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Influence of the method of preparation of clove oil emulsion on the anaesthetic effect in fish (on the example of *Clarias gariepinus*)

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Abstract. In aquaculture, the use of clove oil as a natural anaesthetic for hydrobiontes is gaining popularity. There are several ways to prepare an aqueous emulsion for anaesthesia, which creates a problem for technologists in choosing the most effective one. The purpose of the study was to compare the anaesthetic effect of clove oil in three methods of preparation of the mixture on the youth of the African clary catfish. The study was conducted at the Centre for Aquatic Bioresources and Aquaculture of the National University of Life and Environmental Sciences of Ukraine. Research methods – Ichthyological and statistical. The activity of a water-based clove oil preparation was evaluated using three

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methods: hot, cold, and alcohol. Experimental material – juvenile fish of the clary catfish with an individual body weight from 22 to 285 g. Experimental conditions: the experimental material was kept without feeding for a day before the start of the study; fish anaesthesia was performed in the water at the temperature of +26°C; the duration of manipulations with each fish was minimal. The effectiveness of various methods of preparation of the emulsion was evaluated by the duration of fish entry into the state of anaesthesia and withdrawal from this state. The advantage of the hot method of preparing an aqueous emulsion of clove oil over the other two is established: the duration of fish entry into anaesthesia was 22% shorter compared with the cold method, and 12.3% – with the alcohol method ($p \leqslant 0.05$). The duration of fish recovery from anaesthesia with the hot method of preparation of the mixture was also shorter: compared to the cold method – 18% ($p \leqslant 0.05$), with the alcohol method – 43.2% ($p \leqslant 0.001$). Using correlation analysis, a relationship was established between the duration of fish entry into the state of anaesthesia and the individual body weight of the fish. Degree of binding: high for hot ($r=0.606$) and alcohol methods ($r=0.529$) and medium for a cold method of preparation of an anaesthetic mixture ($r=0.385$). The results obtained are of practical value for improving the method of anaesthesia of fish using clove oil

Keywords: aquaculture, anaesthesia, water emulsion, clary catfish

Introduction

Recent studies have shown that fish experience pain and suffering just like other phylogenetically superior animals (Sneddon, 2015). The use of various methods of calming fish during caviar harvesting, during bonitation, and surgical interventions helps to reduce injury of fish, prevents the manifestation of stress reactions or reduces negative consequences from them, and facilitates the work of the personnel of aquaculture enterprises. Managing stress is one of the main challenges in achieving animal welfare today (Herskin & Nielsen, 2018).

The use of anaesthetic substances in fish handling complies with the requirements of European legislation (Directive 2010/63/EU, 2010), the norms of which should be implemented in the legislation of Ukraine on the way to accession to the EU.

The state of anaesthesia is achieved in various ways, in particular, the effect of low temperatures on the fish body (Olifirenko *et al.*, 2020), or the use of carbon dioxide (Mitsuda & Yoshizawa, 1991). Sometimes these two methods are combined (Tan *et al.*, 2022). However, the most common method of anaesthesia in fish farming is chemical, using anaesthetic drugs, divided into synthetic (MS-222, novocaine, phenasepam, etc.) and natural, such as clove oil and chamomile oil (Ak *et al.*, 2022).

When choosing a drug for anaesthesia, researchers (Park *et al.*, 2017; Bolasina *et al.*, 2017) recommend focusing on the generally accepted requirements for an ideal anaesthetic for fish: rapid anaesthesia (<3 min) and rapid recovery (<5 min) of fish after anaesthesia; solubility in water, affordability and practicality in use, no toxic aftereffect for the fish body and harmful residues in food fish products, no harm to the environment.

The most common anaesthetics in Ukraine are phenasepam, quinaldine, and clove oil. The use of the first two drugs has certain limitations: phenasepam belongs to psychotropic substances and is sold only by prescription; quinaldine has a toxic aftereffect (Zinkovskij *et al.*, 2000).

