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Study of antibacterial effect of *Mentha longifolia* essential oil on *Lactococcus garvieae* in rainbow trout fillet at 4°C

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Abstract

The antibacterial effects of three concentrations of *Mentha longifolia* essential oil on the growth of *Lactococcus garvieae* were studied in fillets of rainbow trout at temperature of 4ºC for 18 days. Although no significant difference was found in bacterial count in different groups immediately post-inoculation, significant difference was observed in growth of *L. garvieae* between treatments with *M. longifolia* essential oil and control group during the experiment ($P<0.05$). The most antimicrobial effect was observed in the fillets containing 80 µg/g *M. longifolia* essential oil. The obtained results indicated that *M. longifolia* essential oil at the concentration of 80 µg/g is suitable to inhibit the growth of *L. garvieae* in rainbow trout fillet stored at 4°C.

Keywords: *Mentha longifolia*; *Lactococcus garvieae*; fish fillet

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Introduction

Keeping in view the increased requirements for fresh water fish, many species of fresh water fish are being farmed in Iran. Rainbow trout is one of the fresh water species being cultured in different parts of Iran to fulfil the fish requirements (Choobkar et al., 2010). Fish is enumerated as highly perishable food, which inflict the development of new preservation technologies to maintain the primary quality.

Use of plant derivatives such as essential oils (EOs) due to their antimicrobial activity of natural preservatives against pathogens and extension in the storage time of foods have been emphasized nowadays (Botsoglou et al., 2003; Chouliara and Kontominas, 2006). Essential oils have been used in preventing or delaying microbial activity and consequently the oxidative spoilage in fish (Burt, 2004; Choobkar et al., 2010).

Among different plant species, *Mentha longifolia* L. (common name: wild mint or horse mint) belongs to mint family Lamiaceae, is known with considerable antibacterial and antioxidant effects. This species is native to Western and Central Asia, Europe and Northern and Southern Africa (Akroum et al., 2009). Antimicrobial effect of this plant is due to pulegone which is found in the essential oils of the mint family (*Mentha* spp.) (Al-Bayati, 2009).

L. garvieae is known as the causative agent of lactococcosis in fish. Many fish species are susceptible to lactococcosis except carp. Among different fish species, rainbow trout is the most sensitive and lactococcosis is accompanied with high mortality and severe economic loss in this species. On the other hand, this pathogen is considered as a zoonotic agent (Vendrell et al., 2006). In recent years, several epizootic outbreaks of lactococcosis caused by *L. garvieae* have been reported in Iran (Raissy and Ansari, 2011; Soltani et al., 2008 & 2011).

The aim of this work was to study the inhibitory effects of *M. longifolia* essential oil (20, 40 and 80 µg/g) on *L. garvieae* in fish fillet stored at 4°C for 18 days.

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Materials and Methods

Sample preparation

Fresh cultured fish (350±15 g) were obtained from a local rainbow trout farm was transported within a short time to the Fishery Research Centre, IAU, Shahrekord. At Iranian Atomic Energy Organization, 12 fillet samples were made sterile by Gamma Ray and were kept in zipped sterile bags. The samples were then randomly divided into four treatments. Each treatment was in tri-replicates. *M. longifolia* essential oil was obtained from Barij Essence (Kashan, Iran) and used at concentrations of 0, 20, 40 and 80 µg/g.

L. garvieae was obtained from the Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Iran. Fish fillets were inoculated in 10 points with 10^3 cfu of the bacteria in each cm^3 of the sample (Barakat et al., 2006). The bags were then carefully zipped, labelled and placed in refrigerator with 4ºC.

Total viable count

Total viable count was determined following Radu et al. (2003). Serial dilutions were prepared after transferring of one gram of each fillet to buffer peptone water. Then 0.1 ml of the medium was spread on Nutrient Agar media. The colonies were counted by using colony counter and the total viable count was presented as $\log_{10}$ (cfu/g) of *L. garvieae*.

Statistical Analysis

The mean $\log_{10}$ (cfu/g) of *L. garvieae* in different days and different treatments were compared using Tukey Test. The differences were considered statistically significant in the case $P<0.05$.

Results

The mean values of *L. garvieae* under the effect of different concentration of *M. longifolia* are presented in Table 1. The results indicated that the growth of *L. garvieae* in fish fillets contained *M. longifolia* essential oil was statistically different compared to control group ($P<0.05$).

No statistical difference was observed between the bacterial growths in different groups on the first day of the experiment. The samples contained 80 µg/g of *M. longifolia* essential oil found to be the most effective against *L. garvieae* compared to other treatments ($P<0.05$) as the bacterial growth in the samples contained 80 µg/g essential oil was statistically lower than in the fillets contained lower concentrations of the essential oil on days 1 and 6 ($P<0.05$).

