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RESEARCH ARTICLE

Investigation of the use of hops (*Humulus lupulus*) and calendula (*Calendula officinalis*) as disinfectants on the hatching efficiency of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

This study investigates the potential application of plant-based resources with antimicrobial properties, namely hops (*Humulus lupulus*) and calendula (*Calendula officinalis*), as viable alternatives to commercial disinfectants for the prevention of fungal growth during the incubation period of rainbow trout (*Oncorhynchus mykiss*) eggs. Fertilized eggs were examined with daily 20-minute baths in 0.25 mg, 0.5 mg, and 1 mg concentrations throughout the incubation period (33-35 days). Control group, commercial formaldehyde, hops, and calendula groups were examined in triplicate for each concentration. As a result, the lowest hatching ratio was observed in the control group with 84.77%, while the hatching ratios in the other groups were as follows: commercial disinfectant group with 86.22%, hops group with 87.44% (0.25 mg), calendula group 88% with (0.25 mg), calendula group with 89% (0.5 mg), calendula group with 89.33% (1 mg), hops group with 89.55% (0.5 mg), and the highest hatching ratio was found in the hops group with 90.66% (1 mg). The lowest survival rate was observed in the control group with 85.22%, with the survival rates of the other groups were 86.44% in the commercial disinfectant group, 87.55% (0.25 mg) in the hops group, 88.55% (0.25 mg) in the calendula group, 88.88% (0.5 mg) in the calendula group, 89.33% (1 mg) in the calendula group, 89.55% (0.5 mg) in the hops group, and the highest survival rate was 90.11% (1 mg) in the hops group. In conclusion, it was specified that plant-based by-products (hops and calendula) could be used as alternative disinfectants to commercial chemicals in the disinfection of rainbow trout eggs and fry, and the most appropriate concentration was 1 mg.

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Introduction

In recent years, trout farming has a magnificent increase in interest, becoming one of the top ventures in aquaculture globally with an accompanying rise in demand for trout fry, which in turn made the production of healthy eggs and fry crucial to enhancing both productivity and quality in trout aquaculture. During the hatching, fish eggs are placed rather densely on incubation racks, creating a high risk of fungal contamination, which can rapidly spread from a single infected egg to other healthy ones. Such a contamination often results in the death of a substantial portion of healthy eggs, and in some severe cases, may lead to the loss of whole batches. This stage of the hatching is when the most significant losses in egg and pry production occur. While a loss rate of 10-20% is during incubation is tolerable, 20-30% is unacceptable (Çelikkale, 1988).

Controlled environmental conditions are required to minimize mortality during the incubation period, and different doses of various disinfectants are used to prevent mortality during the incubation period and larval stages, especially to prevent losses due to fungal and other diseases. The disinfectant substances used are selected because they are abundant, economical and effective. For this purpose, chemicals such as malachite green, formalin, iodine, iodophor, methylene blue, etc. are used in hatcheries (Yanik et al., 2009).

Hatchers can implement various preventive measures to minimize the loss rate during the incubation phase in trout farming. These measures primarily focus on preventing fungal infections during the mentioned period and the subsequent larval phase, all of which include the application of a variety of treatments and chemical disinfectants at different concentrations to some extent. In the context of trout farming, some of the common chemical disinfectants that help protect the eggs during the incubation period and the subsequent fries are malachite green, formalin, iodine, iodophor, methylene blue, bronopol, wescodyne, buffodine, sulformerthiolate, merthiolate, acriflavine, gentian violet, sodium and calcium hypochlorite, chloramine, potassium permanganate, copper sulfate, and salt etc. (Çelikkale, 1988; Barnes et al., 1997; Emre & Kürüm, 1998; Hekimoğlu, 2001; Arda et al., 2002; Timur & Timur, 2003; Alpbaz, 2005; Çağıltay, 2007; Güner et al., 2007; Yılmaz, 2010; Ural et al., 2011a).

