The role of clove (*Sygnium aromaticum*) oil as anaesthetics compound for abalone (*Haliotis squamata*)

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Abstract

Abalone is one of aquaculture commodity that has a high value including in Indonesia due to its price, taste and nutrition content. Unfortunately there is problem in abalone's transportation process which caused stress and even death. Clove oil can be used as anesthetic agent for abalone to obtain a high survival rate because of its eugenol content. This research tried to evaluate the the effect of clove oil as anesthesia agent to determined induction time, survival rate and oxygen cosumption in *H squamata*. This study used an experimental method and used a completely randomized design with treatment concentration of clove oil solution were 0.5 ml/L (A), 0.7 ml/L (B), 0.9 ml/L (C), and 1.1 ml/L (D) which each treatment was repeated three times, and calculated the value of the abalone (*H. Squamata*) survival rate after maintenance for 14 days. The results of the study showed that the survival rate of abalone seeds obtained in the treatment with the concentration of 1.1 ml/L (D). In the treatment also showed the fastest induction time with 96.67 seconds. The lowest oxygen comsumption rate in this study was treatment D with concentration of 1,1 ml/L. It could be concluded that using clove oil with concentration of 1.1 ml/L as anasthesic compound for abalone showed effectiveness in induction time, survival rate, and oxygen consumption rate.

Introduction

Abalone is one of the important commodities in the world. The increase in world abalone production is due to its good taste and high nutrition content (1). Abalone contains protein and carbohydrates reaching 15.87 and 6.36 g / 100g (2). Not only protein, abalone also contains essential amino acids so it is good for consumption (3). Uniquely, abalone is not only eaten but also used as jewelery and furniture (4). In Indonesia, abalone is one of the important non-fish economic commodities. Some abalone species found in Indonesia are *H. asinina, H. varia, H. squamata, H. ovina, H. glabra, H. planata,* and *H. crebrisculpta* (5). One species of native Indonesian abalone developed for aquaculture is *H. squamata* (6). However, in the *H. squamata* aquaculture activity, there are still many problems faced by Indonesian farmer. One of the main problems is the high mortality rate at the time of transfer. In Indonesia, the location of the abalone broodstock as seed producer is usually far from the hatchery, as is the location of the enlargement and market (7). The transportation process is an important basis for aquaculture. Poor transportation processes can cause vibrations, sudden and noisy shock, causing fish to stress, injury, even death (8, 9). Unfortunately there are few reports on how to overcome transportation problems in abalones in Indonesia (7). Therefore it is necessary to make efforts to minimize stress when shipping so that a high survival rate is obtained. One way to overcome the problem is using anesthesia to the abalone. Anesthesia is the provision of chemicals or sedatives given to fish so that it can reduce the negative habits of the fish so that the fish will lose movement, balance, awareness, and become relaxed (10). By

giving anesthesia, the fish will avoid injury, stress and high mortality so that the condition of the aquatic organism remains good (11). The chemicals used for anesthesia can come from both artificial and natural. The use of natural ingredients for anesthesia is widely used because it does not leave residues that are harmful to fish, humans and the environment when compared to synthetics (12). One natural ingredient that is commonly used is clove (*Sygnium aromaticum*) oil (13). Cloves oil contain 70-90% eugenol from several previous studies (14). Eugenol (4-allyl-2-methoxyl) is a phenolic compound that can be used for anesthesia (15). Clove itself has been used as an anesthetic material in various commodities, such as fish (13,16,17), shrimp (18), even abalone (19). Clove oil also has the advantage of being cheap, easy to obtain and safe for the environment (14).

This study aimed to evaluate the efficacy of clove oil as anesthesia in abalone. Tests carried out in this study included anesthesia onset of action speed (induction time), survival rate and water quality. It was hoped that the results could later be utilized by abalone farmers in Indonesia to anesthetize abalones and transported them safely to other places.