Clove oil is a representative of essential oils: it has a pronounced pungent smell and bitter taste. This oil is extracted from the flower buds, leaves, and stems of the clove tree (*Syzygium aromaticum*), which grows on the islands of Java, Zanzibar, Madagascar, Sri Lanka, China, and some other countries. The quality of clove oil depends not only on the part of the tree from which it was extracted but also on the time of year and the climate zone in which the plant grew. Clove oil is also used in the food industry (as a food additive and as a means of extending the shelf life of products), in cosmetology (as a flavouring agent) and in medicine (as an analgesic in dentistry) (Hu *et al.*, 2018; Singh *et al.*, 2022).

In aquaculture, clove oil as an anaesthetic is used in working with a large number of hydrobiontes, both marine and freshwater (Teles *et al.*, 2019; da Silva *et al.*, 2021). Clove oil is also used in amphibian breeding (Sirimanapong, 2020). The anaesthetic effect of clove oil can also be used to reduce fish loss during transportation and to facilitate fish slaughter (Teles *et al.*, 2019; Kovalenko & Kovalenko, 2022).

An important property of clove oil is its safety: the drug does not accumulate in the body of animals and does not affect haematological and biochemical parameters, reproductive function of the body, further behaviour, and growth rates of animals (Aydın & Barbas, 2020). This substance has long been a food additive for various products, so it is considered safe for humans. Therewith, there is a lack of data in the scientific literature on the study of the possible effect of this anaesthetic in the long term on the degree of physiological changes in the fish body, especially if this procedure is repeatedly used on one fish, as is the case in working with the breeding stock of sturgeon with the factory method of reproduction of these fish.

The composition of clove oil includes eugenol (active substance, more than 70%), eugenol acetate (up to 13%), caryophyllene, β-myrcene, α- and β-pinenes, ylangen, γ-selinene, β-elemene, heptanol, nonanol, benzyl alcohol, havicol, benzaldehyde, furfural and furfural alcohol, methyl benzoate, etc. (Nowak *et al.*, 2012). The registration number of the drug is CAS 8000-34-8.

Eugenol can be extracted from clove basil (*Ocimum gratíssimum*) and holy basil (*Ocimum sanctum*). According

to the effect on fish, eugenol from these plants does not differ from eugenol obtained from the clove tree. Eugenol is also part of the AQUI-S anaesthetic (Putland *et al.*, 2020; Sharma *et al.*, 2022).

Clove oil does not dissolve in water and forms large droplets on the surface of the water, which makes it difficult for the active substance to enter the body of fish. This property of clove oil requires careful mixing with water until a fine emulsion is formed (Yostawonkul *et al.*, 2019). Therefore, the search for the optimal method of preparing an emulsion of clove oil is relevant.

There are three main ways to prepare an aqueous emulsion of clove oil: hot, cold, and alcohol. When using the hot method, the required amount of clove oil is collected from the bottle using a syringe and poured into a glass of water heated to +50°C, and then mixed until a fine emulsion is formed. The resulting emulsion is poured into a container for fish anaesthesia (Ross & Ross, 2008).

For the cold method, clove oil is collected in a syringe and poured into a container with water, in which the fish will be anaesthetised. Intensive mixing of oil with water manually or using a technical device, for example, a kitchen mixer, is recommended to form an emulsion (Kovalenko *et al.*, 2022). This method is simpler but requires a careful mixing of water with clove oil because in cold water the process of emulsion formation takes longer than in hot water.

When using the alcohol method, clove oil is dosed using a syringe, poured into a glass with ethyl alcohol (95%), in which the oil is dissolved by careful mixing. The prepared mother liquor is poured into a container for anaesthesia of fish. The recommended ratio of clove oil to alcohol is 1:10 (Öğretmen & Gökçek, 2013; Aydın *et al.*, 2015). The solution is prepared immediately before use. Its long-term storage is unacceptable.