However, some reports indicate that some of the chemical substances used to disinfect eggs as a preventive measure in incubation systems may have harmful effects on healthy eggs as well (Alderman, 1984). For instance, due to its harmful effects and potential risks to human health, many countries have banned the use of malachite green (Emre & Kürüm, 1998; Timur & Timur, 2003; Güner et al., 2007; Yılmaz, 2010). Furthermore, the environmental impact and safety of these chemical substances continue to be subjects of debate. Thus, the research on and the promotion of the natural disinfectants with no harmful effects on the environment, fish, or human health is vital, with researchers worldwide continuing to explore alternative medical plants for use in aquaculture (Cline & Post,1972; Lio-Po et al., 1982; Bayley, 1984).

Hops (*H. lupulus L.*), belonging to the Cannabaceae family, is a dioecious perennial climber native to temperate climates in the Northern Hemisphere. Although it is primarily known as one of four ingredients required to make beer, Hops was initially used as medicinal herb (Bocquet et al., 2018). Nearly all parts of the plant are rich in bioactive compounds. The strong antimicrobial, antioxidant, and antifungal properties of its bitter acids and flavonoids, combined with the growing interest in natural health-promoting substances, offer new and intriguing perspectives on the use of hops beyond brewing. With its recognition as a medicinal plant since ancient times, humanity's cultivation of Hops requires a revisit to with an emphasis on its health-enhancing attributes (Lukešová et al., 2019).

Calendula *(C. officinalis)* is a plant native to the Mediterranean region, with centuries worth of history in ancient medicine, and is commonly referred to as 'aynısafa' in Turkish (Ramos et al., 1998). The English herbalists Gerard and Culpeper were first to discover the medicinal properties of the plant in the 16th and 17th centuries. Another English herbalist Grieves, in the early 20th century, recommended the use of Calendula flowers, nothing that applying the ligulate flowers directly to insect bites could reduce pain and swelling (Della Loggia et al., 1994). In light of these properties, our study investigates the effects of hops and Calendula, which have the potential to serve as natural antimicrobial agents, in preventing fungal growth on trout eggs and their impact on hatching efficiency (Alderman, 1984; Emre & Kürüm, 1998; Timur & Timur, 2003; Güner et al., 2007; Yılmaz, 2010).

Material and Methods

This study was carried out at the Freshwater Fish and the Inland Fish Research and Application Centers at Atatürk University between December 2022 and February 2023, using 2 female and 4 male broodfish, each 3 years old (Table 1). In this research, female and male broodstock fish were stripped according to the standard dry stripping method and the eggs were fertilized under optimal conditions using male sperm. Afterwards, the washed, intact, and fertilized eggs were placed in pre-sterilized incubation baskets according to the volumetric method, each carrying 100 eggs. These included: 24 fiberglass baskets (15×10 cm) placed in 8 all-fiberglass incubation trays (50×40 cm), in 8 aquariums with a capacity of 240 liters each, at the end of which rainbow trout eggs were treated with hops and calendula extract.

In the study, hops and calendula collected naturally and stored under airtight conditions were provided from a herbalist named İpekyolu (Muratpaşa, Haşil Efendi, No: 32, 25100 Yakutiye/Erzurum). Plants were extracted using ethanol as solvent (plant-ethanol ratio, 1:20) and the solutions were filtered using a vacuum pump. A rotary evaporator set at 65°C concentrated the filtered solutions to obtain the extracts. From the beginning of incubation until hatching, eggs were given daily hop and marigold extracts in single doses of 0.25 mg, 0.50 mg and 1.00 mg after the water flow in each aquarium was stopped for 20 min and then the water flow was restarted immediately. Dead and/or bleached eggs were removed with sterilized siphons. In addition, eggs were monitored daily throughout the study. No dose of extracts obtained from yolk sacs was given to the subjects until the larval stage and monitoring continued until the larvae reached 1 gram in weight. Before the completion of the study, the fish subject to the study were stored frozen at -80°C in the Atatürk University Inland Fish Research and Application Center.

$$\text{Incubation Efficiency (\%)} = \frac{\text{Number of live offspring}}{\text{Total number of eggs}} \times 100 \qquad (1)$$

$$\text{Survival Rate (\%)} = \frac{\text{Number of fish at the end of the trial}}{\text{Number of fish at the beginning of the trial}} \times 100 \qquad (2)$$

Water quality parameters (including water temperature, dissolved oxygen, and pH) were measured during study period.