Materials and methods

Material

Abalone seeds (*Haliotis squamata*) measuring 4-4.65 cm and weighting 12-14 g are 300 stocks, consisting of 240 treated and 60 as controls. Abalone seeds used in this study were obtained from cultivation in the village of Musi, Gerokgak, Singaraja, Bali. Abalone used in this study must be off food before anesthesia to reduce feces, stress and death (20). Stocking density used was 20 animals per treatment placed in a basket measuring 39x30x7 cm. This research was conducted on April to May, 2014 in Musi Village, Gerokgak District, Buleleng Regency, Bali. The cloves oil was taken from Laboratory of Fish Production, Faculty if Fisheries and Marine Science, Brawijaya University. The cloves oil was in liquid form. The concentration treatments used in this study were A (0.5 ml/L), B (0.7 ml/L), C (0.9 ml/L) and D (1.1 ml/L) with replications 3 times each. The clove oil was mixed with absolute ethanol to get the desired concentration (19). The rout of administration of clove oil was given by immersion.

Anesthesia effectiveness onset of action

Anesthesia effectiveness test was performed by calculating the induction time in accordance with previous studies (21). Induction time started when the abalones were put into clove oil according to the treatment until they were affected by anesthesia. The characteristics of abalone which had been affected by anesthetic effects were slow, immovable response and loose grip (attachment) from the substrate (fall) (22,23). Induction time was measured when

abalone seeds were immersed in treatment until they lost consciousness. The lost consciousness or the faint state of abalone could be measured by their movement. The moment of abalone fell off the walls of the aquarium was considered as a faint or lost conscious state while the moment of abalone regained an upright position (attached of abalone's ventral side to the aquarium was considered as recovery from anesthesia (24). The time was measured by stopwatch after the abalone seeds reached lost conscious (25).

Survival rate

Survival rate was observed after anesthetic administration for up to 2 weeks. The SR calculation formula was carried out in accordance with previous studies (26): $SR = NT/NO*100$. SR: Survival Rate. N_t: Total number at the end of the experimental. N_o: Total number at the start of the experimental. Oxygen Consumption Rate. Observation of the rate of oxygen consumption seen from the abalone started to move the body and re-attached to the substrate after anesthesia and rinsing with sea water (27). Oxygen consumption rate was calculated according to the formula as $OC = Vx(DO_0-DO_t)/W*T$. OC: oxygen consumption rate (mgO_2 / g / hour). V: volume of water in the container (L). DO_0: dissolved oxygen concentration before anesthesia (mg / L). DO_t: dissolved oxygen concentration when starting to move (mg / L). W: Test fish weight (g). T: observation period (hours). DO in this study was measured by DO meter as previous study (28). DO_0 was measured right before the abalone deeped in the treatment for anesthesia. DO_t was measured when the abalone start to move directly after anesthesia process.

Water quality test

The water quality observed in this study included temperature, pH, salinity according to previous study (29).

Data analysis

This research uses Randomized Complete Design with 3 replications for each treatment. To determine the effect of treatment used diversity analysis or F test. If the F value was significantly different or very real, then to compare the values between treatments continued with the Least Significant Difference test to determine the treatment that gave the best response. The best response at the level or degree of trust was 5%. To find out the relationship between the treatment and the affected outcome, a regression analysis was used which provided information about the effect of the best treatment on the response.

