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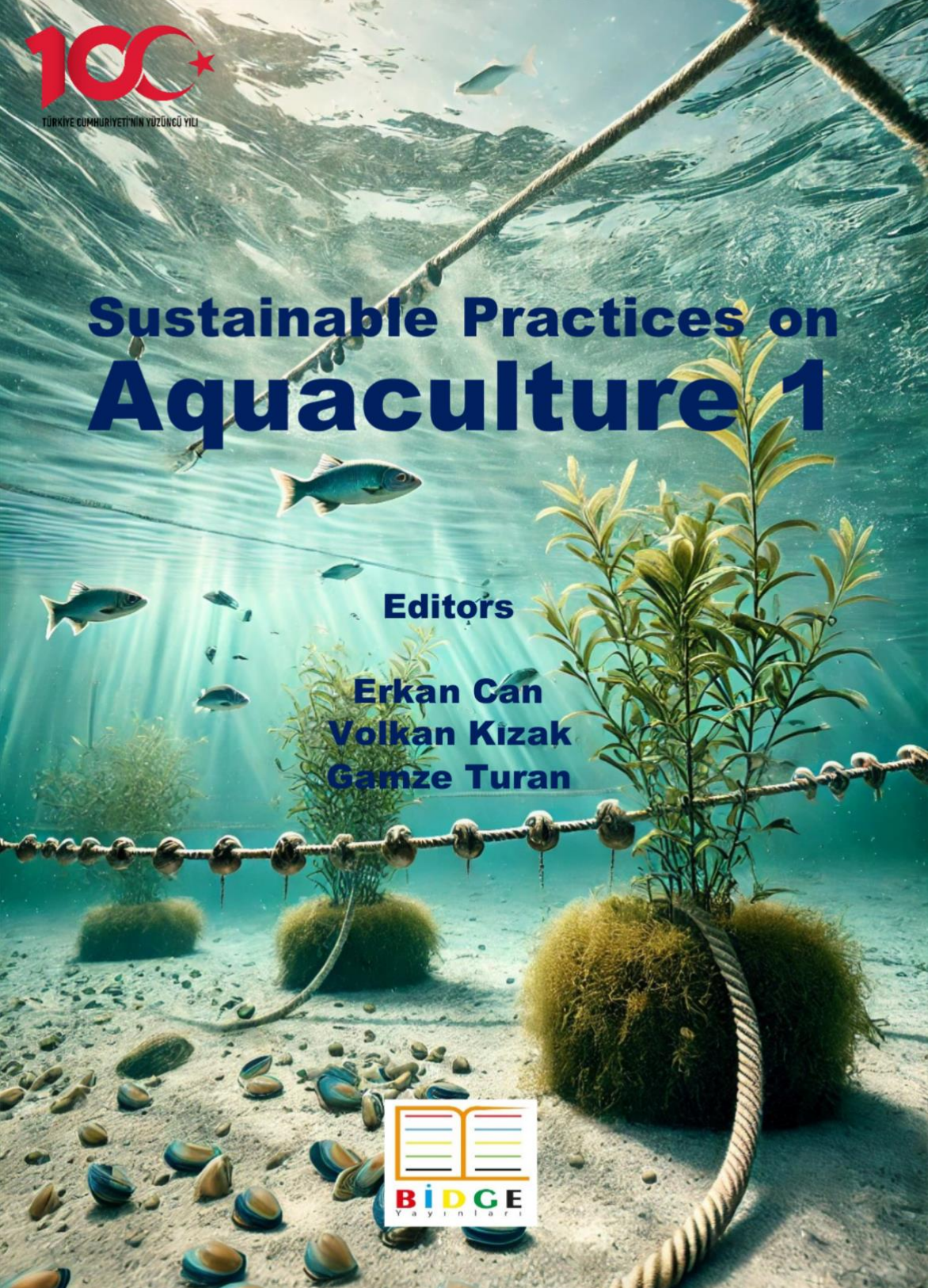
TÜRKİYE CUMHURİYETİ'NİN YÜZÜNCÜ YILI
Sustainable Practices on
Aquaculture 1
Editors
Erkan Can
Volkan Kızak
Gamze Turan
BİDGE
Yayınları

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# PREFACE

In today's world, sustainability is no longer a choice but an imperative for humanity. The rapid growth of the global population, the depletion of natural resources, and increasing environmental challenges highlight the need for innovative and sustainable approaches in various fields, from food production to ecosystem conservation. Aquaculture and marine ecosystems hold critical importance in this regard, as oceans and seas are vital resources for both food security and biodiversity.