In the scientific literature, there is no information on conducting experiments to compare the above-mentioned methods of preparing clove oil for the effectiveness of anaesthetic effects on fish, which was the determining factor for choosing the research subject.

The purpose of this study is a search for the most effective way to prepare an aqueous emulsion of clove oil for the anaesthesia of fish in aquaculture. The task was set to achieve the goal – conduct an experiment to assess the degree of anaesthetic effect of clove oil on juvenile fish of African clary catfish using hot, cold, and alcoholic methods of preparation of the mixture, analyse the results of the experiment, and draw conclusions.

Materials and Methods

An experiment to compare the effectiveness of the anaesthetic effect on fish of clove oil preparation for fish, prepared by three different methods, hot, alcohol, and cold, was conducted in 2022 at the Centre for Aquatic Bioresources and Aquaculture of the National University of Life and Environmental Sciences of Ukraine. The hot method of preparation of the emulsion, the most common in the modern practice of using clove oil for fish anaesthesia (Poplavskaya *et al.,* 2017), was chosen as a control option, cold and alcohol methods – as experimental options.

The object of research is juvenile fish of the African clary catfish (*Clarias gariepinus*), 1.5 months of age, with an individual body weight ranging from 22 to 285 g. The experimental material was kept in an aquarium with a capacity of 400 litres without feeding for one day before the experiment began.

The following laboratory equipment and fish farming equipment were used to conduct the experiment:

- liquid container with lid, working volume 100 ml;
- 1 cm^3 syringe;
- 10 cm^3 syringe
- thermal oximeter;
- stopwatch;
- aquarium aerator;
- aquarium with a capacity of 400 litres – 1 unit;
- aquarium with a capacity of 50 litres – 2 units;
- laboratory glass stick;
- net for catching fish;
- electronic scales;
- bowl for weighing fish.

Clove oil of TM Aromatika (Ukraine) in bottles with a capacity of 5 and 10 ml, purchased in a pharmacy chain in Kyiv was used to prepare an aqueous emulsion of the anaesthetic preparation.

The process of preparing an emulsion of clove oil in three ways included the following steps.

Hot method:

- 1 ml of the drug was collected from a bottle of clove oil with a syringe and poured into a liquid container;
- using a syringe, 10 ml of water heated to a temperature of +50°C was added to the container;
- the container was closed with a lid and shaken manually for 30 seconds until a fine emulsion was formed.

Cold method:

- 1 ml of the drug was collected from a bottle of clove oil with a syringe and poured into a liquid container;
- using a syringe, 10 ml of water at room temperature (+26°C) was added to the container;
- the container was closed with a lid and shaken manually for 30 seconds until a fine emulsion was formed.

Alcohol method:

- 1 ml of the drug was collected from a bottle of clove oil with a syringe and poured into a liquid container;
- using a syringe, 10 ml of 95% ethyl alcohol was added to the container;
- the container was closed with a lid and shaken manually for 30 seconds to better dissolve the oil in alcohol.

Two aquariums with a volume of 50 litres each were prepared for the experiment. Each aquarium was filled with 10 litres of tap water work prepared according to the settling method. One aquarium was used to put fish in a state of anaesthesia, the second – to withdraw fish from a state of anaesthesia. An emulsion of clove oil prepared in one of three ways was poured into an aquarium of water intended for fish anaesthesia and mixed with a glass stick to evenly distribute the emulsion in the water.

The concentration of the drug for the anaesthesia of fish in all variants of the experiment was the same – 0.1 ml of clove oil in 1 litre of water. The choice of such a concentration of the drug for the anaesthesia of clary catfish was made based on the results of a previous study (Kovalenko *et al.*., 2022).

The aquarium for withdrawing fish from the state of anaesthesia was equipped with an aerator to enrich the water with oxygen to speed up the release of fish from anaesthesia in the absence of water flow.