Results

Anesthesia effectiveness test

The results of studies that had been conducted regarding the effectiveness of anesthesia tests using clove oil solutions with different concentration obtained results as in figure 1.
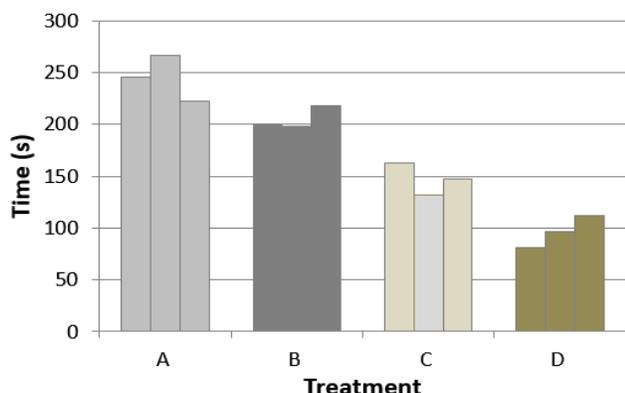
Figure 1: Induction time of clove oil concentrations as anesthetic agent in abalone.

From the picture above it could be seen that the average length of time the abalone starts to faint was shown in the treatment concentration of 0.5 ml/L, which was for 245 seconds. While for the fastest average time of fainting abalone was obtained at a treatment concentration of 1.1 ml/L, which was for 96.67 seconds. To find out more clearly the effect of different concentrations on the time the abalone began to faint, the analysis of variance was performed as presented in table 1.

From the results of the analysis of variance analysis (Table 2) showed the calculated F value> F table of 5%. This mean that giving different concentration of abalone seeds had a very significant effect on the length of time the abalone begins to faint. So the calculation continues with the LSD test. BNT test results could be seen in Table 2 below.

Table 1: Analysis of variety of anesthetic effectiveness tests

Variance	Degree of freedom	Sum of Squares	Middle Squared	F Value	F5%
Treatments	3	38140.92	12713.64	45.86 **	4.07
Random	8	2218	277.25		
Total	11	40358.92			

Table 2: LSD test anesthesia effectiveness test

Average treatment	D = 96.67	C = 147.33	B = 205.33	A = 245	notation
D = 96.67	-	-	-	-	a
C = 147.33	50.67**	-	-	-	b
B = 205.33	108.67**	58**	-	-	c
A = 245	148.33**	97.67**	39.67*	-	d

Note: ns = Non Significant (not significantly different), * = significantly different, ** = very significantly different.

BNT test results showed that the sequence of dosing that gave the fastest time to faint abalone was treatment D (concentration 1.1 ml/L), followed by treatment C (concentration 0.9 ml/L), treatment B (0.7 ml/L) and finally treatment A (0.5 ml/L). It could be seen that the relationship between the treatment of the concentration with the length of time the abalone starts to faint the results obtained $R^2 = 0.9403$ with the equation y = 374.78 - 251.5x. The graph was presented in Figure 2.

Based on the regression chart above, the relationship between the difference in concentration with the length of time the abalone began to faint was directly proportional (Linear). In treatment A (concentration 0.5 ml/L) had the lowest ability to relax muscles quickly. This could be seen from the time it took the abalone to remove from the substrate for longer than the other treatments. Whereas in treatment D (concentration 1.1 ml/L) had the highest ability to relax muscles in abalone seeds.

Survival Rate

The results of studies that had been carried out regarding the viability of abalone shellfish seeds using clove oil

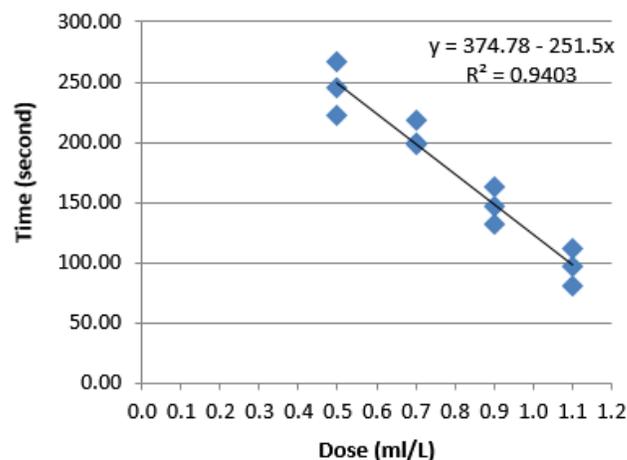solution with different concentrations obtained the following results figure 3.