This book has been prepared to compile the latest advancements and studies on sustainable practices in aquaculture and marine ecosystems. ***Sustainable Practices on Aquaculture 1*** underscores innovative approaches and multidisciplinary solutions while offering practical examples of how sustainability principles can be implemented effectively.

The chapters in this book address diverse aspects of sustainability in the aquaculture sector:

- Integrated Multi-Trophic Aquaculture (IMTA) Case Studies in Türkiye focus on local dynamics within the sector,
- Sustainable Practices in Trout Farming provide a guide for improving current practices,
- Disinfection of Fish Eggs with Medicinal Aromatic Plants introduces an innovative alternative to traditional methods,

- Sustainable Developments in Offshore Aquaculture explore how future technologies can merge with environmentally friendly production, and
- Nanotechnology and Blue Crabs: Carbon Quantum Dots in Marine Conservation highlights pioneering research on the role of technology and innovation in marine conservation.

I would like to extend my gratitude to all the authors and researchers who contributed to the preparation of this book. Their work significantly enhances awareness of sustainability and brings forward practical solutions in this field. I also wish to thank the editorial team and supporters who have played an essential role in bringing this publication to fruition.

***Sustainable Practices on Aquaculture 1*** aims to inspire researchers, industry professionals, and all readers interested in sustainability. I believe this work will not only serve as an academic resource but also contribute to shaping future policies for sustainable aquaculture practices.

With warm regards,

Editors

# CHAPTER III

# Disinfection of Fish Eggs with Medicinal Aromatic Plants: Present and Future Focus

**Mustafa DOĞAN[1]**
**Deniz ÇİRA[2]**
**Erkan CAN[3]**

## 1. Introduction

In the field of aquaculture, the health of fish eggs is the basis of successful fish farming. Traditional disinfection methods using chemical agents negatively affect the environment and cause pathogens to develop resistance to drugs (Semple & Dixon, 2020). There are many studies on the use of various aromatic plant extracts in disinfecting fish eggs and preventing the growth of pathogenic

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microorganisms. Medicinal aromatic plants, rich in bioactive compounds, can be considered as a promising alternative with their strong antimicrobial, antifungal and antiparasitic properties (Dawood et al.,2021).

Fish farming is a rapidly growing sector worldwide, and aquaculture products contribute significantly to people's access to healthy food by increasing supply and demand (FAO, 2018). One of the most important elements of production is the health of fish eggs. Microbial contamination of fish eggs, especially during the incubation period, can cause serious problems. Bacteria and fungi that develop on the surface of fish eggs can negatively affect hatching success and cause hatchling deaths. Egg disinfection has also become mandatory to reduce the transmission of pathogenic microorganisms to other farms via eggs (Can, Saka & Fırat, 2010; Can et al., 2012). Fungi such as Saprolegnia are among the common pathogens that cause significant losses in fish eggs. Traditional disinfection methods usually include chemicals such as formalin, iodine and hydrogen peroxide; however, these substances can cause environmental damage and human health risks (Pirbalouti et al., 2009). Therefore, medicinal aromatic plants and essential oils are attracting attention as environmentally friendly disinfectant alternatives (Austin & Austin, 2016; Austin et al., 2022; Meneses et al., 2022).

Aromatic plants contain essential oils with antimicrobial, antifungal and antioxidant properties (Can et al., 2023). These oils are part of the defense mechanisms of plants and are effective against microorganisms. Bioactive compounds such as thymol, carvacrol and eugenol can prevent the growth of many microorganisms. In

particular, plants such as thyme (*Origanum vulgare*), tea tree (*Melaleuca alternifolia*), garlic extract (*Allium sativum*) and mint (*Mentha piperita*) stand out with their antimicrobial effects (Amiri & Meshkini, 2019; Fierascu et al., 2021). Studies in this field show that medicinal aromatic plants and essential oils are effective in the disinfection of fish eggs **(**Khemis et al., 2016; Doğan, 2024). With a particular focus on the use of medicinal aromatic plants, interest in the research of alternative, environmentally friendly approaches to the preservation of fish eggs is increasing day by day.

The aim of this study was to evaluate the antimicrobial effects of these natural compounds in egg disinfection and to reveal their application potential by reviewing the literature on the use of essential oils of medicinal and aromatic plants in the disinfection of fish eggs.

## 2. Medicinal plants used in fish egg disinfection

### 2.1. Hops (*Humulus lupulus*)

Hops (*H. lupulus* L.) is a perennial plant native to temperate climates in the Northern Hemisphere. Although primarily known as one of the four ingredients needed to make beer, hops were originally used as a medicinal plant (Barnes et al., 2012). Almost all parts of the plant are rich in bioactive compounds. The powerful antimicrobial, antioxidant, and antifungal properties of its bitter acids and flavonoids, combined with the increasing interest in natural health-promoting substances, offer new and intriguing perspectives (Good et al., 2020).

