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The Efficacy of Clove Oil as an Anaesthetic for Wels (*Silurus glanis* L.)

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ABSTRACT

The aim of this study is to establish the effect of different concentrations of clove oil on the time needed for induction and recovery from anaesthesia of two different weight groups one-summer-old wels. Experiments were made with the following four concentrations: 0.02 ml.l^{-1}, 0.04 ml.l^{-1}, 0.06 ml.l^{-1} and 0.08 ml.l^{-1} with the two weight groups (WG) reared up to one-summer-old age in earthen ponds.

1. Weight group I (WG I) – fish weighing 11.92 ± 2.46 g**.**
2. Weight group II (WG II) – fish weighing 128.2 ± 1.65 g**.**

The behavior of the fish was observed and analyzed during the experiment. At lowest concentration the wels from both weight groups do not reach the phase of complete immobilization. Individuals from WG I reach only phase 3 without going into phase 4 of anaesthesia, and with the fish from WG II only a slight decrease in locomotor activity is observed. The application of the next two concentrations 0.04 ml.l^{-1} and 0.06 ml.l^{-1} leads to complete immobilization of fishes from both weight groups. At the same time there is a significant time difference between the two weight groups for reaching the same phase of sedation. At the highest concentration of 0.08 ml.l^{-1} the wels from both weight groups lose equilibrium in the shortest time and in the shortest time reach phase 4 – total loss of reactions. LC_{50} for the two weight groups was determined. For WG I 50% mortality rate was observed when the wels were exposed to the clove oil for a period of ten minutes at a concentration of 0.14 ml.l^{-1}, and for WG II at a concentration of 0.16 ml.l^{-1}.

Key words: Wels, clove oil, anesthetic, toxicity.

INTRODUCTION

The clove oil is a dark brown liquid, which is distilled from the buds, leaves and stems of the clove tree *Eugenia caryophyllata* (Briozza et al., 1989- Keene et al., 1998; Soto and Burhanuddin, 1995). The main compound of the oil is eugenol (4-allyl-2methoxyphenol) 70-90%, followed by acetyl eugenol and caryouphylline.

As a natural product with pleasant aroma the clove oil has been used for a long time in the food industry for flavoring. It is applied in human medicine as a mild anesthetic (Ross and Ross 1999; Taylor and Roberts 1999). It has antibiotic, antiseptic, antimycotic and antibacterial effect (Hamackova et al. 2006).

The first information of clove oil applied for anaesthetizing fish, dates back from more than 30 years ago (Endo et al., 1972). Although there is a wide range of anesthetic substances that can be used for that purpose (Marking and Mayer, 1985; Gilderhus and Marking, 1987; Hamackova et al., 2004; Hamackova et al., 2006), there is a growing interest, in recent years, towards this application of clove oil. The reason for which is that clove oil meets all the main requirements for the modern anesthetics: application of low concentrations, quick induction and recovery of anaesthesia, soluble in water and alcohol, low cost, it does not accumulate in the body of the fish, and last but not least it is harmless to the environment (Brozova and Svobodova, 1986; Ross and Ross, 1999; Hamackova et al., 2006). All these positive qualities determine the clove oil as a feasible alternative to the most widely used anesthetic in fish industry – MS-222 (Anderson et al., 1997; Sink et al., 2007) and give ground for carrying out a number of experiments with various fish species like trout *Oncorhynchus mykiss* (Hoskonen and Pirhonen, 2004; Hamackova et al., 2006; Taylor and Roberts, 1999; Anderson et al., 1997; Keene et al., 1998; Wagner et., 2003; Velisek et al., 2005a), pike *Esox lucius* (Peake, 1998; Hamackova et al., 2006; Zaikov et al., 2008) carp *Cyprinus carpio* (Velisek et al., 2005; Hamackova et al., 2006; Hajek et al., 2006) channel catfish (Waterstrat, 1999; Small, 2003) etc.

The researches on the anesthetic effect of the clove oil on the wels are relatively few. Velisek et al. (2006) determined a concentration of 30 $mg.l^{-1}$ (0.028 $mg.l^{-1}$) as efficient and not having side effects. Hamackova et al. (2006) carried out a series of experiments for establishing the anesthetizing effect of clove oil on some fish species including wels and they determined that at water temperature of 18.4-20^{0}C the efficient concentrations are from 0.033 $mg.l^{-1}$ to 0.050 $mg.l^{-1}$.

The aim of the present study is to establish the effect of different concentrations of clove oil on the time needed for induction and recovery from anaesthesia of two different weight groups one-summer-old wels.

