ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Prospettive sull'utilizzo di fitoterapici in acquacoltura: Luci e Ombre

Incontro tecnico-scientifico SIPI e API

7 giugno 2019

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Vocazione di San Matteo
Caravaggio

Ombre

difficoltà nell'utilizzo del fitoterapico negli animali e in acquacoltura in particolare; carenze relative alla qualità dei fitoterapici; carenze di tipo regolatorio; carenze di studi/pubblicazioni condotti con rigore scientifico; scarse conoscenze sulla cinetica, sui meccanismi di azione e sulla tossicità

Luci

dati dalla letteratura scientifica

- Plants used as medicines for millennia
- First commercial pure natural product introduced for therapeutic use: **morphine** marketed by Merck in 1826
- First semi-synthetic pure drug, **aspirin**, based on a natural product, salicin isolated from *Salix alba*, introduced by Bayer in 1899

- Examples of other natural products:

Cocaine Digitoxin Pilocarpine
Codeine Quinine Statins
Paclitaxel Silymarin Galantamine
Atropine
Camptothecin Capsaicin

Ombre

Tuttora i *medicinali* (principi attivi) derivati da piante e/o parti di esse sono molto numerosi e, anche da tempi molto antichi, rientrano nelle possibilità *terapeutiche* del medico umano e veterinario.

Un *fitoterapico* è diverso da quanto considerato sopra, non può essere definito medicinale e non è possibile attribuire attività terapeutiche

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Ombre

Farmaco (specialità)

Sostanza in grado di modificare QUANTITATIVAMENTE la funzione di un organo, di una cellula (ecc. ecc.)

È soggetto ad Autorizzazione alla Immissione in Commercio (AIC) da parte di Ministero/EMA con procedura regolamentata dal D.Lgs. 193/2006 (Attuazione della Direttiva 2004/28/CE recante codice comunitario dei medicinali veterinari)

Fitoterapico

Sostanza in grado di modificare QUANTITATIVAMENTE la funzione di un organo, di una cellula (ecc. ecc.)

Tutto ciò che è in commercio di fatto è un «alimento», non si può quindi parlare di terapia, non si può fare riferimento a patologie, non si possono usare immagini che richiamino ad es. parassiti o altro

«sostanza che favorisce le normali funzioni dell'organismo quando utilizzato contestualmente a trattamenti di profilassi o prevenzione tradizionali»

GAP DI TIPO REGOLATORIO

Ombre

I prodotti in commercio non seguono/hanno seguito i processi autorizzativi previsti da:

D.Lgs. 193/2006 (Attuazione della Direttiva 2004/28/CE recante codice comunitario dei medicinali veterinari **per le specialità medicinali (Linee Guida EMA)**

EFSA (EFSA Panel FEEDAP, Guidance on the assessment of the efficacy of feed additives, adospted 17 April 2018, EFSA J) per gli additivi ai mangimi

quindi

REGISTRI EU Feed Additives e Food Additives

SYSTEMATIC REVIEW ARTICLE
Front. Vet. Sci., 22 June 2018 | https://doi.org/10.3389/fvets.2018.00140

Ombre

Systematic Review of Phytotherapeutic Treatments for Different Farm Animals Under European Conditions

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necessary. A systematic review of phytotherapy used for the treatment and prevention of infectious diseases in farm animals has been conducted using the PICOS approach.

The systematic review highlighted problems of inadequate study design and lack of reproducibility of studies on phytotherapeutic remedies. Based on the results of the review, it is not currently possible to make definitive conclusions on the effectiveness of phytotherapeutic treatments for diseases in farm practice. This does not mean that there is no

PRINCIPALI LIMITI/CARENZE

Ombre

- non seguite linee guida per autorizzazioni (es. EMA o EFSA)
- non seguito l'approccio Randomized Controlled Trials (RCTs)
- carenze gruppi di controllo (negativo e positivo)
- condizioni ambientali non standardizzate
- numerosità dei gruppi sperimentali e specie animali
- scarsa riproducibilità
- controllo qualità dei *botanicals* utilizzati
- scarsa equivalenza tra differenti prodotti, estratti, stesso lotto contenenti lo stesso principio attivo
- conoscenza dell'esatta concentrazioni dei principi attivi
- conoscenza dei principi attivi presenti
- valutazione della formulazione (> criticità rispetto a farmaco di sintesi)
- valutazione della presenza di eventuali inquinanti (es. metalli pesanti)

