Chapter 29

Role of Essential Oils in Aquatic Health: New Technique for Management of Disease without Inducing Resistance in Pathogens

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ABSTRACT

Because of increased incidence of antibiotic resistance and environmental contamination in aquaculture industry the application of essential oils (EOs) has become a popular alternative approach. This chapter provides comprehensive details about potential of EOs to combat the fish diseases, highlighting the best alternative to traditional antibiotics. This antimicrobial activities of EOs is due to their unique mode of action, as they disrupt regular cellular functions and structures, hence, show equal tendency against Gram-negative and Gram-positive bacteria. Bacterial motility and invasion mechanisms along with virulence is inhibited after the application of EOs, indicating higher efficacy of EOs against these aquatic pathogens. A number of studies have reported the controlling impact of EOs against infections induced by bacterial species including *Pseudomonas, Vibrio, Flavobacterium* and *Aeromonas* species. Other than this, efficacy of EOs against various fish pathogens i.e., *Nocardia, Lactococcus* and *Streptococcus spp* is notable. Anyhow, it is important to consider practical applications of EOs such as selected dose, application methods and expected impacts on aquaculture. The main challenge in establishing the EOs industry is to standardized formulations and optimization of their efficacy with regards to diverse aquatic conditions. Essential oils provide an alternate options for disease management in aquaculture and by harnessing their antimicrobial properties we can overcome antimicrobial resistance along with environmental contamination, ensuring the sustainability of aquatic environments.

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INTRODUCTION

Essential oils (EOs) are those compounds that are derivative of plants and known since ancient times due to its applications in folk medicine and perfumery industries. Numerous parasitic diseases afflict animals, and essential oils play a vital role in their treatments (Toor et al., 2024). EOs compositions depend on various factors including environmental situations (temperature, humidity, sunlight and soil quality), plant part that used for extraction (fruits, leaves, roots, bark and wood), extraction technique and harvesting season. Because of this much variabilities, there always remains a need to adjust doses accordingly to maximize the therapeutic impact of these compounds. Overall, it is important to adjust the required dose carefully and selection should be done by considering composition and efficacy of EOs against selected pathogens accordingly.

Substantial variations have been detected not only among various bacterial species but also among strains within a single species. Essential oils mainly disrupt the bacterial cellular structure, resulting in compromised membrane integrity and the disruption of various cellular functions, together with membrane transport and energy production. Additionally, ruptured cell membrane can result cellular component leakage along with ions loss (Ebani and Mancianti, 2020). Thus, EOs play a significant role in reducing bacterial virulence and impeding mechanisms related to bacterial invasion and motility (Bektaş and Özdal, 2022). The investigation into the antibacterial properties of EOs has garnered growing attention, particularly due

to the need for alternative treatments against antibiotic-resistant strains infections as these strains pose a serious threat in both veterinary and human medicine.

In Gram-positive bacteria, the unique cell wall structure facilitates the diffusion of hydrophobic molecules like EOs, allowing them to be functional both in cytoplasm and on the cell wall (Nazzaro et al., 2013). Anyhow, Gram-negative bacteria due to complex bilayer membrane show resistance against antibacterial compounds when compared with Grampositive bacteria as they have single membrane (Savage, 2001). However, it's noteworthy that the second membrane of Gram-negative bacteria is not entirely impermeable to hydrophobic molecules, as some can slowly penetrate through porins (Sutili et al., 2018).

