Becker (2003). Research has uncovered that goat meat patty oxidation protection can be achieved through *M. oleifera* leaf growth extract (Das et al. 2012).

Due to its numerous benefits, several studies were conducted to isolate bioactive chemicals from different portions of this plant (Gupta et al. 2018). A plant's nutritional composition is crucial for its therapeutic, dietary, and medical benefits (Al-Kharusi et al. 2009). The highest concentration of vitamin A, B, C, D, E, and K can be found in this plant. *M. oleifera* contains calcium, manganese copper, magnesium iron, zinc and potassium among other essential minerals (Richter et al. 2003). More than 40 natural antioxidants are present. Ancient kings and queens have incorporated *M. oleifera* into their diets since 150BC for mental fortitude and healthy skin (Mahmood et al. 2010).

As the global human population increases, there is a corresponding dependence on farmed fish production as a key supply of animal protein (Naylor et al. 2000). By 2006, the aquaculture industry alone consumed 87% of the fish that was used for non-food purposes, or the same amount of fish around 23.8 million metric tons (MMT). Out of the 154 MMT of fish produced worldwide in 2011, 23.2 MMT were used for purposes other than eating (FAO 2014). Fishmeal is therefore heavily used, with 15.0 MMT being used in 2010. However, by 2020, global fishmeal production declined to around 5 MMT, partly due to improved feed efficiency and alternative protein sources. Despite this, aquaculture still relies on wild-caught fish for feed, with roughly 18-20 MMT (20% of global catch) used annually for fishmeal and fish oil production in recent years (FAO 2022). There is a pressing need to explore supplementary products made from understudied plants (Egwui et al. 2013). Several plant-derived protein sources have been tested in the past in numerous fish feed trials to see if they are suitable for different fish species (Ogbe and Affiku 2011; Sirimongkolvorakul et al. 2012). A significant number of these trials centered on plant species including sunflower seeds, groundnut, wheat gluten, palm kernel cakes, soybean, cotton seed, meals having maize, and rapeseed (Francis et al. 2001). It is critical to take various products derived from un-researched and anonymous plants into account for use as constituents in aquaculture feed due to the fact that these plants feed sources are also in great demand for consumption by humans. *Jatropha curcas*, *M. oleifera*, *Mucuna pruriens* spp., and *Sesbania* spp. are a few of these plants (Reyes et al. 2006; Oduro et al. 2008). This might be because it possesses nutritional, medicinal, and preventative characteristics, which boost animal productivity (Fahey 2005).

It has been proven that dried *M. oleifera* leaves have 25 times the iron of spinach, seventeen times the calcium of milk, fifteen times the potassium of bananas, ten times the vitamin A of carrots, nine times the protein of yoghurt, seven times as much vitamin C as an orange (Babu 2000). The leaves of *M. oleifera* receive endorsement from community health workers together with doctors and dietitians who recommend it for protein supplementation to tackle global malnutrition issues.

Multiple researches have confirmed that *M. oleifera* provides nutritional advantages to Rohu and Nile tilapia and Bocourti's catfish diets (Elabd et al. 2019; Hussain et al. 2018). Research has shown that *M. oleifera* enhances feed utilization data with simultaneous increases in digestibility coefficients (Puycha et al. 2017). Studies show *M. oleifera* incorporated into food enhances resistance to diseases along with boosting immune functions (Kaleo et al. 2019; Mansour et al. 2020). El-Gawad et al. (2020) recorded a considerable increase in WBCs ($12.07 \times 10^4/\mu\text{l}$) while feeding 1.5% moringa leaf powder. Thus, being an innovative feed stuff, it is being used to overcome the feed crisis (Su and Chen 2020).

The findings of Hassaan et al. (2018) had shown that adding 10 - 20% MOLM to the diets of *L. rohita* boosted the WG substantially, but adding more MOLM to the diet did not improve the fish growth performance. However, Kasiga and Lochmann (2014) noticed that it is feasible to substitute up to 30% protein source in soybean meal with MOLM without having any negative impact on growth of Nile Tilapia. It has been claimed that moringa leaves can be used in place of 10% of FM in Asian seabass to increase growth performance (Ganzon-Naret 2014). Another research contradicted previous findings that adding *M. oleifera* in the diet inhibits fish growth, this might be due to negative effects of anti-nutrients (saponins, phytates,



tannins and phenols) (Mehdi et al. 2016). The information and research findings on *M. oleifera* as a potential component in fish feed and its impact on the general performance of numerous fish species are compiled in this review article. The main goal of this review is to evaluate the phytochemicals, biological activities, nutritional, and anti-nutritional properties of various forms of *M. oleifera*.

Phytochemical constituents of *M. oleifera* plant

The screening of *M. oleifera*'s roots, stems, gum, bark, pods, leaves, flowers, and seeds for phytonutrients revealed an abundance of several significant chemical constituents within various plant parts are shown in the Table 1.

