**Review: Plant-based anesthesia used in Aquaculture**

**Abstract**

Anesthetics are essential in aquaculture to limit stress throughout the handling and motion approaches. Historically, those included chemical anesthetics like ms-222, benzocaine, phenoxyethanol, and quinaldine, which had been extensively used. But those are artificial substances that accumulate in fish tissues and may pose potential risks to the purchasers, and also show negative reactions in fish, together with hyperactivity, mucus secretion, and damage to the gills and cornea. Thus, a growing interest is being directed toward natural alternatives, especially plant-derived essential oils, as safer and eco-friendly anesthetics in aquaculture. Clove oil as an herbal anesthetic is the most widely used among all the plant-derived oils due to its high content of eugenol and other promising plants such as *Piper betle, Matricaria chamomilla, Aniba rosaeodora, Coriandrum sativum, Mentha piperita, Lavandula angustifolia, Laurus nobilis, Melaleuca alternifolia,* and *Ocimium gratissimum*. This review aimed at illustrating the anesthetic potential, mechanisms of action, and efficacy of various plant-based essential oils with the end goal of promoting the sustainable use of anesthetics in aquaculture.

**Key words:** Aquaculture, Herbal anesthesia, Essential oils, Fish stress, Plant derived compounds

**Introduction**

Aquaculture is a fast-growing area of food production, in which frequent handling and transportation of aquatic organisms may cause significant physiological stress on their part. To minimize the stress response in animals, injury, and management, anesthetics are routinely used (Barcellos *et al*., 2003; Teixeira *et al*., 2017). The chemical anesthetics traditionally utilized, namely MS-222, benzocaine, phenoxyethanol, and quinaldine, have all been shown to exert anesthetic action. However, concerns about their residual toxicity, exorbitant costs, and adverse side effects such as hyperactivity, irritation of gills and mucosa, and potential health hazards to consumers propelled the search for natural alternatives (Ross & Ross 2008; Armani & James 2007). The past few years have seen increasing attention given to essential oils originating from medicinal plants, asserting anesthetic, antimicrobial, and antioxidant properties. Apart from being biodegradable and environment-friendly, herbal anesthetics offer an alternative with a wider safety margin, low side effects to fish and consumers alike. Clove oil containing eugenol as an active compound has been widely studied and employed for its anesthetic effectiveness on several fish species (Vidal *et al*., 2008). But other essential oils derived from plants such as *Piper betle, Matricaria chamomilla, Aniba rosaeodora*, amongst others, have also demonstrated a reasonable anesthetic action in different aquaculture applications. This particular paper will analyze and discuss the pharmacology of such herbal anesthetics with a focus on their composition, efficiency, application dosage, and advantages as compared to synthetic agents.

**1.Cloves (*Syzygium aromaticum*)**

Myrtaceae family belongs to the clove; a pungently aromatic brownish spice of India with therapeutic benefits. Clove oil is obtained by extracting the essential oil from the clove buds. Essential oils are also named as volatile oils. Eugenol is one of the major chemical compounds of clove oil. It acts as an anesthetic, a dental antiseptic, and as an expectorant. Eugenol is extracted from clove by this solvent system consisting of n-hexane, petroleum ether, chloroform, ethanol, and methanol. Phytochemicals in clove are eugenol, acetyl eugenol, alpha and beta caryophyllene, vanillin, and tannins. Clove possesses robust and multiple pharmacological activities including antioxidant, anti-cancer, anti-microbial, anti-inflammatory, anti-pyretic, anti-viral, anti-diabetic, local anesthetic, and pain-relieving, anti-carcinogenic, and antibacterial, antifungal, and antibiotic activities (S. Yadev *et al*., 2020).