MATERIALS and METHODS

The experiments for determining the efficiency of clove oil as an anesthetic for wels were carried out under controlled laboratory conditions. The anesthetizing effect was tested at water temperature of 10^{0}C, at which catching and transporting the wels is most commonly done during autumn and early spring. Experiments were made with the following four concentrations: 0.02 $ml.l^{-1}$, 0.04 $ml.l^{-1}$, 0.06 $ml.l^{-1}$ and 0.08 $ml.l^{-1}$ with two weight groups wels reared up to one-summer-old age in earthen ponds in the experimental base of the Institute of Fisheries and Aquaculture.

1. Weight group I (WG I) – fish weighing 11.92 ± 2.46 g**.**
2. Weight group II (WG II) – fish weighing 128.2 ± 1.65 g**.**

Prior to preparing the solution, the clove oil has been dissolved in ethanol (95%) in a 9:1 ratio, and then was added to experimental tanks with 20l of water. Each concentration was tested with 10 fish from each weight group. Every single wels was placed separately for anaesthesia, and a total of 80 fish were used in the experiment.

For recovering form the anesthetic effect of the clove oil, the fish were transferred into tanks with a volume of 1 m^3, where they were monitored for 24 hours.

The time for induction of anaesthesia and recovery was measured with a stopwatch, and the behaviour of the fish was observed and analyzed according to the phases described in Table 1.

In this study the main focus was on the time needed for the catfish to reach phase 3 and 4 of anaesthesia and respectively the period needed for recovery.

For determining the toxicity of the clove oil, was used guideline for testing of chemicals - "**Fish Acute Toxicity test**" adopted by the Council of the Organization for Economic Co-operation and Development (OECD). The fish were exposed to concentrations of 0.02 $ml.l^{-1}$ – 0.2 $ml.l^{-1}$ for 10 min, with an increase of 0.02 $ml.l^{-1}$ at each step for each following exposition. For each concentration, as well as for the control group, 10 welses from both weight groups were used. Based on the results obtained was calculated the LC50 for a 10 minute exposure period. Student`s T - test for testing the statistical significance of the results was applied.

RESULTS

The results obtained from the experiments are shown in Table 2 for the first weight group, and in Table 3 for the second weight group. The fish from weight group I didn`t reach phase 4 – complete immobilization, when exposed to the lowest concentration of 0.02 $ml.l^{-1}$. They lost equilibrium and entered phase 3 for a period of 2.33 - 4 min. Compared to them the wels from weight group II, when exposed to the same concentration did not reach phase 3 and 4. Only a slight decrease in their locomotor activity was observed. When they were moved to the recovery tanks, the individuals from the two weight groups immediately regained their normal locomotor activity.

Table 1. Phases of induction and recovery from anaesthesia

Anaesthetizing		**Recovery**	
Phase	**Behaviour of the fish**	**Phase**	**Behaviour of the fish**
1	Acceleration of the opercular movements, increased respiratory activity	1	Weak, uncoordinated locomotion.
2	Decresed respiratory activity accompanied by uncoordinated locomotion	2	Regaining of the normal position. Decreased locomotor activity
3	Loss of equilibrium, decreased opercular movements, the fish still react to strong external stimuli	3	Normal position of the body. Normal locomotor activity is regained.
4	Complete immobilization, the fish lie on the bottom and do not react to handling		
5	Complete cessation of opercular movements, the fish die if they remain in the solution.		

Table 2. Time of induction and recovery from anaesthesia in wels (WG I)

Dose		Feature	Induction of anaesthesia					Recovery from anaesthesia		
			Body weight	Body length	Decreased	Total loss	Immobilization	Uncoordinated	Decreased	Normal
ml.l^{-1}			(BW)	(SL)	locomotor activity	of equilibrium		locomotion	locomotor activity	position
			(g)	(cm)	(min)	(min)	(min)	(min)	(min)	(min)
0,02	n=10	x	14,34	11,64	1,42	3,03			2,62	4,22
		SD	5,46	1,78	0,41	0,59			0,68	0,44
		Cv%	38,08	15,29	29,14	19,56			26,07	10,34
		min	8,62	9,70	0,98	2,22			1,57	3,72
		max	22,80	14,70	2,00	4,00			3,90	4,50
0,04	n=10	x	9,80	10,77	1,53	2,77	4,38	1,38	4,33	5,97
		SD	3,68	1,38	0,24	0,29	0,44	0,47	1,06	1,28
		Cv%	37,50	12,82	15,44	10,38	10,12	33,82	24,55	21,42
		min	5,24	9,00	1,23	2,38	3,95	0,75	2,75	3,75
		max	18,80	13,50	1,92	3,20	5,15	2,10	6,00	8,00
0,06	n=10	x	9,83	10,81	1,09	1,82	2,57	2,95	5,72	7,02
		SD	3,69	1,41	0,34	0,44	0,35	1,12	1,14	1,27
		Cv%	37,52	13,04	31,10	24,19	13,50	38,12	19,88	18,05
		min	5,10	9,00	0,67	1,17	2,00	1,67	3,67	4,63
		max	18,50	13,60	1,92	2,57	3,08	4,83	7,18	8,50
0,08	n=10	x	13,74	11,73	0,77	1,23	1,87	3,40	5,93	7,53
		SD	5,19	1,75	0,09	0,23	0,28	1,19	1,27	0,80
		Cv%	37,80	14,95	11,91	18,76	14,89	34,91	21,37	10,61
		min	5,80	9,20	0,60	0,90	1,50	1,17	3,80	6,22
		max	20,84	13,90	0,88	1,63	2,35	5,00	7,67	8,67