Nutrient contents of *M. oleifera*

M. oleifera being a representative of the family Moringaceae has a great potential to overcome problems related to malnutrition (Rockwood et al. 2013). Due to the presence of many micro and macro nutrients essential for human health, it also regarded as a “wonder plant”. Parts like seeds, leaves and flowers of *M. oleifera* have numerous uses in food industry (Oyeyinka and Oyeyinka 2018). Because many vital phytochemical compounds are present in the seeds, leaves and pods is a nutritionally rich plant. Its each part is a magnificent collection of many essential nutrients and anti-nutrients. It also contains beta-carotene and folic acid of vitamin A and B respectively. It also contains vitamin C, D and E along with niacin and pyridoxine (Mbikay 2012). Many researches also revealed the uses of *M. oleifera* plant in enhancing the color, lipid consistency and chemical makeup of meat in livestock sector (Qwele et al. 2013; Hassaan et al. 2018). A recent research study proved that *M. oleifera* delivers better results for treating iron deficiency than standard iron supplements and regulates genes involved in iron response (Saini et al. 2014).

Leaves nutrient contents

According to Kasolo et al. (2010), *M. oleifera* leaves have substantial amounts of zinc together with iron as well as calcium, copper, magnesium and potassium and additional minerals. Its leaves serve as a valuable food alternative to combat infant and childhood malnutrition (Anwar et al. 2007). Its leaves contain extensive quantities of proteins and phenols in addition to manganese, iron and calcium together with other minerals (Hekmat et al. 2015). Percentage of the crude protein in dried out leaves is about 30.3% along with 19 amino acids. Minerals like phosphorus, calcium, potassium, magnesium, sodium, copper and sulfur have percentage like 0.3%, 3.65%, 1.5%, 0.5%, 0.16%, 8.25% and 0.63%, respectively (Moyo et al. 2011). In comparison to fruits like strawberries which have great antioxidant properties, leaves of *M. oleifera* have much greater antioxidant properties (Yang et al. 2006).

Furthermore, having less caloric content, leaves of *M. oleifera* can also be includes in obese person's diet (Oduro et al. 2008). Unlike the fodder of many vascular plants, the leaves of *M. oleifera* contain comparatively higher percentage of lipid content which is 7.09% (Teixeira et al. 2014). Surprisingly, content of unsaturated fatty acids in leaves of *M. oleifera* is about 57% and the one with the largest concentration in these is α -linolenic acid which is 44.57% (Busani et al. 2011). Many vital growth and development inducing minerals are present in *M. oleifera* and one of them most essential for human growth is calcium, which is present in it. *M. oleifera* leaves provide even more calcium i.e. about 1000 mg than milk (300 to 400 mg/8 ounces) and *M. oleifera* powder can provide even more than leaves i.e. about 4000 mg (Fuglie 2005). The nutrients can be condensed when the leaves of *M. oleifera* are dried thus making it easier to consume and store. Due to this, it can be utilized when food supply is short and can also be supplied to places where it doesn't grow naturally. In sub-Saharan countries, it can be employed to enhance both nutrition and health (Moyo et al. 2011).

Seed nutrient contents

Seeds of *M. oleifera* have rich nutrient content along with remarkable antioxidant potential due to hydrolysate and protease enzyme present in them. It contains seven essential amino acids that make the seeds protein density of about 40.34% (Liang et al. 2019). As compare to pods and leaves, the seeds contain much



Table 1 Comprehensive phytochemical profile of *M. oleifera* with corresponding biological activities

