Application of Clove Oil for Transportation and Anesthetic Maintenance of Juvenile Hybrid Catfish (*Clarias macrocephalus* x *C.gariepinus*)

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Abstract

Synthetically chemical anesthetic might affect and accumulate in aquaculture fish when transportation of juvenile hybrid catfish. This study aimed: 1) to investigate the toxicity of clove oil, 2) to test the effectiveness of clove oil in application as an anesthetic in transportation, and 3) to evaluate appropriate concentrations of clove oil in using as an anesthetic. It was found that the cumulative mortality of hybrid catfish increased with an increasing in concentrations and exposure times. The mortality of hybrid catfish exposing to 10 ppm of clove oil was found after 48 h of exposure. For the concentrations of 20, 30 and 40 ppm of clove oil, the mortality was found after 24 h of exposure. The LC_{50} of clove oil in hybrid catfish exposed to clove oil for 24, 48, 72 and 96 h were 66.26 (59.14-77.89), 50.54 (47.83 -54.14), 48.49 (44.63-53.79) and 35.67 (33.65-38.13) ppm, respectively. The concentrations of clove oil causing anesthetic (stage 3, e.g. loss of reflex activity) in the hybrid catfish were only 3 levels: 125, 150 and 175 ppm, respectively. EC_{50} values were calculated in only 2 durations, which were found at 173.73 and 139.63 ppm, respectively. Finally, we found that the fish was recovered in 3 and 5 min. And, the EC_{50} values were 111.96 (88.07-123.52) ppm and 165.09 (159.04-173.43) ppm. After evaluating the effectiveness of clove oil for fish transportation, all the concentrations comprising 2, 4, 6, 8 and 10 ppm did not cause fish mortality. The mucus in exposed fish was excreted more than in the normal condition after 15 h of exposure time and also found lesions on its body. Based on our results, clove oil could be used as an alternative agent in anesthetic maintenance and transportation in juvenile hybrid catfish. The appropriate concentrations of clove oil were in the range of 2-4 ppm.

Keywords: anesthetic, clove oil, hybrid catfish, transportation

Introduction

Hybrid catfish (*Clarias macrocephalus* x *C.gariepinus*) is an economic freshwater fish that Thai people like to consume because of high protein, resulting in expanding of aquaculture in both numbers and areas. The hybrid catfish is increasingly raised because of its good characteristics and having rapid growth rate. It takes only 3 to 4 months before harvesting. Based on these reasons, a demand of hybrid catfish in markets is increased. Currently, it is found that most hybrid catfish raised in farms is purchased from other hatchery farms. They are not in-house breeding, causing problems in transportation. In the case of long transporting being longer than 6 h, their wastes must be managed by changing water during transportation. It may cause stress, injury, weak and dead in fishes. Anesthesia is normally used to calm the fishes, reduces its movement, and decreases excreted wastes during transportation, which is one of the most widely used alternatives. There are many types of anesthetic such as 2-phenoxyethanol metomidate MS-222; moreover, it has been reported that clove oil, an herbal plant extract, can be used as anesthetic in transportation and to reduce stress in aquatic animals (Weber, Peleteiro, Gcarclcc, & Aldegvnde, 2009).

Clove oil is a natural extract from the flower section. It is safe for humans to apply because of having no residue. It is inexpensive and can be degraded naturally. In clove oil, the dominant active ingredients are eugenol (4-allyl-2-meyhoxyphenol) (70-90%), eugenol acetate (no less than 17 %) and cartiofilen (5-12 %), which act as anesthetic in aquatic animals. Clove oil is now widely used in many activities related to aquaculture, especially for transportation in many fish species, such as Carp (*Cyprinus carpio*), Common carp (*Cyprinus carpio* L.) (Velisek, Svobodova, Piackova, Groch, & Nepejchalava, 2005), Trout (*Onecortynchus mykiss*), Goldfish (*Carassius auratus*) (Perdikaris et al., 2010), Chinese Siamese fighting fish (Somjai, Pansue, & Chayvareesajja, 2009) and Shrimp (*Fenneropenaeus indicus*) (Akbari, Khoshnod, Rajaian, & Afsharnasab, 2010). Moreover, its efficacy in using as anesthetic in juvenile (6-10 g) was evaluated and found that the appropriate concentration for complete asphyxiation was 50 mg/L (Ögretmen & Gökçek, 2013) which is especially useful for research, i.e. measuring and weighing.

Recently, the use of clove oil for a fish anesthetic has gained much interest among others. It is extracted from many parts of clove tree, i.e. flowers, leaves and stalks (Eugenia aromaticum or Eugenia caryophyllata). Its principally active ingredients comprise eugenol (76.8-88.58%), eugenyl acetate (1.2-5.62%) and β-caryophyllene (1.39-17.4%). Eugenol has multiple properties being useful for various applications, consisting of an antioxidant, antifungal, antibacterial or an antiparasitic agent. Plentiful researches have indicated that clove oil is an effective agent in the sedation of larvae (Akbulut et al., 2011a), fry, juvenile and adult fish of many species (Aydin, Akbulut, Kucuk, & Kumlu, 2015).