## 2.2. Calendula (*Calendula officinalis*)

Disinfection of fish eggs is important for the prevention of aquatic animal diseases and the healthy development of fry. Different chemical and herbal disinfectants are used to prevent eggs from becoming infected (Syahidah et al., 2015). Generally, chemicals such as formalin, iodophors and chlorine are widely used to disinfect fish eggs. However, excessive use of these chemicals can have negative effects on aquatic ecosystems. Therefore, natural disinfectants, especially herbal ingredients, come to the fore. Many studies show that Calendula has low toxicity and successfully kills pathogens in fish eggs (Singh, Bhandari & Timalsina, 2021). The use of this plant offers both an environmentally friendly solution and protects egg health without creating harmful effects on fish. These completely natural and easily available plants are recommended for the disinfection of trout eggs (Nazımoğulları & Yanık, 2024).

## 2.3. Eucalyptus (*Eucalyptus globulus*)

Eucalyptus oil, known for its antimicrobial properties, has been investigated as a potential disinfectant in aquaculture, including its use in fish farming (Souza et al., 2019). It has been proposed as an alternative to chemical disinfectants to prevent fungal and bacterial infections, especially in species such as rainbow trout (*Oncorhynchus mykiss)*. Studies have shown that eucalyptus oil can effectively inhibit certain pathogens, but its use should be carefully controlled to avoid toxicity in aquatic environments. Although the oil of this plant has been shown to have promising potential, further research is required to determine optimum dosages and evaluate its long-term effects on fish health (Ghiasi et al., 2022).

## 2.4. Laurel (*Laurus nobilis*)

Laurel (*Laurus nobilis*) plant has been traditionally investigated for use in fish egg disinfection. Extracts obtained from laurel leaves have antifungal and antibacterial properties and can inhibit the growth of pathogenic microorganisms in fish eggs. This plant is among the natural disinfectants recommended especially to prevent fungal infections. Studies show that laurel extracts can support the healthy development of fish eggs and can be used as an alternative to chemical disinfectants (Özdemir, Taştan & Güney, 2022; Swamy et al., 2023).

## 2.5. Thyme (*Origanum onites*)

*Origanum onites* (thyme) is used as an effective natural disinfectant in fish egg disinfection. The essential oil obtained from this plant has antibacterial, antifungal and antimicrobial properties and is especially useful in preventing fungal infections (Mabrok & Wahdan, 2018; Yılmaz, Taşbozan & Erbaş, 2018). Studies show that *Origanum onites* oil can support the healthy development of fish eggs and can be applied as an alternative to chemical disinfectants. In particular, *Origanum onites* essential oil prevents the growth of pathogens in fish eggs, contributing to higher fertilization rates of eggs (Rashidian et al., 2021).

## 2.6. Onion (*Allium cepa*)

Onion (*Allium cepa*) extracts are being investigated as a potential natural alternative for fish egg disinfection. Onion exhibits antibacterial, antifungal and antiviral effects thanks to the sulfur compounds and organosulfides it contains (Reda et al., 2024). Studies show that onion extracts can inhibit the development of pathogens in fish eggs and support healthy embryo development of

eggs. In addition, onion extract promotes sustainable fish farming practices by offering an environmentally friendly alternative to chemical disinfectants (Özçelik et al., 2020; Zamini et al., 2023). Therefore, *Allium cepa* can be used as a natural disinfectant and can help avoid the harmful effects of chemical substances, especially in aquatic environments (Elgendy et al., 2023).

### 2.7. Yarpa (*Achillea officinolis*)

*Achillea officinalis* (Yarpa) is another plant that has the potential to be used as an effective natural disinfectant in fish egg disinfection. *Achillea officinalis* has antibacterial, antifungal and antioxidant properties thanks to the flavonoids, tannins and essential oils it contains (Miliauskas et al., 2004). Studies show that this plant can help prevent fungal infections and other microbial contamination by inhibiting the growth of pathogenic microorganisms in fish eggs. In addition, the use of *Achillea officinalis* offers an alternative approach for an environmentally friendly and sustainable fish farming practice (Amiri & Meshkini, 2019; Masigol, Mostowfizadeh-Ghalamfarsa & Grossart, 2021).