Figure 2: Graph of the relationship between concentration treatment and the length of time the abalone begins to faint.
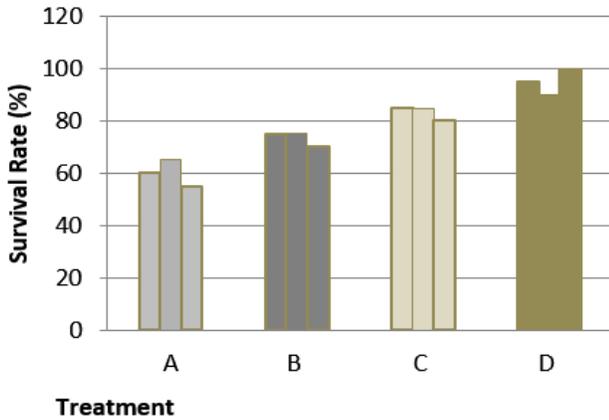
Figure 3: Abalone survival chart diagram. A: 0.5 ml/L; B: 0.7 ml/L; C: 0.9 ml/L; D: 1.1 ml/L.

From the picture above it was known that at a concentration of 0.5 ml/L abalone seeds experienced more deaths compared to the treatment concentration of 0.7 ml/L and 0.9 ml/L. It was indicated that less concentration would be more dangerous to apply as abalone anesthesia agent. Whereas at a concentration of 1.1 ml/L the survival of abalone seeds got the highest average value of 95%. The analysis of variance analysis regarding the survival of abalone seeds was presented in Table 3.

The results of analysis of variance in Table 3 showed that the calculated F value> F table of 5%. This showed that the administration of different dosages had a very significant effect on the survival of abalone seeds. The calculation continues with the LSD test. The LSD test was performed to determine the difference in influence exerted by each treatment. The results of the LSD test were presented in Table 4.

BNT test results showed that the sequence of dosing that gives the best survival rate was D (concentration 1.1 ml/L) followed by C (concentration 0.9 ml/L), B (0.7 ml/L) and finally A (0.5 ml/L). Next to determine the form of the relationship between treatment with the parameters tested, we used orthogonal polynomial analysis. The graph was presented in Figure 4.

Table 3: Analysis of abalone family life variety analysis

Variance	Degree of freedom	Sum of Squares	Middle Squared	F Value	F5%
Treatment	3	1989.58	663.19	39.79**	4.07
Random	8	133.33	16.67		
Total	11	2122.92			

Note: ** = Significantly different.

Table 4: LSD test for survival of abalone seeds

Average	A = 60.00	B = 73.33	C = 83.33	D = 95.00	Notation
A = 60.00	-	-	-	-	a
B = 73.33	13.33**	-	-	-	b
C = 83.33	23.33**	10^s	-	-	c
D = 95.00	35**	21.67**	11,67 ns	-	d

Note: ** = very real different, ns = not significantly different.
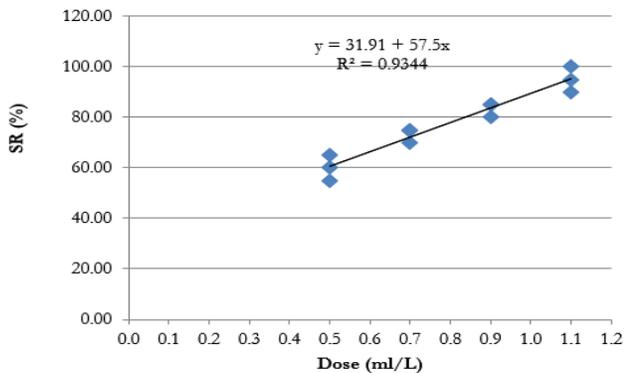
Figure 4: Graph of relationship between dosing treatment and survival.

