Essential oil of *Magnolia denudata* is an effective anesthetic for spotted seabass (*Lateolabrax maculatus*): a test of its effect on blood biochemistry, physiology, and gill morphology

Xiangbing Zeng · Hongbiao Dong · Jingru Wu · Wenhao Wang · Yafei Duan · Jian Chen · Jiasong Zhang

Abstract *Magnolia denudata* is a well-known ornamental tree in China due to its beautiful blossoms, and it has been used as an analgesic to treat human headaches. This study investigated the anesthetic potential and physiological response of the essential oil of *M. denudata* flowers on spotted seabass *Lateolabrax maculatus*. Fish (mean ± SD, 164.16 ± 15.40 g) were individually exposed to different concentrations of *M. denudata* essential oil (MDO, 10, 20, 40, 60, 80, 100, and 120 mg/L) and eugenol (10, 20, 30, 40, 50, 60, and 70 mg/L) to investigate anesthetic efficacy. Based on the ideal time criterion for anesthetic induction (< 3 min) and recovery (< 10 min), the lowest effective concentration for spotted seabass was 100 mg/L for MDO and 60 mg/L for eugenol. The physiological and histopathological damage in the gill of *L. maculatus* after using MDO and eugenol was also evaluated at the minimum dose inducing deep anesthesia, and at 0, 6, and 24 h after recovery. The results showed that MDO and eugenol anesthesia alleviated the levels of cortisol and glucose and the lactic dehydrogenase activity induced by handling. Compared with eugenol, MDO also caused secondary stress to the body, but MDO caused minor physiological responses and histological changes in the gills. This study suggests that MDO is an effective anesthetic for spotted seabass.

Keywords Cortisol · Anesthesia · Stress · Physiological · Histopathology

Introduction

In recent decades, an increasing number of fish anesthetics have been used in animal welfare to reduce the trauma caused by handling (Wang et al. 2019; Jerez-Cepa et al. 2021; Taheri Mirghaed et al. 2018; Aydn and Barbas 2020). Fish anesthetics are currently classified as synthetic anesthetics or plant-derived...
anesthetics (Purbosari et al. 2019). Chemically synthesized anesthetics have several adverse effects on fish, including reproductive suppression, liver function changes, and mucus secretion (Purbosari et al. 2019; Dong et al. 2020a; Wang et al. 2020; Teixeira et al. 2017; dos Santos Teixeira et al. 2021; Ouyang et al. 2020), and the United States Food and Drug Administration (FDA) has approved only tricaine methanesulfonate (MS-222) for use on food fish (Carter et al. 2011). Moreover, there is growing concern about the adverse impact of synthetic anesthetics on human and fish health as well as on the environment due to tissue residue.

For the reasons stated above, less expensive, safer, and more natural anesthetics are needed. Plant-derived anesthetics are anti-bacterial, anti-oxidant, anti-stress, and low risk to human health (Aydın and Barbas 2020; Purbosari et al. 2019, 2021). Essential oil anesthetics have been considered the most effective plant-derived anesthetics (Aydın and Barbas 2020). Plant essential oil is a type of volatile liquid with aromatic properties derived from aromatic plant parts such as leaves, flowers, fruits, seeds, buds, and stems (Baptista-Silva et al. 2020). *Magnolia denudata* is a well-known ornamental tree species in China due to its attractive flowers, with a cultivation history of over 2500 years (Park et al. 2018). Its dried flower buds, known as Xin Yi in traditional Chinese herbal medicine, have been used as an analgesic agent for treating headaches (Wang et al. 2010). Furthermore, *M. denudata* flowers can be consumed as a food (Lu et al. 2016; Zhang et al. 2021). However, the anesthetic properties of *M. denudata* essential oils have not been assessed in fish.

Although anesthetics are used to reduce stress, the anesthetic itself may also be a source of stress (Readman et al. 2013; Barata et al. 2016). When stress factors stimulate the body, it releases a large amount of catecholamine hormone which then activates the hypothalamic-pituitary-interrenal gland (HPI) axis to produce a neuroendocrine cascade reaction (Schreck and Tort 2016). As a result, corticosteroid-releasing hormone (CRH) and pituitary gland-adrenocorticotropic hormone (ACTH) are released successively and pass through the blood circulation to reach the renal/interrenal tissue to stimulate the production of cortisol (Martos-Sitcha et al. 2014; Mommsen et al. 1999). Therefore, blood hematological and biochemical parameters are good indicators to evaluate fish stress and health (Roche and Bogé 1996; Fazio 2019).

The multifunctional fish gills are essential for gas exchange, osmoregulation, acid–base regulation, and nitrogenous waste excretion (Evans et al. 2005; Jiao et al. 2019). Furthermore, the gill is the main organ that comes into direct contact with and absorbs anesthetics (Hunn and Allen 1974; Ferreira et al. 1984). Many anesthetics have adverse impacts on gills, such as lamellar epithelium hyperplasia, ionocyte apoptosis, and necrosis (Wang et al. 2020; Afifi et al. 2000; Brandão et al. 2021; de Lima et al. 2021). Therefore, it is necessary to evaluate biochemical parameters in blood and the physiological changes in gills after anesthetic exposure.

Dosage recommendations may differ between and within species, increasing the necessity for research on specific fish species to prevent the adverse effects from a high dose (Zahl et al. 2012). Spotted seabass (*Lateolabrax maculatus*) is widely distributed in China, Korea, and Japan. It has the advantages of fast growth, adaptability to a wide salinity range, high breeding survival rate, and delicious meat, and it is popular among farmers and consumers (Yokogawa and Seki 1995; Dong et al. 2020b). However, the poor stress tolerance of spotted seabass, especially the stress caused by handling during culturing and transportation, seriously affects its growth and survival (Sun et al. 2020; He et al. 2020). MS-222 and eugenol are anesthetics used in aquaculture to reduce fish activity (Bahrekazemi and Yousefi 2017; Skår et al. 2017; Aydn et al. 2015; Purbosari et al. 2019). They have been tested on *L. maculatus* and have been shown to cause gill and liver damage in recent studies (Wang et al. 2010; Wang et al. 2020). In addition, there are no reports regarding the anesthetic effect of essential oils on *L. maculatus*. However, some previous studies have shown that eugenol is more effective and safer than MS-222 (Wang et al. 2010; Wang et al. 2020; Dong et al. 2020a; Cao et al. 2019). Therefore, our study aimed to compare the anesthetic effects, blood parameters, and gill physiological changes after exposure to *M. denudata* essential oil and eugenol.
Materials and methods