### 2.8. Chamomile (*Matricaria chamomilla*)

*Matricaria chamomilla*, namely the chamomile plant, is being investigated as an alternative disinfectant in the field of aquaculture. Chamomile is a herbal product that is being studied as an alternative to traditional chemical disinfectants due to its natural antimicrobial properties. The antimicrobial components of extracts obtained from the flowers of *Matricaria chamomilla* offer a potential natural solution for the disinfection of fish eggs (Das et al., 2019; Larcombe et al., 2024). Considering the environmental and biological side effects of disinfection with chemicals in particular,

the use of herbal disinfectants to ensure biological safety is gaining importance (Salehi, Soltani & Islami, 2015). Studies show that chamomile extract has an effective antimicrobial effect against pathogenic bacteria and can be used as an environmentally friendly method for the protection of eggs. However, further research is required to determine an effective usage dose and to understand the long-term effects of herbal compounds on fish embryos.

### 2.9. Tea Tree Oil (*Melaleuca alternifolia*)

Tea tree (*Melaleuca alternifolia*) is a plant native to Australia and is known for its medicinal properties. Tea tree oil obtained from this original plant has strong antibacterial, antifungal, and anti-inflammatory properties. It has been traditionally used for skin diseases, wound care, and fungal treatments. Tea tree oil, which is widely used in cosmetic and pharmaceutical products today, also stands out as a natural disinfectant (Carson, Hammer & Riley, 2006; Brun et al., 2019). In recent studies, herbal disinfectants have shown promising anthelmintic activity against monogenean infections in fish (Doan et al., 2020; Zhang et al., 2022).

### 2.10. Garlic extracts (*Allium sativum*)

It is known for its antifungal properties. Garlic contains a compound called allicin, which has an inhibitory effect on the growth of fungal pathogens (Baylan et al., 2018). Garlic extracts are distinguished by their low toxicity to fish. However, determining the correct concentrations and continuous monitoring are necessary to ensure effective disinfection (Al-Janae'e, Ali & Al-Edany, 2017). It has been shown to be effective against fungal pathogens, especially Saprolegnia in various studies (Özçelik et al., 2020).

These herbal disinfectants have significant potential to both reduce environmental impacts and support sustainable aquaculture practices. However, more research is needed to determine the efficacy and safety of each herbal product. Optimizing the application conditions and dosages of these products will help increase their effectiveness at water temperatures above 7-12 °C and minimize their possible side effects (Valenzuela-Gutiérrez et al., 2021).

## 3. Application Doses

Fish egg disinfection using medicinal and aromatic plants attracts attention as environmentally friendly and sustainable products instead of traditional chemical disinfectants (Özçelik et al., 2020). Various medicinal plants known for their antimicrobial properties are being tested for their effectiveness in preventing microbial contamination in fish eggs. Application concentrations of plant extracts can vary significantly depending on the fish species, the type of plant used, the part of the plant (seed, leaf, stem, shoot, etc.) and the amount of metabolites contained in the plant.

In current research, the most commonly used herbs for fish roe disinfection include *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano), *Lavandula angustifolia* (lavender), and *Rosmarinus officinalis* (rosemary), all of which contain essential oils with strong antibacterial and antifungal properties. Recommended application dosages for these herbs generally range from 1% to 5% concentration of the plant extract, although some studies have investigated higher concentrations of up to 10% for more severe contaminations (Doan et al., 2020).

For example, a study using *Thymus vulgaris* essential oil found that a 2% concentration was effective for disinfection without significantly damaging eggs. However, the exact dosage should be adjusted according to the specific characteristics of the fish species and the pathogen in question, as higher concentrations could potentially cause toxicity or damage to developing embryos (Turan, Gürağaç & Sayın, 2012; Çalışıcı-Narin, 2019).

**4. Application Periods**

Application time is one of the critical factors affecting disinfection effectiveness and survival rates of fish eggs. Typically, the exposure time to the plant extract varies from 10 minutes to 60 minutes, depending on the type of plant extract used and the severity of contamination (Doğan, 2024).

Shorter exposure times, usually around 10-20 minutes, are usually sufficient for disinfection with essential oils such as thyme or marjoram. However, in some cases where there is heavy contamination or more persistent pathogens, longer exposure times (30 to 60 minutes) may be required. It is important to time the exposure correctly, as prolonged contact with plant extracts can have adverse effects on egg viability, including low hatching rates or delayed development.

Studies have shown that immersion in a 2% oregano oil solution for 30 minutes can effectively reduce the bacterial load in fish eggs and maintain a high hatching rate (Can & Kaşıkçı, 2018). For example, Atlantic salmon (*Salmo salar*) eggs showed effective disinfection results when exposed to rosemary extract for 30 minutes, indicating a potentially ideal exposure time for this species.

