Comparative Analysis of Antifungal Properties of Zataria multiflora Boiss, Eucalyptus spp Essence and Malachite Green on Eggs of Kutum (Rutilus frisii Kutum)

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Abstract: The objective of this study was to assay the effectiveness of Malachite green, Zataria multiflora and Eucalyptus Spp essence on the fungal CFU (in eggs and water) and hatching success of Rutilus frisii kutum eggs. Eggs were artificially fertilized and transferred to a static bath treatment in different concentrations of Eucalyptus (50 and 100 mg/l), Z. multiflora (100 and 150 mg/l) essence and Malachite green, for 1 h daily, during 3 days. Treatment efficacy was investigated by comparing the percentage of hatched eggs in the treatment group to the untreated control group 1–3 days after fertilization. All treatments demonstrated decreasing fungal CFU and lowest CFU was recorded in eggs treated with Eucalyptus essence (100 mg/l) (p<0.05). But there were no remarkable effect of different antifungal on water. Eggs treated with Eucalyptus essence at 100 mg/l recorded greater mean percentage of hatching compared to the control (p<0.05). In conclusion Eucalyptus Spp was more effective than other antifungal for the treatment of fungus-infected kutum eggs.

Key words: Egg • Eucalyptus Spp • Zataria Multiflora • Malachite Green • Rutilus Frisii Kutum

INTRODUCTION

Recently, more efficient drugs, derived from herbal resources, against microbial infections have been recommended by investigators [1]. Use of chemical substances such as malachite green, formalin and sodium chloride to control diseases and aquatic pests has been banned worldwide because of high residue levels in the animals [2, 3]. Unlike chemical materials, plant immunostimulants have no residuals in the environment and more attention has been payed to these plants for their immune stimulating functions in fish culture [4-10]. Water mold infections cause casualties of fish and their eggs in both nature and commercial fish farms [11]. Hence, use of plant extracts can play a role in supporting both performance and health status of the animals [12–14]. Unfertilized eggs are susceptible to fungal infection during incubation; mycelium of fungi soon spreads to nearby, healthy eggs causing mortality [15]. There are many reports from all over the world that chemical drugs have been replaced by herbal medicine in aquaculture [6, 16]. Also, numerous studies have demonstrated stimulating role of plant extracts in fish immune system challenged with bacterial and fungal agent's [16-20].

Since, plant extracts are considered as a prominent antimicrobial agent, eucalyptus and Zataria multiflora with medical properties have been used in the treatment of infectious diseases [21-23]. Zataria multiflora with the common Persian name “Avishan-e Shirazi” belongs to the family of Labiatae which is a native plant of Iran, Pakistan and Afghanistan. Furthermore, it is one of the plants with characterized antimicrobial properties [22]. Moreover, there are reports indicating that Z. multiflora has immunostimulatory effects in common carp [24]. In this regard, Z. multiflora could be safely used to protect the fish as an antimicrobial agent [25]. Eucalyptus (Family: Myrtaceae) all around the world have more than 700 species, among them at least 500 species have essence [28]. Essence of some species of
Eucalyptus is used in the treatment of some diseases [29]. Several studies have been documented the essence of some eucalyptus species as an agent with antimicrobial and remedial activities [1, 30-34]. Kutum, *Rutilus frisii kutum* belonged to Cyprinidae family with an ecological and economic importance [35]. Kutum is in great demand by virtue of its high taste quality and the cuisine customs of the local residents. Available evidence showed that natural reproduction of this species in recent years declined markedly due to overfishing, increasing pollution, river body degradation, migration and lack of security area [35-38]. So, the Iranian fisheries organization release up to 200 million fry (average weight, 1g) into the Caspian Sea annually to restock this valuable species. In spite of the positive effects of medicinal plants in controlling some microbial diseases [28, 39-44], there is a lack of data about their antibacterial effects. Hence, the aim of the present study was to evaluate the effect of two important and traditional medicinal plants (eucalyptus and *Z. multiflora*) on fungal agents in Kutum (*Rutilus frisii kutum*) egg.

MATERIALS AND METHODS

This research was carried out in the reproductive season of kutum in Shahid Ansari Fish Propagation and Rearing Center, Guilan, Iran from March until May 2011. In this study *Eucalyptus Spp* and *Zataria multiflora* extracts were purchased from Zardband Pharmaceutical Company, Tehran, Iran. For this purpose 60 mature females (average body weight: 960±142.5 g and length: 44.4±3.9 cm) were caught in the Sefidroud River estuary by seine nets. The eggs were collected and poured into plastic jars with pressure on the abdominal. Then stripped eggs were mixed with sperm that obtained from three males. Following fertilization, the eggs were stirred for 1 h and then eggs rinsed with hatchery water and placed into the incubator. Hatching occurred between 1 – 3 days after fertilization. The mean water temperature was maintained within 10±2°C, dissolved oxygen concentration and pH were calculated approximately 7 ppm and 7.8-8.2 during the experiment, respectively. The egg batches were randomly assigned in triplicate to static bath treatments of given concentrations of either *Eucalyptus Spp* essence (50 and 100 mg/l), *Zataria multiflora* essence (100 and 150 mg/l), Malachite green and a control (0 ppm) for 1 h daily during 3 days exposure before being transferred to randomized compartments of the incubation tank. The fungal infection of egg was estimated as follows: A total of 150 infected eggs were sampled and placed in sterile glass bottle with closed plug. Afterwards, the eggs were washed 3-5 times with distilled water and then the obtained suspension composed of water and fungus was diluted ten-fold with distilled water in sterile glass pipes. After that, 0.5 mL of diluted suspension was incubated in culture mediums i.e. SabroDextrose Agar (SDA) and corn meal containing Chloramphenicol and Gentamicin. To calculate the fungal colony numbers, the inoculated plates were incubated for a period of 48-72 h and 3-5 days at 25°C, respectively. After this period, the colonies were counted and the CFU (Colony-Forming Unit) values were calculated on the basis of the mL of water and egg. For CFU calculation, the arithmetical average of two fungal counting was multiplied at the dilution rate. All data were subjected to a one-way analysis of variance (ANOVA) after confirmation of normality and homogeneity of variance. Significance of the differences between means was tested using Duncan’s multiple range test (*P*<0.05). All assays were performed in triplicates and data were shown as mean ± SE for each treatment.