Plant Parts	Pictures	Phytochemical constituents	Biological Activities	References
Root		Benzyl Isothiocyanate, Spinachin, Deoxy-niazimicin, Moringine, Moringinine, p-cymene, Alpha- phellandrene, 4-(alpha-L-rhamnopyranosyloxy) benzyl glucosinolate	Antioxidant, Anti-inflammatory, Anti-microbial, Anti-fertility	Foild et al. (2001) Amaglo et al. (2010) Sharma et al. (2011)
Stem		Moringine, Moringinine, Benzyl glucosinolate, Octacosanoic acid, β -Sitosterol, 4-Hydroxymellein, Vanillin, Beta- sitosterol, Beta-sitosterone	Anti-cancer, Anti-microbial, Cardioprotective, Anti-cholesterol	Foild et al. (2001) Anwar et al. (2007) Amaglo et al. (2010) Sharma et al. (2011)
Gum		Mannose, Arabinose, Rhamnose, Galactose, Leucoanthocyanin, Glucoronate acid	Anti-rheumatic, Anti-syphilitic, Anti-asthmatic, Anti-dysenteric, Astringent	Fuglie (2001)
Bark		Octacosanoic acid, Vanillin, Moringine, β -Sitosterol, Moringinine, 4-(alpha-L-rhamnopyranosyloxy) Benzyl glucosinolate.	Anti-inflammatory, Anti-uro lithiatic, Anti-microbial	Foild et al. (2001) Siddharaju and Becker (2003) Fahad et al. (2010) Sharma et al. (2011)
Pods		Benzyl glucosinolate, Methyl-p-hydroxybenzoate, Isothiocyanate, Thiocarbamates, Beta- sitosterol, nitrites, O-(1-heptenyloxy) propyl undecanoate, O-ethyl-4-[(α -L-rhamnosyloxy)-benzyl] carbamate	Anti-skin cancer, Anti-inflammatory, Anti-diabetic	Foild et al. (2001) Bhanali et al. (2003) Roy et al. (2007) Amaglo et al. (2010) Sharma et al. (2011)
Leaves		Ethyl palmitate, Ethyl ester, 7-Octadene-3-ol, 3-Cyclohexyliden-4-ethyl-E2-dodecenylacetate, 2-Hexanone, 2,6-Dimethyl-1, Palmitic acid, p-Cresol, Guanosine, Hexadecanoic acid, 4-Hexadecen-6-yne, pyrrolenarumine 40(0- α -L-rhamnopyranoside, 40- hydroxyphenyl ethanamide, 4-Aminobutyrate, Adenosine, Tyrosine, 4-Aminobutyrate, Kaempferol, Quercetin, Benzyl glucosinolate, Niazimicin, Niazirin and Niazirinin, Niazirinin A and B	Anti-cancer, Antioxidant, Anti-epileptic, Anti-Inflammatory, Anti-Diabetic, Anti-Microbial, Neuroprotective, Hepatoprotective, Cardio protective, Anthelmintic	Foild et al. (2001) Nepolean et al. (2009) Sharma et al. (2011) Annutia et al. (2011) Mishra et al. (2011) Joy et al. (2013) Coppin et al. (2013) Tayo et al. (2014) Mahmud et al. (2014)
Flowers		D-glucose, Ocenol, Decanoate acid, Dodecanal, Oleol, Benzyl glucosinolate, Quercetin, Kaempferitin, Isoquercetin, D-mannose, Ascorbic acid, Moringine, Sipo and Satol, cis-9-Octadecen-1-ol	Anti-inflammatory, Ant-bacterial, Anti-cholera, Anti-ulcer	Foild et al. (2001) Sanchez-Machado et al. (2006) Amaglo et al. (2010) Sharma et al. (2011)
Seeds		Roridin E, Veridiflorol, 9-Octadecenoate acid, Niazimicin, Niazirin, Methionine, 4-(alpha-L- rhamnopyranosyloxy) Benzyl glucosinolate, Cysteine	Anti-cancer, Anti-microbial, Hepatoprotective, Anti-asthmatic	Foild et al. (2001) Agrawal and Mehta (2008) Amaglo et al. (2010) Sharma et al. (2011) Mishra et al. (2011)

great amount of fats about 38% along with vitamin E, magnesium and copper (Gopalakrishnan et al. 2016). Large number and amount of micro and macro nutrients are also present in seeds. The level of potassium; a macronutrient is about 2,357.7 mg per kilogram and that of iron; a micronutrient is about 36.2 mg per kilogram (Liang et al. 2019).

Pods nutrient contents

According to a study, the percentages of fibers and proteins in immature pods of *M. oleifera* are 46% and 20%, respectively. The amount of amino acids represents in flowers, pods, and leaves of *M. oleifera* are 31%, 30% and 44%, accordingly. Almost equal amount of lipids including oleic acid, omega-6 fatty acid, palmitic acid and omega-3 fatty acid, etc. are found in flowers and younger pods of *M. oleifera* (Sanchez-Machado et al. 2010). Being fibrous in nature, pods of *M. oleifera* are used to cure gastrointestinal issues and cancer of colon, etc. (Oduro et al. 2008).

Flower nutrient contents

M. oleifera's flowers have great nutrient contents along with great medicinal importance. They are especially beneficial for old age people, women and children being rich in calcium and iron. Large quantity of vitamin C, protein and fiber is present in powder form of flowers of *M. oleifera* (Rani and Vijayarani 2019). A study conducted by Fuglie (2005) shows that nutrient content of *M. oleifera* is affected by seasonal changes. For instance, during cold and dry weather conditions, the amount of iron and vitamin C is high and during hot and wet weather conditions, the level of vitamin A is high (Yang et al. 2006). The study conducted by Richter et al. (2003) demonstrates that adding 10% raw *M. oleifera* leaves to Nile tilapia fish feed promotes their growth and behavioral activity. The organic compound from *M. oleifera* leaves which has essential amino acids makes it a valuable additive to fish feeds because it presents outstanding nutritional properties (Richter et al. 2003). The fish activity remains effective when *Nile tilapia* consume 30% *M. oleifera* leaf food extract as part of their daily 100% fish feed diet (Afuang et al. 2003).