In disinfection, as in fish anesthesia applications (Can & Sümer, 2019), contact with the least damaging substance in the shortest time can be considered as the most important factor to prevent work and time loss. Disinfection applications exceeding 30 minutes may pose a risk as they may also lead to water quality changes during application. Controllability decreases for disinfection personnel, therefore, the selection of the best disinfecting plant materials in the shortest time is important. In light of the studies conducted, it is revealed that future studies should focus on increasing the efficiency of disinfection and the survival rates of embryos. In this context, it is essential to conduct comparative trials on different species on concentration and duration.

**5. Effect on Survival Rate**

Medicinal and aromatic plants for disinfection is the potential impact on the survival rate (hatching rate) of fish eggs. The aim is to effectively disinfect the eggs without harming the developing embryos.

Several studies have shown that medicinal plant extracts, when used at optimum concentrations and exposure times, can effectively disinfect fish eggs without significantly affecting their viability. For example, in a study using *Thymus vulgaris* essential oil, the hatching rate of fish eggs remained high (approximately 85–90%) when eggs were disinfected at a 2% concentration for 20 min. Similarly, lavender extract has been shown to have antimicrobial effects on *Oncorhynchus mykiss* (rainbow trout) eggs and has minimal effects on survival rates (Turan, Gürağaç & Sayın, 2012; Çalışıcı-Narin, 2019; Pirinççioğlu, 2022).

However, excessive or prolonged exposure to plant extracts may lead to a decrease in hatching success. For example, higher essential oil concentrations or prolonged exposure times have been associated with lower survival rates due to the toxicity of some compounds to developing embryos (Conti et al., 2014; Ferraz et al., 2022). Therefore, careful optimization of dosage and exposure time is crucial to maintain high survival rates while effectively controlling microbial contamination.

## 6. Techniques Used in Impact Assessment

Medicinal and aromatic plants on fish eggs involves a variety of techniques, ranging from microbiological analysis to embryo development assessments (Lindholm-Lehto & Pylkkö, 2024). Commonly used methods to evaluate disinfection effectiveness are:

**Microbial Load Analysis:** One of the primary techniques used to assess the antimicrobial effect of medicinal plant extracts on fish eggs is the microbial load test. This involves culturing microbial samples from eggs to measure bacterial and fungal populations. The reduction in microbial load indicates the effectiveness of the disinfection procedure. Standard methods such as the plate count technique are often used to measure bacterial growth on selective agar media.

**Hatching and Survival Rate Analyses:** The ultimate measure of the effectiveness of a disinfection protocol is the survival rate of fish eggs and the percentage of successful hatching. Hatching analyses involve monitoring the number of eggs that hatch successfully after disinfection compared to controls. Survival rates are usually assessed by counting the number of live embryos after treatment and comparing them to untreated controls.

**Histological Examination:** Histological techniques can also be used to evaluate the effect of plant extracts on the morphology and development of eggs. These techniques provide the demonstration of possible toxicity at the cellular level that may occur with the use of plant products. It allows the evaluation of possible damage to the egg membrane or embryo before and after treatment.

These analyses help researchers identify and quantify specific bioactive compounds responsible for antimicrobial activity, thus facilitating the finding of the most suitable formulation for egg disinfection.

**Microscopic Observation:** Microscopic methods can be used to observe the physical and chemical changes in the egg membrane after treatment. This is important to understand whether the plant extracts cause any damage to the eggs or embryos, which in turn affects the hatching rate.

**7. Efficiency**

Disinfection of fish eggs with medicinal and aromatic plants is preferred over chemical disinfectants due to both antimicrobial efficacy and minimal toxicity to developing embryos.

Application dosages, exposure times, survival rates, and evaluation techniques are critical factors that need to be optimized for each fish species and plant extract used. Future research will likely focus on improving these parameters, developing standard protocols, and exploring synergies between different plant species for more effective and sustainable fish egg disinfection strategies (Kumar & Bossier, 2018; Brun et al., 2019).

Below are tables containing optimum hatching rates and microbial findings obtained from studies conducted on the disinfection of fish eggs. These tables include fish egg type, application type, duration, plant material used, optimum hatching rate, microbial load at optimum dose, and bibliography information. Studies are listed in the table as in vivo (Table 1) and in vitro (Table 2).