SCIENTIFIC OPINION

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EFSA Journal

ADOPTED: 1 December 2016 (EFSA BIOHAZ Panel), 8 December 2016 (EMA CVMP)

doi: 10.2903/j.efsa.2017.4666

Ombre

EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA)

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Ombre

- Botanicals ….most of the studies do not provide any data about the chemical composition of the tested plant products..

- Essential oils (EOs) …it is difficult to predict the efficacy of EOs because the active components can vary depending on the method of extraction, geographical origin, plant genothype, the harvesting season and length storage…

- ….administration with feed, the reduction of the antimicrobial activity could be mainly associated with the volatility and poor solubility of the active compound,….rapid clearance and short half-lives…

inoltre ATTENZIONE A

- REALE POSSIBILITA' DI UTILIZZO IN ALLEVAMENTO E SOPRATTUTTO IN ACQUACOLTURA
- CINETICA DEL PESCE
- POTENZIALE TOSSICITA'
- PRODUTTORE DEL FITOTERAPICO

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Luci

Antimicrobial activity of essential oil of *Eucalyptus globulus* against fish pathogenic bacteria

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College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea

The antibacterial activities of the essential oil of *Eucalyptus globulus* (EOEG) was determined against 7 fish pathogenic bacteria (*Edwardsiella tarda*, *Streptococcus iniae*, *S. parauberis*, *Lactococcus garviae*, *Vibrio harveyi*, *V. ichthyoenteri* and *Photobacterium damselae*) obtained from farmed olive flounder. The inhibitory activity was evaluated by three methods: Disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). According to the disc diffusion test, as the concentration of EOEG (5-40 μg) rises, the inhibitory zone increases in size. Compared with amoxicillin, tetracycline and chloramphenicol, EOEG showed similar antibacterial activity. The MIC of EOEG ranged from 7.8 to 125 mg/mL and MBC values ranged from 62 to 250 mg/mL. These results show that EOEG has antimicrobial activity against all seven bacteria, but there was no marked difference between each genus. From these results, it is suggested that EOEG can be used as an antimicrobial agent against fish bacterial diseases in the fish industry.

Keywords: *Eucalyptus globulus*, antibacterial activity, fish pathogenic bacteria

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E. globus è caratterizzato da:

-attività repellente verso gli insetti

-attività sull'apparato respiratorio

-attività antiinfiammatoria

-attività antibatterica

-attività antitumorale

LIMNOFISH-Journal of Limnology and Freshwater Fisheries Research 4(2): 67-74 (2018)

Journal of Limnology and Freshwater Fisheries Research

limnofish

http://www.limnofish.org

Luci

Antibacterial Activity of Some Aromatic Plant Essential Oils Against Fish Pathogenic Bacteria

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ABSTRACT

Essential oils of twenty-four plant species were obtained by hydrodistillation and investigated for their antibacterial effects against seven fish pathogenic bacteria (*Aeromonas hydrophila*, *Aeromonas salmonicida*, *Vibrio anguillarum*, *Yersinia ruckeri*, *Enterococcus faecalis*, *Lactococcus garvieae* and *Streptococcus agalactiae*). The antibacterial activity results of the essential oils obtained by disc diffussion method showed strong activities against all pathogens. In general, whole essential oils except *Artemisia absinthium* exhibited strong antibacterial effects against the most of the fish pathogens. However, the essential oil of *A. absinthium* showed weak antibacterial effect against only *A. hydrophila*. Mostly seven essential oils of the plants (*T. spicata*, *T. vulgaris*, *L. nobilis*, *C. verum*, *H. plicatum* and *A. citriodora* Paláu) among twenty-four essential oils exhibited good antibacterial activity against all fish pathogens. When compared to the tested antibiotics (furazolidon, oxytetracycline, cephalothin, and trimethoprim/sulfamethoxazole), the antibacterial effects of essential oils were mostly obtained equivalent or stronger. Considering the antibacterial activity results of the essential oils, their alternative use in lieu of antimicrobial agents against bacterial fish diseases might be convenient in the aquaculture.