Data Analysis

In this study, the significance of the difference data of all groups determined as hatching efficiency (%) and survival rate (%) of different concentrations of hops and calendula in egg, eyed egg, and larval stages was determined using One Way ANOVA and DUNCAN tests.

Results

The eggs turned into eyed eggs between days 16 and 19, and the larvae hatched between days 33 and 35. In the two female and four male broodstock used in the study, the average weight and length of the females reached 0.790 kg and 37 cm respectively, and the average egg yield was 1.576 per kg, while the average weight and length of the males were 0.735 kg and 39 cm, respectively (Table 1).

Table 1. The brood stock in the study (Mean±SD)

Gender	Value	Weight (kg)	Length (cm)	Egg yield/kg
FEMALE	N	2	2	2
	Min - Max.	0.755 – 0.825	33-41	1479 – 1673
	Mean±SD	0.790±0.3	37±1.8	1576±217
MALE	N	4	4	
	Min-Max.	0.674 – 0.796	36 - 42	
	Mean±SD	0.735±0.2	39±1.7	

Table 2. Change in hatchability rate depending on groups (Mean±SD, %)

Groups	Hatching efficiency of the groups (%)
Hops 0.25 mg	87.33±2.08 ^{C*}
Hops 0.5 mg	89.00±1.00 ^{B}
Hops 1 mg	90.33±0.58 ^{A}
Calendula 0.25 mg	87.67±0.58 ^{C}
Calendula 0.5 mg	88.67±0.58 ^{B}
Calendula 1 mg	89.00±1.00 ^{B}
Commercial Disinfectant	86.33±0.58 ^{D}
Control	84.67±0.58 ^{D}

Note: $^{*\ A,\ B}$: Uppercase letters denote significant differences in hatchability rates between groups. Groups within the same row that are marked by different uppercase letters exhibit statistically significant differences (p<0.05).

The results indicated significant differences among the groups (p<0.05), with the control group having the lowest hatching efficiency at 84.67%, while the hops group had the highest efficiency at 90.33% (1 mg). The hatching efficiency of the remaining groups was as follows: commercial disinfectant group 86.33%, the hops group 87.33% (0.25 mg), the calendula group 87.67% (0.25 mg), the calendula group 88.67% (0.5 mg), the calendula group 89% (1 mg), the hops group 89% (0.5 mg) (Figure 1 and Table 2).

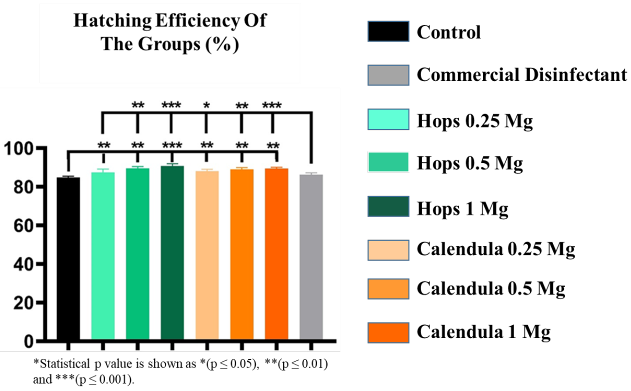

Figure 1. Hatching efficiency of the groups (%)

The analysis of survival rate (%) revealed significant differences between the groups (p<0.05), with the control group having the lowest survival rate of 85.22%, while the hops group had the highest survival rate. The survival rates of the other groups were as follows: commercial disinfectant group 86.44%, the hops group 87.55% (0.25 mg), the calendula group 88.55% (0.25 mg), the calendula group 88.88% (0.5 mg), the calendula group 89.33% (1 mg), the hops group 89.55% (0.5 mg) (Figure 2 and Table 3).