There are some studies of clove oil in Thailand. Tungsitthiwat & Sriveerachai (2013) showed that clove oil is used in aquaculture for many purposes including fish stress decreasing and anesthetic in transportation, such as baby coral transportation. Also, Imjai, Kanchan, and Chaiyara (2017) used clove oil to faint Clarias gariepinus at 20 ppm, that was recovered the consciousness after 5 min, and Aiuyamnouy, Sungpetch, Muntiprasert, and Yomlar (2016) investigated the efficiency of clove oil in transportation and reported that the transportation time was 16 h when applying the clove oil at 15 mg/L. Furthermore, Wongkaew & Nonwachai (2015) found that clove oil at 48 ppm could faint *Labe rohita* within 2 min.

However, the data of clove oil application in controlling asphyxiation, especially for transporting hybrid catfish to control panic, waste excretion and water quality are still inadequate. Moreover, the studies on the appropriate concentration of clove oil for asphyxiation in other activities such as blood collection and marking for research purposes have been performed in some fish species. These factors, i.e. concentration level, exposure time, species and age of aquatic animals, can affect asphyxiation and recovery. Therefore, this research has realized the possibility of using clove oil in solution form that is convenient and suitable for using in the transportation of juvenile hybrid catfish in reducing the trauma, stress and process of water exchange. Moreover, median lethal concentration (LC_{50}), the concentration in asphyxiation and recovery time were studied for usefulness of transportation and other activities in hybrid catfish.

Methods and Materials

Experimental animal

Juvenile hybrid catfish with an average length of 5.12 ± 1.65 cm, an average width of 1.4 ± 0.2 cm and an average weight of 5.34 ± 1.1 g were used in this study.

Determination of median lethal concentration (LC_{50}) of clove oil by Probit analysis

For studying the toxicity of clove oil solution (commercial form, Piping rock company), juvenile hybrid catfish were acclimatized in a glass tank of 8 L (n=10). The fainting times were set to 2 ranges comprising 0, 10, 100 and 1000 ppm and 0, 10, 20, 30 and 40 ppm. Next, clove oil was filled until the designed concentrations at 10, 20, 30, and 40 ppm. The mortality rates were recorded at 0, 24, 48, 72, and 96 h. The accumulative mortality rate in each concentration and exposure time were calculated. After that, the cumulative mortality rate percentage was used to analyze the toxicity of clove oil causing 50% mortality (LC_{50}) by Probit analysis using the Mini tab software. All experiments were performed triplicately. The clove oil used was in the form of a pure solution from Piping Rock Company.

Study of clove oil efficacy in anesthesia and recovery of juvenile hybrid catfish

Fish behavior was assessed according to anesthesia phases suggested by Hamachkova et al. (2006):

Phrase 1: Acceleration pursued by slowing down of opercular movements, partial loss of reactivity to external stimuli

Phrase 2: Loss of equilibrium, decreased opercular movements, fish react to strong external stimuli

Phrase 3: Complete loss of reactivity, fish lie on the bottom and do not react to manipulations

Phrase 4: Complete cessation of opercular movements, the fish die if they remain for a longer period in the anesthetic solution

Half-maximal effective concentration (EC_{50}) by Probit analysis

The study of asphyxiation of juvenile hybrid cat fish was tripicately performed in various concentrations, i.e. 0, 25, 50, 75, 100, 125, 150, and 175 ppm (n = 10). It was found that the fish was asphyxiated for 5 min. Then, they were assessed for asphyxia duration and the lowest concentration, causing 50% asphyxia (EC_{50}). The method was performed according to the method of Kulkham, Champasri, and Vanichkul (2017) modified from the study of McFarland (1959). The asphyxia is divided into 4 phases:

Phase 1 Sedation: The fish do not have any reaction to external stimuli except the pressure. The opening rate of operculum is slightly slower than normal and the fish swims slower.

Phase 2 Loss of equilibrium: The fish loses control of all their muscles. They react with strong stimuli. The opening rate of operculum is slightly slower than the normal.

Phase 3 Loss of reflex reactivity: the fish loses all reactions to stimuli. Respiration rate and heart rate are very slow

Phase 4 Medullary collapse: the fish stop breathing and after minutes the heart will stop beating.

After that, the recovery time in 10 min was studied and then the percentage of anesthesia and anesthesia recovery were calculated. Next, it was converted to Probit value and reported in the form of EC_{50} (Half maximal effective concentration). After evaluating the anesthesia and its duration, the juvenile hybrid catfish was further fed to assess the survival rate.