After the above preparation, the experiment began:

1) using a net, one by one, specimens of fish was caught from a 400-litre Aquarium and placed in an aquarium with an aqueous emulsion of an anaesthetic;

2) the changes were observed in the state of fish;

3) a stopwatch was used to record the time when the fish entered a state of anaesthesia, which, in accordance with the protocol of induction of anaesthesia for fish (Zahl *et al.*, 2012), has the following signs: loss of reflex activity, position with the abdomen up, movement of the gill covers – barely noticeable, lack of response to strong external stimuli;

4) the immobilised fish was taken out of the aquarium and weighed on a laboratory scale with an accuracy of ±0.1 g;

5) immediately after weighing, the fish was placed in an aquarium with clean aerated water;

6) changes in the condition of fish were observed and the time of recovery from anaesthesia was recorded for signs of respiratory activation, complete recovery of motor activity and normal body position – with back up, and the presence of a reaction to external stimuli.

Thus, an experiment was conducted on three variants, with the corresponding use of a clove oil preparation prepared in 3 different ways. The total number of clary catfish used in the experiment was 90 fishes. 30 of them were used in each version of the experiment.

The results of the experiment were processed using generally accepted methods of variation statistics. Univariate dispersion analysis was used to assess the effect of the method of preparation of an aqueous emulsion on the time of entry into anaesthesia and the time of recovery from this state.

A correlation analysis of experimental data was performed to assess the relationship between the duration of individual stages of fish anaesthesia (the processes of fish entering and withdrawaling the state of anaesthesia) and individual fish body weight.

The mathematical processing of the digital data of the experiment was conducted using methods generally accepted in animal husbandry (Ruban *et al.*., 2020).

Results and Discussion

The effectiveness of the anaesthetic effect on clary catfish juvenile fish of clove oil prepared by three methods was investigated: hot, cold, and alcohol. The results of the experiment on the comparative evaluation of these three methods of preparation of the mixture for the achieved effect of fish anaesthesia are presented in Table 1.

Table 1. *Influence of the method of preparation of clove oil emulsion on achieving the effect of anaesthesia in clary catfish juvenile fish*

Method of preparation of water emulsion of clove oil	Duration of changes in the condition of fish, seconds	
	Entering a state of anaesthesia	Recovery from anaesthesia
Hot	94.1±7.49	111.7±3.86
Alcohol	116.5±8.81*	196.5±21.10***
Cold	120.2±8.09*	136.1±8.83*

Note: * – $p \leq 0.05$, *** – $p \leq 0.001$ (compared to the hot method)

As can be seen from Table 1, for all variants of preparation of clove oil mixture, the effect of an anaesthetic effect on the condition of experimental biological material of fish was obtained. Therewith, the time interval during which clary catfish juvenile fish were first introduced into a state of anaesthesia, and then, after recovery from the drug and weighing, withdrawed from this state, varied substantially: from 94.1±7.49 to 120.2±8.09 and from 111.7±3.86 to 196.5±21.10 seconds, respectively. The difference between the extreme values of these indicators was 27.7% and 75.9%, respectively, which is objective evidence of the influence of the method of preparing clove oil on the effectiveness of the anaesthetic effect of this drug on fish. Univariate analysis of variance showed that the strength of the influence of the method of preparation of clove oil for anaesthesia factor on the duration of entry of clary catfish juvenile fish into the state of anaesthesia was 6.4% ($p \leq 0.05$), and for the time to withdrawal this state – 19.6% ($p \leq 0.001$).

Evaluation of the results of the experiment based on compliance with existing recommendations for choosing the ideal anaesthetic for fish (Park, *et al.*, 2017; Bolasina, *et al.*, 2017) on the duration of the processes of entering the object of influence into the state of anaesthesia (<3 min) and recovery after this state (<5 min) showed that for all three variants of preparation, the mixture of clove oil met the established criteria for effectiveness.