Table 3. Time of induction and recovery from anaesthesia in wels (WG II)

Dose		Feature	Induction of anaesthesia					Recovery from aneasthesia		
			Body weight	Body length	Decreased	Total loss	Immobilization	Uncoordinated	Decreased	Normal
ml.l^{-1}			(BW)	(SL)	locomotor activity	of equilibrium		locomotion	locomotor activity	position
			(g)	(cm)	(min)	(min)	(min)	(min)	(min)	(min)
0,02	n=10	x	127,30	26,50	5,83					
		SD	26,03	2,45	0,89					
		Cv%	20,45	9,24	15,24					
		min	98,00	23,20	4,50					
		max	170,00	30,00	7,30					
0,04	n=10	x	127	26,19	1,50	3,02	5,52	2,32	4,88	7,05
		SD	30,28	2,57	0,20	0,75	0,87	0,45	1,20	1,28
		Cv%	23,84	9,81	13,50	24,81	15,79	19,28	24,61	18,15
		min	93,00	23,50	1,28	2,17	4,25	1,67	3,50	5,00
		max	167,00	30,00	1,83	3,50	7,17	3,08	6,83	9,50
0,06	n=10	x	127,90	26,25	1,18	2,25	3,57	4,88	6,98	9,18
		SD	30,37	1,78	0,21	0,39	0,42	0,65	1,31	1,78
		Cv%	23,74	6,79	17,73	17,37	11,69	13,24	18,76	19,38
		min	91,00	23,50	0,85	1,83	2,95	3,50	4,17	5,50
		max	180,00	29,00	1,50	2,92	4,30	5,83	9,08	11,00
0,08	n=10	x	130,60	26,28	1,00	1,43	2,88	7,45	8,90	11,08
		SD	28,69	2,14	0,15	0,28	0,30	2,09	2,03	2,26
		Cv%	21,97	8,14	14,60	19,29	10,56	28,06	22,86	20,41
		min	98,00	23,60	0,77	1,00	2,33	5,17	6,25	8,75
		max	179,00	29,50	1,17	1,83	3,33	12,50	13,17	16,00

With concentration of 0.04 ml.l^{-1} the fish from WG I lose equilibrium in 2.38 to 3.2 min, and became completely immobilized in 4 to 5.15 min. Recovery from anaesthesia and regaining normal activity was done in an interval of 3 to 8 min. At the same concentration the wels from WG II reached phase 3 for 2.17 to 4.67 min, and complete anaesthesia (phase4) was induced in 4.83 to 7.83 min. The time for restoring their normal locomotor activity was 3.5 to 13 min.

At concentration of 0.06 ml.l^{-1} the fish reached phase 4 in an interval of 2 to 3.08 min for WG I, and respectively 2.95 - 4.3 min for WG II, and their recovery was accomplished in the interval between 3.67 - 8.45 min for the first weight group, and 4.17 - 11 min for the second weight group.

At the highest concentration of the clove oil of 0.08 ml.l^{-1}, the fish ware anaesthetized in a period of 1.5 to 2.35 min (WG I) and 2.33 - 3.22 min (WG II), and the time for recovering and regaining normal position was 3.67 - 7.18 min (WG I) and 4.17 - 9.08 min (WG II).

Based on the researches carried out was determined the LC50 for the two weight groups. In weight group I a 50% mortality rate was observed when the wels were exposed to the clove oil for a period of ten minutes at a concentration of 0.14 ml.l^{-1}, and for weight group II at a concentration of 0.16 ml.l^{-1}.