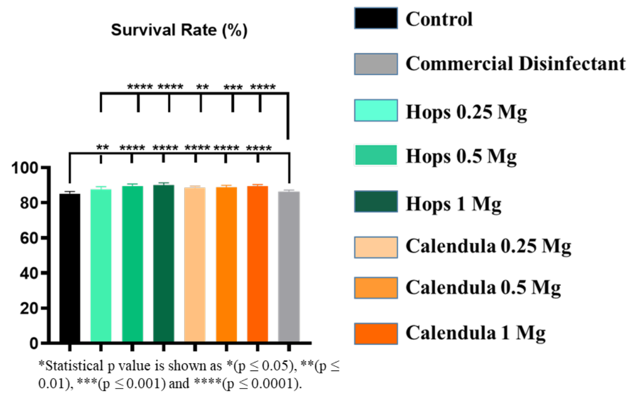

Figure 2. Survival rate (%)

The average water temperature, pH, and dissolved oxygen (DO) values measured during the incubation period are given in Table 2. The changes between these parameters measured daily were found to be statistically insignificant (p>0.05), and the means values of water temperature, pH, and dissolved oxygen (DO) were measured 10, 8.65 and 7.1, respectively.

Table 3. Change in survival rate depending on groups (Mean±SD, %)

Groups	Survival rate (%)
Hops 0.25 mg	87.33±2.08 ^{C*}
Hops 0.5 mg	89.00±1.00 ^{B}
Hops 1 mg	90.33±0.57 ^{A}
Calendula 0.25 mg	87.67±0.57 ^{C}
Calendula 0.5 mg	88.67±0.57 ^{B}
Calendula 1 mg	89.00±1.00 ^{B}
Commercial Disinfectant	86.33±0.57 ^{D}
Control	84.67±0.57 ^{D}

Note: $^{*\ A,\ B}$: Uppercase letters denote significant differences in hatchability rates between groups. Groups within the same row that are marked by different uppercase letters exhibit statistically significant differences (p<0.05).

Table 4. Water quality parameters in the hatching aquariums (Mean±SD)

Parameter	Control Group	Commercial Disinfectant Group	Hops Group	Calendula Group
Water Temperature (°C)	9.8±0.2	9.9±0.2	10.1±0.1	10.3±0.3
pH	8.74±0.4	8.73±0.4	8.65±0.2	8.63±0.2
DO (mg/L)	7.0±0.3	7.61±0.8	7.08±0.3	6.70±0.5

Discussion

Hatchers use a wide variety of chemical agents during the incubation phase of rainbow trout for the disinfection of eggs and fry. However, there are significant variance in the methods of applications and concentrations of these chemical substances, with persisting debates on their safety on water and living organisms. Therefore, the importance of the use of entirely natural disinfectants and preventive measures that do not cause any harmful effects – either respiratory or acute and chronic – on the environment and other living beings has become increasingly recognized (Can et al., 2012).

Many studies indicate the destructive effects of plant extracts, their main components and essential oils on fish eggs or fish egg-preserved *Saprolegnia parasitica* fungi (in vitro) (Khallil, 2001; Mori et al., 2002; Udomkusonsri et al., 2007; Ghasemi Pirbalouti et al., 2009; Khosravi et al., 2012; Xue-Gang et al., 2013).

In this study, the extracts of the two plants mentioned were extracted and applied to the eggs of rainbow trout immediately after fertilization. The concepts of time in the embryonic stages were examined on the trout eggs. As a result; the embryological development, fertilization and survival rates of the experimental groups after short-term preservation were examined. When the obtained data were examined, it was reported that no difference was observed in the fertilization rate after storage for up to 6 hours compared to the control group, that lemon balm (*Melissa officinalis*) showed a protective effect without losing the ability of the eggs to be fertilized after short-term storage, and that it reduced losses due to fungus by 10%, and that coriander (*Coriandrum sativum*) showed a mortal effect at the applied concentration and should be used at a lower concentration (Özdemir, 2018).

In the study, the number of eggs that died due to infection in the control group was determined as 635.00±54.049. The number of these dead eggs was 325.00±31.943, 393.67±3.283 and 420.00±80.829 in the groups which garlic peel extract was applied at concentrations of 0.4, 0.8 and 1.6 g/L, respectively. Similarly, the number of eggs that died in the 0.8 and 1.6 g/L garlic stalk groups were reported as 370.67±24.333 and 166.00±28.478, respectively. The findings suggest that garlic peel extract used at concentrations of 0.4, 0.8 and 1.6 g/L reduced the number of eggs that died due to fungus compared to the control group ($p<0.05$). It was also reported that although there was a slight increase in the number of dead eggs in the 3.2 g/L group compared to the control group, this increase was not statistically significant ($p>0.05$) (Özçelik, 2019).