Analysis of nutrient content

M. oleifera leaves maintain substantial levels of polyphenols along with minerals and vitamins and fatty acids and crude protein and several more micro-elements (Gopalakrishnan et al. 2016). Different important phytochemicals including anthraquinones, anthocyanins, proanthocyanidins along with numerous other phytochemicals exist within *M. oleifera* roots, leaves, flowers, fruits and seeds (Goyal et al. 2007).

Crude proteins (CP) and amino acid contents

The amount of CP found in *M. oleifera* leaves were found ranged from 23% to 30.3% by Wu et al. (2013). Ten essential amino acids, including threonine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, histidine, lysine, and tryptophan, were found in *M. oleifera* after phytochemical investigation by Sanchez-Machado et al. (2010).

Crude fiber (CF)

The crude fiber composition of *M. oleifera* leaves are less than 5.9% (Wu et al. 2013). Generally, low content of CF suggests suitable appetizing for fish and livestock (Su and Chen 2020).

Mineral contents

M. oleifera leaves possess high mineral values with ash content of up to 12 percent (Wu et al. 2013). Its leaves are numerous in minerals like potassium, zinc, phosphorus, calcium and iron (Su and Chen 2020).



Anti-nutrient contents of *M. oleifera*

The compounds generated by plant's additional metabolic processing are known as anti-nutritional compounds and they have the potential to exert detrimental impacts upon the animal's dietary needs and overall health which feed on them, via a variety of processes (Nouman et al. 2014). On the basis of their mode of action, anti-nutritional elements are divided by most scientists into four categories: 1) protein consumption and metabolism inhibitors, 2) energy usage inhibitors, 3) animal's vitamin needs boosters, and 4) host's immune system suppressors (Soetan and Oyewole 2009). Many types of animal food material contain compounds like amino acids (non-proteinous in nature), terpenes, saponins, glycosides, tannins and alkaloids, etc. which all are anti-nutritional. Because of their unpleasant flavor, low palatable quality and poor digestion ability, these compounds are typically unsuitable for animals (Nouman et al. 2014).

M. oleifera has very few compounds which are anti-nutritional in nature (Pérez et al. 2010). On the basis of plant's genetic composition like the variety being grown and cultivation conditions, *M. oleifera*'s percentage of anti-nutritional compounds has a lot of fluctuations (Nasrin et al. 2014). With the exception of phenols and saponins, leaves of *M. oleifera* are generally devoid of anti-nutritional components (Egwui et al. 2013). Although seeds of *M. oleifera* contain many proteins, vital minerals and nutritious oils and is valuable in preparing and enriching animal diets due to its dietary advantages, but the reduced-fat seeds are preferred to be employed for animal meal preparation as their anti-nutritional components are minimum (Olagbemide and Philip 2014).

Leaves of *M. oleifera* possess a great deal of raw fiber material and anti-nutritional components which have difficult in digestion in fish body like saponins and tannins and are thus not used extensively as a constituent of meals for fish (Hussain et al. 2018). Some anti-nutritional compounds present in *M. oleifera* are discussed below:

Tannins

Phenolic molecules on combination with amylase or trypsin or their corresponding targets results in the formation of complex compounds which are known as tannins. The resulting complex compounds are difficult to digest and leads to low appetizing ability and less consumption of food (Vitti et al. 2005). Tannins constitute about 12 grams per kilogram of dry matter in *M. oleifera* (Udom and Idiong 2011). In contrast to newly harvested leaves, tannins can be reduced from 15 to 30% by employing process like ensilage, dehydration and evaporation, etc. (Vitti et al. 2005).

Saponin

Saponins, extracted from *M. oleifera*'s pods contain antimicrobial and chemotherapeutic properties (Paliwal and Sharma 2011). Leaves extracts of *M. oleifera* do not pose any harmful effect to animals which ingest them because they contain very less i.e. about 4.7-5 grams per kilogram of dry matter content of saponin (Foild et al. 2001; García et al. 2008). Many detrimental impacts will be shown on fish and other farm animals feed with leaves of *M. oleifera* as they contain extremely high saponins ratio (Busani et al. 2011).

Oxalates and phytates

As compared to other green plants, the oxalate as well as phytates concentration of leaves of *M. oleifera* is significantly low (Shih et al. 2011). Amount of oxalate present in leaves of *M. oleifera* is roughly about 27.5 milligrams per gram of dry matter content (Radek and Savage, 2008; Stevens et al. 2015) while in case of leaves extracts, *M. oleifera* contain 21 grams of phytates per kilogram of dry matter (Udom and Idoing 2011).