The general anesthetic effect was induced by clove oil in doses ranging from 30 to 200 mg, with 40 mg being the least amount that induced a general anesthetic effect in less than 3 minutes on average induction time. Recovery lasted approximately 4 minutes, independent of concentration, in experiment 1. The time for ventilation during anesthesia decreased with increasing concentrations, whereas the time for recovery increased in experiment 2. Concentrations of clove oil considered safe and effective in carp seemed to lie between 30 and 50 mg, which should be applied in aquaculture practice where the procedure requires exposure to anesthetic for more than 5 minutes (Hajek *et al*.,2006).

S. Kaewmalun *et al*., 2022 worked with *Penaeus vannamei*, a species of whiteleg shrimp particularly subjected to stress induced by various aquaculture activities, such as capture, handling, and shipping. For the specific purpose of increasing water solubility and thereby enhance anesthetic action on the whiteleg shrimp, the clove oil-nanostructured lipid carrier (CO-NLC) was designed. Assessment of physicochemical characteristics, stability, and drug release profiles was then achieved in vitro. The acute-pathway toxicity study and the anesthetic effect and biodistribution in the shrimp body were extensively studied. The average size of the CO-NLC was approximately 175 nm, with a polydispersity index of 0.12 and a zeta potential determination of 48.37 mV characterizing a spherical shape and remaining stable for three months under storage conditions. It possessed an average entrapment efficiency of about 88.55%. The CO-NLCs also released 20% less eugenol than the standard (STD)-CO after the first 2 hours. In terms of anesthetic effects on the shrimp body, CO-NLC at 50 ppm had the lowest period of anesthesia (2.2 min), the shortest time to recover (3.3 min), and fastest clearance (30 min). This finding implies that CO-NLC is a potentially efficient alternative nanodelivery platform for the increase of clove oil.

**2.Betle leaves (*Piper betle*)**

*Piper betle* L., a member of the Piperaceae family, is a well-known traditional herbal medicine plant utilized for a variety of health advantages in Asian nations. The demand for its goods has grown recently, including for its herbal medications, drugs, and natural herbal formulations. Betel leaves and its products' health benefits have been traditionally used to treat a variety of illnesses, including bad breath, wounds, inflammations, colds and coughs, indigestion, and more. The essential oil (EO) and extracts of betel leaves have so far been used to identify a wide variety of bioactive substances, such as polyphenols and terpenes. By using a variety of cutting-edge standard techniques, the structural and functional characterisation of the extract and EO bio-actives has been obtained. Betel leaves' bioactive compounds have been linked to the majority of its health advantages (Madhumita. M, Guha. P, & Nag. A, 2020). Eugenol, isoeugenol, methyl eugenol, safrole, chavicol, hydroxychavicol, chavibetol, anethole, estragole, germecrene-D, etc. are some of the 30–60 chemicals that make up betel oil (P. Guha & S. Nandi, 2019). Chewing betle leaf causes numbness in the mouth and the perception of taste and sensibility of the buccal mucous membrane becomes temporarily dulled indicating the presence of anesthetic effect (Chopra *et al*., 1958).

According to Bond, M. M., & Senggagau, B. 2019, Betel leaf extracts demonstrate considerable natural antimicrobial activity. These extracts are used as an antimicrobial agent by an aqua farmer in Indonesia for an alternative method against fish diseases. The objective of the study is to determine the effects of betel leaf extract in respect of fish pathogenic bacteria distinguishing different bacteria in-vitro. The extracts of betel leaves were prepared with three kinds of solvents: ethanol, n-hexane, and ethyl-acetate. Extraction was performed by macerating about 100 g of betel leaf simplicia in 1000 ml of solvent contained in a stainless container for 24 hours. The extract was filtered through a 0.42 μm paper filter and then evaporated at about 50C for 30 minutes with vacuum rotary evaporation at 50 rpm. This concentrated extract was then used for antimicrobial testing. The next study was pathogenic bacteria for in-vitro testing comprising of Gram-negative bacteria: *Aeromonas hydrophila*, *Vibrio alginolyticus*, and *Edwardsiella tarda*. In the in-vitro test, betel extract against pathogenic Gram-negative bacteria indicated that betel leaf extract could inhibit the growth of pathogenic bacteria with an average of inhibition zone diameter greater than 14 mm. Whereas, this solvent variation was not significantly different (p>0.05) to diameter inhibition zones of *V. alginolyticus* and *A. hydrophila*, it was significantly different (p<0.05) to *E. tarda*.