In research examining the effects of vinegar disinfection following the fertilization of trout eggs through milking, it was reported that the application of a 12 mL/L vinegar solution produced favorable results (Ural et al., 2011b). The study employed various concentrations of vinegar and was conducted in a controlled hatchery environment where water temperature was kept at 8.4±0.2°C in fiberglass tank systems. The pH of the water in the control group was measured at 7.6±0.4, while the pH in the group treated with 12 mL/L vinegar decreased to 4.0±0.1. As a result of these data, the mortality rate in the control group was recorded at 20.2%, whereas the group exposed to vinegar treatment exhibited a significantly lower mortality rate of 12.1%.

In this study, 35 mL/L of vinegar stock solution was applied, resulting in a pH of 5.9 in the incubation water. The expert posited that the differences in mortality rates observed in both studies could be attributed to the variations in pH levels and water temperatures, indicating a potential correlation between these factors and the effectiveness of vinegar as a disinfectant in egg incubation.

Recent works in the field show that there are very few studies focusing on the use of completely natural substances in combating fungal infections in trout eggs. The studies, or lack thereof, inspired the study on the potential use of hops and calendula, both medicinal plants, as disinfectants. The use of hops dates back to ancient times and it is a well-known preservative. In addition to its antimicrobial properties, it has widespread industrial uses because almost all parts of the plant are rich in bioactive compounds. The strong antimicrobial, antioxidant, and antifungal properties of its bitter acids and flavonoids, combined with the growing interest in natural health-promoting substances, offer new and intriguing perspectives on the use of hops beyond brewing. The cultivation of hops by humanity, which has been considered a medicinal plant since ancient times, should be reconsidered by emphasizing its positive effects on health (Lukešová et al., 2019).

In light of these properties, our study investigated the effects of hops and calendula, which have the potential to be natural antimicrobial agents, on preventing fungal growth in trout eggs and their impacts on hatching efficiency (Alderman, 1984; Emre & Kürüm, 1998; Timur & Timur, 2003; Güner et al., 2007; Yılmaz, 2010).

During the incubation stages of trout eggs, it is crucial to use entirely natural products, which have no adverse effects, as alternatives to chemical disinfectants. The works in the field so are insufficient to explore the potential of the availability of plant-based medicinal disinfectants, and warrants further research. In this context, the study investigated the feasibility of using hops and calendula, known for their antifungal disinfectant properties, on trout eggs.

Conclusion

This study involved the application of hops and calendula to trout eggs in doses of 0.25 mg, 0.50 mg, and 1.00 mg/L. The results showed that the group with the 1.00 mg concentration of hops had the best hatching efficiency (%), while the control group had the lowest hatching efficiency. Similarly, in terms of survival rate, the same group with the 1.00 mg concentration of hops had the highest survival rate, with the control group having the lowest, thus revealing significant statistical differences between the groups ($p<0.05$). This research showed that increasing the concentration values gave positive results in hatching efficiency and survival rate. Therefore, it is recommended to determine the best rate by trying concentrations above 1 mg on future researches.

The water temperature in the incubation system during the study was at approximately 9.9°C. Maintaining the optimal levels for water temperature and other parameters is crucial during the incubation phase, since water quality, particularly the water temperature, are variables that can result in fungal growth in eggs. Meanwhile, the groups in the study had no significant fungal growth during the study. The findings suggest that the results may be applicable to facilities with similar water temperatures and quality. Higher or lower concentrations of hops and calendula can neutralize fungal growth in eggs that might occur due to changes in water temperature. Given that hops and calendula are entirely natural, readily available, this study recommends these medicinal plants for the disinfection of trout eggs.

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Compliance With Ethical Standards

Authors' Contributions

MUN: Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing

TY: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Alderman, D. J. (1984). The toxicity of iodophors to salmonid eggs. *Aquaculture*, *40*(1), 7-16. https://doi.org/10.1016/0044-8486(84)90211-4

Alpbaz, A. (2005). *Su Ürünleri Yetiştiriciliği.* Alp Yayınları.

Arda, M., Seçer, S., & Sarıeyyüpoğlu, M. (2002). *Balık Hastalıkları.* Medisan Yayın Serisi: 56.