Based on the regression chart above, it showed that increasing the concentration of clove oil to a concentration of 1.1 ml/L can improve the survival rate of abalone seeds after anesthesia linearly with the equation y = 31.91 + 57.5x with R^2 equal to 0.9344. This was because the clove oil had eugenol compounds that function as an anesthetic ingredients.

Oxygen consumption rate

Based on the results of the study the level of oxygen consumption of abalone after anesthesia treatment result showed in Figure 5.
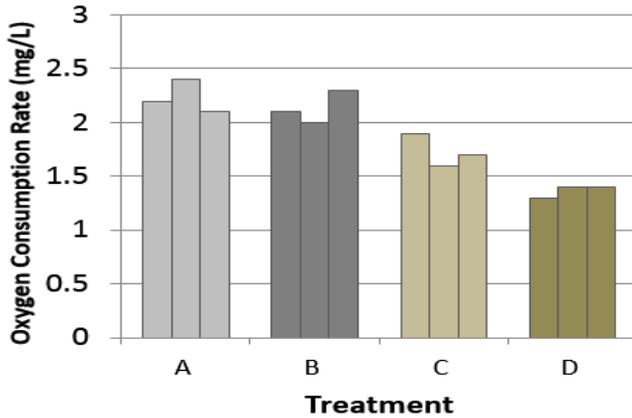
Figure 5: Diagram of oxygen ab post-anesthesia seed oxygen consumption rate. A: 0.5 ml/L; B: 0.7 ml/L; C: 0.9 ml/L; D: 1.1 ml/L

Based on the table above showed that the average oxygen consumption value of post-anesthetic abalone in treatment A (0.5 ml/L) was the highest value of 2.23 mg/L and treatment D (1.1 ml/L) was the value the lowest consumption of abalone oxygen after the anesthetic process was 1.37 mg/L. This also showed a connection with Figure 3 that the lower concentration would increase oxygen consumption and reduce survival rate. To find out more clearly the effect of different dosage treatments on the level of oxygen consumption, analysis of variance was performed as presented in Table 5.

From the results of the analysis of variance analysis (Table 8) showed the calculated F value> F table of 5%. This meaned that giving different concentration of abalone seeds had a very significant effect on the level of oxygen consumption after anesthesia. So that the calculation was continued with the LSD test, the LSD test results could be seen in Table 6 below.

Table 5: Analysis of variation rates of consumption of abalone seed oxygen

Variance	Degree of freedom	Sum of Squares	Middle Squared	F Value	F5%
Treatments	3	1.42	0.47	25.82**	4.07
Random	8	0.14	0.02		
Total	11	1.57			

Table 6: LSD test of abalone seed oxygen consumption rate

Average	D = 1.37	C = 1.73	B = 2.13	A = 2.23	Notation
D = 1.37	-	-	-	-	a
C = 1.73	0.37**	-	-	-	b
B = 2.13	0.77**	0.40*^{s}	-	-	c
A = 2.23	0.87**	0.50**	0.10^{ns}	-	c

Note: ns is not significantly different, * significantly different, ** very real different.

The sequence of treatments that provide the highest level of oxygen consumption was treatment A (0.5 ml/L), followed by treatment B (0.7 ml/L), C (0.9 ml/L) and D (1.1 ml/L). It could be seen that the relationship between the treatment of concentration with the level of oxygen consumption after anesthetic abalone seeds obtained R^2 = 0.8617 with the equation y = 3.198 -1.66x. The graph was presented in Figure 6.

