

## Determination of lethal concentration (LC<sub>50</sub>) values of *Cinnamomum zeylanicum* hydrosol on carp fish

Kuukgul Gulec A.<sup>1\*</sup>; Altinterim B.<sup>2</sup>; Aksu O.<sup>1</sup>

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### Abstract

In this study, lethal concentration (LC<sub>50</sub>) values of cinnamon hydrosol (*Cinnamomum zeylanicum*) on carp (*Cyprinus carpio*) were investigated. In practice, experimental setup was constituted 30 fish (a total of 180 fish with 30 control fish) to be placed in three replicates. Hydrosol was added into aquariums at the doses of 0.5, 1, 2.5, 5 and 10% and determined mortality times of carp exposed to these concentrations. Percentage death of fish calculated in these concentrations. Mortality was observed at all treatments exception of dose of 0.5 %. The results indicate that the hydrosol had swimming changes, lethargy, lack of breath and leaning to the depth of the aquariums at all of the concentrations. The results of regression analysis indicated that the mortality rate (Y) is positively correlated the concentration (X) having a regression coefficient (R) close to one in each case. While the 1 h LC<sub>50</sub> value (with 95% confidence limits) of the safe dose of *C. zeylanicum* hydrosol was estimated at LC<sub>50</sub>=4.39%, 2 h LC<sub>50</sub> value=2.629%, and 12 h LC<sub>50</sub> value= 1.027%.

**Keywords:** Hydrosol, *Cinnamomum zeylanicum*, Mortality, *Cyprinus carpio*

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1-Tunceli University, Fisheries Faculty, TR62000, Tunceli/Turkey

2-Firat University, Fisheries Faculty, TR23100, Elazığ/Turkey

\*Corresponding author's email: agulec@tunceli.edu.tr

## Introduction

In Turkey, Cyprinids are the richest and the most important family of fish, and its members are distributed world-wide. These family members are distributed widely in fresh water sources (Demirsoy, 1988; Geldiay and Balık, 1998). Common carp (*Cyprinus carpio* L., 1758) are members of the Cyprinidae family are frequently cultured and of great commercial value as a food fish both over their native and introduced range (Berra, 2001).

Freshwater Cyprinid fish dominate world aquaculture production and the production has been increasing rapidly. The increase of productivity in fish culture has been accompanied by stressful conditions and problems related to fish diseases. Nowadays, the practically of using chemotherapeutics specially antibiotic to eliminate these problems has not been evaluated including use of natural antioxidant and antimicrobial agents as use of natural spices.

*Cinnamon* is one of the natural antioxidants and antimicrobial agents that is a member of Lauraceae family which occur in Asia and Australia. In traditional medicine *Cinnamomum zeylanicum* is used for thousands of years for medicinal purposes as analgesic, antiseptic, antispasmodic, aphrodisiac, astringent, carminative, haemostatic, insecticidal and parasitic. There are few studies on effect of this plant in aquaculture. Previous research has revealed interesting hypoglycemic effect (Khann et al., 2003; Kim et al., 2005; Verspohl et al., 2005),

antioxidant effect (Lin et al., 2003; Blomhoff 2004), antibacterial effect (Chang et al., 2001; Sagdic and Ozcan, 2003; Singh et al., 2007; Abasali and Mohammad, 2010) antifungal effect (Singh et al., 1995; Mishra et al., 2009) and cytotoxicity effect (Sharififar et al., 2009) of *C. zeylanicum*. In these studies, *C. zeylanicum* has been used as extract with several soluble or as essential oil. Scientific evaluation of hydrosols is necessary before medical application, but in this case, little work has been published so far.

Hydrosols, also known as floral water, distillate water or aromatic water, are the co-products or the byproducts of hydro- and steam distillation of plant material. Hydrosols are quite complex mixtures containing traces of the essential oil and, of course, several water-soluble components as well. They have practically been used as a beverage for a long time in many areas of Turkey (Sagdic 2003; Tajkarimi et al., 2010). Inhibitor effects of hydrosols of various spices and derivatives were reported by some researchers (Sagdic, 2003; Sagdic and Ozcan, 2003; Chorianopoulos et al., 2008). In previous studies, some researchers determine LC<sub>50</sub> values of herb extracts in cytotoxicity effect (Sharififar et al., 2009; Altınterim et al., 2012), larvicidal activity of shrimps (Patil and Magdum, 2011) and freshwater fish and zooplankton community (Promsiri et al., 2002; Mousa et al., 2008; Rana and Rana, 2012).