On day 12, although the microbial load was higher, antimicrobial effects of the treatments was the same with no statistical difference. On day 18, the effects of treatments contained 80 and 40 µg/g essential oil on the bacterial growth were statistically higher than the other treatment.

Discussion

There are many studies dealing with the use of chemical and natural preservatives for decreasing microbial growth and increasing storage time in fish fillets (Chouliara and Kontominas, 2004; Choobkar et al., 2010). Considering the side effects of chemical compounds, there is an increasing interest in plant essential oils or extracts which are safer (Burt, 2004). On the other hand, the inhibitory effects of many plants and their derivates have been proven in many studies (Al-Bayati, 2009). Garlic oil, allylisothiocyanate, carvacrol, cinnamaldehyde, citral, cuminnaldehyde, eugenol, isoeugenol, linalool and thymol have been effective against the population of the micro flora of common carp. Carvacrvl, thymol and cinnmaldehyde were found to be more effective against some of the bacteria (Barakat et al., 2004). In another study, the effects of 6 different concentrations of the essential oils of *Zataria multiflora* on *Staphylococcus aureus* in silver carp salted fillets were studied for 21 days. Their results showed significant difference between treatment contained 0.135 µg/g of *Z. multiflora* and control group on 2 and 6 days post-storage (Choobkar et al., 2010).

Antimicrobial effects of menthol which is the known compound of *M. longifolia* have been studied in a previous study. Antimicrobial activity of *M. longifolia* extracted menthol against seven pathogenic and non-pathogenic bacteria including *Staphylococcus aureus, Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenes, Lactobacillus acidophilus, Pseudomonas aeruginosa* and the yeast *Candida albicans* was evaluated using the disc diffusion method. Menthol (1:1, 1:5, 1:10, and 1:20) was found to be effective against the examined bacteria except *P. aeruginosa*, and it was mostly effective against *S. mutans* (Al-Bayati, 2009).

Niksic et al. (2012) studied the antimicrobial effects of *M. longifolia* on some bacteria including *Escherichia coli, Bacillus subtilis, Pseudomonas aeroginosa* and *Salmonella enterica.* Their results showed that essential oils of *M. longifolia* had inhibitory effect on the growth of the studied bacteria.

Antimicrobial activity of the methanolic and ethanolic extracts of *M. longifolia* against some clinical bacteria was examined by Akroum et al. (2009). The ethanolic extract was reported to be effective on *Staphylococcus aureus, Bacillus cereus* and *B. subtilis.*

In this study, *M. longifolia* was effective against *L. garvieae* in rainbow trout fillet. Three concentrations of the essential oil including 20, 40 and 80 µg/g were added to fillets. The results of this study revealed that

Table 1: Log_{10} (cfu/g) of *L. garvieae* in fish fillets with different concentrations of *M. longifolia*

Essential oil	Day				
Concentration (µg/g)	0	1	6	12	18
0 (control)	3.71±0.07[a]	5.25±0.01[a]	7.46±0.00[a]	10.16±0.19[a]	11.47±0.00[a]
20	3.58±0.15[a]	4.47±0.08[b]	6.36±0.02[b]	8.12±0.05[b]	10.06±0.02[b]
40	3.20±0.07[a]	4.04±0.05[c]	5.98±0.02[c]	8.29±0.01[b]	9.33±0.04[c]
80	3.44±0.04[a]	3.56±0.05[d]	5.48±0.01[d]	7.67±0.10[b]	9.11±0.01[c]

Different letters in a row differ significantly (P≤0.05)

the growth of *Lactococcus* in fillets contained *M. longifolia* essential oil was statistically higher than control group (P<0.05). According to the results, the samples treated with concentration of 80 µg/g of *M. longifolia* essential oil significantly decreased the growth of the bacteria compared with control samples during the experiment. These findings are in agreement with the results of Akroum et al. (2009) and Al-Bayati (2009).

It should be considered that the inhibitory effects of different essential oils increase by decreasing the storage temperature (Burt, 2004). The comprehensive inhibitory effect of *Z. multiflora* essential oils on bacterial growth at 8ºC was better than 25ºC (Moosavy et al., 2008).

In addition, the results of other researchers signify that the antimicrobial effects of essential oils may differ *in vitro* and *in vivo* as the essential oil may be affected by protein or fat contents of meat resulting in lower inhibitory effects of the essential oil (Tassou et al., 2000; Gutierrez et al., 2008). Regardless of the food nature, pH of food is also an important factor affecting the activity of essential oils (Tassou et al., 2000; Burt, 2004). This can explain the differences in antimicrobial activity of essential oils in different food with different conditions.

Conclusion

It is concluded that *M. longifolia* can inhibit growth of *L. garvieae* in fish fillet especially at the concentration of 80 µg/g. However, further studies are needed to evaluate flavour, odour or possible chemical alternations in fish fillets.

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