Keywords: Antibacterial activity, disc diffusion, fish pathogens, essential oils

ARTICLE I

RESEARCH ARTI

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Yayım : 17.08

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Table 1. List of the studied plant species, plant parts used and essential oil yields.

Family and plant species	Common name	Part used	Yield (ml)*
Lamiaceae			
Lavandula angustifolia	Lavender	Flower	3.3
Lavandula stoechas	French lavender	Flower	0.7
Menthax piperita	Pepper mint	Leaves	0.6
Ocimum basilicum	Sweet basil	Leaves	0.5
Origanum majorana	Wild marjoram	Leaves	0.05
Thymus vulgaris	Thyme	Leaves	0.7
Rosmarinus officinalis	Rosemary	Leaves	2.0
Thymbra spicata	Spiked thyme	Leaves	0.6
Salvia officinalis	Sage	Leaves	2.2
Lauraceae			
Laurus nobilis	Bay laurel	Leaves	1.1
Cinnamomum verum	Cinnamon	Bark	3.0
Geraniaceae			
Pelargonium graveolens	Rose geranium	Leaves	0.2
Piperaceae			
Piper nigrum	Black pepper	Seed	4.6
Verbenaceae			
Aloysia citriodora Paláu	Lemon verbena	Leaves	0.4
Zingiberaceae			
Zingiber officinale	Ginger	Root	0.7
Apiaceae			
Coriandrum sativum	Chinese parsley	Seed	0.5
Foeniculum vulgare	Common fennel	Leaves	1.2
Petroselinum sativum	Parsley	Leaves	0.1
Pimpinella anisum	Anise	Leaves	3.5
Asteraceae			
Helichrysum plicatum	Everlasting	Flower	0.03
Achillea millefolium	Yarrow	Flower	0.5
Artemisia absinthium	Wormwood	Flower	0.2
Myrtaceae			
Eucalyptus camaldulensis	River red gum	Leaves	1.6
Syzygium aromaticum	Clove	Flower buds	3.4

* Yield (ml) = weight of essential oil (ml)/ 100 g of powdered plant sample.

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Research Article

Antibacterial Activity of *Citrus limon* Peel Essential Oil and *Argania spinosa* Oil Against Fish Pathogenic Bacteria

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Abstract

The main objective of the study was the identification of antibacterial activity of lemon (*Citrus limon* L.) peel essential oil and argan (*Argania spinosa* L.) oil against fish pathogenic bacteria. Antibacterial activity was determined against six different fish pathogens (*Yersinia ruckeri, Aeromonas hydrophila, Listonella anguillarum, Edwarsiella tarda, Citrobacter freundii and Lactococcus garvieae*). Essential oil derived from lemon peel and argan oil were applied against the bacteria using the disc diffusion and micro dilution method under in vitro conditions. The disc diffusion results indicated that essential oil of naturally *C. limon* peel and argan oil significantly inhibited the growth of *Y. ruckeri, A. hydrophila, L. anguillarum* and *C. freundii*. Our results suggested that the use of lemon peel essential oil and argan oil induced a stronger antibacterial effect.

Keywords: Essential oil, Citrus limon, Argania spinosa, Fish pathogen, Antibacterial

Minimum Inhibitory Result (MIC)

The result of minimum inhibitory concentration (MIC) of oil of lemon and argan is shown in *Tables 7* and *8*. The lemon peel essential oil against *Y. ruckeri* and *L. anguillarum* showed a higher MIC values (62.5 µL/mL), followed by *A. hydrophila* and *C. freundii* (125 µL/mL), *E. tarda* and *L. garvieae* (250 µL/mL) *(Table 7)*. Argan oil against *Y. ruckeri* showed a higher MIC values of 62.5 µL/mL followed by *L. anguillarum, A. hydrophila* and *C. freundii* (125 µL/mL), *E. tarda* and *L. garvieae* (250 µL/mL) *(Table 8)*.