In the control version of the experiment (hot method of preparation of clove oil mixture), the highest effect of anaesthetic effects on clary catfish juvenile fish was obtained, both in terms of the time of introduction of young catfish into the state of anaesthesia (94.1±7.49) and the duration of the fish recovery process after this state (111.7±3.86). Comparison of the results obtained

in two experimental versions of the experiment with the control showed that the time of the entry of fish into the state of anaesthesia for both methods substantially increased (p≤0.05): for the alcohol method – by 22.4 seconds (12.3%), for the cold method – by 26.1 seconds (22%). The duration of fish recovery from the state of anaesthesia, in comparison with the control option, also increased: with the alcohol method of preparation of the anaesthetic emulsion – by 84.8 seconds (43.2%) (p≤0.001), in the cold method – by 24.4 seconds (18%) (p≤0.05).

The results of the search for a correlation between the live weight of clary catfish juvenile fish and the duration of fish entry into the state of anaesthesia and calculations of the regression coefficient are shown in Table 2.

Table 2. *Correlation and regression coefficients between the live weight of fish and the duration of entry of clary catfish juvenile fish into the state of anaesthesia for various methods of preparation of the anaesthetic mixture*

Method of preparation of clove oil mixture	Correlation coefficient (r)	Regression coefficient, s/10 grams
Hot	0.606***	3.91***
Alcohol solution	0.529***	5.01**
Cold	0.385*	2.96*

Note: *– p≤0.05; **– p≤0.01; ***–p≤0.001

As can be seen from Table 2, the degree of correlation between the live weight of clary catfish juvenile fish and the duration of entry of these fish into the state of anaesthesia was generally positive, but substantially varied depending on the method of preparation of the mixture for anaesthesia: from high, using hot (r=0.606) and alcohol methods (r=0.529) to medium – using the cold method of preparing an aqueous emulsion of clove oil (r=0.385). It should also be noted that there is a substantial correlation between the live mass of clary catfish and the duration of induction of the state of anaesthesia in fish for all variants of the experiment, the value of which was: for hot and alcoholic methods of preparation of the anaesthetic emulsion – p≤0.001, for the cold method – p≤0.05.

Analysis of the degree of dependence between the live mass of clary catfish and the duration of the induction process of anaesthesia in fish showed that an increase in the mass of clary catfish by 10 g leads to an increase in the duration of fish entry into the state of anaesthesia by 3.91 seconds for hot, 5.01 for alcohol, and 2.96 for cold methods of preparation of clove oil. The substantiality of the reliability of the regression coefficients for the experimental variants was: for the hot method of preparation of the anaesthetic mixture – p≤0.001, for the alcohol method – p≤0.01, for the cold method – p≤0.05.

The results of determining the correlation coefficients between the live weight of clary catfish and the time of recovery of these fish from the state of anaesthesia and the degree of regression changes in these indicators for each method of preparation of the anaesthetic mixture are shown in Table 3.

Table 3. *Correlation and regression coefficients between the live weight of clary catfish juvenile fish and the time of recovery from anaesthesia for different methods of preparation of the anaesthetic emulsion*

Method of preparation of clove oil mixture	Correlation coefficient (r)	Regression coefficient, s/10 grams
Hot	0.111	0.37
Alcohol solution	-0.368*	-8.36*
Cold	0.306	2.57

Note: *– p ≤ 0.05

As can be seen from Table 3, the search for a correlation between the live weight of fish and the duration of their recovery from anaesthesia showed radically different results, depending on the experiment option: the practical absence of such a relationship (r=0.111) for the hot method of preparing an aqueous emulsion of clove oil; a weak direct connection (r=0.306) – for the cold method; inversely proportional relationship, with a degree below average (r=-0.368) – for the alcohol method of preparing a solution of an anaesthetic preparation. The substantiality of correlation ties was established only for the alcohol method of preparation of the anaesthetic mixture (p≤0.05).