***Table 1.*** *Optimum hatching rates and microbial findings obtained from some studies conducted on fish eggs (in vivo)*

| Fish Species | Herbal Plants | Studied Pathogen | Application Method | Exposure Time (Min) | Optimal Application Dose | Total Pathogen Load (CFU/ml) | Hatching efficiency (%) | Survival rate (%) | References |
|---|---|---|---|---|---|---|---|---|---|
| Rainbow trout (*Oncorhynchus mykiss*) | Hops (*Humulus lupulus*) | Saprolegnia | Bath Treatment | 20 | 1 mg | NM | %90,33 | %90,11 | Nazımoğulları, M. U., & Yanık, T. (2024) |
| Rainbow trout (*Oncorhynchus mykiss*) | Calendula (*Calendula officinalis*) | Saprolegnia | Bath Treatment | 20 | 1 mg | NM | % 89.00 | % 89.33 | Nazımoğulları, M. U., & Yanık, T. (2024) |
| Persian sturgeon (*Acipenser persicus Borodin*) | *Eucalyptus globulus* | Overall fungal and bacterial loads | Short-term bath | 30 | 100 mg/L | Bacterial count: 5.92 log CFU/g. Fungal count: 10 CFU/g, | 62.66±2.51 | 3133.33 healthy larvae | Zamini et al., 2023 |
| Persian sturgeon (*Acipenser persicus Borodin*) | *Allium sativum* (garlic) | Overall fungal and bacterial loads | Short-term bath | 30 | 100 mg/L | Bacterial count: 6.26 log CFU/g Fungal count: 16 CFU/g | 55.33±1.52 | 2766.66 healthy larvae | Zamini et al., 2023 |
| Rainbow trout (*Oncorhynchus mykiss*) | *Oregano (Origanum onites)* | *Saprolegnia* infections | Daily drip application | 5 | 500 ppm | NM (Complete inhibition of fungal growth) | 82.11%, | 81.39% | Özdemir et al., 2022 |
| Rainbow trout (*Oncorhynchus mykiss*) | Laurel *(Laurus nobilis)* | *Saprolegnia* infections | Daily drip application | 5 | 500 ppm | NM (Complete inhibition of fungal growth) | 79.87% | NM | Özdemir et al., 2022 |
| Angelfish (*Pterophyllum scalare*) | *Terminalia catappa* (Indian almond leaf) aqueous extract | *Saprolegnia parasitica* | The eggs were placed in water containing the extract (hot extract) | 72 hours | 50 mg/L and 100 mg/L | NM | Egg hatching ↑ | Survival rate of larvae↑ | Meneses et al., 2021 |
| Rainbow trout (*Oncorhynchus mykiss*) | Wastes of onion (*Allium cepa*) and garlic (*Allium sativum*) methanolic extracts | *Saprolegnia parasitica* | Bath Treatment | 10 | 0,4 g/L (only garlic skin extract) 0,8 and 1,6 g/L (both Garlic skin and Garlic stem) Onion extract ↔ | NM | Dead eggs compared to control with: Using Garlic Skin Extract ↓ Garlic Stem Extract ↓ Onion extract ↔ | Garlic Skin Extract: 0,4, 0,8 and 1,6 g/L ↑ Garlic Stem Extract: 0,8 and 1,6 g/L ↑ Onion extract ↔ | Özçelik et al., 2020 |
| Rainbow trout (*Oncorhynchus mykiss*) | *Achillea officinalis* | *Saprolegnia parasitica* | *Constant Flow* | 60 | 150 and 200 mg/mL | NM | 71.28±0.30 | Mortality 28.6±0.05 | Amiri and Meshkini, 2019 |
| Rainbow trout (*Oncorhynchus mykiss*) | *Matricaria chamomilla* | *Saprolegnia parasitica* | *Constant Flow* | 60 | 100 and 150 mg/mL | NM | 75.90±0.26 | Mortality 23.67±0.76 | Amiri and Meshkini, 2019 |
| Rainbow trout (*Oncorhynchus mykiss*) | *Daenensis thyme(T.daenensis) and Mentha (M.longifolia)* | *Saprolegniasis* | Constant flow treatment method | 30 | Daenensisthyme at20 mgL-1 Mentha 10 mgL-1 | NM | Hatching success ↑ | Mortality ↓ | Salehi et al.,2016 |

**NM: This information has non-mentioned in referenced article, ↑ : Effective, increasing; ↔ : No significant differences; ↓ : Reducing**

***Table 2:*** *Results of some studies on plant-based products and their effective values applied to fish pathogens (in vitro)*