In this study, we have demonstrated death percentages of *C.*

*zeylanicum* hydrosol at different concentrations in cultured carp and have determined LC<sub>50</sub> values for each time (1h, 2h and 12h).

## Materials and methods

### Experimental animals

*Cyprinus carpio* with an average weight of 47.44±1.96 g and lengths of 13.64±0.25 cm were used in this study. Fish were acclimated for 1 week in these aquariums. After which a total 180 fish were transferred to 50 L aquariums.

### Preparation of *C. zeylanicum* hydrosol

Cinnamon (*C. zeylanicum*) was purchased from a local firmin Elazig/Turkey. *C. carpio* was obtained from Pertek Dam Lake in April, 2010. Hydrosols were prepared by the hydro-distillation according the method described by Sagdic (2003). About 100 g of *C. zeylanicum* was ground in a mixer. The hydrosol of *C. zeylanicum* was obtained for 1 h in a hydro distillation apparatus with 500 ml of double distilled water (1:10; w/v). After hydro-distillation, essential oil was separated by separation funnel and the mixture without essential oil identified as hydrosol. The hydrosol was then filtered and kept in sterile dark bottles (500 ml) a cool environment (4 °C) until use.

### Experimental Design

A total of 180 fish were used in the experiment. The fish were divided into 6 groups of 30 specimens (10 fish of each group, in 3 replicates) each as follows: Group 1 (Non-hydrosol normal fish, control group) received normal

commercial diet. Group 2 (the lowest concentration): hydrosol-added group at the level of 0.5%, Group 3 (low concentration): hydrosol-added group at the dose of 1%, Group 4 (mid concentration): hydrosol-added group at the level of 2.5%. Group 5 (high concentration): hydrosol-added group at the level of 5%. Group 6 (the highest concentration): hydrosol-added group at the level of 10%. All of the experimental groups received the treatments for a period of 1 week. During the experiment, the fish of each group were examined.

### Statistical analyses

All experiments were repeated three times and performed in triplicate. Data were analyzed with SPSS statistical analysis software (Version 10.0) using Probit Analysis Statistical Method. The LC<sub>50</sub> values (with 95% confidence limits) were calculated. Differences among the results were considered to be statistically significant when *P* value was < 0.05. Also, the MS Excel 2007 was used to find regression equation (Y=mortality; X=concentrations), the LC<sub>50</sub> was derived from the best-fit line obtained.

## Results

After determination of temperature, pH, hardness and dissolved oxygen level of water, hydrosol was added and *C. carpio* transferred into aquariums (Table 1). Temperature was 17 °C during experiment period. Dissolved oxygen increased when compared to the initial value (Table 1). The hydrosol concentrations were set up using the 0.5, 1.0, 2.5, 5.0, and 10% levels

of hydrosol to determine mortalities and effects of the hydrosol concentrations (Table 2). These results indicate that the

hydrosol had swimming changes, lethargy, lack of breath and leaning to the depth of the aquariums at all of the concentrations.

**Table 1: Physical characteristics observed in fish exposed to different hydrosol concentrations**

Concentrations of <i>C. zeylanicum</i> oxygen (mg/l)	Hydrosol (%)	Temperature (°C)	pH	Dissolved
10 (Group 6)	17	8.18	12.0	
5.0 (Group 5)	17	7.40	13.5	
2.5 (Group 4)	17	7.33	12.0	
1.0 (Group 3)	17	8.20	14.0	
0.5 (Group 2)	17	7.98	13.6	
Cnt. (Group 1)	17	6.94	6.67	