Study on the concentration of clove oil in the transportation of juvenile hybrid catfish

Juvenile hybrid catfish were placed in a plastic bag and then filled with clove oil at concentrations of 2, 4, 6, 8, and 10 ppm, dissolved in 2 L of water for 20 fish. The air was filled up and then closed the bag. Mortality rate was recorded at 12, 18 and 24 h in each experiment. And, behavioral change was studied by Probit analysis, which evaluates the change of 50% of the sample. After that, the survival juvenile hybrid catfish were further cultured in order to study behavior and survival rate compared to the control set.

Results

Median lethal concentration (LC_{50}) of clove oil om juvenile of hybrid catfish

Toxicity testing was set into two ranges: 0, 10, 100 and 1000 ppm as well as 0, 10, 20, 30 and 40 ppm. However, in the range of 10-100 ppm, the hybrid catfish were all died then we studied LC_{50} from cumulative mortality at the concentrations of 0, 10, 20, and 40 ppm for 24, 48, 72 และ 96 h. After converting the cumulative mortality to a percentage for applying in Probit analysis, it was found that at the lowest concentration (10 ppm) the cumulative mortality of juvenile hybrid catfish was 13.33 ± 5.77%. The cumulative mortality at 96 h was 30 ± 10%. The juvenile hybrid catfish exposed to clove oil at concentrations of 20, 30 and 40 ppm (cumulative mortality at 24 h) were consecutively 10.00 ± 5.77%, 16.67 ± 5.77% and 23.33 ± 5.77%. Besides, juvenile hybrid catfish exposed to clove oil showed the highest mortality rate at 96 h in all clove oil concentrations studied (Figure 1).

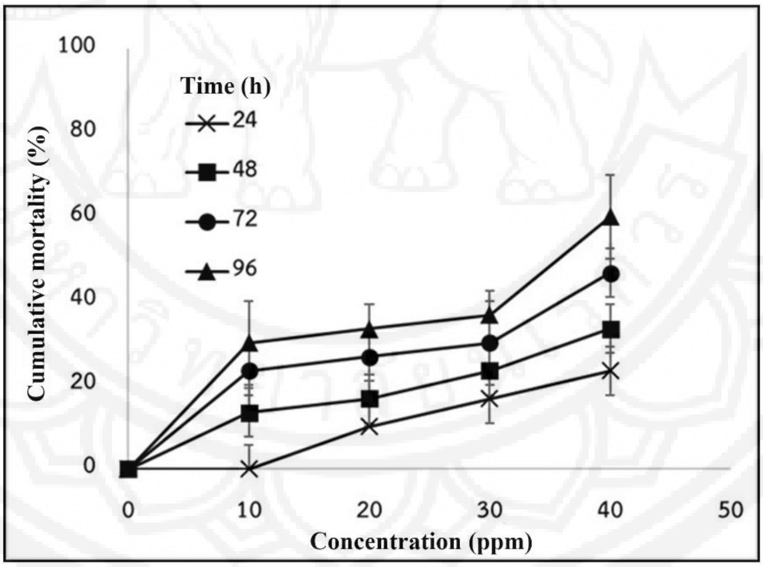

Figure 1 Cumulative mortality of hybrid catfish after exposure to clove oil in different concentrations and exposure times

After the mortality level of juvenile hybrid catfish was studied and the mortality rate was calculated by Probit analysis using the Minitab program to determine LC_{50}, it was found that LC_{50} values were calculated in all concentrations (Figure 2). The LC_{50} of hybrid catfish when exposed to clove oil at 24, 48, 72 and 96 h were 66.26 (59.14-77.89), 50.54 (47.83 -54.14), 48.49 and 35.67 (44.63- 53.79) ppm, respectively (Table 1).