| Fish Species | Herbal Plants | Studied Pathogen | Application Method | Exposure temperature and time | MIC | MLC | Results and Effective consantrations | Reference |
|---|---|---|---|---|---|---|---|---|
| Rainbow trout (*Oncorhynchus mykiss*) | *Oregano (Origanum onites)*and *Laurel (Laurus nobilis)* | *Saprolegnia* infections | Conducted in petri dishcontaining Potato dextrose agar (PDA) | 20°C 96 hr | 250 ppm | 500 ppm | Growth of the fungus was 100% inhibited for 500 ppm both herbals. | Özdemir et al., 2022 |
| Angelfish (*Pterophyllum scalare*) | Terminalia catappa (Indian almond) | Saprolegnia parasitica | Conducted in solid (potato dextrose agar) and liquid (potato dextrose broth) mediums | 25°C 96 hours | NM | NM | 5 g/L and 10 g/L hot extracts : inhibited mycelial growth and showed fungisidal effect | Meneses et al., 2021 |
| Rainbow trout (*Oncorhynchus mykiss*) | *Eucalyptus globulus*, *Myrtus communis* (Myrtle), *Thymus daenensis* (Thyme), *Matricaria recutita* (Chamomile), *Satureja bachtiarica* (Savory), *Mentha longifolia* (Pennyroyal) | *Saprolegnia parasitica and Aspergillus species* | Incubation | 25°C 24 hours | Myrtus communis and Mentha longifolia (Yarpuz): (Mersin): 10 µL mL⁻¹ | Study look for MFC and it is Same as MIC. | For Saprolegnia parasitica:<br>Pennyroyal 5 µL mL⁻¹↑<br>Thyme and Myrtle 10 µL mL⁻¹↑<br>For Aspergillus species: 10-20 µL mL⁻¹ ↑ | Salehi et al., 2015 |
| Rainbow trout (*Oncorhynchus mykiss*) | Essentialoils: Thymus daenensis, Thymus khuzestanicum<br>Ethanol extracts: Mentha longifolia, Satureja bachtiarica, Tanacetumpartheniu m, Myrtus communis | *Saprolegnia parasitica* | Incubation | 18°C 7 days | Thymus daenensis and Thymus khuzestanicum: 0.63 µL mL⁻¹<br>Tanacetum parthenium: 31.25 µg mL⁻<br>Mentha longifolia: 62.5 µg mL⁻¹<br>Satureja bachtiarica: 125 µg mL⁻¹ | Thymus daenensis, Thymus khuzestanicum: 22 µL mL⁻¹<br>Tanacetum parthenium:600 µg mL⁻¹<br>Mentha longifolia: 550 µg mL⁻¹<br>Satureja bachtiarica: 750 µg mL⁻¹ | Thymus daenensis Thymu khuzestanicum Mentha longifolia↑ | Pirbalouti et al., 2009 |

**NM: This information has non-mentioned in referenced article, ↑ : Effective, increasing; ↔ : No significant differences; ↓ : Reducing**

## 8. Advantages and Disadvantages of Herbal Disinfectants

### 8.1. Advantages

Herbal disinfectants used in fish and fish egg disinfection have many advantages over traditional chemical disinfectants in aquaculture (Can et al., 2023). First of all, natural extracts obtained from plants such as garlic, chamomile, and thyme contain biodegradable components that do not harm the environment and

aquatic organisms. Unlike chemical substances, these herbal disinfectants reduce environmental pollution and contribute to the protection of water resources. These herbal extracts, which have antimicrobial and antifungal properties, prevent the proliferation of pathogens and support the healthy development of eggs (Nazımoğulları & Yanık, 2024). The non-toxic effects of natural plant extracts on fish embryos provide important protection in terms of egg and offspring health. While chemical disinfectants have the risk of causing resistance development, the use of herbal disinfectants also minimizes the risk of developing resistance. In addition, essential oils decompose rapidly in nature and the risk of leaving residue is low. With these properties, herbal disinfectants stand out as a sustainable and ecologically appropriate disinfection method (Salehi, Soltani & Hosseini-Shekarabi, 2016; Austin et al., 2022).

### 8.2. Disadvantages

There are also some disadvantages of herbal disinfectants in fish and fish eggs disinfection. First of all, the effectiveness of herbal essential oils may be lower than chemical disinfectants and may not be sufficient to completely eliminate pathogens. Since the mechanisms of action of herbal disinfectants are complex, the effectiveness of different plants against different pathogen types may vary. Therefore, stable and reliable results may not be obtained when these herbal products are used. In addition, some of the components contained in natural extracts may have toxic effects on fish embryos if the appropriate dose is exceeded. Furthermore, the specific antimicrobial compounds in many plant extracts are not well understood, and isolating pure compounds from them poses a significant challenge (Li et al., 2024). The lack of standardized

formulations of herbal disinfectants may lead to difficulties in determining the dosage, and it may be difficult to achieve the same effect every time. Another important challenge is that the application time and form of herbal disinfectants may differ from each other.

In order for herbal disinfectants to be effective, they sometimes require long-term contact and this can slow down the cultivation process. For these reasons, more research is needed to use herbal disinfectants safely and effectively (Nazımoğulları & Yanık, 2024).