**3.Chammomile (*Matricaria chamomilla*)**

*Matricaria chamomilla* L (family asteraceae), also known as Chamomile, camomille, italian camomilla, german chamomile, wild chamomile, or hungarian chamomile, is some of the most utilized plants in remedy. Its miles used for various illnesses like gastrointestinal issues, colic, spasms, stomachaches, irritable bowel syndrome, inflammatory ailments, and insomnia (LawGow B, 2007). Various pieces of evidence have shown that chamomile has antispasmodic and anti-inflammatory properties and can aid gastrointestinal motility disorders (Vissiennon C *et al*.,2017). Quercetin, patuletin, apigenin, apigenin-7-O-glucoside, luteolin, luteolin-7-O-glucoside, caffeic acid, chlorogenic acid, bisabolol, farnesene, chamazulene, various coumarins, and a volatile oil consisting of various monoterpene and sesquiterpene types were found in chamomile (Mustafa Cemek *et al*.,2010; Carnat *et al*.,2004). Other effects include sedative, anti-inflammatory, antiseptic, healing, carminative, and spasmolytic (Salamon, 1992). It is thought in general to be a mild tranquilizer and sleep inducer. The sedative effect may be due to the presence of a flavonoid acting on benzodiazepine receptors in the brain (Avallone *et al*., 1996). Chamomile extracts also contain substances capable of binding BDZ and GABA receptors in the brain and may contribute to the sedative effect (Park *et al*. 1999). Nonetheless, many of these substances remain unidentified (Srivastava *et al*., 2010). Anesthetic potential of chammomile (*Matricaria chamomilla*) oil for rainbow trout was evaluated by Kubra *et al*. in 2022, where they demonstrated that chamomile oil in concentrations of 150 and 200 μl/L negatively impacted the health of rainbow trout with 100μl/L of chamomile oil being the ideal anesthetic dose.

**4.Rosewood (*Aniba roseadora*)**

Rosewood oil is extracted from the wood of the rosewood tree (*Aniba rosaeodora* Ducke). It may range from colorless to pale yellow and has a pleasant odor. As reported in the earlier literature, rosewood essential oil contains up to 86.23 percent linalool, which is a strong anesthetic compound Kizak *et al*., (2018). The chemical acts as an inhibitor of the glutamatergic system within the central nervous system, thereby inducing anesthetic effects in animals (Pankevich *et al*.,2011).

The characteristic of aromaticity is displayed by all the parts of the tree, but only the trunkwood is obtained and subjected to hydrodistillation for the extraction of rosewood oil, a high-priced commodity (J.G.S. Maia *et al*., 2007). The species has been employed in the Amazon region for ornamental purposes, medicinally for treatment of epileptic convulsions, and as raw material for local perfumes (J.G.S. Maia *et al*., 2003).

The anesthetic effect of rosewood oil was studied in goldfish. Rosewood oil is novel as an anesthetic for fish species. It induces rapid induction and recovery, and is therefore an effective natural anesthetic. The effective doses of 2-phenoxyethanol and rosewood oil were found to be 250 μl/l and 700 μl/l, respectively. Rosewood oil can thus be applied at least three times less than the dose of 2-phenoxyethanol. Apart from the fact that it is economical and nontoxic (so no side effects on goldfish have been noticed), the other two natural oils investigated did not show any side effects in terms of fish mortality (V. Kizak *et al*., 2018).