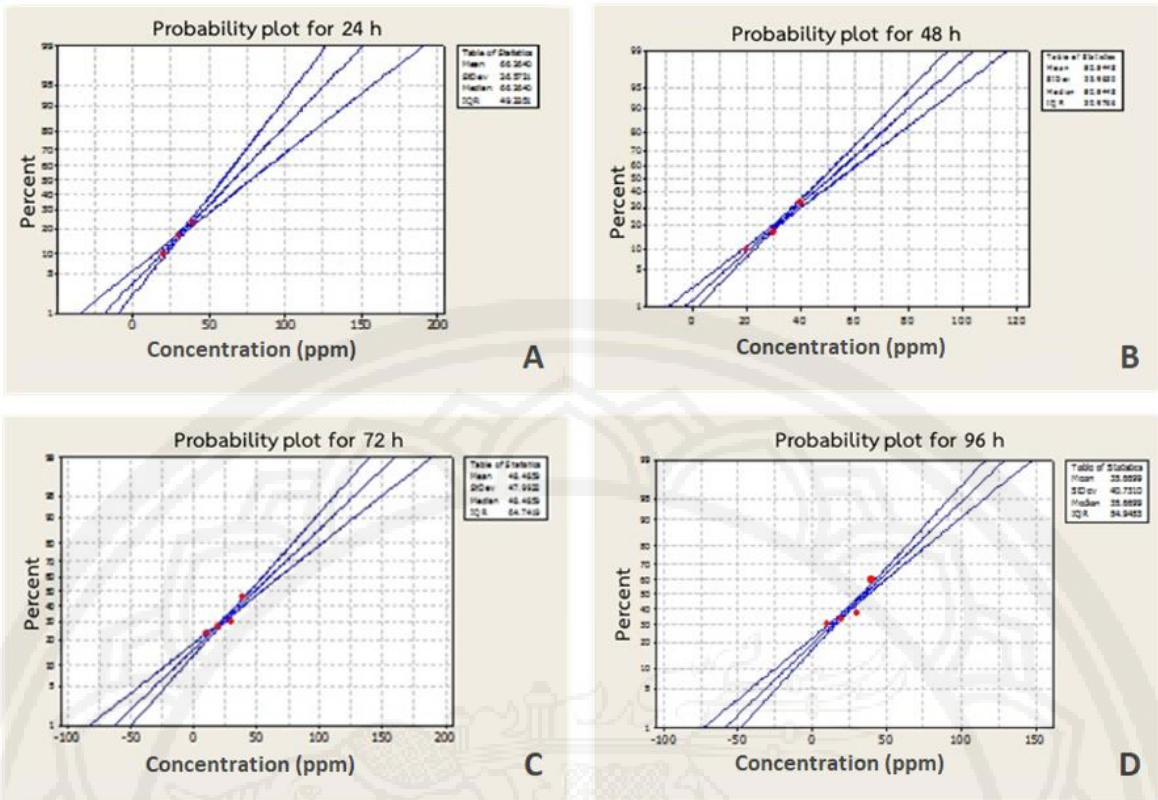

Figure 2 Analysis of LC_{50} by cumulative mortality calculation to Probit values using Minitab program with hybrid catfish exposing clove oil at 24, 48, 72 and 96 h

Table 1 LC_{50} in hybrid catfish after exposure to clove oil for 24, 48, 72 and 96 h

Time (h)	LC_{50} (95% confidence) (ppm)
24	66.26 (59.14-77.89)
48	50.54 (47.83-54.14)
72	48.49 (44.63-53.79)
96	35.67 (33.65-38.13)

Study on appropriate concentration of clove oil in anesthesia maintenance and recovery time in juvenile hybrid catfish

Study on appropriate concentration of clove oil in anesthesia maintenance in juvenile hybrid catfish

To study the appropriate concentration of clove oil for asphyxiation and the recovery time in juvenile hybrid catfish, 7 levels clove oil concentrations were applied, i.e. 25, 50, 75, 100, 125, 150 and 175 ppm. However, there were three levels of concentration causing asphyxiation in hybrid catfish, i. e. 125, 150 and 175 ppm. The concentration applying in assessing asphyxiation (10 min) and recovery (5 min) were illustrated in Table 2.

Table 2 Effect of clove oil in different concentrations on anesthesia in hybrid catfish referenced from Kulkham et al. (2017)

Concentration of clove oil (ppm)	Behaviors of juvenile hybrid catfish
125	In the first minute, the catfish slowly swam and gradually tranquil. Within 5 min, they remained motionless and then entered into phase 3 in 6-7 min.
150	In the first minute, the catfish became motionless and lost their balance. The body slowly weakened. Catfish began to turn over within 3 - 5 min and entered the phase 3 of asphyxiation in 5-7 min.
175	At first, catfish fainted, lost their balance and did not move. All of them felt in asphyxiation within 3 min and entered in phase 3 in 4-6 min.

Note: Phase **3** Loss of reflex reactivity: the fish loses all reactions to stimuli. Respiration rate and heart rate were very slow.

For obtaining the appropriate concentrations of clove oil for applying to anesthesia in juvenile hybrid catfish, three different concentrations of clove oil (125, 150 and 175 ppm) were investigated at 1, 3 and 5 min. The results indicated that the rate of anesthesia depended on exposure time and concentration. The percentage of cumulative asphyxiation of clove oil in concentration of 125 ppm examined at 1, 3 and 5 min were 10%, 20 ± 28.28% and 100.00%, respectively. At the concentration of 150 ppm, cumulative asphyxiation at 1, 3 and 5 min were consecutively 30 ± 14.14%, 70% and 100%. Finally, at the concentration of 175 ppm, the cumulative asphyxiation at 1, 3 and 5 min were 50 ± 14.14%, 100% and 100%, respectively (Figure 3).