Fish maintenance and anesthetic agents

Juvenile *L. maculatus* (164.16 ± 15.40 g) were purchased from a fish hatchery in Zhuhai, Guangdong, China. The fish were acclimated in plastic tanks (4 m × 4 m) for 2 weeks. The daily water exchange rate was 100% of the tank volume. The water quality parameters were maintained at 6.9 ± 0.2 mg/L dissolved oxygen, 28 ± 0.5 °C temperature, and 7.7 ± 0.1 pH. The natural photoperiod was used, and the water quality indicators were checked daily to maintain them within the safety range. Eugenol (purity 99%; Wuhan Kangchun Perfume Co., Ltd., Wuhan, China) and *M. denudata* essential oil (MDO, purity 98%; Wuhan Kangchun Perfume Co., Ltd., Wuhan, China) were first diluted 1:9 in 95% ethanol to better mix with the tank water. The main MDO components were linalool (47.8%), terpineol (9.9%), neral (7.0%), geranial (6.9%), and cinnamaldehyde (3.7%), as determined by GC–MS. Essential oil samples were analyzed on an Agilent 7820A GC (USA) equipped with a 19091S-433UI HP-5MS Ultra Inert chromatographic column. Identification of constituents was carried out with the help of retention times of standard substances by composition of mass spectra with the data given in the NIST library.

Experiment I: Anesthesia efficacy evaluation

In this experiment, the anesthetic efficacy of MDO was compared to that of eugenol. Before the experiment was started, the fish were fasted for 24 h. Then, fish (*n*= 10) were individually observed and used only once during the test. Each fish was netted quietly and placed in an induction barrel (10 L) containing one of the seven MDO concentrations (10, 20, 40, 60, 80, 100, and 120 mg/L) or one of the seven eugenol concentrations (10, 20, 30, 40, 50, 60, and 70 mg/L). The anesthetic induction and recovery process of *L. maculatus* was divided into five stages according to Wang et al. (2010) and He et al. (2020) (Table 1). The induction and recovery times were recorded by using a digital stopwatch. After 0.5 h, when the fish stopped breathing or were unable to enter more profound anesthesia, the fish were immediately transferred into the anesthetic-free and aerated recovery tank, and the time to restoration of regular swimming activity was recorded. The survival rate after induction, recovery, and at 72 h after recovery were recorded.

Experiment II: Anesthetic anti-stress and secondary stress evaluation

The ideal anesthetic effect allows a fish to enter anesthesia in less than 3 min and recover in less than 10 min (Hanggono 2006; Aydin and Barbas 2020; Ross and Ross 2009; Purbosari et al. 2019) because these concentrations have a short induction time and may not cause damage to the fish. In experiment I, the appropriate concentrations of MDO and eugenol for fast anesthesia (induction time < 180 s) were 100 and 60 mg/L, respectively. After fasting for 24 h, the fish were individually transferred to three aquaria containing either 100 mg/L MDO, 60 mg/L eugenol or no anesthetic (control). After anesthetic exposure, when fish lost body balance and had slow opercular movement (deep anesthesia stage), five fish per aquarium were sampled at this time point. The remaining fish in each aquarium were then transferred to a new anesthetic-free aquarium to recover and were sampled at 0 h (immediately after recovery) and at 6 h and 24 h after recovery. The fish in the recovery stages and those in the control group were euthanized via cranial concussion according to the protocol approved by the Animal Ethical Committee of the South China Sea Fisheries Institute.

Table 1 Behavioral characteristics of *L. maculatus* during anesthesia and recovery stages

| Stage | Behavioral characteristics |
|-------|---------------------------|
| A1    | Deep sedation: equilibrium normal; total loss of reactivity to external stimuli |
| A2    | Swimming ability disrupted and loss of equilibrium but fish respond to pressure on the caudal peduncle |
| A3    | Deep anesthesia: completely loss of reflex activity or failure to respond to strong external stimuli |
| A4    | Medullary collapse: asphyxia; opercular movements cease |
| Recovery | Complete recovery of equilibrium; ability to remain upright and normal swimming behavior |
fish were hit in the head with a mechanical hammer for cranial concussion, resulting in syncope but not death. This cranial concussion lasted approximately 30 s in air exposure. Subsequently, the fish were held with a damp cloth, and blood was collected by caudal venipuncture. Gill samples were removed and immediately frozen at −80 °C for later analyses.

Serum biochemistry

After centrifugation at 1000 × g at 4 °C for 10 min, the blood serum was frozen and stored at −20 °C until further analysis. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose, cortisol, and lactic dehydrogenase (LDH) were measured by using a BS-200 automatic biochemical analyzer and kits (Shenzhen Mindray Biomedical Electronics Co., Ltd. Shenzhen, Guangdong, China). In brief, ALT activity was determined by the catalysis of ALT of the conversion of amino acids between alanine and α-ketoglutarate to produce pyruvate and glutamate. Then, phenylhydrazine was added to produce red–brown compounds, and the absorbance was measured at 505 nm. The AST activity was determined as the transfer of an amino group from aspartate to α-ketoglutarate, resulting in the production of a colorimetric product proportional to the AST enzymatic activity and was the absorbance was measured at 510 nm. In the ALP assay, disodium phenyl phosphate is transformed into free phenol and phosphoric acid by ALP. In alkaline solutions, phenol and 4-aminoantipyrine produce red–brown compounds. The malondialdehyde (MDA) concentration was measured by the thiobarbituric acid method. MDA was condensed with thiobarbituric acid during lipid peroxide degradation to produce a red product for determination of optical absorbance at 532 nm. HSP70 and cytochrome C (Cyt-c) were determined with commercial test kits based on the manufacturer’s protocol (MLBio, Shanghai, China). The enzymatic activities of cysteine-aspartic protease-9 (Caspase-9) and cysteine-aspartic protease-3 (Caspase-3) were measured with Ac-LEHD-pNA and Ac-DEVD-pNA as substrates, respectively (Beyotime, Haimen, China). These parameters were measured in a microplate reader (Bio-Rad, USA).