Harnessing the Power of Essential Oils for Enhanced Aquatic Health

Essential oils usually extracted from geranium (Pelargonium graveolens) and lemongrass (Cymbopogon citratus) leaves by using hydro-distillation method, and through gas chromatography/mass spectrometry (GC-MS) their chemical compositions analyzed. The findings from Al-Sagheer et al. (2018) study indicate notable improvements in growth performance, enhanced antioxidant and immune activities and reduced pathogenic bacterial loads in Oreochromis niloticus fish through the supplementation of their diets with essential oils extracted from P. graveolens and C. citratus. Both Geranium and Lemongrass EOs in vitro application revealed inhibitory effects against pathogenic Aeromonas sobria and A. hydrophila. Moreover, there was a clear reduction of oxidative stress in fish that were receiving EOs as indicated by increased catalase activity and reduced glutathione activity in fish plasma. Similarly, EOs applications enhanced IgM level and lysozyme activity in fish. Water and intestinal analysis indicated reduced bacterial counts, specifically Aeromonas and Coliform species. The beneficial effects of dietary supplementation of Thymus vulgaris EOs is remarkable in rainbow trout (Oncorhynchus mykiss). Thymol, cymene and terpinene are the key compounds and use of *Thymus vulgaris* EOs significantly enhanced the growth of fish as indicated by parameters such as weight gain, length gain, daily growth coefficient and specific growth rate. In fish, gene expression analysis indicated a high regulation of immune related genes including interleukin-1ß (IL-1ß), component C3, CD4, and lysozyme. Moreover, serum lysozyme activity was notably higher and enzyme assays indicated varying levels of aspartate transaminase, alkaline phosphatase and alanine transaminase. Following challenge with Aeromonas hydrophila, fish fed TVEO-supplemented diets exhibited significantly higher relative percent survival (RPS) compared to the control group, suggesting enhanced disease resistance (Zargar et al., 2019).

The microbial presence in fish is significantly influenced by the level of microbiological contamination in their aquatic habitats and the hygienic conditions maintained during various stages such as cultivation, harvesting, during processing, in storage and in transportation process (Alerte et al., 2012; Fuertes Vicente et al., 2018). Various bacterial infections affecting fish, such as Aeromonas septicemia, Edwardsiellosis, Columnaris, Streptococcosis, and vibriosis, pose significant challenges to the aquaculture industry. Despite the diverse range of pathogens, few of these are responsible for substantial economic losses globally (El-Ekiaby, 2019).

Targeting Aeromonas Species: Harnessing Herbal Essential Oils for Pathogen Control

Aeromonas species, for instance, are prevalent bacterial pathogens found in freshwater and tropical fish species, often leading to bacterial hemorrhage in fish populations. Among these, *Aeromonas salmonicida* stands out as one of the oldest and most widespread fish pathogens, affecting both freshwater and marine species, infection commonly include skin ulceration and hemorrhages (Menanteau-Ledouble et al., 2016). This bacterial species has long been identified as the primary agent responsible for induction furunculosis in fish. Motile Aeromonas septicemia in fish is commonly caused by bacteria such as *A. hydrophila*, *A. veronii* and *A. sobria*. Moreover, various *Aeromonas* species are known to induce disease in fish (Mzula et al., 2019). Infections caused by *A. hydrophila* are linked to a range of symptoms, including red body disease, hemorrhagic septicemia, epizootic ulcerative syndrome, edema and hemorrhagic enteritis. These infections impact various cultivable finfish species such as Goldfish, Common Carps, Catfish, Eel, and Tilapia (Thirumalaikumar et al., 2021).

Antimicrobial properties of certain herbs derived EOs have been evaluated by certain experts against *A. salmonicida*. Hayatgheib et al. (2020) reported minimum inhibitory concentration and minimum bactericidal concentration fall between 113 to \geq 3628 µg/mL. Those herbs which shown maximum efficacy include *Thymus vulgaris, Eugenia caryophyllata, Cinnamomum zeylanicum/verum, Origanum compactum, O. heracleoticum* and *O. vulgare.* Moreover, against *A. salmonicida* 18 isolates, antimicrobial properties of essential oils derived from *O. vulgare, O. onites* and *Thymbra spicata* have been reported. In a study, Tural et al. (2019) reported that essential oils collected from *T. vulgaris, Petroselinum crispum, Laurus nobilis* and *Laurus nobilis* have significant inhibitory effects against *A. salmonicida* with a zone diameter of 30mm. Starliper et al. (2015) reported a higher inhibitory activity of *Cinnamomum cassia* EO against *A. salmonicida* with zone diameter of 56mm.

Essential oil collected from *T. vulgaris* shown efficacy against *A. sobria* and *A. veronii* (Tural et al., 2019). Additionally, Gulec et al. (2014) determined 32.7mm zone diameter of *Origanum acutidens* essential oil against *A. hydrophila*. Moreover, *C. aromaticum*, *C. cassia*, *O. vulgare* and *Cymbopogon citratus* essential oils have found to be effective to control various strains of *Aeromonas*, including *A. salmonicida*, *A. hydrophila* and *A. veronii* with mean percentage minimum bactericidal concentration (MBC) values ranging from 0.02% to 0.65% ((Starliper et al., 2015).