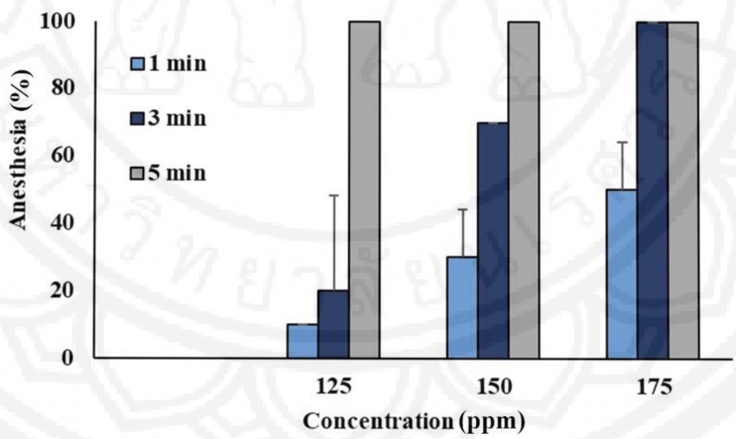

Figure 3 Cumulative anesthesia in hybrid catfish after exposure to clove oil in different concentrations and exposure times

After studying cumulative anesthesia level and calculating cumulative anesthesia rate of juvenile hybrid catfish by Probit analysis using the Minitab software to study the EC_{50} of juvenile hybrid catfish were performed (Figure 4), it was found that in the study that EC_{50} values could be calculated for only 2 periods, which were at 1 and 3 min. The EC_{50} values of juvenile hybrid catfish, when exposed to clove oil and 50% of the total number of fish were in anesthesia, were 173.73 (166.39-185.49) ppm and 139.63 (136.52-142.62) ppm at 1 and 3 min, respectively (Table 3).

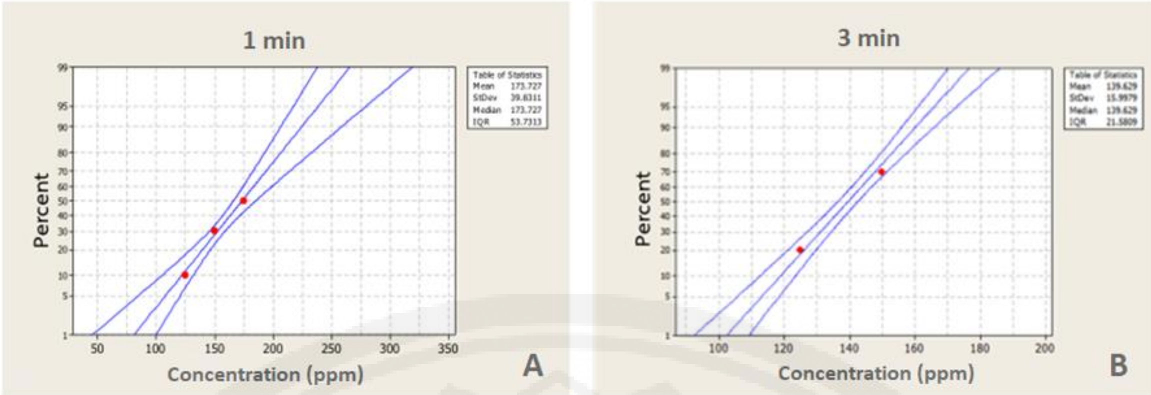

Figure 4 EC_{50} analysis by changing anesthesia percentage of hybrid catfish to Probit values using Minitab, anesthesia at 1 min (A), and at 3 min (B)

Table 3 EC_{50} values calculated based on anesthesia in hybrid catfish after exposure to clove oil by Probit analysis

Anesthesia (min)	EC_{50} (95% confidence) ppm
1	173.73 (166.39-185.49)
3	139.63 (136.52-142.62)
5	ND

Note: ND = Not detectable because percentage of asphyxiation in every concentration at 5 min is equal to 100%

Recovery time of juvenile hybrid catfish after exposed to clove oil

Clove oil at concentrations of 125, 150 and 175 ppm caused asphyxiation in juvenile hybrid catfish, and when studying the recovery, it was found that the recovery occurred at 3 min in all exposure concentrations. After 5 min, the recovery rate gradually increased and reached to 100% at 10 min (Figure 5).

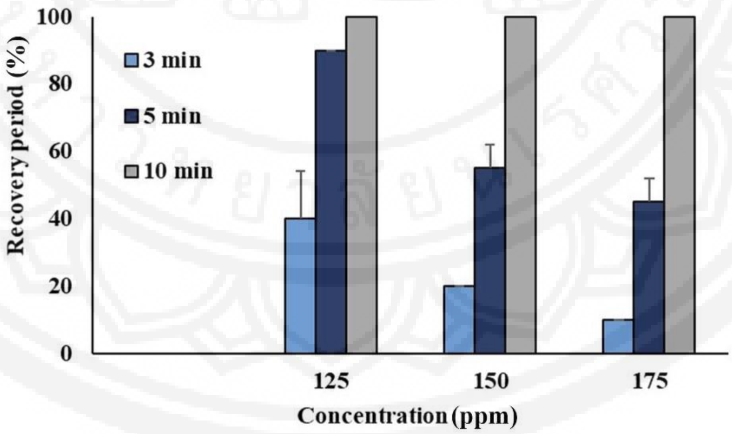

Figure 5 EC_{50} graph showing Probit analysis being converted from recovery percentage by using Mini tab program

Probit analysis was applied to indicate clove oil exposure in order to assess recovery as well as anesthesia (Figure 6). After calculating the percentage of cumulative recovery and analyzing the recovery time based on EC_{50} value, it was found that the recovery of catfish occurred after 3 min and 5 min with EC_{50} values of 111.96 (88.07-123.52) ppm and 165.09 (159.04-173.43) ppm, respectively (Table 4). According to the study of asphyxiation and study of recovery time of hybrid catfish for applying in culture and activities, it was found that the concentration of 125 ppm was appropriate to apply because it gave appropriate asphyxiation and recovery time.