Histopathology

To further compare the adverse effects of MDO and eugenol on _L. maculatus_, the gills were analyzed, and the histological changes in the gills were examined at 6 h. Gills were fixed with 10% formaldehyde and washed with a 4 °C physiological saline solution. Subsequently, the samples were gradually dehydrated with ethanol (70–100%), clarified with xylene, embedded in paraffin, and cut into 5–6 µm thick slices for H&E staining and neutral resin sealing. An optical microscope was used for staining observation and imaging. Image-Pro Plus 6.0 software was used to process the images. The gill alterations were classified into three stages: stage I (alterations that do not affect organ function), stage II (moderate alterations that affect organ function) and stage III (severe and irreversible alterations), according to de Lima et al. (2021).
Statistical analysis

All data are expressed as the mean ± standard deviation. Statistical analysis was performed using SPSS 22.0 software. Data normality and homogeneity of variances were tested and confirmed using the Shapiro–Wilk and Levene tests, respectively. After single-factor analysis of variance (ANOVA), Duncan’s multiple comparison test was used to identify which groups differed. In all cases, the minimum level of significance was set to \( p < 0.05 \).

Results

Anesthetic efficacy of MDO and eugenol

No mortality was observed during the entire experimental period. When the anesthetic concentration was increased, the time of the induction stages decreased and the recovery time was prolonged. All concentrations induced the fish to enter the deep sedation stage. When the concentration of MDO was increased to 100–120 mg/L and the concentration of eugenol was increased to 60–70 mg/L, the induction time and recovery times reached the ideal criterion of less than 3 min and less than 10 min, respectively. No fish entered the A4 stage at any concentrations of MDO; conversely, when the concentration of eugenol was increased beyond 40 mg/L, fish opercular movement ceased at 0.5 h. In addition, the recovery times of the experimental fish that were anesthetized with MDO (72–110 s) were shorter than those of the fish anesthetized with eugenol (170–269 s) \( (p < 0.05) \) (Table 2).

Serum biochemical parameters

The serum biochemical parameters of fish anesthetized with MDO and eugenol are shown in Fig. 1. The cortisol concentration significantly decreased at the deep anesthesia stage with both anesthetics tested but increased at 0 h after recovery compared to the control group \( (p<0.05) \). The glucose of fish anesthetized with MDO and eugenol was significantly increased at 0 h, and the glucose level in the MDO group was higher than that in the control group at 6 h after recovery \( (p<0.05) \). LDH activity significantly decreased at the deep anesthesia stage and significantly increased at 0, 6, and 24 h in the eugenol group and at 0 h in the MDO group, and the LDH activity in the eugenol group was higher than that in the MDO group at 0 h \( (p<0.05) \). ALT activity increased at 6 h in eugenol group \( (p<0.05) \). AST activity significantly increased at 6 and 24 h after recovery in both anesthetic groups. In addition, the AST activity in the eugenol group was higher than that in the MDO group at 6 and 24 h.

Table 2 Induction and recovery times of fish anesthetized by MDO and eugenol

| Concentration of anesthetic (mg/L) | Average time for reaching different anesthesia stages | Recovery time \( (R) \) | Survival rate after 72 h |
|-----------------------------------|-----------------------------------------------------|------------------------|-------------------------|
|                                   | A1 | A2    | A3 | A4 |                                |                        |                         |
| MDO                               |    |       |    |    |                                |                        |                         |
| 10                                 | 515 ± 144 | _ | _ | _ |                                |                        | 100%                    |
| 20                                 | 210 ± 35 | _ | _ | _ | _ |                                |                        | 100%                    |
| 40                                 | 57 ± 12 | 275 ± 33 | * | _ | 72 ± 13 | 100% |                         |
| 60                                 | 47 ± 17 | 134 ± 33 | 907 ± 172 | _ | 76 ± 13 | 100% |                         |
| 80                                 | 24 ± 7 | 95 ± 28 | 412 ± 89 | _ | 78 ± 16 | 100% |                         |
| 100                                | 12 ± 3 | 72 ± 25 | 183 ± 54 | _ | 90 ± 19 | 100% |                         |
| 120                                | 16 ± 7 | 43 ± 8 | 116 ± 33 | _ | 110 ± 33a | 100% |                         |
| EUGENOL                           |    |       |    |    |                                |                        |                         |
| 10                                 | 129 ± 29 | * | _ | _ | _ |                                |                        | 100%                    |
| 20                                 | 50 ± 23 | 83 ± 18 | 677 ± 108 | _ | 170 ± 20b | 100% |                         |
| 30                                 | 27 ± 5 | 51 ± 11 | 295 ± 36 | _ | 209 ± 29 | 100% |                         |
| 40                                 | 26 ± 26 | 40 ± 12 | 225 ± 42 | _ | 222 ± 45 | 100% |                         |
| 50                                 | 22 ± 6 | 34 ± 12 | 220 ± 36 | 1298 ± 115 | 265 ± 29 | 100% |                         |
| 60                                 | 19 ± 4 | 26 ± 5 | 167 ± 24 | 777 ± 86 | 269 ± 33 | 100% |                         |
| 70                                 | 14 ± 3 | 27 ± 8 | 128 ± 26 | 451 ± 51 | 208 ± 34 | 100% |                         |

Water temperature was 28 ± 0.5°C. A1–A4 and recovery correspond to anesthesia stages in Table 1. "_" indicates that it was not observed corresponding anesthesia state in the experiment within 0.5 h. "*" indicates that only a few go into anesthesia within 0.5 h. Different letters mean significant difference \( (n = 10; \ p < 0.05) \).
Antioxidative stress and physiological response of gills

The antioxidative stress parameters of L. maculatus in gills after anesthesia with MDO and eugenol are shown in Fig. 2. SOD activity significantly increased at 6 h after recovery in both the eugenol and MDO groups, and the SOD activity in the eugenol group was higher than that in the MDO group (p < 0.05). CAT activity significantly increased at 0 and 6 h after recovery in the eugenol group and at 6 h after recovery in the MDO group, and the CAT activity in the eugenol group was higher than that in the MDO group at 6 h (p < 0.05). POD activity did not change throughout the experimental period (p > 0.05). The GSH concentration significantly increased at 6 h after recovery in both anesthetic groups, and the GSH concentration of the eugenol group was higher than that of the MDO group at 6 h. However, the GSH concentration of both anesthetic groups significantly decreased at 24 h after recovery (p < 0.05). The MDA concentration significantly increased at 6 h in both the eugenol and MDO groups and significantly increased at 24 h in the eugenol group (p < 0.05).