Based on the regression chart above, the relationship between differences in concentration with the level of oxygen consumption after anesthetic abalone seeds was directly proportional (linear). The highest level of oxygen consumption was obtained in treatment A (0.5 ml/L) of 2.23 mg/L and the lowest was obtained in treatment D (1.1 ml/L) of 1.37 mg/L. The high level of oxygen consumption also affected the survival of abalone seeds. The higher the level of oxygen consumption would decrease the survival rate of abalone.
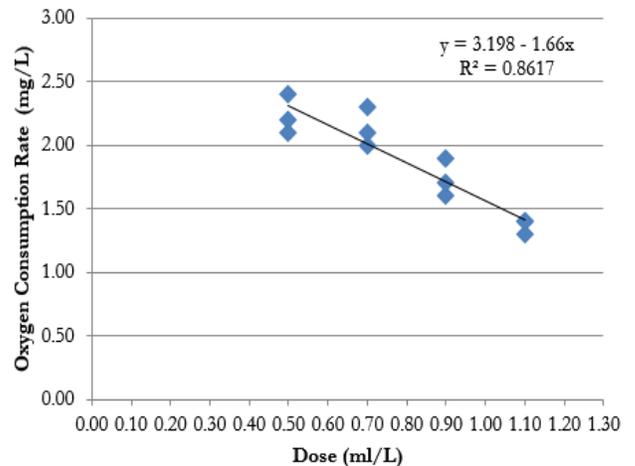

Figure 6: Graph of relationship between dosage treatment and oxygen abalone seeds oxygen consumption post.

Water quality

In aquaculture activities water quality was an important thing that must be considered. Water was a living medium and very influential on the survival of fish. Water quality parameters observed during the study included temperature, pH, salinity and dissolved oxygen (DO). Water quality measurements were carried out every morning and evening. During the maintenance period of abalone seed, water quality showed temperature of 27.5 - 30.8 °C, pH of 7.7 - 8.2, salinity of 32 - 36 ppt and DO 7.8 - 9.3 ppm.

Discussion

This research showed that the use of clove oil could have anesthetic effect on abalone. This has been proven also in previous studies in abalone (19). Even clove oil has recently been studied as a potential anesthetic agent for some ornamental fish and several farmed cold and warm water fish species (30). This was because clove oil has eugenol content. Eugenol was absorbed through the gills and quickly enter the bloodstream, where it functioned to inhibit the brain cortex and then act on the basal ganglia, the cerebellum and finally the spinal cord (31). The higher the concentration given, the less time it took for the abalone to faint and escape the substrate. Previous study stated that the use of clove oil to fish anesthetics at higher concentration would stimulate the fish fainted because of it reacted faster (32).

The high survival rate of abalone in treatment D compared to other treatments because clove oil worked as an anesthetic material (33,34). Anesthesia could improve survival rate by reducing mechanical damage and physiological stress (33). Anesthesia decreased fish stress levels by reducing potential negative effects on fish homeostasis, thereby reducing mortality (34). This faint abalone condition would lessen stress conditions. While the large number of deaths in treatment A was suspected to be due to stress that occurred in abalone during anaesthetic process. Because of the low content of the anesthetic concentration in treatment A, the stress level of the abalone in that treatment was higher than the others. Anesthesia could reduce stress levels in organisms by reducing the activity of metabolic, enzyme and ions in plasma (35). Stress was a trigger for death, including abalone, by affecting the blood which leading to reduce fitness, behaviour and immediate mortality (36). Anesthesia should not be overused. Excessive use of anesthesia severely suppressed fish, causing abnormal metabolic rates and oxygen consumption. In addition, these side effects could last for hours after the fish recover from anesthesia (8).

This study showed that the higher the concentration given, the lower the value of oxygen consumption. This was because the anesthetic content caused disorientation, loss of equilibrum, reduced swimming activity, and reduced respiration in stage III of anesthesia (37). The equilibrum loss caused respiration to decrease significantly after losing the ability to respond through the five senses (38). So that the increase in the concentration value was inversely proportional to the increase in oxygen consumption of post-anesthetic abalone seeds. High metabolic processes in abalone seeds made abalone seeds consume higher oxygen, therefore abalone seeds in treatment A (0.5 ml/L) the level of oxygen consumption was higher than the level of oxygen consumption in treatment D (1.1 ml/L). Eugenol could reduce oxygen consumption by 15.4% (39).