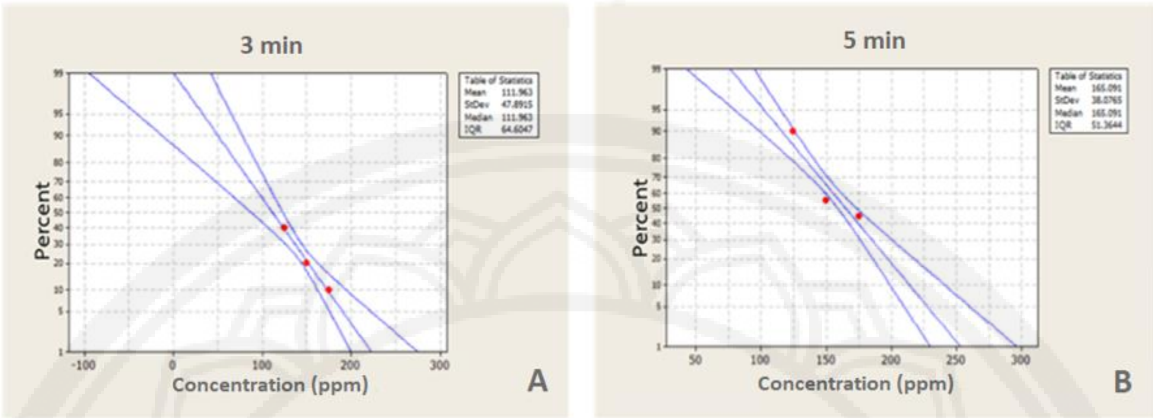

Figure 6 Graphs of EC_{50} analysis by changing recovery periods of hybrid catfish with Probit analysis at 3 min (A), and at 5 min (B)

Table 4 EC_{50} based on recovery percentage in hybrid catfish after exposure to clove oil analyzed by using Probit analysis

Recovery period (min)	EC_{50} (μl/L) (95% confidence)
3	111.96 (88.07-123.52)
5	165.09 (159.04-173.43)
10	ND

Note: ND = Not detectable because percentage of asphyxiation in every concentration at 5 min was equal to 100%.

The efficiency of clove oil on the transportation of hybrid catfish and the behavior of fish during transportation

After packing 10 juvenile hybrid catfishes (4 replications) and added clove oil at concentrations of 2, 4, 6, 8 and 10 ppm and oxygen to evaluate the efficiency of clove oil for transportation, it was found that the bags with clove oil at concentrations of 2, 4, 6, 8 and 10 ppm the catfish moved slower following the time after 12, 18 and 24 h of exposure. However, mortality was not found. In the control group, the catfish could survive for only 8 - 10 h. Moreover, the result of morphological study performed in clove oil exposed catfish (in concentration) indicated that the catfish had more mucus excretion after 15 h and found minor injuries. After that, the hybrid catfish exposed to clove oil at all concentrations had recovered to normal movement and a 100% survival rate. The behavior of hybrid catfish exposed to clove oil at the concentrations of 2, 4, 6, 8 and 10 ppm was not different and they did not cause mortality; therefore, we selected clove oil at the concentration of 2 ppm to further apply. Fish behavior was traced and analyzed according to anesthesia phases reported by Hamachkova, Kouril, Kozak, and Stupka (2006).

Discussion

Anesthetic in fish has been applied in plentiful purposes, e.g. artificial insemination, egg collection, weighing and length measurement, fertility testing, marking, sizing, transferring and taking photo. Anesthetic research in various areas has been investigated in many countries. Most of anesthetics applied wre synthetic chemicals, imported from foreign countries with expensive price, such as Quinaldine and MS-222. These cause limits for applying in Thailand. Aquaculture, especially in freshwater fish, has started to provide technical documents confirming the use of anesthetic in the last 10 years. For coastal aquaculture, most species being transported have been treated by temperature reduction (Tangsitthiw & Sriveerachai, 2013). At present, it is found that an interesting alternative in decreasing chemicals application is an application of clove oil which is a Thai herb.