Barnes, M. E., Corde, R. J., & Sayler, W. A. (1997). Use of formol solution during incubation of eyed eggs of inland fall chinook salmon. *The Progressive Fish-Culturist, 59*(4), 303-306. https://doi.org/10.1577/1548-8640(1997)059%3C0303:UOFDIO%3E2.3.CO;2

Bayley, A. C. (1984). Aggressive Kaposi's sarcoma in Zambia, 1983. *The Lancet*, *323*(8390), 1318-1320. https://doi.org/10.1016/S0140-6736(84)91818-X

Bocquet, L., Rivière, C., Dermont, C., Samaillie, J., Hilbert, J. L., Halama, P., Siah, A., & Sahpaz, S. (2018). Antifungal activity of hop extracts and compounds against the wheat pathogen *Zymoseptoria tritici. Industrial Crops and Products,* 122, 290–297. https://doi.org/10.1016/j.indcrop.2018.05.061

Çağıltay, F. (2007). *İçsu Balıkları Yetiştiriciliği.* Nobel Yayın No: 58.

Can, E., Karacalar, U., Saka, Ş., & Fırat, K. (2012). Ozone disinfection of eggs from gilthead seabream *Sparus aurata*, sea bass *Dicentrarchus labrax*, red porgy, and common dentex *Dentex dentex*. *Journal of Aquatic Animal Health,* *24*(2), 129-133. https://doi.org/10.1080/08997659.2012.675925

Çelikkale, M. S. (1994). *İç Su Balıkları ve Yetiştiriciliği.* KTÜ Sürmene Deniz Bilimleri Fak. Genel Yayın, (124), 37.

Cline, T. F., & Post, G. (1972). Therapy for trout eggs infected with *Saprogenia*. *Progressive Fish-Culturist, 34*(3), 148-151. https://doi.org/10.1577/1548-8640(1972)34[148:TFTEIW]2.0.CO;2

Della Loggia, R., Tubaro, A., Sosa, S., Becker, H., Saar, S., & Isaac, D. (1994). The role of triperpenoids in the topical anti-inflammatory activity of *Calendula officinalis* flowers. *Planta Medica, 60*(6), 516-520. https://doi.org/10.1055/s-2006-959562

Emre, Y., & Kürüm, V. (1998). *Alabalık Yetiştiriciliği.* Minpa Matbaacılık.

Ghasemi Pirbalouti, A., Taheri, M., Raiesi, M., Bahrami, H., & Abdizadeh, R. (2009). In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. *Journal of Food, Agriculture & Environment, 7*(2), 94–96.

Güner, Y., Güzel, Ş., Kayım, M., Serezli, R., Öksüz, A., Bircan, R., Atamanalp, M., Tokşen, E., & Kocabaş, M. (2007). *Balık Üreticisi El Kitabı.* Y.Y.Ü. Ziraat Fakültesi, Proje Koordinasyon Merkezi Yayını, SAS AJANS.

Hekimoğlu, M. A. (2001). *Benekli deniz alası (Cynoscion nebulosus Cuvier 1830) yumurtalarda mantar görülmesine karşı formol çözeltisi ve iyot çözeltisi kullanılmasının etkilerinin araştırılması* [Effects of different formalin and iodine concentration usage for control of fungus on spotted seatrout egg]. *Ege Üniversitesi Su Ürünleri Dergisi, 18*(3-4), 503-507.

Khallil, M. A. (2001). Phytofungitoxic properties in the aqueous extracts of some plants. *Pakistan Journal of Biological Sciences, 4*, 392–394.

Khosravi, A. R., Shokri, H., Sharifrohani, M., Mousavi, H. E., & Moosavi, Z. (2012). Evaluation of the antifungal activity of *Zataria multiflora*, *Geranium herbarium*, and *Eucalyptus camaldulensis* essential oils on *Saprolegnia parasitica*-infected rainbow trout (*Oncorhynchus mykiss*) eggs. *Foodborne Pathogens and Disease, 9*(7), 674–679.