Potential of Essential Oils in Mitigating Vibrio-induced Vibriosis

Throughout history, members of the Vibrionaceae family have posed significant infectious threats to marine fish species (Schiewe et al., 1981). Vibriosis poses a significant challenge to fishery market worldwide, impacting a diverse group aquaculture animals. Two main species including *Vibrio anguillarum* and *V. harveyi* are survivor of both marine and freshwater environments and induce vibriosis in a number of economically important fish species. The more severe hemorrhagic septicemia cases in Rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Japanese seaperch (*Lateolabrax japonicus*) are usually induced by *V. anguillarum*. In aquaculture industry, its outbreaks usually result in extensive fish mortality and substantial economic losses (Yang et al., 2007; Yang et al., 2021).

Research investigating the antimicrobial properties of EOs from *C. verum*, *C. citratus*, *T. vulgaris*, *M. alternifolia* and *O. vulgare* against *V. parahaemolyticus*, *V. harveyi*. *V. campbellii* and *V. vulnificus* and the results against *Vibrio* spp. have revealed moderate to weak inhibitory impacts of these selected EOs (Domínguez-Borbor et al., 2020). Similarly, Wei and Wee (2013) demonstrated that EO from *Cymbopogon nardus* revealed potent inhibitory effects against *Vibrio damsel* and *Vibrio spp*. with 0.488µg/mL and 0.244µg/mL MIC values, respectively. Likewise, *T. vulgaris* EO with MIC values 80 µg/mL against *V. ordalii* was reported to induce strong inhibitory effect (Navarrete et al., 2010). Lee et al. (2009) reported a significant activity of *Syzygium aromaticum* EO against six different *Vibrio* spp. isolates, with 0.015µg/mL MIC values.

On the other hand, EOs of O. vulgare subspecies including O. marjorana, O. Hirtum and O. onites EOs shown inhibitory effects on Listonella anguillarum, V. splendidus and V. alginolyticus with 9.1-14.1mm, 7.3-14.3mm and 7.8-13.6mm zone diameters, respectively (Stefanakis et al., 2013). Additionally, Öntaş et al. (2016) reported marked activity for EO of Argania spinosa, with 62.5µL/mL MIC value against L. anguillarum.

Potential of Essential Oils in Combating Pseudomonas Infections in Aquaculture

Pseudomonas anguilliseptica is commonly categorized as opportunistic pathogen that impacts a number of fish species in both brackish and marine water aquaculture operations globally. Initially in Japanese eel farming it was identified as the causative agent behind red spot disease, since then it has been detected in various other regions, infecting both wild and cultured fish. Among affected species are Black Sea Bream, European Eel, Atlantic Salmon, Whitefish, Ayu, Sea Trout, Rainbow Trout, Baltic Herring, Orange-Spotted Grouper and Striped Jack (López-Romalde et al., 2003; Fadel et al., 2018). Moreover, *P. fluorescens* poses a threat to various farmed fish species. EI-Ekiaby (2019) testified that EO of *Ocimum basilicum* with 9 µL/mL MIC value displayed potent inhibitory activity against *P. fluorescens*. Additionally, Wei and Wee (2013) and Lee et al. (2009) found EOs from *Syzygium aromaticum* and *Cymbopogon nardus* shown marked activity against *P. aeruginosa* and *Pseudomonas spp.*, respectively. The EO from *T. vulgaris* with 640 µg/mL MIC value against *Pseudomonas spp.* exhibited moderate inhibitory effect (Navarrete et al., 2010). In a study, Tural et al. (2019) compared the activity of EOs of *L. nobilis*, *T. vulgaris*, *P. crispum* and *R. officinalis* against *P. fluorescens*, and the EO of *T. vulgaris* demonstrated 26.5mm the highest zone diameter among all. Moreover, EO of *T. vulgaris* also shown 13mm zone diameter and prove to be most effective against *P. aeruginosa*.

Essential Oils in Mitigating Citrobacter Infections in Diverse Fish Species

Citrobacter spp. poses a threat to fish species as an opportunistic pathogen. *C. freundii* is frequently isolated in fish leading to significant damage and high mortality rates. Notable among the *Citrobacter* species, two species *C. freundii* and *C. braakii* cause diseases in fish species (Jeremić et al., 2003; Lü et al., 2011), former is associated with various fish afflictions, including gill lesions, severe kidney disease, severe enteritis and episodes of hemorrhagic septicemia, particularly in catfish species such as *Pseudoplatystoma spp.* (Pádua et al., 2014).