**5.Coriander (*Coriandrum sativum*).**

Coriander, known for its fruits and essential oil, is cultivated worldwide mainly for linalool, its principal constituent, which in addition to being used as a flavoring agent also acts as a sedative and analgesic (Msaada *et al*., 2009; Ghobadi and Ghobadi, 2012; Mandal and Mandal, 2015) (Sugawara *et al*., 1998; Gastón *et al*., 2016; Satyal and Setzer, 2020). There seems to be only one study documenting the anesthetic effect of coriander oil on fish. According to Can *et al*. (2019), the essential oil of coriander can act as an anesthetic on prisoner cichlids (*Amatitlania nigrofasciata*).

Yigit and Kocaayan in 2023 compared the anesthetic effect of thyme essential oil (*Origanum* *onites*) and coriander essential oil (*Coriandrum sativum*) on rainbow trout (*Oncorhynchus mykiss*). A concentration of 50 mgL-1 of coriander essential oil induced deep sedation, while 200 mgL-1 produced deep anesthesia. This was contrary to thyme, as fish in the coriander group did not exhibit any abnormal signs in any organ, but those in the thyme group died. Fish exposed to thyme essential oil manifested edema, inflammatory cell infiltrations, desquamation, and hyperemia in the gills, liver, and kidney. Therefore, this study demonstrated that thyme essential oil cannot be used as an anesthetic for rainbow trout, while coriander essential oil can be effectively used at 200 mgL-1**.**

**6.Peppermint (*Mentha piperita*)**

The extracted oil of peppermint, regarded scientifically as *Mentha piperita*, shows antioxidant, antibacterial, antiviral, and fungicidal results (Mckay and Blumberg, 2006). This oil particularly includes menthol, which makes up among 33-60% of its composition (Pittler and Ernst, 1998). Research has shown that menthol can induce anesthesia in certain fish species. The anesthetic effect of menthol is believed to be partly linked to the activation of GABAA receptors (Kasai *et al*., 2014).

In 2019, Spanghero DBN *et al*., analyzed the peppermint (*Mentha piperita*) essential oil toxicity as well as the anesthetic effect in young silver catfish (*Rhamdia quelen*). Two hundred ten fishes were treated to 0, 20, 50, 80, 110, and 140 mg L-1 essential oil for 4 hours to assess fatal concentration (LC50-4h). Nine fist-scaled fish were individually subjected to the four different oil concentrations to assess narcotic potential: 50, 80, 110, and 140 mg L-1 Water pleasant parameters have been saved constant. Increasing concentrations of peppermint essential oil induced better mortality charges and expanded severity and volume of gill lesions in juvenile silver catfish over a 4h publicity period. The LC50-4h became calculated to be 75.06 mg l-1. Most of the gill injuries observed were as follows: edema at the vicinity of lamella, aneurism, epithelial detachment, hyperplasia interlamellar, and fusion of the lamellae, congestion of the venous sinus of the primary lamella and at the base of the secondary lamella, and congestion of the venous sinus of both primary and secondary lamellae. This oil concentration of 80 mg L-1 acts as a very effective anesthetic for young silver catfish since it anesthetizes only for 4 minutes and takes 10 minutes for recovery without any mortalities.

**7.Lavander (*Lavandula agustifolia*)**

Lavender (*Lavandula angustifolia*) is a flowering plant within the family Lamiaceae. Lavender oil has sedative, antidepressant, antispasmodic, antibacterial, and local anesthetic results (Can and Sümer, 2019). Linalyl acetate and linalool is considered the number one active constituent. Each additive is accountable for the sedative results (Denner, 2009). Lavander vital oil has been showed an anesthetic effect on blue dolphin cichlid, *C. moorii* (Can and Sümer, 2019), convict cichlid fish, *Amatitlania nigrofasciata* (Can *et al*., 2019), common carp, *Cyprinus carpio* (Beheshti *et al*., 2018), European catfish, *Silurus glanis* (Krasteva *et al*., 2021b) and rainbow trout, *O. mykiss* (Metin *et al*., 2015).