Ways to reduce anti-nutrient contents

One approach to lower the amount of raw fiber along with the elimination of anti-nutritional compounds from leaves of *M. oleifera* is the use of *Aspergillus niger* bacteria in the process of fermentation. The di-



gestion of food and nutrients is enhanced by the activity of cellulase, amylase, protease and other enzymes generated by *A. niger* (Masood et al. 2020). A latest investigation reveals that the use of *A. niger* as catalyst in fermentation process can enhance the nutritional value of leaves of *M. oleifera*. *A. niger*'s optimal dose was reported 6% by Suharman et al. (2022) for lowering the raw fiber content present in leaves of *M. Oleifera*. In another study conducted to treat *M. oleifera* leaves by Afuang et al. (2003) an identical approach was developed but by using methanol as a substitute. They discovered that in comparison to general fish feed category even if 33% of refined leaf feed of *M. oleifera* will be given to fish no appreciable variation in development and growth outcomes of fish will be observed (Afuang et al. 2003).

Another research shows that the anti-nutrient contents present in leaves of *M. oleifera* breakdown by using processes like high temperature, heating, bubbling and bleaching and the concentration of inhibitors of trypsin, phytate, cyanides and oxalate, etc. also reduce considerably (Sallau et al. 2012). A previous investigation also demonstrate that the leaves of plants become more appetizing and digestible for different animals when undergoes high temperature heating and this also demolish a few anti-nutritional compounds (Nwaogu and Udebuani 2010). Mbah et al. (2012) proved that the use of various drying strategies like direct use of sunlight, air drying at cool shady places and microwave processing lower the levels of many anti-nutritional compounds appreciably.

Comparison of *M. Oleifera* with Neem and Aleo vera

A detailed comparison of *M. Oleifera* with Neem (*Azadirachta indica*) and *Aleo vera* is given in the Table 2.

Dietary applications and beneficial uses of *M. oleifera* in fish diet

Numerous studies have demonstrated the value of adding *M. oleifera* to the diets of many fish species, including the enhancement of *Nile tilapia*'s growth performance (Elabd et al. 2019) and Rohu (Hussain et al. 2018), enhancement of feed utilization indices and nutrient digestibility coefficients of Bocourti's catfish (Puycha et al. 2017). The dietary applications and beneficial uses of *M. oleifera* in fish diet is briefly described in Table 3.

Effect of *M. oleifera* on fish growth

The increased development and widely growing aquaculture field has also impacted the greater needs and demands of the fish meal (FM) and to fulfill these demands, there is an increased need for innovative alternatives of the FM (Abdel-Latif et al. 2022). Innovative additives are being added to fish feed to improve the efficiency of feeding conversion and, ultimately, fish growth (Nasreen and Tayar 2022). Plant protein sources are preferred by fish due to their consistency, affordability, lack of phosphorus, a substantial amount of protein, and many different amino acid combinations (Shahzad et al. 2022). Experts in fish nutrition have addressed the introduction of alternative energy meals in fish diets.

M. oleifera has received considerable attention as a substitute for protein in fish diets and appears to be a potential protein source. Its leaves supplementation in the diet improves growth performance (Muhammad et al. 2016). The effects of *M. oleifera* in the diets and the food, as a supplement, in a variety of animals, including fish has been assessed and studied, which proved that *M. oleifera* can be consumed by the fish as a substitute and replacement of protein (Afuang et al. 2003). A certain range of *M. oleifera* concentrations included in the meal boosted the growth performance of various aquaculture species (Abdul-Lateef et al. 2022). *M. oleifera* leaf can be used to supplement traditional food in the diets of *Oreochromis niloticus* with increasing their growth performance (Richter et al. 2003; Elabd et al. 2019). Moreover, its leaf meal improves fish immunological response considerably (Adeshina et al. 2018). Because of it said nutritional content and low level of anti-nutrient factors its seeds and leaves are the most commonly used plant-based components in food preparation (Trigo et al. 2020). Its leaves have large concentrations of proteins and minerals together with vital vitamins which enhance fish health while promoting growth and sustaining the blood system (Aiyelari and Adeyeye 2022). Research shows that the consumption of *M. oleifera* improves both growth performance and health benefits of *Clarias gariepinus* juvenile stages (Chabi et al. 2015). Its leaves meal demonstrates effective results when applied to aqua feeding for different fish species because



Table 2 Comparison of *M. Oleifera* with Neem and *A. vera*

Plants	<i>M. oleifera</i>	Neem (<i>Azadirachta indica</i>)	<i>A. vera</i>
Common Name	Drumstick tree	Neem	<i>A. vera</i>
Parts Used	Leaves, pods, seeds	Leaves, bark, seeds	Leaves, gel
Main Nutrients	Vitamin A, C, calcium, protein (Fahey 2005)	Nimbin, nimbidin, azadirachtin (Subapriya and Nagini 2005)	Vitamins, enzymes, amino acids (Surjushe et al. 2008)
Health Benefits	Nutritional supplement, antioxidant, anti-inflammatory (Fahey 2005)	Antibacterial, antifungal, immune-boosting (Subapriya and Nagini 2005)	Skin healing, digestion aid (Surjushe et al. 2008)
Medicinal Uses	Diabetes, high BP, anemia (Fahey 2005)	Skin diseases, dental care (Subapriya and Nagini, 2005)	Burns, constipation (Surjushe et al. 2008)
Geographical Adaptability	Tropical & subtropical	Tropical	Semi-tropical, dry climates