Medicinal aromatic plants in fish egg disinfection is attracting attention as an environmentally friendly and sustainable alternative. The use of herbal products instead of traditional chemical disinfectants is increasing, which offers the opportunity to both protect the environment and increase biodiversity in fish species. However, there are several important focal points for further development of this field.

## 9.Future focuses

### 9.1. In-Depth Study of Plant Extracts and Active Compounds

Efficiency of medicinal and aromatic plants depends on the type and concentration of active plants they contain. The effects they show against these genetic microorganisms need to be better understood. In particular, the study on the effectiveness of plants with antibacterial, antifungal and antiviral products should be deepened. In addition, the effects of these relationships on fish eggs (possible harmful difficulty, negative effects on egg development, etc.) should be examined in detail and optimal doses should be determined. In the future, these studies will enable the development

of more sensitive formulations that will minimize potential damage to direct microbial control and other embryos (Austin et al., 2022).

### 9.2. Development of Standardized Application Protocols

Determining appropriate disinfection protocols for different fish species is of great importance for increasing treatment processes. In the future, more standardized application dosages and durations will need to be determined for disinfection processes with medicinal aromatic plants (Varlı, Hancı & Kalafat, 2020; Rhodes et al., 2023). These protocols should not harm the egg development of different fish species and provide effective protection against microorganisms. This is especially important for ensuring the widespread use of fish products.

### 9.3. Use of Multiple Herb Mixtures

Herbal extracts used alone may not be effective in all cases. In this way, traditional combined use should be investigated further. By taking advantage of different spectrum synergistic effects, more powerful and broad-spectrum disinfection solutions can be developed. For example, the combined use of lavender, rosemary, and thyme is not only suitable for families, but also effective against fungal and viral diseases (Aydın, 2022). Thus, it is possible to protect fish eggs by taking advantage of multiple mechanisms of action.

### 9.4. Biotechnological and Nanotechnological Approaches

In the future, there are biotechnological and nanotechnological applications to increase the amount of herbal extracts (Jeyavani et al., 2022). For example, combining herbal extracts with nanoparticles, providing more effective disinfection. Nanotechnology allows active systems to reach the egg faster and more effectively, allowing higher efficacy to be achieved with lower

doses (Austin et al., 2022; Adetuyi et al., 2024). However, research on nanotechnology and biological safety also needs to be deepened.

### 9.5. Integrated Solutions for Sustainable and Ecological Cultivation

Focus should be placed on developing integrated solutions for fish egg disinfection for sustainable and ecological aquaculture (Ahmed, Thompson & Glaser, 2019). Adopting herbal alternatives instead of chemical disinfectants can protect aquatic ecosystems as well as protecting biodiversity (Arthur et al., 2008). In addition, the use of such biological disinfection methods will contribute to healthier and more efficient production by reducing the destruction of water used in fish resources (Özçelik et al., 2020).

The use of native plant species in fish egg disinfection can be encouraged in the future. This can both increase biodiversity by preserving native plant diversity and provide new perspectives for research. Using them for this purpose can provide a more economical disinfection solution by reducing transportation costs.

### 9.6. Assessment of Environmental and Economic Impacts

It is possible to evaluate the economics and effects of disinfection with medicinal plants in detail. The extent to which it is economically suitable for medical production services, fish production, and the level of symptoms (e.g. effects on biodiversity) should be one of the most important research focuses in this field (Atique, 2023). In addition, herbal disinfectants should be comprehensively presented in terms of sustainability compared to chemical alternatives (Can et al., 2023).

Thus, the use of environmentally friendly methods in the fish products sector will become widespread and replace traditional chemical disinfectants.

## 10. Conclusion and Recommendations

Plant-based disinfectants have been gaining more attention in recent years as an environmentally friendly alternative for fish and fish eggs disinfection. Medicinal aromatic plants and essential oils are attracting attention as promising natural alternatives for fish egg disinfection. International studies show that these compounds can be used effectively in eggs of different fish species. However, the most important factors to be considered during application are appropriate concentration and effective application time. Since there may be a risk of toxic effects at high concentrations, it is necessary to determine safe ranges. Therefore, it is very important to conduct toxicity tests and economic analyses of plants in order to increase the use of medicinal aromatic plants in fish egg disinfection. By focusing future studies on this area, practical and safe uses of natural products will be determined and their commercial use will increase.

Medicinal aromatic products is a method that is compatible with ecological systems, environmentally friendly, does not leave residue, and is economical and practical. It ensures that similar studies in the future will make this field more efficient, safe and sustainable.

As a result, further research is required to investigate the effectiveness and toxicity of herbal disinfectants, to eliminate the deficiencies encountered in their standardization, and to determine their practical use.

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