**Table 2: Macroscopically observing according to hydrosol concentrations**

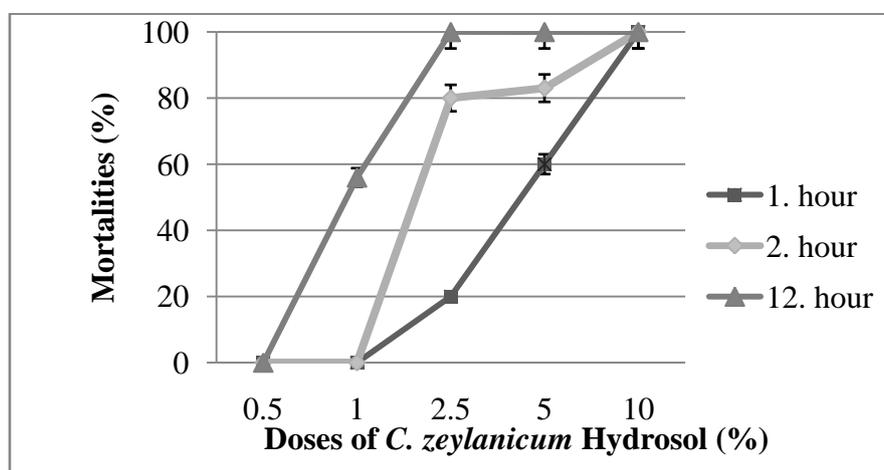
Hydrosol Concentration (%)	Macroscopic Observations
10	Immediately swimming changes in fish were observed after 1 minute. Breathless and lethargy were seen after 5 minutes. Operculum motilities of fish became irregular within 8 minutes and finally all of the fish died after approximately 1h.
5	Immediately swimming changes in fish were observed after 1 minute. Fish went to deep part of aquarium and maintained calmly in there after 3 minutes. Operculum motilities of fish were seen often after 23 minutes. While breathless was observed after 28 minutes, breathing stopped in some of fish after about 1h. 18 of 30 fish died within 1h, 25 of 30 fish died after 2 h and finally all of fish died after 12 h.
2.5	Immediately jumping in fish was seen after 1 minute. Fish maintained in the deep part of aquarium after 3 minutes. Breathing stopped after about 1h. 6 of 30 fish died in 1 h, 24 of 30 fish died in 2 h, finally all of fish within approximately 12 h.
1	Lethargy and calm in fish motilities were observed in 4 h. and, mortalities were seen in 17 of 30 fish after 12 h.
0.5	Lethargy was observed in 12h. But, all of fish lived after 4 days. During the experiment, mortalities were not observed in all of fish.

Dead specimens were removed immediately after death and their numbers registered and calculated percentage death. Mortalities were observed at all treatments exception of dose of 0.5 % (Figure 1). All

fish died within 1 h at the concentration of 10 % while fish deaths depending on the time showed an increase at the concentrations of 5 and 2.5 % (Table 3).

**Table 3: Percentage death of fish exposed to different hydrosol concentrations in different times (ND: no data because of 100 % mortality)**

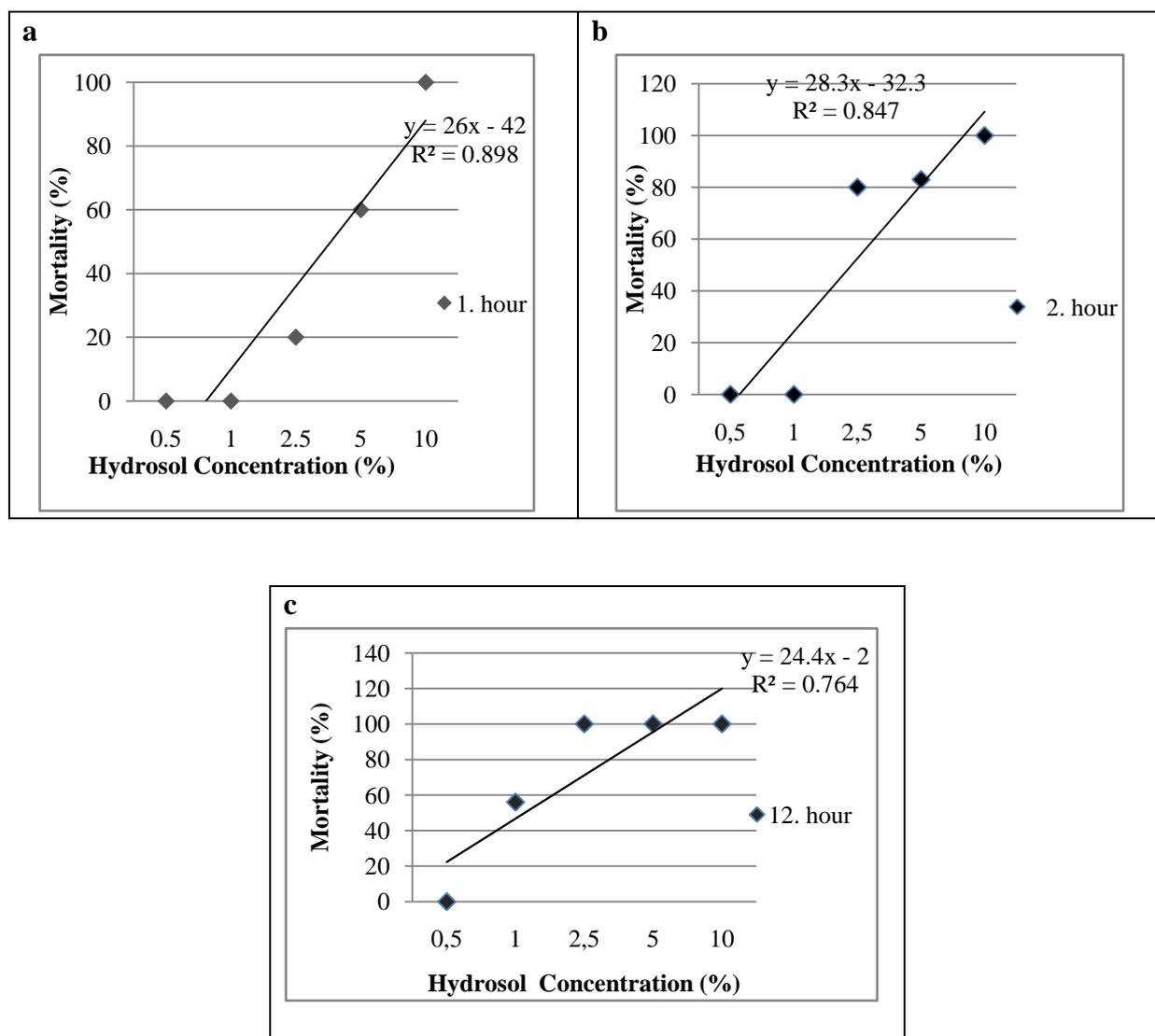
Concentration (%)	Mortality (%)		
	1 h	2 h	12 h
10	100	ND	ND
5.0	60	83	100
2.5	20	80	100
1.0	0	0	56
0.5	0	0	0