Table 3 Overview of *M. oleifera* supplements in aquaculture: feed forms, recommended doses and biological responses by different species

Fish species	<i>M. oleifera</i> forms	Effects	Recommended dose	References
Bocourti's catfish (<i>Pangasius bocourti</i>)	Seed cake	No variations between serum biochemistry and SR percent No variations between HIS (hepatosomatic index) and digestibility In the distal intestines and liver, there are no histological changes ↑ <i>M. oleifera</i> seed cake = ↑FCR (feed conversion ratio), little poor growth, feed utilization	Not over 500g per kg diet	Yuangsoi et al. (2014)
Mozambique tilapia (<i>Oreochromis mossambicus</i>)	Leaf powder	Maximum WG (Weight gain) and SGR (Specific growth rate)	5 g per kg diet	Karpagam and Krishnaveni (2014); Mbokane and Moyoy (2018)
African Cat fish	leaves	Better WG and SGR in 15% group	Upto 15 percent	Eyo and Iyon (2017)
Hybrid tilapia	leaf powder	Alternate upto 20 percent of sardine meal protein without have an effect on growth.	Substitute upto 20% of sardine protein	Rivas-Vega et al. (2012)
Red breast tilapia	leaves	Intestinal villi that are shorter as <i>M. oleifera</i> levels rise	25 percent fish meal replacement	Hlophe and Moyoy (2014)

it improves growth rate along with survival rates and addresses other health concerns. Ochang et al. (2015) reported that the optimal growth performance for *C. gariepinus* occurs when using 20% *M. oleifera* leaf meal as feed. Its nutritional value consists of important minerals including Calcium (Ca), Iron (Fe), Potassium (K), Phosphorus (P) and Zinc (Zn) that enhance fish growth (Su et al. 2020). Research showed that *M. oleifera* leaf powder elevation of intestinal villi length and inter villus space length significantly speeds up fish growth and performance (El-Kassas et al. 2020).

The *M. oleifera* seed meal shows high absorption in diets while remaining safe for use as a plant-based protein that reduces fish dietary production expenses (Hashem et al. 2017). The aqueous extract demonstrated superior effectiveness than its leaves since it produced stronger effects on fish performance and growth together with stress resistance (Khalil and Kornilov 2017). The fish fed with the diet containing 100 g/kg *M. oleifera* leaf achieved the fastest growth rate among the tested groups. Testing *M. oleifera* leaves on Common carp diets proved beneficial by improving every growth parameter according to Mizory and Altaee (2023).

Assessments on aquatic animals reveal that *M. oleifera* leaf extract additions to food enhance developmental outcomes along with growth and physiological functions. Raising *Ctenopharyngodon idella* (Grass Carp) with a 15% *M. oleifera* extract within their diet enhanced both their growth development and their immunological system responses (Faheem et al. 2020). The use of 0.5% *M. oleifera* extract in the meal corresponded to increased development and growth rates of *Macrobrachium rosenbergii* (Kaleo et al. 2019). At a dose of 2.5 g per kg, *M. oleifera* supplementation improved growth performance in *Litopenaeus vannamei* (Abidin et al. 2022).

The dietary implementation of *M. oleifera* leaves at varying proportions generated findings which improved the growth and general performance of *Heterobranchus longifilis* according to Eyo and Ivon (2017). A 10% inclusion of *M. oleifera* leaf meal in Heteroclarias diets produced the best performance which led to weight increases (Aiyelari and Adeyeye 2022). Due to the presence and occurrence of tannins and secondary metabolites in *M. oleifera* in undesirable quantities may prevent the working of enzymes that help in digestion and other dietary proteins if used in a high concentration (Dongmeza et al. 2006). Fortunately, if used in right amount, with the inclusion of plant extracts in the food of aquatic animals promotes the stimulation of digestive enzymes and works as hunger enhancer, thereby boosting growth, development, and diet consumption (Radhakrishnan et al. 2014).