As a result, this study showed that lemon essential oil and argan oil had antibacterial potentials against some fish pathogens. Since the lemon essential oil mainly obtained from fruit peels which are discarded as waste, the essential oil to be used for fish fed will be produced economically cheaper than those of other plants. Therefore, both can be used to prevent fish diseases by adding to fish fed or to prepare solutions for immersion treatment. However, further studies, particularly *in vivo* studies are necessary.

Luci

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Effect of Australian tea tree oil on *Gyrodactylus* spp. infection of the three-spined stickleback *Gasterosteus aculeatus*

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ABSTRACT: *Gyrodactylus* spp. infections of commercially farmed fishes are responsible for significant economic losses. Existing treatments have proved uneconomic, stressful to the fishes, and ecologically damaging. Essential oils are naturally occurring compounds that exhibit a wide range of anti-microbial and anti-fungal activities. This study explored the possibility of using Australian tea tree (*Melaleuca alternifolia*) oil (TTO) to treat *Gyrodactylus* spp. infection on the three-spined stickleback *Gasterosteus aculeatus*. In the presence of 0.01% Tween 80 as an emulsifier, TTO treatments at concentrations between 3 and 30 ppmv (parts per million by volume) lowered the prevalence and significantly reduced the parasite burden of sticklebacks naturally infected with *Gyrodactylus* spp. In addition, Tween 80 alone exhibited parasiticidal activity against *Gyrodactylus* spp. These findings show the potential of TTO in combination with Tween 80 as an effective treatment of *Gyrodactylus* spp. infection of fishes.

KEY WORDS: Australian tea tree oil · Tween 80 · *Gyrodactylus* · Monogenea · Three-spined stickleback

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Legislation regarding the use of EO in aquaculture

Some EOs have achieved the generally recognized as safe label to be used as food additives in the United States, but no clear information existed in the European legislation (Carocho *et al.* 2015). The Brazilian legislation (interministerial normative instruction No. 28) establishes some standards for organic aquaculture production in the country and includes EOs in the list of permissible substances to be used in the control of pests and diseases (MAPA, Ministry of Agriculture, Livestock and Food Supply 2011).

Journal of Applied Microbiology

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REVIEW ARTICLE

The effects of essential oils and their major compounds on fish bacterial pathogens – a review

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Keywords
alternative treatment, antibacterial, fish-farming, isolated compounds, natural compounds.

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doi:10.1111/jam.13911

Summary
The increased resistance of fish pathogens to conventional treatments has lead researchers to investigate the antibacterial properties of natural resources, such as essential oils (EOs) of plants, in an effort to find products that are less harmful to the environment. The objective of this review is to provide an overview of the studies, *in vivo* and *in vitro*, that addressed the use of EOs and their major compounds as antimicrobial agents in fish, to identify the best EOs and compounds to investigate considering feasibility of application and suggest possible future studies. To date, studies suggest that the use of EOs in the prevention and/or treatment of infectious diseases in fish may be a promising strategy to reduce the use of conventional antibiotics in aquaculture, since several EOs effectively reduce or avoid the effects of bacterial infections in fish. The use of EOs through nanotechnology delivery systems, especially in dietary supplementation experiments, is promising. This form of application of the EOs allows a potentiation and targeting of the desired effect of the EOs and also allows the protection of EOs active constituents against enzymatic hydrolysis, deserving further study.

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molecules may modulate the activity of the main components. Moreover, it is likely that several components of the EOs play a role in cell penetration, in lipophilic or hydrophilic attraction and fixation on cell walls and membranes, and in cellular distribution. This last feature is very important because the distribution of the oil in the cell determines the different types of radical reactions produced, which depend on their compartmentation in the cell. In this regard, for biological purposes, it is more informative to study an entire oil rather than some of its components, because the concept of synergism appears to be more meaningful (Bakkali et al., 2008).