Only one substantial indicator was considered to analyse the degree of dependence between the weight of fish and the time of its release from the state of anaesthesia – from the option of alcohol method for preparing a mother liquor of clove oil for fish anaesthesia, with a regression coefficient of -8.36 at the substantiality level at p≤0.05. This indicator shows that with an increase in the body weight of the clary catfish by 10 grams, the time when the fish comes out of the state of anaesthesia decreases by 8.36 seconds.

Based on the results obtained, it can be concluded that there is a moderate tendency to increase the time of the recovery of the African clary catfish from the state of anaesthesia with a decrease in the live weight of the object of anaesthetic action only when using an alcoholic method of preparing the mother liquor of clove oil for anaesthesia of this type of fish.

The establishment of a positive correlation between the individual weight of clary catfish juvenile fish and the duration of fish introduction into anaesthesia confirmed the results of the first study of the authors conducted on this fish species earlier (Kovalenko *et al.*, 2022). Thus, in the previous experiment conducted on fish with an average body weight of 928 g, against 575 g in the second experiment, under similar conditions (cold method of preparation of clove oil emulsion for anaesthesia, option with a water temperature of +26°C), the time of entry of fish into the state of anaesthesia was recorded, almost one and a half minutes longer than in this study, confirming the identified dependence. However, the conclusion made in the previous study about the presence of an inverse relationship between the individual weight of fish and the time of its release from the state of anaesthesia was confirmed only in the version with the alcohol method of preparing the mother liquor of clove oil. In the version with the cold method of preparing the emulsion for anaesthesia, the effect of body weight on the duration of fish recovery from the state of anaesthesia was the opposite – a direct weak relationship between these indicators was established. Such different results of assessing the degree of correlation between the live mass of fish and the duration of their withdrawal from the state of anaesthesia, depending on the method of preparation of clove oil mixture, dissonant with the indicators obtained when searching for links between the mass of fish and the duration of induction of their state of anaesthesia, indicate the feasibility of conducting a second experiment.

Thus, according to the results of the experiment, it can be stated that the hot method of preparing an aqueous emulsion of clove oil for fish anaesthesia (on the example of clary catfish), in comparison with alcohol and cold methods, demonstrated its advantages, both in the shorter duration of fish entry into the state of anaesthesia and in the time of fish withdrawal from this state. This conclusion is generally consistent with the conclusions of other researchers (Ross, 2008; Aydın, 2020) regarding the feasibility of preparing a mother's liquor of a fine clove oil emulsion in high-temperature water (about 50°C), due to which, the drug is quite easily dispersed during intensive shaking. Therewith, this method loses its advantage when it is necessary to work with fish in conditions of low water temperatures (below 10°C), for example, when manipulating cold-loving fish species (salmon, whitefish) or with heat-loving objects, but at low water temperatures (for example, when conducting spring bonitation of breeding carp fish species). Under such working conditions, an alcohol method for preparing a mother liquor of clove oil, followed by bringing the concentration of the drug in water to working values can be proposed (Javahery, 2012; Öğretmen, 2013; Aydın, 2015).

Unfortunately, the authors of the study were not able to compare the results of an experiment to test the effectiveness of using clove oil from different methods of preparing a mixture for anaesthesia of 1.5-month-old clary catfish juvenile fish with materials from similar studies by other researchers due to the lack of relevant information. The following is a likely explanation for this state of affairs: physiological features of the clary catfish, as a representative of lungfish with exceptional survivability and a wide range of ecological adaptations (Weyl *et al.*, 2016), primarily due to oxygen deficiency, until recently did not encourage researchers to search for ways to anaesthetise this strong and hardy fish during technological manipulations or lifetime laboratory tests with it. Only the growing attention to the subject of the humane treatment of animals throughout the civilised world and the introduction of appropriate national and international standards and protocols for working with biological material have led to certain changes in this issue.

Conclusions

As a result of the experiment, new data were obtained on the technique of using clove oil for anaesthesia of African clary catfish, which have no analogues according to the search data in sources of scientific and technical information. The anaesthetic effect of using three methods of preparation of this anaesthetic, hot, cold, and alcohol, on 4-5-month-old juvenile fish of clary catfish was investigated and the influence of individual fish mass on the duration of different stages of the anaesthesia process was evaluated.