In catfish (*Rhamdia quelen*), this bacterial group induce leukopenia with neutropenia, anemia degenerative disease, reduced renal hematopoietic tissue, leucoblastosis, protein loss, lymphocytosis, liver degeneration, and the presence of melanomacrophage centers in cephalic kidney and spleen, ultimately result in mass mortality of fish (Junior et al., 2018). Tilapia species, including *Oreochromis mossambicus*, often exhibit symptoms such as body reddening, septicemia, tail necrosis and hemorrhage. Due to these infection the Nile tilapia (*O. niloticus*) frequently experiences high mortality rates. Similarly, economically important Rainbow trout (*O. mykiss*) may display dark pigmentation, gastroenteritis, unresponsiveness to external stimuli, incoordination, intestine petechial hemorrhage, bilateral exophthalmia, pale spleen and ultimately mass mortality. Moreover, in cyprinids like *Cyprinus carpio*, the main infection includes systemic infection and hemorrhagic gastroenteritis (Puello-Caballero et al., 2018). For other species such as Eels (*Anguilla japonica*), Angelfish (*Pterophyllum scalare*), Freshwater Rays (*Potamotrygon motoro*), Pacu (*Piaractus mesopotamicus*) and shrimp (*Litopenaeus vannamei*) infection can lead to bleeding and the formation of hepatic and splenic granulomas (Bandeira et al., 2017).

To overcome various bacterial strains application of essential oils is a better option due to attack mechanism. Anyhow, it is crucial to find out which essential oil is effective against which bacterial strain and species before the selection. It is possible that one specific EO shows moderate, low or high inhibitory effects at the same time against different species. For example, against *C. freundii*, the *O. gratissimum* and *H. ringens* essential oils revealed weak to moderate inhibitory impacts (Bandeira et al., 2017). Nonetheless, *L. origanoides* EO showed moderate inhibitory effect (Majolo et al., 2019). Similarly, Öntaş et al. (2016) testified that *Argania spinosa* EO is operational to overcome *C. freundii* as demonstrated by zone diameter of 15mm. Furthermore, it was reported that *C. nardus* EO revealed MIC=0.244µg/mL for *C. freundii* (Wei and Wee, 2013).

The applications of Essential Oil to Mitigate Risks associated with Raoultella ornithinolytica

Raoultella ornithinolytica, is an encapsulated bacterium that belong to family Enterobacteriaceae. It is an aerobic gramnegative bacillus that is non-mobile and frequently establish its colonies in aquatic habitats. This bacterial species displays high resilience to maintain viability even at 4°C remarkably (Silva et al., 2016). This bacterium is recognized worldwide as a freshly evolving and potentially life-threatening pathogen. Its impact are not only limited to aquatic environments, but also induce infections in humans. It has the conversion ability and do convert histidine to histamine, ultimately induce skin flushing and poisoning leading to scombroid syndrome. The damage induced because of this bacterium create complications and there is urge need to understand and manage *R. ornithinolytica* induced infections (Kanki et al., 2002; Haruki et al., 2014). This pathogen cause considerable losses to aquaculture operations. There are a number of antibiotics available in market to treat this pathogen but there always remains considerable evidence of drug resistance. Anyhow, essential oil derived from *Ocimum gratissimum* shown significant inhibitory effects against *R. ornithinolytica*.