The physiological stress parameters of L. maculatus in gills after anesthesia with MDO are shown in Fig. 3. Caspase-3 activity significantly increased at 6 h after recovery in both anesthetic groups (p < 0.05). Caspase-9 activity significantly increased at 6 h in both anesthetic groups and at 24 h after recovery in the eugenol group (p < 0.05). The Cyt-c concentration significantly increased at 0, 6, and 24 h after recovery in the eugenol group and at 6 h after recovery in the MDO group, and the concentration in the eugenol group was higher than that in the MDO group at 6 h (p < 0.05). The HSP70 concentration significantly increased at 6 h and 24 h in the eugenol and MDO groups at 6 h after recovery (p < 0.05). ALP activity was not affected by the treatments (p > 0.05).

Discussion

To date, a large number of essential oils have been reported to have anesthetic effects on fish, such as Lippia alba, Cymbopogom flexuosus, Myrca flexio, Ocimum gratissimum, Aloysia triphylla, Lippia sidoides, and Mentha piperita. The active ingredients in these essential oils are linalool, geranial, β-pinene, thymol, and menthol (Junior et al. 2018; dos Santos Batista et al. 2018; Brandão et al. 2021; Saccal et al. 2017; Boaventura et al. 2020; Souza et al. 2017). Souza et al. (2017) found that L. alba essential oil of the linalool chemotype could induce anesthesia in fish. In addition, another study showed the use of C. flexuosus essential oil for anesthesia in Orechromis niloticus. Notably, the main active ingredients in C. flexuosus essential oil are geranial and neral (Netto et al. 2017). Cinnamicdehyde, a Trpa1 agonist, acts as a local anesthetic by inhibiting voltage-gated sodium channels in sensory neurons (Boonen et al. 2014). Thus, we speculate that the active substances in MDO inducing anesthesia in L. maculate are linalool, neral, geranial, and cinnamaldehyde.
It is critical to determine the effective concentration of anesthetics in each fish species to protect the welfare of both fish and the environment (Aydın and Barbas 2020). The ideal anesthetic effect allows the fish to enter anesthesia in less than 3 min and recover in less than 10 min (Hanggono 2006; Aydın and Barbas 2020; Ross and Ross 2009; Purbosari et al. 2019). Based on the ideal time criterion, the lowest effective concentrations were 100 mg/L for MDO and 60 mg/L for eugenol in L. maculate juveniles. As the concentration of the essential oil was increased, the time to induce anesthesia diminished, and the recovery time was prolonged. In addition, MDO has a shorter recovery time than eugenol. This may indicate that MDO is easier for fish to eliminate. In addition, no mortality was observed during the entire experimental period. These results

**Fig. 2** Antioxidant stress parameters of L. maculatus in gills after anesthesia with essential oil of M. demudate (MDO; 100 mg/L) and eugenol (60 mg/L). Different letters above the bars mean significant difference (n=5; p<0.05). (A) SOD; (B) CAT; (C) POD; (D) GSH; (E) MDA; DA, deep anesthesia stage; R0, 0 h after recovery; R6, 6 h after recovery; R24, 24 h after recovery

**Fig. 3** Physiological stress parameters of L. maculatus in gills after anesthesia with essential oil of M. demudate (MDO; 100 mg/L) and eugenol (60 mg/L). Different letters above the bars mean significant difference (n=5; p<0.05). (A) Caspase-3; (B) Caspase-9; (C) Cyt-c; (D) HSP70; DA, deep anesthesia stage; R0, 0 h after recovery; R6, 6 h after recovery; R24, 24 h after recovery
showed that MDO is a safe and efficient natural anesthetic based on the parameters analyzed in this study.

The blood biochemical parameters of fish are valuable markers and are very sensitive to anesthesia protocols (Yousefi et al. 2018, 2019; Fazio 2019; Saccol et al. 2018). To further evaluate the anti-stress effect and potential secondary stress of MDO on fish, the cortisol, glucose, LDH, AST, ALT and ALP levels in the blood were investigated in the present study. Cortisol and glucose are the most common stress indicators in fish (Ainsworth et al. 1985; Semenkova et al. 1999; Martinez-Porchas et al. 2009). Silva et al. (2015) found that the essential oil of Ocimum americanum effectively prevented cortisol from increasing in Rhamdia quelen after air exposure. Nile tilapia anesthetized with A. triphylla essential oil and then exposed to air for 1 min had lowered plasma cortisol levels (Teixeira et al. 2017). In the present study, fish in the deep anesthesia stage had lower cortisol levels than those in the cranial concussion stage, confirming the stress-reducing properties of MDO and eugenol. We also found that anesthesia elevated cortisol and glucose levels after recovery, but these parameters declined back to control levels after 24 h in the MDO group. Bodur et al. (2018) found that higher plasma cortisol was observed at the recovery stage after exposure to Origanum sp. and Eucalyptus sp. essential oil used as anesthetics. Toni et al. (2015) also found that L. alba essential oil induced higher cortisol at 4 h after recovery. These results suggest secondary stress of eugenol and MDO anesthesia on fish. The ALT, AST and LDH activities can be used as sensitive biomarkers in ecotoxicology because they provide early warnings for aquatic organisms (Hoseini et al. 2016; Haschek et al. 2009). Our results showed that the ALT, AST and LDH activities increased after MDO and eugenol exposure, indicating hepatotoxicity. Similar to our study, Toni et al. (2014) found that glucose levels and AST and ALT activities were significantly increased during the recovery period after exposure to different concentrations of essential oils of Hesperozygis ringens.