It was identified that the induction of the state of anaesthesia in clary catfish juvenile fish and the recovery of fish from the state of anaesthesia occurs most quickly using a fine aqueous emulsion prepared using a hot method of diluting clove oil. In the version with the alcohol method of preparing a solution of clove oil for anaesthesia, the second result was obtained in terms of the rate of introduction of fish into the state of anaesthesia and the third – in terms of the duration of recovery of fish from this state.

A direct relationship was identified between the duration of entry of clary catfish into the state of anaesthesia and the individual body weight of fish, with the degree of connection, depending on the method of preparation of clove oil for anaesthesia: high – for hot and alcoholic methods, medium – for cold method. Regression coefficients are calculated for each method of preparing clove oil for anaesthesia, which is of practical importance – it allows predicting the time of this stage of anaesthesia for each object of influence, depending on its individual body weight.

Divergent data were obtained on various variants of the experiment regarding the nature of the influence of individual body weight of clary catfish on the duration of the stage of withdrawal of clary catfish from the state of anaesthesia: from weak positive – for the cold method of preparation of the anaesthetic mixture, to average inversely proportional – for the alcohol method. This result makes it necessary to conduct additional research on this issue.

A promising area for deepening the research of the use of clove oil as a natural anaesthetic for various fish species should be considered the assessment of the consequences for fish health from repeated use of the anaesthesia procedure with this drug, in particular, for the implementation of reproductive function in the mother material of the valuable aquaculture objects.

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Вплив способу приготування емульсії гвоздичної олії на анестезуючий ефект у риб (на прикладі *Clarias gariepinus)*

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Анотація. В аквакультурі набуває популярності використання гвоздичної олії в якості природного анестетика для гідробіонтів. Існує декілька способів приготування водної емульсії препарату для анестезії, що створює для технологів проблему вибору найбільш ефективного. Метою дослідження була порівняльна оцінка анестезуючого впливу гвоздичної олії за трьох способів приготування препарату на молодь африканського кларієвого сома. Дослідження проведено у Центрі водних біоресурсів та аквакультури Національного університету біоресурсів і природокористування України. Методи дослідження – іхтіологічні та статистичні. Оцінено активність препарату гвоздичної олії, приготовленого на водній основі за трьома способами: гарячим, холодним і спиртовим. Дослідний матеріал – цьоголітки кларієвого сома з індивідуальною масою тіла від 22 до 285 г. Умови експерименту: дослідний матеріал до початку дослідження утримували без годівлі протягом доби; анестезію риб проводили у воді однакової температури +26 °С; тривалість маніпуляцій з кожною рибою була мінімальною. Ефективність різних способів приготування препарату оцінювали за тривалістю входження риби у стан анестезії і виходу із цього стану. Встановлено перевагу гарячого способу приготування водної емульсії гвоздичної олії над двома іншими: тривалість входу риби в анестезію була меншою у порівнянні з холодним способом на 22 %, із спиртовим способом – на 12,3 % ($p \leq 0,05$). Тривалість виходу риби зі стану анестезії за гарячого способу підготовки препарату також виявилася меншою: у порівнянні з холодним способом – на 18 % ($p \leq 0,05$), із спиртовим способом – на 43,2 % ($p \leq 0,001$). За допомогою кореляційного аналізу встановлено зв'язок між тривалістю входження риби у стан анестезії та індивідуальною масою тіла риби. Ступінь зв'язку: високий для гарячого ($r=0,606$) та спиртового способів ($r=0,529$) і середній – для холодного способу підготовки препарату анестетика ($r=0,385$). Отримані результати становлять практичну цінність для удосконалення методу анестезії риб з використанням гвоздичної олії

Ключові слова: аквакультура, анестезія, водна емульсія, кларієвий сом