Can E & Sumer E study the anesthetic action of lavender Essential oil (*Lavandula angustifolia*; LAEo) and peppermint Eo (*Mentha piperita*; MPEo) in 2019. Appropriate induction time (AIt) and ready recovery time (RRt) criteria were used for effective concentrations of three anesthetic agents (MPEo, LAEo, and MS222) in juvenile blue dolphin cichlid (*Cyrtocara moorii*) (4.92 1.03 g and 7.13 1.22 cm) (Trial 1). For transportation (Trial 2), animals were divided into 4 groups under 3 different concentrations of anesthetics (10, 20, and 30 ppm) versus a control group without any anesthetic agent. It is concluded that MPEo and LAEo can be safely used as anesthetic and sedative agents in tropical fish species. For deep anesthesia, MPEo (100 µL L-1: AIt 183.5s and RRt 227.8s) and LAEo (300 µL L-1: AIt 109.2s and RRt 420.0s) were sufficient; conversely, doses in the range of 10-30 µL L-1 are suggested for fish transport.

**8.Laurel (*Laurus nobilis*)**

The laurel (*Laurus nobilis*), an evergreen plant, that is a member of the Lauraceae family, is a plant that grows in the Mediterranean region. It has been reported that laurel plant has antimicrobial, antioxidant and sedative effects (Sayyah *et al*., 2002) and dominant components of the essential oil are 1,8-cineole, α-terpinene and sabinene (Dadalioglu and Evrendilek, 2004). The main component, cineole, is responsible for the serenity of laurel essential oil (Sayyah *et al*., 2002). One research realized was the anesthetic effect of laurel essential oil on fish (Kızak *et al*., 2020). The effect of the anesthetic of laurel (*L. nobilis*) essential oil on blue dolphin chiclid, *Cyrtocara moori* has been established.

In the study, conducted by N.O.Yigit *et al*. in 2022, the authors investigated the effects of the essential oils of laurel (*Laurus nobilis*) and lavender (*Lavandula angustofolia*) as anesthetic agents in rainbow trout (*Oncorhynchus mykiss*). Fish which weighed an average of 10 g went through the various concentrations of essential oils (5, 10, 20, 50, 70, 100, 150, 200, 300, 400, 500, and 600 mg L-1). The duration of anesthesia induction and recovery were recorded. Post deep anesthesia, tissue of the fish was analyzed histologically to assess the action of the essential oils. It was concluded that laurel essential oil (400 mg L-1) and lavender essential oil (200 mg L-1) were the most effective in inducing anesthesia in rainbow trout. The periods for induction of deep anesthesia (Stage 4) were above for lavender 258.0 s and for Laurel 189.5 s at these doses. Recovery times for laurel were observed to be 129.5 s while lavender at 41 s at these doses. There were no histopathological findings in kidney, liver or gills. This suggests that lavender and Laurel essential oils can be used as efficient anesthetics in rainbow trout.

**9.Tea tree oil (*Melaleuca alternifolia*)**

The Australian native plant *Melaleuca alternifolia* is used extensively in medicine because of its strong antiseptic properties. It is a powerful agent against a variety of microorganisms, including bacteria, viruses, parasites, and fungi, and it also has antioxidant properties (Baldissera *et al*., 2017). TTO is an effective antibacterial and antiparasitic agent in aquaculture (Souza *et al*., 2016). Furthermore, research has shown that TTO can be used as an anesthetic for gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) (Hajek, 2011) (Golomazou *et al*., 2016).