### Table 3 Qualitative analysis of the histological alterations observed in the gills of juveniles of L. maculatus exposed to eugenol and MDO

| Histopathological alterations               | Degree | Anesthetics |
|---------------------------------------------|--------|-------------|
|                                            |        | MDO Eugenol |
| Lamellar epithelium hypertrophy             | I      | + +         |
| Lamellar epithelium hyperplasia             | I      | + +         |
| Lamellar fusion                             | I      | - -         |
| Epithelial detachment                       | I      | - +         |
| Proliferation of ionocytes                  | I      | + +         |
| Mucous cell proliferation                   | I      | + +         |
| Edema                                       | I      | + +         |
| Epithelial rupture                          | II     | - +         |
| Lamellar aneurysm                           | II     | - -         |
| Necrosis                                    | III    | - +         |

+ and—mean that the occurrence of the phenomenon was observed or not

### Fig. 4 Observation on gills in different groups. CG, control group; MG, MDO group; EG, eugenol group; PVC, pavement cells; BV, blood vessel; ER, erythrocytes; scale bar: 50 μm (10×40 times); solid arrow (epithelial rupture); dotted arrow (edema); double solid arrow (hyperplasia); triangle (proliferation of chloride cells)
and *L. alba*. In addition, MDO had a less adverse impact on *L. maculatus* than eugenol. In recent studies, eugenol also showed a more severe impact than other anesthetics (Yousefi et al. 2018, 2019; de Lima et al. 2021).

Antioxidant enzymes play a critical role in responding to oxidative stress during intracellular redox balance in vertebrates (Zhang et al. 2020; Wang et al. 2020; Jiao et al. 2019). SOD, CAT, and POD are important enzymes involved in anti-oxidant defense systems and represent the cell’s first line of defense against oxidative stress (Wang et al. 2020). In the present study, increased anti-oxidant enzyme activity (POD, SOD, and CAT) implies that anesthesia stress stimulates the anti-oxidant system during the recovery period. Wang et al. (2020) found that the SOD, CAT, and POD activities in the gills of *L. maculatus* were significantly increased at 6 h after eugenol and MS-222 treatment. GSH is an important nonenzymatic anti-oxidant in cells that can neutralize free radicals or oxidants (Galano and Alvarez-Idaboy 2011). Moreover, MDA is the most important marker of lipid peroxidation (Liu et al. 2011). GSH and MDA have been commonly used as biomarkers for the evaluation of oxidative stress. The higher lipoperoxidation observed at 6 h after recovery in fish anesthetized with MDO and eugenol probably led to the increase in these anti-oxidant enzymes.

Apoptosis, a type of cell death, is regulated by a series of molecules induced by environmental, physical, or chemical stress. These molecules can mediate phagocytosis and clearance of dead or infected cells and prevent autoimmunity (Cheng et al. 2018; Cifuentes-Rius et al. 2021). Cyt-c and caspases play an essential role in cell apoptosis (Sun et al. 2020; Liang et al. 2019). In addition, HSP70 is a stress-induced molecular chaperone that aids in protein structural folding and transport in response to environmental stress (Maniya and Srivastava 2020). Our study showed that eugenol and MDO induced apoptosis and HSP70 increased in the gills of *L. maculatus*. These results are consistent with another study on the effects of eugenol and MS-222 on the gills of spotted sea bass (Wang et al. 2020).

In some studies, extensive histopathological abnormalities were observed in the gills, including hyperplasia, epithelial lifting, and curling of lamellae after exposure to anesthetics (essential oils of *A. triphylla, L. sidoides,* and *M. piperita, MS-222, eugenol*), indicating that the gill is a sensitive organ for responding to anesthetics (Brandão et al. 2021; Wang et al. 2020; Lima et al. 2021). In the present study, changes in gill morphology were identified using H&E staining and were classified into three degrees, according to de Lima et al. (2021). We observed lamellar epithelium hyperplasia in the gill after exposure to MDO and eugenol. The hypertrophy of the gill epithelium and hyperplasia of epithelial cells dilate the space between the exterior medium and the blood flow, reducing the direct contact of unwanted substances (Ghayyur et al. 2021). Furthermore, more severe pathology, such as epithelial rupture and necrosis, was found in gills exposed to eugenol but not to MDO. These results are similar to a recent report by de Lima et al. (2021), which found that eugenol caused more irreversible damage than *L. alba* essential oil to the gills of *Potamotrygon wallacei*. In another study, no irreversible damage was found in gills after exposure to three essential oil anesthetics (Brandão et al. 2021). Combined with the apoptotic and antioxidative results, we speculate that the use of eugenol caused more damage to the gills than MDO. In recent studies, eugenol also showed more stress, anti-oxidant impact, and tissue damage than thymol, citronellal, and *L. alba* essential oil (Yousefi et al. 2018, 2019; de Lima et al. 2021).

In conclusion, the lowest effective concentration of MDO for *L. maculatus* was 100 mg/L. MDO at 100 mg/L effectively reduced stress during handling and caused less secondary stress in gills than eugenol. These results show that MDO has the potential to be a safe and efficient natural anesthetic for *L. maculate* handling. However, further analyses are necessary using techniques such as electroencephalogram, electromyogram, and electrocardiogram at deep anesthesia stage. Additionally, measuring the relative gene expression changes as well as the physiological changes in other tissues after MDO exposure is also important.
Author contribution X.Z., H.D. and J.Z. designed the experiment. X.Z. and H.D. performed the experiments, data analyses, and drafted the manuscript. W.J., W.W., Y.D. and J.C. contributed with essential materials. All authors have given approval to the final version of the manuscript, decided to submit the work for publication. The authors like to thank all the laboratory members for experimental material preparation and technical assistance, and thank to Prof. Jian Qin, Flinders University, for providing revisions and suggestions in the manuscript writing.

Funding This research was supported by the fun of Key-Area Research and Development Program of Guangdong Province (2021B0202030001), China—ASEAN Maritime Cooperation Fund Project “China—ASEAN Modern Marine Fishery Technical Cooperation and Industrial Development Demonstration,” the fund of Central Public-interest Scientific Institution Basin Research Fund, South China Sea Fisheries Research Institute, CAFS (2021SD19), the fund of Guangdong Province Modern Agricultural Industrial Technology System Innovation Team Building Project (2022KJ150), the fund of Guangzhou Science and Technology Planning Project (201904010169), the fund of National Key Research and Development Plan Projects (2019YFD0900500), and the fund of Guangdong Province Supporting Town and Villages Technology Commissioner Project (KTP20210259).