The strength of the synergistic effects of total EO varies between plants and types of assay. Thus, the antiparasitic effects of the EO will depend on both the presence of major compounds and the parasite species. The presence of synergistic compound combinations in a total EO may therefore augment the antiparasitic activity of these natural products. Therefore, synergistic interactions, in which the total EO is more effective than a single major compound, are of particular clinical, veterinary and ecological interest, given that synergistic combinations of total EOs may have greater efficacy against fish parasites or achieve clinical effects at a

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REVIEW ARTICLE

Current knowledge on use of essential oils as alternative treatment against fish parasites

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Handling Editor: Ryan B. Carnegie

Abstract – This review article focuses on current knowledge about *in vitro* and *in vivo* experimentation relating to use of essential oils (EOs) to combat fish parasites. In addition, we discuss the existing methodologies used in studies to determine the antiparasitic activity of EOs, along with their toxicity and major compounds. The methodological approaches used to describe the anthelmintic properties of EOs were demonstrated. The consistency of their activity and thus their potential use for fish ectoparasites (*in vitro* and *in vivo*) and endoparasites (*in vitro*) control was reviewed. There is a clear need to find EOs and active agents of EOs to treatment *in vivo* against endoparasites of fish. Thus, progress may be achieved through considering the beneficial aspects of EOs when their complementarity and potentiality are exploited. EOs are therefore viable alternative sources of therapeutic products against fish parasites. On the other hand, use of chemotherapeutics has been increasingly questioned, such that constant environmental and consumer concerns regarding them now exist. The synergistic functions of EOs, in comparison with the action of one or two major compounds of these oils, seems unquestionable. It is possible that their activity is modulated by several molecules of the major compounds. Lastly, EOs are bioactive products that are viable sources of therapy against fish parasites. Although more than 3000 EOs are known, less than 0.4% of them have been tested on fish parasites. Thus, it has become clear that more studies testing these therapeutic alternatives are required, in order to evaluate the antiparasitic potential of other EOs for controlling fish parasites and to maximize their benefits to hosts.

Keywords: Aquaculture / fish / parasites / essential oils / toxicity / treatment

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Luci

Effects of *Zataria multiflora* Essential Oil on Innate Immune Responses of Common Carp (*Cyprinus carpio*)

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Abstract: Influence of dietary administration of *Zataria multiflora* was evaluated on some immunological factors of common carp (*Cyprinus carpio*) under temperature less than optimum (16-17°C). Fish weighing 30-35 g were fed with *Z. multiflora* at different doses of 30, 60 and 120 ppm for a period of 8 continuous days. Serum lysozyme activity, bactericidal activity, total white blood cell population, total protein, globulin and albumin levels were measured on days 1, 2, 8, 15 and 23 after the essential oil administration. On day 23 post-administration the remaining fish from each group were intraperitoneally injected with formalin-killed whole cells of *Aeromonas hydrophila* (6×10^8cells mL^{-1}) and antibody titer was measured 3 weeks later. The obtained results showed that this essential oil had some immunostimulatory effects on these immunological factors such as antibody titers, total white blood cells and serum bactericidal activity in some test groups especially at 30 and 60 ppm.

Key words: *Zataria multiflora*, common carp, essential oil, dietary administration, immunological factors

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Efficacy of *Ocimum gratissimum* essential oil against the monogenean *Cichlidogyrus tilapiae* gill parasite of Nile tilapia

[*Eficácia do óleo essencial de* Ocimum gratissimum *contra o monogenea* Cichlidogyrus tilapiae *parasita de brânquias de tilápia-do-nilo*]