Lio-Po, G. D., Sanvictores, M. E. G., Baticados, M. C. L., & Lavilla, C. R. (1982). In vitro effect of fungicides on hyphal growth and sporogenesis of *Lagenidium* spp. isolated from *Penaeus monodon* larvae and *Scylla serrata* eggs. *Journal of Fish Diseases, 5*(2), 97-112. https://doi.org/10.1111/j.1365-2761.1982.tb00463.x

Lukešová, H., Andersen, H. L., Kolínová, M., & Holst, B. (2019). Is it hop? Identifying hop fibres in a European historical context. *Archaeometry, 61*(2), 494–505. https://doi.org/10.1111/arcm.12437

Mori, T., Hirose, H., Hanjavanit, C., & Hatai, K. (2002). Antifungal activities of plant extracts against some aquatic fungi. *Biocontrol Science, 7*(3), 187–191. https://doi.org/10.4265/bio.7.187

Özçelik, H., Taştan, Y., Terzi, E., & Sönmez, A. Y. (2020). Use of onion (*Allium cepa*) and garlic (*Allium sativum*) wastes for the prevention of fungal disease (*Saprolegnia parasitica*) on eggs of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases, 43*(10), 1325-1330. https://doi.org/10.1111/jfd.13229

Özdemir, R. C. (2018). *Oğul otu (Melissa officinalis) ve kişniş (Coriandrum sativum) bitki özütleri kullanılan gökkuşağı alabalığı (Oncorhynchus mykiss) yumurtalarının kısa süreli muhafazası sonrası embriyolojik gelişimin izlenmesi ve yaşama oranlarının belirlenmesi* [Monitoring embryological development and determination of survival ratios after short-term storage of rainbow trout (*Oncorhynchus mykiss*) eggs using lemon balm (*Melissa officinalis*) and coriander (*Coriandrum sativum*) plant extract]. *Menba Su Ürünleri Fakültesi Dergisi, 4*(2), 14-19.

Ramos, A., Edreira, A., Vizoso, A., Betancourt, J., López, M., & Décalo, M. (1988). Genotoxicity of an extract of *Calendula officinalis* L. *Journal of Ethnopharmacology, 61*(1), 49-55. https://doi.org/10.1016/S0378-8741(98)00017-8

Timur, G., & Timur, M. (2003). *Balık Hastalıkları.* İstanbul Üniversitesi Su Ürünleri Fakültesi Yayın No: 5.

Udomkusonsri, P., Trongvanichnam, K., Limpoka, M., Klangkaew, N., & Kusucharit, N. (2007). In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, *Saprolegnia parasitica* H2, from fish. *Kasetsart Journal (Natural Science), 41*(5), 56–61.

Ural, M. Ş., Çalta, M., & Celayir, Y. (2011b). *Gökkuşağı alabalığı (Oncorhynchus mykiss Walbaum, 1972) yumurtalarının dezenfeksiyonunda sirkenin kullanılabilirliği* [Usability of vinegar in disinfection of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1972) Eggs]. *Karadeniz Fen Bilimleri Dergisi, 2*(3), 81-88.

Ural, M. Ş., Çalta, M., Celayir, Y., & Aydın, R. (2011a). *Gökkuşağı alabalığı (Oncorhynchus mykiss Walbaum, 1972) yumurtalarının dezenfeksiyonunda kullanılan bazı kimyasal maddelerin kuluçka parametrelerine etkileri* [The effects of some disinfection chemicals on hatchery parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1972) eggs]. *BİBAD-Biyoloji Bilimleri Araştırma Dergisi, 4*(1), 37-41.

Xue-Gang, H., Lei, L., Cheng, C., Kun, H., Xian-Le, Y., & Gao-Xue, W. (2013). In vitro screening of Chinese medicinal plants for antifungal activity against *Saprolegnia sp*. and *Achlya klebsiana*. *North American Journal of Aquaculture, 75*(4), 468–473. https://doi.org/10.1080/15222055.2013.808298

Yanik, T., Hisar, O., Hisar, S. A., Bayir, A. K., Sirkecioglu, A. N., Ayik, O., & Aras, M. S. (2009). Intensive and semi-intensive culture probability of caner (*Barbus capito capito*, Güldenstädt) of Aras River, Erzurum, Turkey. *Journal of Animal and Veterinary Advances*, *8*(5), 829-834.

Yılmaz, C. (2010). *Alabalık Yetiştiriciliği.* Elazığ Tarım İl Müdürlüğü.