Role of *M. oleifera* in boosting immunity and disease control in fish

The therapeutic and medicinal uses of *M. oleifera* have been proven due to abundance of its antioxidant-rich phytochemicals that reduce oxidative stress (Kou et al. 2018). Fish protect itself through a process called phagocytosis, which may be affected by immune-stimulating agents by raising or elevating the number of phagocytes, or by encouraging the production of chemicals that contribute to defensive mechanism (Gultepe et al. 2014). Thus, in fish's natural immune response, these phagocytic cells thus perform a vital biological role (Zhang et al. 2009). Khalil and Kornilov (2017) stated that supplementation of *M. oleifera*

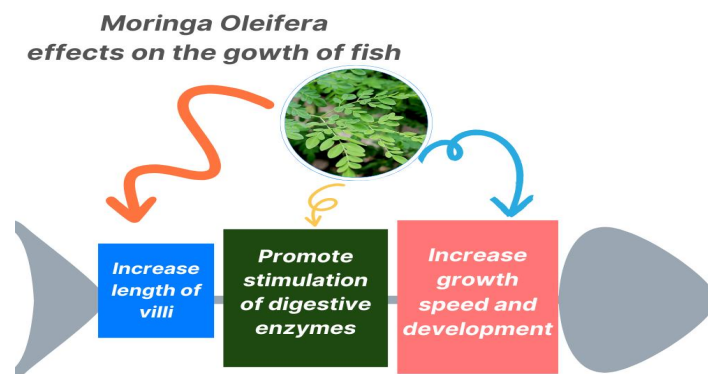


Fig. 1 Effects of *M. oleifera* supplementation on fish growth metrics

era leaves greatly boosted the activity of the phagocytic cells in fish. According to several other studies, feeding *M. oleifera* could enhance mucosal membrane protection in Guppies as well as the immunological responses and disease resistance in *O. niloticus*, *O. mossambicus*, *C. carpio*, *Sparus aurata*, *C. gariepinus*, *M. rosenbergii* and also in *Carassius gibelio*.

According to Bisht et al. (2020), feeding guppies with 15% *M. oleifera* leaf powder per kg of diet can greatly increase their skin mucus defensive mechanism by increasing the amount of mucus production. Elgendy et al. (2021) findings suggest that adding crude *M. oleifera* to the diet of *N. tilapia* improves antioxidant properties, growth, immunological proficiency, and boosts resistance to *Aeromonas hydrophila*. Tekle and Sahu (2015) resulted that adding *M. oleifera* flower ethanolic extract into *N. tilapia* food at a concentration of 0.25% can boost the fish's immune system and, as a result, increase their growth potential as well as resistance to different diseases. Monir et al. (2020) proved that tilapia fed with 0.25 mg/kg *M. oleifera* leaf extract in their diet experienced enhanced serum lysozyme activity and increased immunity levels.

The researchers from Mbokane and Moyo (2018) reported that *M. oleifera* food consumption enhanced immune system function by improving lysozyme serum activity along with better resistance capabilities and increased Mozambique tilapia survival under *A. hydrophila* bacterial infections in their experimental studies. Furthermore, the presence of chemically active substances or immuno-stimulants is believed to majorly contribute to resistance to different diseases as phytoconstituents of *M. oleifera* includes high amounts of total phenols, total polyphenols, total carotenoids, flavonoids, as well as vitamins E and C. When Common Carp were exposed to *A. hydrophila*, an aqueous extract of *M. oleifera* incorporated into the diet could boost serum Lysozyme activity and increase resistance.

García-Beltrán et al. (2020) experimentally treated pathogenic strains of *Vibrio anguillarum* and *Photobacterium damsela* with *M. oleifera*, the ethanolic and, especially, the aqueous extracts exhibited significant bactericidal action. Additionally, it was shown by Mbokane and Moyo (2020) that supplementing food with *M. oleifera* essential oil extracts significantly boosted blood lysozyme activity and increased survival rates from 3% to 12%.

Antioxidative potential of *M. oleifera*

Its leaves possess a variety of phytochemicals with antioxidant qualities and functions in preventing and treating a wide range of illnesses, such as diarrhea, asthma, and various malignancies (Gupta et al. 2018). They also possess high concentration of phytonutrients, including tocopherols, carotenoids, and ascorbic acid, which are beneficial dietary antioxidants (Qwele et al. 2013). The chemical substances that can donate hydrogen to free radicals to reduce rancidity and delay lipid peroxidation without affecting the normal functionality are referred as antioxidants (Lahucky et al. 2010). Ahmed et al. (2020) noticed that Nile Tilapia given with a supplement high in *M. oleifera* showed a substantial decrease in MDA levels and a significant

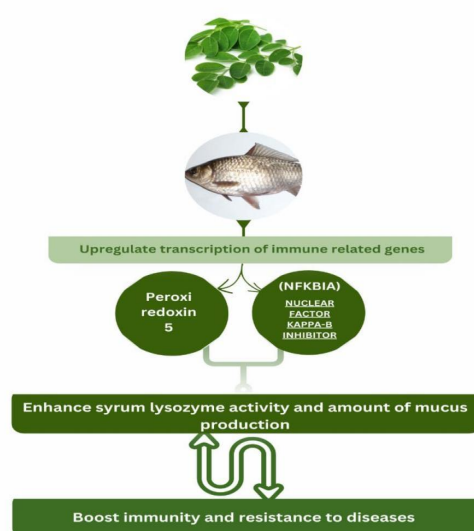


Fig. 2 Schematic representation of *M. oleifera* feed-mediated immune activation in fish

rise in the enzyme activity of GPx, GSH, CAT, and SOD. Furthermore, oxidative stability in oils utilizing antioxidants is crucial since it delays fat and oil deterioration and extends their functional lives. The *M. oleifera* components (seeds, leaves and flowers) oxidative stability was also investigated by Nascimento et al. (2013) with leaves having the highest stability due to an elevated amount of phenolic compound, supporting their higher antioxidant capability. Other findings on fish fed plant-based dietary supplements have found that the changes seen are related to the nature of the ingredients, dosages, and time period (Van-Doan et al. 2017).