The application of Essential Oils to Combat Nocardia seriolae Infections

In fish the nocardiosis disease is primarily caused by *Nocardia seriolae*, linked through production of granulomas in kidney, spleen, epidermis and gills of crowded fish species. The spreading speed of this disease is slow, hence cause mortalities throughout the culture period gradually (Chen and Tung, 1991; Wang et al., 2007). Due to this disease significant loses have been reported in aquaculture and fisheries sector each year (Wang et al., 2009). A number of fish species including Gray Mullet (*Mugil cephalus*), Japanese Perch (*Lateolabrax japonicus*) and Striped Bass (*Morone saxatilis*) faces mass mortality because of *N. seriolae* infections. Moreover, both wild and culture species have faced an upsurge of *N. seriolae* induced infections. Most commonly affected species include *Trachinotus blochii*, *Epinephelus spp., Scatophagus argus*, *Terapon jarbua*, *Lutjanus erythropterus Leionathus equulus*, and *Lates calcarifer* (Tanekhy et al., 2010). Ismail and Yoshida (2017) conducted a study to assess the efficacy of various essential oils (EOs) against 80 isolates of *Nocardia seriolae*. Their investigation unveiled a broad spectrum of minimum inhibitory concentration (MIC) values for EOs of *Thymus vulgaris*, *Cinnamomum zeylanicum*, *Melaleuca alternifolia* and *Cymbopogon flexuosus* ranging from 5 to >5120 µg/mL. Notably, EOs of *C. zeylanicum* and *T. vulgaris* emerged as the most potent among the tested herb species to overcome the bacterial species.

Confronting Flavobacterium Challenges by Essential Oils for Aquaculture Health

In marine and freshwater environment the Flavobacterium species are commonly found, presenting significant challenges in aquaculture industry. Within the genus Flavobacterium, three distinct species have been identified as pathogens affecting freshwater and wild fish populations on a global scale. Among this group *F. columnare* cause columnaris disease, while bacterial gill disease induced by *F. branchiophilum* and similarly for cold-water disease *F. psychrophilum* is well known. Research has highlighted that these pathogens exhibit a remarkably broad spectrum of hosts and geographic distribution, making them significant threats to aquatic ecosystems (Orieux et al., 2013; Viel et al., 2021).

The essential oil of *Thymus vulgaris* has been shown to possess potent inhibitory effects with 320µg/mL MIC value against *F. psychrophilum* (Navarrete et al., 2010). Furthermore, *Syzygium aromaticum* EO demonstrated high efficacy against *Flavobacterium spp.*, exhibiting 0.031µg/mL MIC value (Lee et al., 2009), while *Cymbopogon nardus* EO showed susceptibility at 0.977µg/mL MIC value (Wei and Wee, 2013). Moreover, *Rosmarinus officinalis* EO displayed a moderate zone of inhibition, measuring over 18 mm against *F. psychrophilum* (Ostrand et al., 2012). Notably, *Allium tuberosum* EO exhibited significant activity against various isolates of *Flavobacterium columnare*, within 20µg/mL-80µg/mL MIC value range (Rattanachaikunsopon and Phumkhachorn, 2009).

Applications of Essential Oils to Combat Staphylococcus Challenges

The members of *Staphylococcus* presents a notable challenge as a Gram-positive opportunistic pathogens within aquaculture settings. Noteworthy species from both clinical and food safety perspectives include *Staphylococcus aureus*, *S. epidermidis*, *S. cohnii* subsp. *urealyticum*, *S. lugdunensis*, *S. intermedius*, *S. capitis* subsp. *ureolyticus*, *S. haemolyticus*, *S. hominis* subsp. *hominis*, *S. saprophyticus*, *S. warneri*, *S. simulans* and *S. auricularis* (Sánchez et al., 2017). Among these *S. aureus* stands out as significant opportunistic pathogen prevalent in natural environments. Known for its virulence factors, *S. aureus* when coupled with compromised host defenses, leads to colonization and various diseases. These diseases encompass septic arthritis, pneumonia, abscesses, osteomyelitis, meningitis, pericarditis, empyema, endocarditis and most importantly the toxin-mediated conditions like scarlet fever, food poisoning, toxic shock syndrome and scalded skin syndrome (Arteaga Bonilla and Arteaga Michel, 2005). Notably, *S. aureus* considered to be accountable for numerous instances of foodborne illnesses, including food poisoning, which is usually spread by consuming contaminated food. Consumption of contaminated fish has also been implicated in inducing poisoning (Macori et al., 2016). It was observed that *Ocimum acutidens* EO demonstrated a substantial inhibitory effect, evidenced by a zone diameter of 28mm against *S. aureus* (Shehata et al., 2013). Anyhow, *Syzygium aromaticum* and *Eruca sativa*, showed no discernible inhibitory effects on *S. aureus* (Shehata et al., 2013).