**Figure 1: Mortalities of fish exposed to different hydrosol concentrations in different times**

Different concentrations of cinnamon hydrosol depending on the time on cultured fish were tested and regression equations were showed in Figure 2. The results of regression analysis indicated that

the mortality rate (Y) is positively correlated the concentration (X) having a regression coefficient (R) close to one in each case.



**Figure 2.** Mortality curve of fish exposed to different *C. zeylanicum* hydrosol concentrations in different times (1. hour - a; 2. hour - b; 12. hour - c)

The LC<sub>50</sub> values and their upper and lower fiducially limits and the number of dead fish at certain *C. zeylanicum* hydrosol doses was examined in related to duration (1, 2 and 12h) of exposure (Table 4). The number of dead fish significantly increased in response to *C. zeylanicum* hydrosol

concentrations of 2.5 and 5.0 % ( $P \leq 0.001$  for each case, Table 4). The 1, 2 and 12h LC<sub>50</sub> values (with 95% confidence limits) of *C. zeylanicum* hydrosol for common carp were estimated as 4.390% (3.48-5.49), 2.629% (1.25-4.33) and 1.027% (0.83-1.30) respectively (Table 4).

**Table 4. LC<sub>50</sub> of *C. zeylanicum* hydrosol on common carp (n=30 each)**

Concentration (%)	Number of dead fish		
	1h	2h	12h
0.5	0	0	0
1.0	0	0	17
2.5	6	24	30
5.0	18	25	30
10	30	ND	ND
Control	0	0	0
<i>P</i>	>0.05	≤0.001	>0.05
LC <sub>50</sub> value with 95% confidence limits*	% 4.390 (3.48-5.49)	% 2.629 (1.25-4.33)	% 1.027 (0.83-1.30)

ND: no data because of 100 % mortality

\*LC<sub>50</sub> values with different superscripts are significantly different ( $p < 0.05$ ,  $p \leq 0.001$ ).

### Discussion

Cyprinidae is the largest family of fish in the world (Berra, 1981). *C. carpio* is an economically important freshwater fish cultured in Turkey. But, outbreaks of diseases and stress in cultured common fish are very serious problems. These problems are mainly controlled by chemotherapeutics and antibiotics. However, recently the use of antibiotics and chemotherapy have been criticized, because their use have created problems with drug resistance bacteria, toxicity and accumulation both in fish an environment (Farag et al., 1989; Citarasu et al., 2002, Sagdic and Ozcan, 2003).

Some spices used in place of antibiotics are rich sources of compounds like volatile oils, saponins, phenolics. These natural plant products have various activities like anti stress, appetizer, tonic, antimicrobials and immunostimulants (Citarasu et al., 2003). Recently, in aquaculture, there has been considerable

emphasis on studies involving essential oils, extracts and decoctions of spices on inhibiting the growth of microbes (Rao et al., 2006; Pachanawan et al., 2008).