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ABSTRACT

The phythotherapy is an alternative to use of chemotherapeutical agents against monogenean infection. This study evaluated the anthelmintic activity of essential oil *Ocimum gratissimum* against monogenean *Cichlidogyrus tilapiae* as well as its acute toxicity in tilapia juveniles. The mean lethal concentration (LC_{50}) and different concentrations of the essential oil, both *in vitro* and *in vivo* assays (short and long-term baths) were assessed. The LC_{50} was 40.70mg.L^{-1} and in the *in vitro* assay this concentration showed 80% efficacy at the last two hours and in the *in vivo* assay 65.87% efficacy in long-term bath. However, it provoked morphological alterations on the gills such as hyperplasia and edema. The parasites exposure at the highest concentration (320mg.L^{-1}) showed 100% mortality after 2h exposure in the *in vitro* assay, whereas in the *in vivo* assay, short-term baths of 5min for 3 consecutive days showed an efficacy of 87.71% without gills damage. These results demonstrate the anthelminthic activity of essential oil *O. gratissimum* and the safety concentration to use in Nile tilapia.

Keywords: disease, freshwater fish, helminth, natural product, treatment

Luci

Iranian Journal of Aquatic Animal Health 2(2) 97-107 2016

Effects of antifungal activity of Daenensis thyme (*Thymus daenensis*)and Mentha (*Mentha longifolia*)essential oils on rainbow trout(*Oncorhynchus mykiss*) eggs hatchability

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Received: April 2016 Accepted: September 2016

Abstract

In this study, rainbow trout (*Oncorhynchus mykiss*) eggs were treated with effective doses of Daenensis thyme, *Thymus daenensis* (20, 10 and 5 mgL^{-1}) and Mentha, *Mentha longifolia* (10, 5 and 2.5 mgL^{-1}), during incubation period (20 days) until hatching for 30 minutes per every other day as constant flow bath treatment method. The mortality level in normal control (without any treatment) was significantly higher than other groups ($p<0.05$). Level of mortality in egg treated with Daenensis thyme at 20 mgL^{-1}(12.01±0.357%) was significantly lower than both normal control (33.35±0.889%) and Mentha groups until the eyed-egg stage ($p<0.05$). No significant different was seen between Mentha at 10 and 5 mgL^{-1}mortality untileyed-egg stage($p>0.05$).

The highest hatching rate (78.36±0.340%)was recorded in Daenensis thyme at 20 mgL^{-1}concentrations compared to other groups except Malachite green ($p<0.05$). Eggs treated with Daenensis thyme and Mentha essence showed greater mean percentage of survival and hatching rates compared to the normal control ($p<0.05$). However, essential oil derived from Daenensis thyme at 20 mgL^{-1} probably has the potential to be used as health control of rainbow trout eggs against fungal contamination.

Keywords: Antifungal activity, Daenensis thyme, Essential oil, Malachite green, Mentha.

Introduction

One of the main types of fungal diseases in farmed salmonid fish is saprolegniasis and

SIPI-API – 7 giugno 2019

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Antifungal and toxicity effects of new combined essential oils on *Oncorhynchus mykiss* in comparison with malachite green

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Abstract

Mold infection is one of the most important problems in aquaculture. Recently, administration of some chemicals such as malachite green in order to control mold infections has been limited in aquaculture. In the current decade, researchers have been more focused on using herbal extracts and essential oils in aquaculture. In this study, Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of a new combination of four essential oils (*Eucalyptus globulus*, *Mentha piperita*, *Salvia officinalis* and *Thymus vulgaris*) were determined by serial dilution method. LC50 of the combined essential oils for 48 and 96 hours were then determined on *Oncorhynchus mykiss* fingerlings. MIC and MFC of combined essential oils were 0.025µl/ml and 0.050 µ l/ml for *F. solani* and 0.018 µ l/ml and 0.035 µ l/ml for *S. parasitica,* which was lower compared to malachite green. It was also 0.060µl/ml and 0.300 µ l/ml for *F. solani* and 0.045 µ l/ml and 0.120 µ l/ml for *S. parasitica* respectively ($p<0.05$). The results of LC50 for 48 and 96 hours were calculated equal to 34.98ppm. Based on these results, the combination usage of essential oils can be proposed as a good antifungal therapeutic strategy in hatcheries.

Keywords: mold infection, salmonid hatcheries, essential oil, MIC, MFC, LC50

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