Clove oil is an essential oil obtained from dried cloves by steam distillation. Clove oil and derivatives (eugenol and iso - eugenol) have become popular anesthetic for fish because it is inexpensive and safe for fish and humans. In many countries such as Australia, New Zealand and other countries in the southern and western islands of the Oceanic countries, eugenol and clove oil derivatives (iso-eugenol) produced in a commercial form has been certified for use as anesthetic for various purposes in fish because it is safe. Especially for fish released into natural water sources and raised for human consumption without need to stop applying before consuming or releasing, like other chemicals. In general, 1 ml of clove oil contains approximately 1 g or 1,000 mg of clove oil. Clove oil has unique properties that are insoluble, but can be dissolved with 95 % ethyl alcohol into clove oil solutions or ethyl alcohol in the ratio of 1: 9 by volume (Tangsitthiwat & Sriveerachai, 2013)

Wongtavatchai (2006) confirmed that that clove oil could be applied for anesthtic. The clove oil is a natural product derived from plants with biological activity, causing aquatic animals such as various kinds of fish to have drowsiness, calm, suppress muscle relaxation and become unconscious. It is useful for aquatic management such as controlling or reducing aggression, artificial insemination, weighing and transportation. Although clove oil can be biologically decomposed and environmental-friendly; however, its application in aquatic animals has efficiency limitations because clove oil is not water-soluble, resulting in poor distribution and uneven action. It floats as oil film covering the water surface. From the above information that illustrates the problem, Wongtavatchai (2006) studied and developed the formulation of ready-to-use clove oil products in lotion form to increase the effectiveness of application and safety for aquatic animals. Initially, it must be evaluated acute toxicity of clove oil solutions in various aquatic animals when exposed to the substance for 24 h, which was found that the concentration of clove oil that caused 50% of exposed animals; abalone size 8-12 mm to die (Lethal Dose 50%) was 13.18 ppm. And, it was 11.45 ppm and 15.07 ppm for tilapia (0.4 g) and cichlids (20.32 g).

Moreover, Wongtavatchai (2006) studied the sub-acute toxicity of clove oil in cichlids (20.32 g) and found that after exposed to clove oil (4-6 ppm) for 48 h, 50% of fish population was died. From the importance above, the objective of this study was to investigate the toxicity level (LC_{50}) and to test the efficacy of clove oil as anesthetic in the transportation of hybrid catfish. In addition, the appropriate concentrations of clove oil were studied for asphyxiation and the recovery time of juvenile hybrid catfish (estimated from the EC_{50}) because at present, hybrid catfish is an important economic fish and utilized in education and research proposes.

Although it has been reported that clove oil could be used to asphyxia fish, the initial assessment of toxicity as Wongtavatchai (2006) reported is necessary. This may be because each animal has different tolerances. And, the concentration of the substance, exposure time, and route also affect the response and mortality rate (Walker, Hopkin, Sibly, & Peakall, 2006)

In addition, Urumnouy, Sungpetch, Suntiprasert, and Yomlar (2006) reported that the appropriate concentration that caused anesthesia in African catfish (*Clarias gariepinus*) were 15 mg/L. The hybrid catfish firstly showed asphyxia at 37.44 ± 3.7 min and had a recovery time from asphyxia within 1.27 ± 0.4 min.

after 24 of experiment, it was found that the temperature and pH values at before and after exposure were changed in a narrow range. And, when African catfish were transported for 16 h, the survival rate in the control group and the group exposing clove oil solution (15 mg/L) was not statistical different. However, ammonia concentration in the control group was higher than those of clove oil ($p < 0.05$). All of the researches reported in the same way that clove oil could calm aquatic organism, suspend and reduce movement as well as could reduce the excretion of fish waste. Therefore, it can be used in transporting fish for a period of 12-24 h in containers with limited size and without changing water.

Siamese fighting fish has also been used to study the efficacy of clove oil for asphyxiation. Somjai et al. (2008) studied the appropriate concentration of clove oil solution for asphyxiation. It was found that the appropriate concentration was 15 mg/L while it was 30-50 mg/L for common carp (*Cyprinus carpio* L.) with 5 of anesthetic application. The anesthesia lasted for 2 and 4 min, respectively. The concentration was the dominant factor that affects fish asphyxia. The appropriate concentration was in the range of 20-50 mg/L. However, the suitable concentration varied according to species and size of fish and water temperature (Urumnouy et al., 2006).

In this study, there were three levels of clove oil concentration that caused hybrid catfish to fall in anesthesia: 125, 150 and 175 ppm. The asphyxiation occurred was in three phases. However, after considering the concentration of clove oil that can cause fish asphyxiation to carry out fishery activities, such as tagging or blood collecting for research purpose, an appropriate concentration was 125 ppm because it makes a good asphyxiation and recovers in a timely manner. This is different from the study of Penprapat, Wongsathein, Sang suttiwongsa, and Pikulkaew (2007) which reported that the appropriate concentration of clove flower oil for the study of asphyxia and recovery in carp (*Cyprinus carpio*), were 50 and 100 ppm because it makes the time to reach the 4^{th} phase of asphyxiation significantly shorter than the concentration of 25 ppm. However, it was not found the difference in between the concentration of 50 and 100 ppm.