*C. zeylanicum* used our study is an important spice and aromatic crop, having wide applications in flavoring and medicines. Effects (antimicrobial, antioxidant, antifungal e.g.) of *C. zeylanicum* were reported by some researchers (Smith-Palmer et al., 1998; Agaoglu et al., 2006). Agaoglu et al. (2006) obtained that *C. zeylanicum* had the most effective spice against all of the test strains. Smith-Palmer et al. (1998) found that the oils of cinnamon were the most inhibitory, each having a bacteriostatic concentration of 0.075% or less against all of five pathogens (*S. aureus*, *L. monocytogenes*, *Camphylobacter jejuni*, *Salmonella enteritidis*, *E. coli*).

Hydrosols, also known as floral water, distillate water or aromatic water are steam

distillation of plant material. They practically were used as beverages for a long time. But, now they have been used alternative medicine (Sagdic and Ozcan, 2003; Trombetta et al., 2005; Bourgou et al., 2010). In a study conducted by Sađdic and Ozcan (2003), researchers investigated antibacterial activity of hydrosols against microorganisms including *E. coli* and they found that basil (*Ocimum basilicum*), laurel (*Laurus nobilis*), mint (*Mentha spicata*), rosemary (*Rosmarinus officinalis*), sage (*Salvia aucheri*) and sumach (*Rhus coriaria*) hydrosols were ineffective.

In previous literatures, many researchers focused on the therapy of herb extracts and determined LC<sub>50</sub> values of herb extracts in cytotoxicity effect (Promsiri et al., 2002; Mousa et al., 2008; Patil and Magdum, 2011; Rana and Rana, 2012). Sharififar et al. (2009) assessed cytotoxicity effects of four plant essential oils and various extracts. They obtained that *Heracleum persicum* and *C. zeylanicum* had the most cytotoxicity (LC<sub>50</sub> values showed 0.007 and 0.03 µg/ml, respectively). Cytotoxicity effects of *Euphorbia hirta* and *E. nerifolia* extracts were examined in other study. This experimentation showed that the LC<sub>50</sub> of ethyl acetate and acetone extract of *E. hirta* and methanolic extract *E. nerifolia* were found to be 71.15, 92.15 and 49.55 µg/ml respectively (Patil et al., 2011). Rana and Rana (2012) reported larvicidal activity of aromatic plant the essential oils against *Culex quinquefasciatus*. They suggested the use of these essential oils as a potentially alternative source for developing of *C. quinquefasciatus* larvae. There are a limited

number of researches on the therapy of spice hydrosols (distilled spice water). Only few studies have reported safety dose determination of spice hydrosol (Altinterim and Dorucu, 2007). In a separate study done by Altinterim and Dorucu (2007) was showed LC<sub>50</sub> value of *E. camaldulensis* hydrosol was 93 ml L<sup>-1</sup> (0.03-0.11). Similarly, Altinterim et al. (2012) investigated that LC<sub>50</sub> value of *Eucalyptus camaldulensis* hydrosol on carp and they reported that the 5h LC<sub>50</sub> value (95 % confidence limit) of the safe dose of *E. camaldulensis* hydrosol estimated to be 0.93 ml L<sup>-1</sup> (0.03-0.11).

We studied lethal concentration (LC<sub>50</sub>) values of cinamon hydrosol at different concentrations on carp. Our results confirmed that mortality was observed at all treatments exception of dose of 0.5% and regression analysis indicated that regression coefficient had close to one in each case. LC<sub>50</sub> values of *C. zeylanicum* hydrosol were 4.390 % for 1h, 2.629 % for 2 h, and 1.027 % for 12 h. Different of LC<sub>50</sub> determination studies on fish may be due to the concentration differences in the methods applied or different herbs used.

Based on the results of this study, death percentages and LC<sub>50</sub> values of the hydrosol on carp exposed *C. zeylanicum* hydrosol determined. Mortality was observed at all treatments exception of dose of 0.5 %. The results of this work indicated that the use of *C. zeylanicum* hydrosol in different concentrations occurred increases in oxygen values of aquarium water. However, fish deaths were higher due to the increase in the concentration. Fish

mortalities may be due to the hydrosol concentrations or other components of hydrosol. The data reported in this study shows that the 0.5 % concentration of *C. zeylanicum* hydrosol has better impact and it can potentially be used in aquaculture.

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