Siddhuraju and Becker (2003) tested the radical-scavenging and antioxidant properties of extracts freeze-dried *M. oleifera* leaf in aqueous ethanol, water and aqueous methanol. The extracts from *M. oleifera* leaves showed dose-dependent radical scavenging consequence in the carotene linoleic acid system after 14 days of incubation. It should be mentioned that some fish species' lipid profiles are characterized by the existence of oxidatively degradable long-chain polyunsaturated fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Izquierdo et al. 2005). Several investigations have indicated that muscle tissue of seabream is particularly susceptible to changes in fatty acid content caused by food (Benedito et al. 2010). Based on the aforementioned, considering the muscle of seabream fed with *M. oleifera* enriched diets shows an apparent rise in levels of both oleic and linoleic acid.

Future perspective and conclusion

The global market for fish and seafood has expanded greatly over the last few decades, driven by a growing worldwide population and increased knowledge of the health advantages of eating fish. With this upsurge in demand, there has also been a rise of market demand for fish food. Fish feed is critical to the aquaculture business because it offers the nutrients required for fish to grow, develop, reproduce and flourish in captivity. Traditional fish feed sources, such as fishmeal and fish oil, are becoming increasingly scarce and costly. So, something which is natural and economical should be used to fulfill this need. Scientific research has demonstrated that *M. oleifera* shows promise for improving fish growth together with higher development rates and increased health outcomes. The nutritional composition makes *M. oleifera* work as an eco-friendly natural replacement for both synthetic additives and antibiotics in fish feed. The full utilization of *M. oleifera* in fish feed requires additional scientific research to establish optimal application doses and processing protocols. *M. oleifera* serves as a safe and environmentally friendly approach to produce fish feed which brings nutritional benefits to the aquaculture industry. The use of *M. oleifera* in fish feed composition can potentially reduce the need for conventional fish feed components that industries acquire through environmentally harmful procedures including overfishing and overhunting and natural resource exploitation and deforestation. Further research needs to advance the realization of *M. oleifera*'s potential although safety testing must take place to ensure its security and effectiveness.

Numerous studies reported positive effect of *M. oleifera* meal on fish immunity and development but at various percentages. So, a proper research work should be carried out to determine a safe, specie-specific inclusion level that enhance growth, FCR, survival and nutrient utilization in fish. Different processing investigations to reduce anti-nutritional content of this plant should be required to assess their impact on

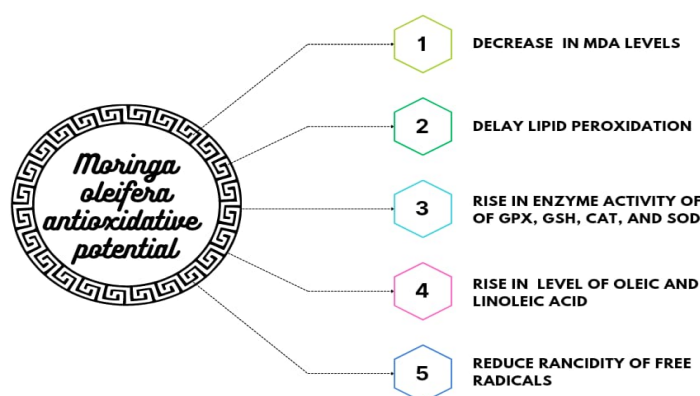


Fig. 3 Metabolic impacts related to antioxidant potential of *M. oleifera*

digestibility, gut histology and nutrient absorption in fish that ultimately lead to the selection of one appropriate processing technique. The studies reported in this article, were limited to some specific fish species. Therefore, there is a need to expand trials to other fish species to have a holistic understanding about incorporation of *M. oleifera* in fish feed. Research gaps related to cost-effectiveness, reproductive performance, fillet quality and feed palatability of *M. oleifera* should be addressed to improve its use as potential fish feed supplement.

Authors' contributions The study idea originated from TA and HN. The review article for this idea was written and surveyed from literature by AS, SA, IA, HA, AI, MHB and RR. MA along with HN and TA performed a final checkup on the manuscript before its publication. Every author examined and approved the last revisions made to the manuscript.

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