Data availability The authors confirm that all the data involved in the manuscript are available for publication.

Code availability Not applicable.

Declarations

Ethics approval The collection and handling of the animals in this study were approved by the Animal Care and Use Committee at the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (SCSFRI-CAFS), and all experimental animal protocols were carried out in accordance with national and institutional guidelines for the care and use of laboratory animals at the SCSFRI-CAFS.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Afifi SH, Al-Thobaiti S, Rasem BM (2000) Multiple exposure of Asian sea bass, (Lates calcarifer, Centropomidae) to clove oil: a histopathological study. Ass Vet Med J 42(84):166–174

Ainsworth AJ, Bowser PR, Beleau MH (1985) Serum cortisol levels in channel catfish, from production ponds. Prog Fish-Cult 47(3):176–181. https://doi.org/10.1577/1548-8640(1985)47<176:SCLICC>2.0.CO;2

Aydin B, Barbas L (2020) Sedative and anesthetic properties of essential oils and their active compounds in fish: a review. Aquaculture 520:734999. https://doi.org/10.1016/j.aquaculture.2020.734999

Aydin İ, Akbulut B, Küçük E, Kumlu M (2015) Effects of temperature, fish size and dosage of clove oil on anesthesia in turbo (Psetta maxima Linnaeus, 1758). Turk J Fish Aquat Sci 15(4):899–904. https://doi.org/10.4194/1303-2712-v15_4.13

Bahrekazemi M, Yousefi N (2017) Plasma enzymatic, biochemical and hormonal responses to clove oil, 2-phenoxyethanol, and MS-222 exposed to Caspian brown trout (Salmo trutta caspius, kessleri). Iran J Aquat Anim Health 3(1):47–60. https://doi.org/10.18869/acacadpub.ijaah.3.1.47

Baptista-Silva S, Borges S, Ramos OL, Pintado M, Sarmento B (2020) The progress of essential oils as potential therapeutic agents: A review. J Essent Oil Res 32(4):279–295. https://doi.org/10.1080/10412905.2020.1746698

Barata M, Soares F, Aragão C, Almeida AC, Pousão-Ferreira P, Ribeiro L (2016) Efficiency of 2-phenoxyethanol and clove oil for reducing handling stress in reared meagre, Argyrosomus regius (Pisces: Sciaenidae). J World Aquacult Soc 47:82–92. https://doi.org/10.1111/jwss.12245

Boaventura TP, Souza CF, Ferreira AL, Favero GC, Baldissera MD, Heinzenmann BM, Baldisserotto B, Luz RK (2020) Essential oil of Ocimum gratissimum (Linnaeus, 1753) as anesthetic for Lophiosilurus alexandri: induction, recovery, hematology, biochemistry and oxidative stress. Aquaculture 529:735676. https://doi.org/10.1016/j.aquaculture.2020.735676

Bodur T, León-Bernabeu S, Navarro A, Tort L, Afonso JM, Montero D (2018) Effects of new plant based anesthetics Origanum sp. and Eucalyptus sp. oils on stress and welfare parameters in Diplarchus labrax and their comparison with clove oil. Aquaculture 495:402–408. https://doi.org/10.1016/j.aquaculture.2018.06.021

Boonen B, Alpizar YA, Benoy V, Van Den Bosch L, Voets T, Talavera K (2014) The Trpa1 agonist cinnamaldehyde acts as a local anesthetic inhibiting voltage-gated sodium channels in sensory neurons. Biophys J 106(2):326a–327a. https://doi.org/10.1016/j.bpj.2013.11.1879

Brandão FR, Farias CFS, de Melo Souza DC, de Oliveira MB, de Matos LV, Majolo C, de Oliveira MR, Chaves FCM, de Almeida O’Sullivan FL, Chagas EC (2021) Anesthetic potential of the essential oils of Aloysia triphylla, Lippia sidoides and Mentha piperita for Colossoma macropomum. Aquaculture 534:736275. https://doi.org/10.1016/j.aquaculture.2020.736275

Cao X, Wang Y, Yu N, Le Q, Hu J, Yang Y, Kuang S, Zhang M, Sun Y, Gu W, Yan X (2019) Transcriptome analysis of the immune system of crucian carp (Carassius auratus) under the process of treatment and low concentration transport by MS-222 and Eugenol. Aquaculture 50(11):3138–3153. https://doi.org/10.1111/are.14268

Carter KM, Woodley CM, Brown RS (2011) A review of tricaine methanesulfonate for anesthesia of fish. Rev
Fish Physiol Biochem (2022) 48:1349–1363

Fish Biol Fisher 21(1):51–59. https://doi.org/10.1007/s11160-010-9188-0

Cheng CH, Guo ZX, Luo SW, Wang AL (2018) Effects of high temperature on biochemical parameters, oxidative stress, DNA damage and apoptosis of pufferfish (Takifugu obscurus). Ecotoxicol Environ Saf 150:190–198. https://doi.org/10.1016/j.ecoenv.2017.12.045

Cifuentes-Rius A, Desai A, Yuen D, Johnston AP, Voelcker (1984) The uptake of the fonio. Fish Physiol Biochem 1:20. https://doi.org/10.1007/s10695-021-01029-1

Dong H, Wang W, Duan Y, Li H, Liu Q, Sun Y, Zhang J (2020a) Transcriptomic analysis of juvenile Chinese sea bass (Lateolabrax maculatus) anesthetized by MS-222 (tricaine methanesulfonate) and euugenol. Fish Physiol Biochem 1–12. https://doi.org/10.1007/s10695-019-00755-x

Dong H, Sun Y, Duan Y, Li H, Li Y, Liu Q, Wang W, Zhang J (2020b) The effect of teleost in the intestinal morphology and microbially community of Chinese sea bass (Lateolabrax maculatus) under intermittent hypoxic stress. Fish Physiol Biochem 46(5):1873–1882

dos Santos Batista E, Brandão FR, Majolo C, Inoue LAKO, Maciel PO, de Oliveira MR, Chaves FCM, Chagas EC (2018) Lippia alba essential oil as anesthetic for tambaqui. Aquaculture 495:545–549. https://doi.org/10.1016/j.aquaculture.2018.06.040

dos Santos Teixeira N, Marques LS, Rodrigues RB, Gusso D, Pinheiro PO, Machado TLF, Streit DP Jr (2021) Effects of anesthetic MS-222 on stress and reproduction of South American silver catfish (Rhamdia quelen) males. Anim Reprod Sci 225:106669. https://doi.org/10.1016/j.anireprosci.2020.106669

Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish Gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev 85(1):97–177. https://doi.org/10.1152/physrev.00050.2003

Fazio F (2019) Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture 500:237–242. https://doi.org/10.1016/j.aquaculture.2018.10.030

Ferreira JT, Schoonbee HJ, Smit GL (1984) The uptake of the anesthetic benzocaine hydrochloride by the gills and the skin of three freshwater fish species. J Fish Biol 25(1):35–41. https://doi.org/10.1111/j.1095-8649.1984.tb04848.x

Galano A, Alvarez-Idaboy JR (2011) Glutathione: mechanism and kinetics of its non-enzymatic defense action against free radicals. RSC Adv 1(9):1763–1771. https://doi.org/10.1039/C1RA00474C

Ghayyar S, Khan MF, Tabassum S, Ahmad MS, Sajid M, Badshah K, Muhammad AK, Saira Ghayyur S, Khan NK, Ahmad B, Qamer S (2021) A comparative study on the effects of selected pesticides on hemato-biochemistry and tissue histology of freshwater fish Cirrhinus mirigala (Hamilton, 1822). Saudi J Biol Sci 28(1):603-611

Hanggono B (2006) Application of clove oil as anesthetic for sea bass (Lates calcarifer Bloch) Jurnal Perikanan. J Fish Sci VIII(1):9–16

Haschek WM, Rousseaux CG, Wallig MA (2009) Fundamentals of Toxicologic Pathology. Academic Press, San Diego

He R, Lei B, Su Y, Wang A, Cui K, Shi X, Chen X (2020) Effectiveness of euugenol as an anesthetic for adult spotted sea bass (Lateolabrax maculatus). Aquaculture 523:735180. https://doi.org/10.1016/j.aquaculture.2020.735180

Hoseini SM, Rajabisterabadi H, Kordrostami S (2016) Chronic exposure of Rutillus rutillus castus fingerlings to ambient copper: effects on food intake, growth performance, biochemistry and stress resistance. Toxicol Ind Health 32(2):375–383. https://doi.org/10.1177/074823731500825

Hunn JB, Allen JL (1974) Movement of drugs across the gills of fishes. Annu Rev Pharmacol 14(1):47–54. https://doi.org/10.1146/annurev.pa.14.040174.000403

Jerez-Cepa I, Fernández-Castro M, Alameda-López M, González-Manzano G, Mancera JM, Ruiz-Jarabo I (2021) Transport and recovery of gilthead seabream (Sparus aurata L.) sedated with AQUI-S® and etomidate: effects on intermediary metabolism and osmoregulation. Aquaculture. 530:735745. https://doi.org/10.1016/j.aquaculture.2020.735745

Jiao W, Han Q, Xu Y, Jiang H, Xing H, Teng X (2019) Impaired immune function and structural integrity in the gills of common carp (Cyprinus carpio L.) caused by chlorpyrifos exposure: through oxidative stress and apoptosis. Fish Shellfish Immunol 86:239–245. https://doi.org/10.1016/j.fsi.2018.09.060

Junior GB, de Abreu MS, da Rosa JGDS, Pinheiro CG, Hein- zmann BM, Caron BO, Baldisserotto B, Barcellos LJJG (2018) Lippia alba and Aloysia triphylla essential oils are anxiolytic without inducing aversiveness in fish. Aquaculture 482:49–56. https://doi.org/10.1016/j.aquaculture.2017.09.023

Liang T, Xu X, Ye D, Chen W, Gao B, Huang Y (2019) Caspase/ALF/apoptosis pathway: a new target of puerarin for diabetes mellitus therapy. Mol Biol Rep 46(5):4787–4797. https://doi.org/10.1007/s11303-019-04925-1

Liu XL, Xi YQ, Yang L, Li HY, Jiang QY, Shu G, Wang S, Gao P, Zhu XT, Zhang YL (2011) The effect of dietary Panax ginseng polysaccharide extract on the immune responses in white shrimp, Litopenaeus vannamei. Fish Shellfish Immunol 30(2):495–500. https://doi.org/10.1016/j.fsi.2010.11.018

Lu B, Li M, Yin R (2016) Phytochemical content, health benefits, and toxicology of common edible flowers: a review (2000–2015). Crit Rev Food Sci Nutr 56(sup1):S130–S148. https://doi.org/10.1080/10408398.2015.1078276

Maniya NH, Srivastava DN (2020) Fabrication of porous silicon based label-free optical biosensor for heat shock protein 70 detection. Mater Sci Semicond Process 115:105126. https://doi.org/10.1016/j.mspproc.2020.105126

Martínez-Porchas M, Martínez-Córdova LR, Ramos-Enriquez R (2009) Cortisol and glucose: reliable indicators of fish stress? Pan-Am J Aquat Sci 158–178. https://
Saccol EM, Toni C, Pês TS, Ouiriche GM, Gressler LT, Silva LV, Mourão R, Oliveira R, Baldisserotto B, Pavaroni MA (2017) Anaesthetic and antioxidant effects of Myrcia sylvatica (G. Mey.) DC. and Curcuma longa L. essential oils on tambaqui (Colossoma macropomum). Aquacult Res 48(5):2012–2031. https://doi.org/10.1111/are.13034

Saccol EM, Jerez-Cepa I, Ouiriche GM, Pês TS, Gressler LT, Mourão RH, Martínez-Rodríguez G, Mancera JM, Baldisserotto B, Pavaroni MA, Martos-Sitcha JA (2018) Myrcia sylvatica essential oil mitigates molecular, biochemical and physiological alterations in Rhamdia quelen under different stress events associated to transport. Res Vet Sci 117:150–160. https://doi.org/10.1016/j.rvsc.2017.12.009