After introducing them in the solution the catfish moved vigorously for about 1 minute, and then they calmed down. Visually the effect of the clove oil was established through an initial increase of opercular movements, and consequently a partial loss of reaction to external stimuli was observed, but the effect was most apparent in phase 3 – loss of equilibrium. In this phase the fish lay on their back or to their side and periodically turned over. Gradually the intensity of their movements decreased and they entered phase four. The fish

lay on the bottom, they did not move and did not react to external stimuli. Various manipulations can be carried out, including surgical intervention, injecting, etc.

The recovery of the wels from the anaesthesia is relatively quick once they were transferred in fresh water. For a short time they lay on the bottom motionless and after a while started to move their fins and made uncoordinated movements. At a certain point they regain equilibrium, which they may lose several times until their complete recovery.

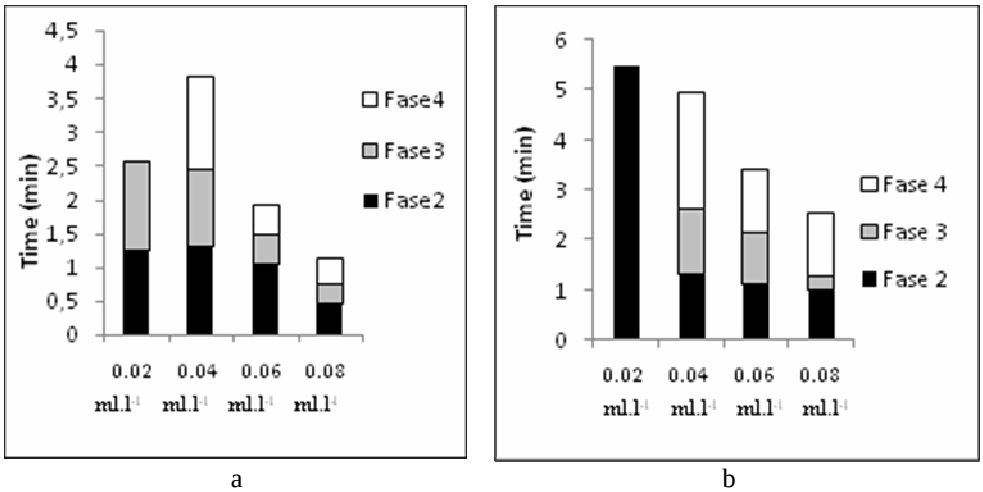
DISCUSSION

The results have shown that the lowest concentration tested, 0.02 $ml.l^{-1}$, cannot be used for anaesthetizing the fish as the individuals from WG I reach only phase 3 without going into phase 4 of anaesthesia, and with the fish from WG II only a slight decrease in locomotor activity is observed. From this point of view a concentration of 0.02 $ml.l^{-1}$ has only a sedative effect on the treated fish. Same and lower concentrations are proven to be safe for live fish transportation (Kaiser et al., 2006). The time it takes for the clove oil to induce phase 3 and 4 as well as the time for recovery, at the mentioned concentration, varies in relatively small range for the individuals. Similar information has been announced by Hajek et al. (2006) for carp.

The effect of the clove oil on the wels at the next two concentrations 0.04 $ml.l^{-1}$ and 0.06 $ml.l^{-1}$ was approximately the same. At the same time there was a significant time difference between the two weight groups for reaching the same phase of sedation. The average values showed that the fish from WG I lost equilibrium after 2.77 and 1.82 min, and reached phase 4 after 4.38 and 2.57 min respectively at the first and second (higher) concentrations. Their recovery of normal body position and locomotor activity were accomplished in an average of 5.97 min at 0.04 $ml.l^{-1}$, and 7.03 min at 0.06 $ml.l^{-1}$. With the wels from WG II the time to enter phase 3 was 3.02 min at 0.04 $ml.l^{-1}$, and 2.25 min at 0.06 $ml.l^{-1}$, and complete anaesthesia was achieved in 5.52 and 3.57 min for the lower and higher concentrations respectively. Their recovery was slower compared to the fish from weight group I.