In 2018, C.F. Souza *et al*. study the anesthetic potential lasting efficacy in silver catfish (*Rhamdia quelen*) for three major components in tea tree oil (TTO; *Melaleuca alternifolia*): terpinen-4-ol, γ-terpinene and α-terpinene; being postulated to be responsible for the anesthetic effect; will thus be set to influence oxidative parameters with prolonged exposure. Anesthesia induction time was timed according to the deviant behavior exhibited by the silver catfish within defined concentrations (100, 300, 500, 800, and 1000 µL L-1) of TTO and its three products-terpinen-4-ol, γ-terpinene and α-terpinene). Whereas the induction of anesthesia with terpinen-4-ol took 103-630s, TTO in concentrations of 300-1000 µL L-1 induced anesthesia in 185-1.512s. Recovery time for both anesthetics took 134-673s. While the monoterpenes γ-terpinene and α-terpinene had no sedative or anesthetic effect on *R. quelen*, they acted antagonistically to as with these two other anaesthetics. It can, therefore, be concluded from this data that the anesthetic effect is directly due to terpinen-4-ol and thus accounts for the anesthetic effects of TTO. There were reduced amounts of thiobarbituric acid-reactive species in the liver and levels of lipid hydroperoxide in liver and kidney after six hours of exposure to TTO (25 µL L-1) and terpinen-4-ol (42 µL L-1). The liver of silver catfish exposed to terpinen-4-ol (20 and 42 µL L-1), as well as the kidney of silver catfish treated with TTO at a concentration of 25 µL L-1, exhibited significantly high levels of glutathione-S-transferase activity when compared with its control. Considering that TTO and terpinen-4-ol showed increased antioxidant status in silver catfish, they are recommended for application in promoting rapid anesthesia and could be applied in sedation for transport.

**10.Basil (*Ocimium gratissimum*)**

The plant *Ocimum gratissimum* L. (Lamiaceae family), popularly known as “alfavaca” or basil, is a native species of Africa that is being used in different countries as a condiment, sedative, and stress reducer and to treat headaches in humans (Albuquerque *et al*. [2007](https://link.springer.com/article/10.1007/s10695-020-00900-x#ref-CR2)). The essential oil of the leaves of this plant showed safety and efficacy as anesthesia for some species of fish, such as the Brazilian flounder *Paralichthys orbignyanus* (Benovit *et al*. [2012](https://link.springer.com/article/10.1007/s10695-020-00900-x#ref-CR11)), silver catfish *Rhamdia quelen* ( Bandeira *et al*. [2017](https://link.springer.com/article/10.1007/s10695-020-00900-x#ref-CR6)), matrinxã *Brycon amazonicus* (Ribeiro *et al*. [2016](https://link.springer.com/article/10.1007/s10695-020-00900-x#ref-CR53)).

The study elaborates on the anthelmintic and anesthetic action of clove basil essential oil (*Ocimium gratissimum*) in tambaqui (*Colossoma macropomum*) farming. The first experiment assessed the anthelmintic effects on tambaqui subjected to 0, 5, 10, and 15 mg L-1 *Ocimium gratissimum* essential oil (OgEO) in a series of 15-min immersion baths. In the second experiment, fish blood parameters were evaluated after 15-minute baths in 15 and 60 mg L-1 OgEO. Anesthesia potentials of OgEO with the animals were assessed in the third experiment. OgEO showed anthelmintic and anesthetic effects at doses above 15 mg L-1. Fish serum ammonia increased due to stress caused by handling and OgEO bathing, but within 24 hours the ammonia levels returned to those in fish under no stress conditions. Overall, the results show that the essential oils represent a safe and effective component for developing natural solutions for anthelmintic and anesthetic treatments to tropical fish aquaculture (Boijink.C *et al*.,2016).

**Conclusion**

The increasing demand for practices in aquaculture that is stress residue-free and environmentally friendly considerations triggers dependence on alternative anesthetic agents. Herbal anesthetics from medicinal plants like clove, betel leaf, chamomile, rosewood, etc., are more effective, inexpensive, and eco-friendly alternatives according to available literature. The evidence is mounting in support of these natural agents having a significant anesthetic activity with minimum side effects; thus, they become applicable in a wide range of aquaculture species and practices. Further confirmation of their efficacy and safety will include plant-based anesthetic agents as an integral part of aquaculture, impacting greatly on the welfare of the fish employed while also facilitating the establishment of sustainable aquafarming systems.

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