Schreck CB, Tort L (2016) The concept of stress in fish. In: Fish physiology (vol 35, pp 1–34). Academic Press. https://doi.org/10.1016/B978-0-12-802728-8.00001-1

Semkenova TB, Bayunova LV, Boev AA, Dyubin VP (1999) Effects of stress on serum cortisol levels of sturgeon in aquaculture. J Appl Ichthyol 15(4–5):270–272. https://doi.org/10.1111/j.1439-0426.1999.tb00249.x

Silva LDL, Garlet QI, Koakoski G, Abreu MSD, Malmann CA, Baldisserotto B, Barcellos LG, Heinzmann BM (2015) Anesthetic activity of the essential oil of Ocimum americanum in Rhamdia quelen (Quoy & Gaimard, 1824) and its effects on stress parameters. Neotrop Ichthyol 13:715–722. https://doi.org/10.1590/1982-0224-20150012

Skár MW, Haugland GT, Powell MD, Wergeland HI, Samuelsen OB (2017) Development of anaesthetic protocols for lumpfish (Cyclopterus lumpus L.): effect of anaesthetic concentrations, sea water temperature and body weight. PLoS One 12(7):e0179344. https://doi.org/10.1371/journal.pone.0179344

Souza CDF, Baldissera MD, Salbego J, Lopes JM, Vaucher RDA, Mourão RHV, Caron BO, Heinzmann BM, da Silva LFV, Baldisserotto B (2017) Physiological responses of Rhamdia quelen (Siluriformes: Heptapteridae) to anaesthesia with essential oils from two different chemotypes of Lippia alba. Neotrop Ichthyol 15. https://doi.org/10.1590/1982-0224-20160083

Sun Y, Dong H, Zhan A, Wang W, Duan Y, Xie M, Liu Q, Li H, Zhang J (2020) Protection of teprenone against hypoxia and reoxygenation stress in stomach and intestine of Lateolabrax maculatus. Fish Physiol Biochem 46(2):575–584. https://doi.org/10.1007/s10695-019-00732-4

Taheri Mirghaed A, Ghelichpour M, Zargari A, Yousefi M (2018) Anaesthetic efficacy and biochemical effects of 1, 8-cineole in rainbow trout (Onchorhynchus mykiss, Walbaum, 1792). Aquac Res 49(6):2156–2165. https://doi.org/10.1111/are.13671

Teixeira RR, de Souza RC, Sena AC, Baldisserotto B, Heinzmann BM, Couto RD, Copatti CE (2017) Essential oil of Aloysia triphylla in Nile tilapia: anaesthesia, stress parameters and sensory evaluation of fillets. Aquac Res 48(7):3383–3392. https://doi.org/10.1111/are.13165

Toni C, Becker AG, Simões LN, Pinheiro CG, de Lima Silva L, Heinzmann BM, Caron B, O., Baldisserotto, B. (2014) Fish anaesthesia: effects of the essential oils of Hesperozygis ringens and Lippia alba on the biochemistry and physiology of silver catfish (Rhamdia quelen). Fish Physiol Biochem 40(3):701–714. https://doi.org/10.1007/s10695-013-9877-4

Toni C, Martos-Sitcha JA, Baldisserotto B, Heinzmann BM, de Lima Silva L, Martínez-Rodríguez G, Mancera JM (2015) Sedative effect of 2-phenoxyethanol and essential oil of Lippia alba on stress response in gilthead sea bream (Sparus aurata). Res Vet Sci 103:20–27. https://doi.org/10.1016/j.rvsc.2015.09.006

Wang R, Jia H, Wang J, Zhang Z (2010) Flowering and pollination patterns of Magnolia denudata with emphasis on anatomical changes in ovule and seed development. Flora-Morphol Distrib Funct Ecol Plants 205(4):259–265. https://doi.org/10.1016/j.flора.2009.04.003
Wang W, Dong H, Sun Y, Cao M, Duan Y, Li H, Liu Q, Gu Q, Zhang J (2019) The efficacy of eugenol and tricaine methanesulphonate as anaesthetics for juvenile Chinese sea bass (Lateolabrax maculatus) during simulated transport. J Appl Ichthyol 35(2):551–557. https://doi.org/10.1111/jai.13844

Wang W, Dong H, Sun Y, Sun C, Duan Y, Gu Q, Li Y, Xie M, Zhang J (2020) Immune and physiological responses of juvenile Chinese sea bass (Lateolabrax maculatus) to eugenol and tricaine methanesulfonate (MS-222) in gills. Aquacult Rep 18:100554. https://doi.org/10.1016/j.aqrep.2020.100554

Yokogawa K, Seki S (1995) Morphological and genetic differences between Japanese and Chinese sea bass of the genus Lateolabrax. Jpn J Ichthyol 41(4):437–445. https://doi.org/10.11369/jji19950.41.437

Yousefi M, Hoseini SM, Vatnikov YA, Nikishov AA, Kulikov EV (2018) Thymol as a new anesthetic in common carp (Cyprinus carpio): efficacy and physiological effects in comparison with eugenol. Aquaculture 495:376–383. https://doi.org/10.1016/j.aquaculture.2018.06.022

Yousefi M, Vatnikov YA, Kulikov EV, Ghelichpour M (2019) Change in blood stress and antioxidant markers and hydromineral balance of common carp (Cyprinus carpio) anaesthetized with citronellal and linalool: comparison with eugenol. Aquacult Res 50(4):1313–1320. https://doi.org/10.1111/are.14007

Zahl IH, Samuelsen O, Kiessling A (2012) Anaesthesia of farmed fish: implications for welfare. Fish Physiol Biochem 38(1):201–218. https://doi.org/10.1007/s10695-011-9565-1

Zhang T, Yan Z, Zheng X, Wang S, Fan J, Liu Z (2020) Effects of acute ammonia toxicity on oxidative stress, DNA damage and apoptosis in digestive gland and gill of Asian clam (Corbicula fluminea). Fish Shellfish Immunol 99:514–525. https://doi.org/10.1016/j.fsi.2019.11.056

Zhang J, Wu M, Zhang M, Chen X, Yang J (2021) Analysis on aroma components of flowers of five Magnolia denudata cultivars. IOP Conf Ser: Earth Environ Sci 705(1):012007. IOP Publishing

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.