Confronting Streptococcus, Lactococcus and Vagococcus salmoninarum Challenges in Aquaculture

Streptococcus species pose significant concerns for both cold and warm water salmonid species, having zoonotic implications too. One important species include *Lactococcus garvieae*, which is linked to warm-water streptococcosis, results in considerable mortalities, amounting to thousands of tons. On the other hand, cold-water streptococcosis is induced by *Vagococcus salmoninarum*, which causes chronic disease as well as significant broodstock mortality, leading to devastating impacts on aquaculture operations (Saticioglu et al., 2021).

Species belonging to the Streptococcaceae family represent significant Gram-positive pathogens in fish species. Various *Streptococcus* species, including *S. iniae*, *S. parauberis*, *S. agalactiae*. *S. dysgalactiae*, *V. salmoninarum* and *L. garvieae* have been documented across various regions globally (Baeck et al., 2006; Agnew and Barnes, 2007; Nho et al., 2013; Li et al., 2015). Streptococcosis in fish is a complex disease influenced by factors such as host species, pathogen type (strain and species), age, immune status and importantly environmental conditions (Vendrell et al., 2006). Initially, Streptococcal infections in fish manifest on the fins, skin, external organs and gills. It was determined that among nanoemulsions and EOs of *Lavandula angustifolia*, *Eucalyptus globulus*. *Melaleuca alternifolia* and *O. vulgare*; nano-emulsion exhibited the highest effectiveness against *S. iniae* (Gholipourkanani et al., 2019). Additionally, EO of *Oliveria decumbens* displayed 69mm zone of inhibition, with 0.5mg/mL MIC and 2mg/mL MBC values against *S. iniae*, as reported by Vazirzadeh et al. (2019).

Lactococcus spp. have been identified as causative agents of fish diseases. L. garvieae is a gram-positive bacterium characterized by hemolytic, chain-forming cocci. Severe hemorrhagic septicemia has been associated with this bacterial species and it can also lead to meningoencephalitis in a number of fish species. Hence, it poses a significant threat, impacting a wide array of freshwater and marine species and ultimately leading to economic losses. Emergence of warm water lactococcosis, primarily attributed to *L. garvieae*, has become a major concern, especially during summer when temperature of water increase directly above 21°C. Because of this illness significant loss of production of rainbow trout occur during last few decades (Halimi et al., 2020). To control the *Lactococcus garvieae* by application of *Thymus vulgaris* EO a study was conducted by Gulec et al. (2014). Results of this experiment shown a zone diameter of 36.7mm against *L. garvieae*. Furthermore, Tural et al. (2019) compared the activity of essential oils collected from *T. vulgaris, Rosmarinus officinalis, Petroselinum crispum* and *Laurus nobilis* against *L. garvieae*. Outcome of this study exhibited *T. vulgaris* have more potential to control the colonies of *L. garvieae* with a zone diameter of 29.5mm.

One more genus of Gram-positive cocci is *Vagococcus* that have been discovered recently and is famous because of absence of catalase enzyme activity. Within this genus, the total identified species includes; *V. acidifermentans, V. salmoninarum, V. fluvialis, V. lutrae, V. elongates, V. fessus, V. penaei* and *V. carniphilus*. Of these, the most popular bacterium is *V. salmoninarum* because it not only causes disease outbreaks in rainbow trout, but also have been associated with widespread incidence among salmonid species across the globe (Didinen et al., 2014). A number of studies have been conducted to evaluate the impact of essential oils on this selected genus of bacterium. Effects of essential oils including *Eugenia caryophyllata, Zingiber officinale, Rosmarinus officinalis, Origanum vulgare, Lavandula hybrid, Hypericum perforatum, Nigella sativa* and *Mentha piperita;* importantly *O. vulgare* and *E. caryophyllata* EOs shown high potential to control colonies of *V. salmoninarum*.

Conclusion

In aquaculture industry, the role of essential oils to combat the aquatic diseases is undebatable as it is safe and environmental friendly method. The use of antibiotics pose environmental pollution and chances of antibiotic resistance are maximum, while at the same time, the essential oils provides an alternative approach to control fish diseases without the fear for induction of resistance in pathogens. Essential oils shown efficacy against both Gram-negative and Grampositive bacteria because of its unique mode of action including disruption of structures and functions of host pathogens. It is crucial to understand efficacy and composition of EOs against specific host pathogen along with the need of proper information regarding application method and standardized formulations. Application of EOs induce disease resistance in fish and continuous research is required in this area to explore the full potential of EOs for wellbeing of different aquatic environments.

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