After Probit analysis was performed, it was found that the EC_{50} value of clove oil was 173.73 (166.39-185.49) ppm at 1 min and the EC_{50} value in clove oil exposed hybrid catfish at 3 min was 139.63 (136.52-142.62) ppm. For studying the recovery phase, the recovery of exposed catfish could be detected after 3 and 5 min. The observed EC_{50} values were 111.96 (88.07-123.52) ppm and 165.09 (159.04-173.43) ppm, respectively. After comparing to all the reported data, it was found that this is inconsistent with this study. It might be caused by many factors, such as the type of clove oil used and species, age, sex and size of fish.

Urumnouy et al. (2016) studied acute toxicity of clove oil causing 50% mortality rate in African catfish larvae in 24 h (24-h LC_{50}). They tested the effect of clove oil in the concentrations of 15.0, 18.8, 18.6, 20.4, and 22.2 mg/L. At the end of experiment, mortality rates of larvae in each concentration were 0.0, 16.7, 93.3, 100.0 and 100.0%, respectively. Then, these mortality rates were used to calculate LC_{50} value at 24 h. and it was 17.37 (15.12-19.61) mg/L at a confidence level of 95%. And, the study of Somjai et al. (2008) who studied the acute toxicity of clove oil solution in Siamese fighting fish (*Bettas plendens*), that has respiratory organs and can live in the low oxygen content condition, as same as catfish, the LC_{50} value at 24 h was 21.7 mg/L. It showed that clove oil was more toxic in hybrid catfish than Chinese fighting fish. In this study, acute toxicity values being assessed based on LC_{50} value of clove oil causing mortality in hybrid catfish exposed to clove oil for 24, 48, 72 and 96 h, were consectutively 66.26 (59.14-77.89), 50.54 (47.83 -54.14), 48.49 (44.63-53.79) and 35.67 (33.65-38.13) ppm. Our results were differed from the study of African catfish and Chinese fighting fish (Urumnouy et al., 2006; Somjai et al., 2008). This might be due to the form of clove oil used because it was in solution form in this study, and the species of fish used was different; therefore, it resulted in different mortality rates.

Seetapan, Mahawong, Bookong, Moojareinsup, and Kongha (2010) reported that duration of asphyxiation and the recovery time of Mekong giant catfish (*Panggasisnodon gigas*) in the small size significantly depended on anesthetic type and concentration ($p<0.05$). In increased concentration of clove oil and MS-222, the hybrid catfish was asphyxiated faster. It was found that clove oil caused the catfish to become asphyxiation faster than MS-222 at the same level ($p<0.05$).

In this study, hybrid catfish were tested with clove oil at concentrations of 2, 4, 6, 8 and 10 ppm for transportation. It was found that after 12, 18 and 24 h, hybrid catfish movement was slower in every hour of study but no mortality was observed. Therefore, clove oil could be used in the transportation of hybrid catfish in order to reduce movement rate and stress during transportation. After comparing all concentrations studied, there was no mortality of hybrid catfish found. Therefore, the application of clove oil in only 2-4 ppm is suitable because it is not wasteful and reduces transportation costs. The concentration of clove oil used in this study is similar to the study of Wongtavatchai (2006) which used clove oil as a solution to test in different fish species for transportation. It was found that the suitable clove oil concentration for anesthesia maintenance was 4 ppm for Sea Bass larvae (1 g weight), 5-10 ppm for tilapia (*Oreochromis niloticus*) larvae (0.3-5 g weight) and 4-6 ppm for cichlids (8-30 g weight) with the experimental period of 12-24 h

Conclusion and Suggestions

The study of the efficacy of clove oil for anesthesia maintenance in the 3rd phase of juvenile hybrid catfish for fishery activities such as tagging and blood collection indicated that the concentrations of clove oil at 25, 50, 75, and 100 ppm were not able to asphyxiate juvenile hybrid catfish. There were only 3 concentrations of clove oil being able to asphyxiate, consisting of 125, 150 and 175 ppm. The anesthesia lasted for 5 min and recovery within 10 min. However, the appropriate concentration was 125 ppm. And, after studying the concentration of clove oil used to transport juvenile hybrid catfish, it was found that after exposed to clove oil at concentrations of 2, 4, 6, 8 and 10 ppm for 12, 18 and 24 h, hybrid catfish had slower movement but no mortality found. After that, the exposed hybrid catfish (at all concentrations) had 100% survival rate after recovery. Therefore, all concentrations studied could be used for transportation; however, in order to reduce the cost, it should be applied in the concentrations of 2-4 ppm. Thus, we concluded that clove oil can be applied for transportation and asphyxiation of juvenile hybrid catfish.

A future interesting research should be an investigation of concentration of clove oil in transportation and asphyxiation with other fish species because different species have different tolerance for the benefit of aquaculture in the future.

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