RESULTS AND DISCUSSION

Fungal CFU in eggs and water for all treatments were shown in Figs. 1 and 2, respectively. Significant difference was observed between control eggs and treatment groups in terms of CFU (*p*<0.05). Significant decrease of fungal CFU for *Eucalyptus Spp* was measured in eggs treated with 100 mg/l (Fig. 1) and the highest fungal CFU was significantly observed in control group (*p*<0.05).

The obtained results showed that the fungal growth in water treated with different concentrations of *Z. multiflora*, *Eucalyptus Spp* essence and Malachite green had not significant difference compared with control group (*p*>0.05) (Fig. 2).

![Fig. 1: Fungal CFU (ml⁻¹) of Kutum eggs after exposure to different treatments. Data are expressed as mean ± SE. Mean values bearing same superscript are not statistically significant (*p*>0.05).](image-url)
Fig. 2: Fungal CFU (ml⁻¹) of incubation water of Kutum after exposure to different treatments. Data are expressed as mean ± SE. Mean values bearing same superscript are not statistically significant (p<0.05).

Fig. 3: Infected eggs by fungi (abundance) in Kutum after exposure to different treatments. Data are expressed as mean ± SE. Mean values bearing same superscript are not statistically significant (p>0.05).

Fig. 4: Hatching rate in different treatments (%) in Kutum after exposure to different treatments. Data are expressed as mean ± SE. Mean values bearing same superscript are not statistically significant (p>0.05).

In line with these results a study done by others [45], reported that eucalyptus essence the most effective against some fungi such as Aspergillus flavus. Similar results were also obtained by Mousavi et al., [15], they demonstrated the inhibitory effects of Eucalyptus essence in control of fungal growth and increasing the hatching rate of rainbow trout (Oncorhynchus mykiss) eggs. Data analysis revealed no significant difference between control group and treatment (p>0.05) in terms of infected eggs by fungi (abundance) (Fig. 3). In this experiment, there was a significant effect on hatching rate based on Eucalyptus treatment (p<0.05) (Fig. 4).

Several studies have investigated using natural products such as Z. multiflora and Eucalyptus Spp as fungicidal agents on fish eggs [46-49] and they have proved antifungal activity against different fungal agents such as Aspergillus and Fusarium species [50, 51]. In interaction effect, the highest hatching rate was significantly observed in group treated with 100 mg/l of Eucalyptus. Also, Ahmadi et al. [52] reported that Eucalyptus essence in dose of 200 ppm is effective in control of Saprolegniasis and resulted in higher hatching rate in Persian sturgeon, Acipenser Persicus. The control of eggs infected with Saprolegnia by dosage of 100 ppm Eucalyptus essence has also been recorded for Kutum by Najafi et al. [53]. Similar results were reported in other literature, Macchioni et al. [54] and Rai et al. [47] found that extract of some plants such as Artemisia verlotorum, Antolina etrusca, Guizotia abyssynica and Tagetes erecta could be used as antifungal agents. Use of chemical materials such as malachite green has routinely been used in Iran for controlling of eggs infection against fungi for several years [52]. According to literature, malachite green has proved to be an excellent antifungal agent in fish eggs at given concentrations [56]. On the other hand, from the results of some studies, different dosages of malachite green decreased hatching rates of Chinese sucker (Myxocyprinus asiaticus) eggs [57] and in another study by Marking et al. [58], presented delayed hatching of rainbow trout eggs. In surveying of disinfection material, the lowest percentage of hatch was recorded in malachite green treatment (Fig. 3). In spite of antifungal effects of malachite green, it causes cancer and environment pollution [52]. Meanwhile, higher concentrations of malachite green are toxic to the eggs [59] and damage egg membrane and chorion [60]. In this regard, Ahmadi et al. [52] reported that the highest unhatched eggs treated with malachite Green, as we showed in the present experiment.

As a conclusion, essence of plants has greater antimicrobial activity than chemical components and several experiments have shown that chemical drugs in aquaculture system have been replaced by herbal
antimicrobials. The present study confirmed antifungal properties of Eucalyptus essence that showed significant growth inhibition for fungal agents and the highest hatching rate in treated eggs.

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REFERENCES