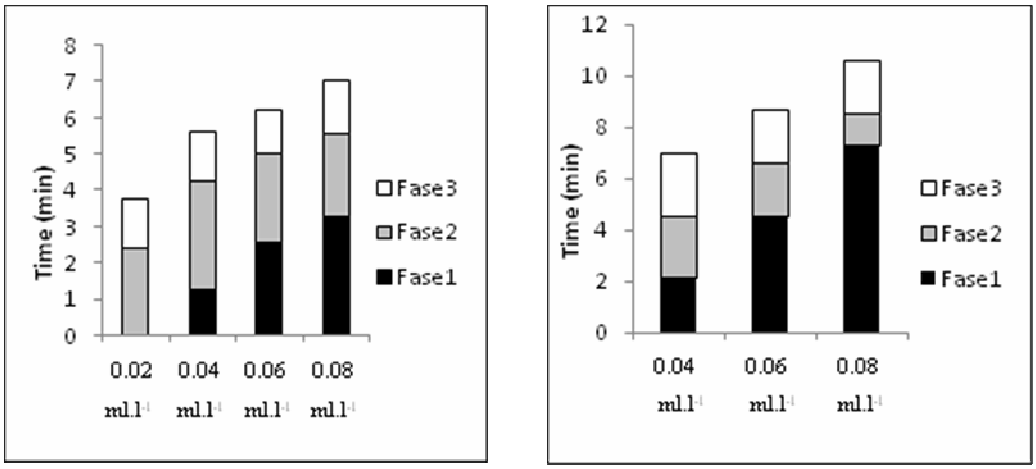
At the highest concentration of 0.08 $ml.l^{-1}$ the wels from both weight groups lost equilibrium in the shortest time and in the shortest time reached phase 4 – total loss of reactions – for an average of 1.87 min (WG I) and 2.88 min (WG II). The average time for regaining normal position was longest; for WG I it was 7.53 min, and for WG II – 11.08 min.

An important point to be noticed is that when the concentration of the clove oil is increased the necessary time for losing equilibrium and reaching phase 4 and total loss of reaction (Fig 1a and 1b), was decreased. The reverse dependency was observed when the wels recovered from anaesthesia and regained their normal position. At the higher concentrations this process took longer, and the fish that recovered in the shortest times were those exposed to the lowest concentration (Fig 2a and 2b). Similar dependencies have been established by other authors for clove oil, used for anaesthetizing various fish species (Bosworth et al., 2007: Hoskonen and Pirhonen, 2004: Keene et al., 1998).



a b

Figure 1. Time for reaching each of the phases of anaesthesia of wels using different clove oil concentrations (a-WG I; b-WG II)



a b

Figure 2. Time necessary for recovering and regaining equilibrium for wels anaesthetized at various concentrations (a- WG I; b-WG II)

When the weight of the fish is taken into consideration in respect to the time necessary for anesthetizing and recovery, it is clear that at equal concentrations, the wels from WG I reach phase 4, complete immobilization, quicker (with probability value $P<0.01$ at concentration of 0.04 $ml.l^{-1}$ and $P<0.001$ at concentration of 0.06 and 0.08 $ml.l^{-1}$). The fish from this weight group recover faster compared to larger fish, which can be explained with the shorter period of exposure to the anaesthetizing effect of the clove oil (with probability value $P<0.05 – P<0.001$ at different concentrations).

Considering the necessary time for achieving complete immobilization and the time for recovery, and based on the results obtained the efficient concentrations recommended are 0.04 $ml.l^{-1}$ for weight group I and 0.06 $ml.l^{-1}$ for weight group II. The second concentration is higher that the values recommended by Hamackova et al. (2006) for the wels (0.033 $ml.l^{-1}$-0.050 $ml.l^{-1}$), their experiments, however, were conducted at higher water temperatures between +18.4, $+20^0$C. The concentration of 0.04 $ml.l^{-1}$ is similar to the lowest effective concentration causing general anaesthesia in common carp juveniles (Hajek et al., 2006) and pike (Zaikov et al., 2008).

The therapeutic indexes, i.e. the ratio between the therapeutic and the toxic concentrations, established for the concentrations recommended in this researches are 3.5 for the fish from the first weight group and 2.6 for the fish from the second. For the smaller fish it is near the optimal values of 1:4 (Svobodova and Vykusova, 1991) and for the larger catfish it is identical with that obtained by Velisek et al. (2006).

CONCLUSION

This research confirms the positive qualities of the clove oil as an anaesthetic in aquaculture and proves the possible applications when carried out various manipulations on the wels.

In the conditions of the experiment, the concentrations recommended are 0.04 $ml.l^{-1}$ for fish weighing 11.92 ± 2.46 g, and 0.06 $ml.l^{-1}$ for fish weighing 128.2 ± 1.65 g, at which concentrations the fish from WG I reach phase 4 of anaesthesia in an average of 4.38 min, and those from WG II in 3.57 min. Recovery of their normal locomotor activity is achieved in 5.97 min for the first group and 9.18 min for the second.

With increasing the concentration of the clove oil, the time necessary for anaesthetizing the fish and reaching phase 4, total loss of reaction, is decreased, and the time necessary for their recovery is increased.

The weight of the fish effects the time for anaesthesia and recovery. At equal concentrations the smaller catfish reach phase 4 of anaesthesia in a shorter period and recover faster compared to the larger catfish.

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