During the maintenance period of abalone, temperature, pH and salinity values were 27.5-30.8 °C, 7.7-8.2 and 32-36 ppt. This value was still optimal according to the abalone environment with temperature, pH and salinity ranging from 30 °C, 7.5-8.7 and 30-35 ppt (40). Whereas dissolved oxygen (DO) showed 7.8 - 9.3 ppm. The DO results in this study was better than previous studies with DO 5.9 - 6.11 mg/L (41).

Based on the results of research that had been done, it could be concluded that the use of clove oil with different concentration as an anesthetic material had a very significant effect on the survival of abalone seeds. In this study the highest survival rate obtained in treatment D of 95% with an average time to start fainting 96.67 seconds. Supporting parameters in this study was the level of oxygen consumption where the higher the concentration given the level of oxygen consumption would be lower. As for the water quality parameters obtained results where the temperature value ranges from 27.5 - 30.8 °C, salinity ranges between 32-36 ppt, pH ranges between 7.7 - 8.2 and DO ranges from 7.8 to 9.3 ppm.

Based on observations during the study, clove oil could be used as anasthetic compound for abalone. It was recommended to use a solution of clove oil at a concentration of 1.1 ml/L as an anesthetic, and this study could be continued to use simulation of transportation like car to evaluate the effect of clover oil in abalone accurately.

Conclusion

It could be concluded that clove oil with concentration of 1.1 ml/L could be used as anasthesic compound for abalone because of its effectiveness in induction time, survival rate, and oxygen consumption rate.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Acknowledgement

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دور زيت القرنفل (*Sygnium aromaticum*) كمادة مخدرة لاذن البحر (*Haliotis squamata*)

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الخلاصة

يعد إذن البحر أحد الأحياء المائية ذو القيمة العالية في اندونيسيا نظرا لسعره وطعمه وقيمته الغذائية المرتفعة. لكن لسوء الحظ، فإن عملية نقله يمكن أن تؤثر على قيمته العالية بما تسببه من إجهاد وحتى الموت أحيانا، وللتغلب على هذه المعضلة يمكن استخدام زيت القرنفل كعنصر مخدر أثناء مرحلة النقل للحصول على أعلى معدل بقاء ومقاومة نظرا لمحتواه العالي من مادة الاوجينول. حاول هذا البحث تقييم تأثير زيت القرنفل كعامل تخدير لتحديد وقت استحداث التخدير، نسبة المقاومة والبقاء ومعدل استهلاك الأوكسجين في إذن البحر. هذه الدراسة اعتمدت الطريقة التجريبيه واعتمدت التقسيم العشوائي التام باستخدام تراكيز علاجية مختلفة من زيت القرنفل بتركيز ٠,٥ مل/لتر مجموعة (ا), ٠,٧ مل/ لتر مجموعة(ب)، ٠,٩ مل/ لتر مجموعة (ج)، و١,١ مل / لتر مجموعة (د)، مع تكرار كل معالجة ثلاث مرات. تم حساب نسبة مقاومة إذن البحر بعد مرور ١٤ يوم من البقاء. أظهرت نتائج هذه الدراسة ان معدل البقاء لبذور إذن البحر تم الحصول عليه عند استخدام تركيز ١,١ مل/ لتر مجموعة (د)، كما وان هذه المجموعة أعطت أسرع وقت لأستحداث التخدير بزمن ٩٦,٦٧ ثانية، وأقل معدل استهلاك الأوكسجين. يمكن الاستنتاج ان إستخدام زيت القرنفل بتركيز ١,١ مل / لتر كمادة مخدرة لاذن البحر كان التركيز الأمثل الذي أعطى فعالية أفضل فيما يتعلق بوقت أستحداث التخدير، نسبة المقاومة والبقاء، ومعدل استهلاك الأوكسجين.