Available online at www.garmian.edu.krd

Passer Journal

Passer 6 (Issue 2) (2024) 252-258

http://passer.garmian.edu.krd/

Impact of Oak Seed Extract and Virkon on *Saprolegnia* Prevention in Common Carp

Makwan Saeed Qadir[1*], Trifa Kamal Jalal[1], Sulaf Mustafa Mohammed[1]

[1]*Department of Biology, College of Science, University of Sulaimani, Sulaimani, Kurdistan Region, Iraq*

Received 19 January 2024; revised 12 April 2024;
accepted 13 April 2024; available online 06 May 2024

DOI: 10.24271/PSR.2024.436692.1479

ABSTRACT

Fungal diseases pose a severe threat to freshwater fish, resulting in considerable losses and high mortality rates. The objective of this study was to assess how an extract from the acorn oak species (*Quercus aegilops L*) affects oxidative stress and the responses of common carp to their diet. Fish weighing sixty, seventy, and eighty grams were divided into eight groups and placed in 70-L tanks filled with water at a density of 3 g/L for the low-density group or ten g/L for the high-density group. Fish were given meals supplemented with 0.0, which acted as a control without exposure to zoospores. The following seven groups were exposed to *Saprolegnia* zoospores in different amounts of oak extract, namely 5%, 10%, and 15%. Moreover, three groups received treatment for 14 days using a combination of virkon and oak extract. Findings indicated a noteworthy decrease in the amounts of liver catalase (CAT), glucose-6-phosphate dehydrogenase (G6PD), superoxide dismutase (SOD), glutathione peroxidase (GTP), and Glutathione-s transferase (GTST) (U/I) in all fish groups relative to the control group. Alternatively, there was a notable elevation in the concentration of liver malondialdehyde (MDA) (µmol/L) in fish in contrast to the control group. Oak extract led to considerable increases in the level of liver MDA, whereas the control fish exhibited their lowest levels. Conclusion, incorporating oak extract into the diets of the fish groups led to significant decreases ($P < 0.05$) in liver levels of CAT, SOD, GTP, G6PD, and GTST. Simultaneously, there was a significant ($P < 0.05$) increase in the liver MDA level compared to the control group. The outcomes of this study reveal that the groups receiving oak and Virkon exhibited considerable modulation in liver enzymes compared to the groups fed only oak extract.

Keywords: Common Carp, Oak Seed Extract, Oxidative Biomarkers, Oxidative Stress, Virkon Antifungal, and Aquatic Fungus.

1. Introduction

Fungal diseases pose significant challenges for freshwater and agricultural fish, leading to substantial losses and high fatality rates. The pathogenic potential of aquatic fungi in fish is well-known. The majority of freshwater fish, along with a small number of marine species and developing eggs, are susceptible to fungal infection. Intensive aquaculture settings have been found to facilitate the transmission of fish infections, especially fungal diseases. This has resulted in economic losses, making studies on these diseases increasingly crucial in the last two decades. Zoosporic fungi are well-known pathogens that infect fish epithelial surfaces as secondary invaders. Their infectivity increases in situations characterized by low water quality or overall immunosuppression[1]. Saprolegniasis is a prevalent infection that affects freshwater fish, as well as some estuarine species, in warm and tropical regions, and it is found worldwide. *Saprolegnia parasitica* is an opportunistic fish pathogen that thrives under fish physiology disruption, typically caused by stressful circumstances resulting from inadequate handling, management, and transportation. It is one of the primary fungi responsible for saprolegniosis. Fish exteriors are the only target of a common fungus species called *Saprolegnia parasitica*. It is easy to remove sickness if the underlying cause has been found and fixed[2]. The *Saprolegnia parasitica* infection has led to an imbalance between oxidants and antioxidants, causing oxidative damage in the fish liver, potentially contributing to disease development. The liver is vital to the body and is susceptible to hepatic damage[3]. Oxidative stress refers to the equilibrium between oxidants and antioxidants, where oxidants have the upper hand and can disrupt the redox state of cellular compartments. This condition is considered reversible[4]. Oxidative injury, sometimes referred to as biomolecular damage, is produced due to the assault of free radicals. Specifically, the formation of reactive oxygen species (ROS) happens when the antioxidant system fails to keep up with the rate of oxidation of cellular components[3]. Organisms utilize the antioxidant protection system to mitigate prooxidant activity and prevent or decrease the formation of free radicals, such as ROS[5]. Oxidative stress occurs when the fish's antioxidant system becomes unable to neutralize prooxidants effectively. Oxidative stress triggers the process of fatty acid oxidation, resulting in the production of

* Corresponding author
E-mail address: Makwanqadir@yahoo.com (Instructor).
Peer-reviewed under the responsibility of the University of Garmian.

malondialdehyde (MDA). Thus, a robust antioxidant system safeguards the fish's fatty acids from oxidation, ensuring the well-being of the fish[6]. The defense mechanisms against antioxidants in fish encompass both enzyme systems and low molecular weight antioxidants, resembling those present in mammals. However, the distinct isoforms of enzymes in various fish species have not been comprehensively characterized[7]. SOD, CAT, GPx, and GST, enzymes serve as the main antioxidant enzymes and noteworthy indicators of oxidative stress[8]. SOD, CAT, and GPx serve as vital antioxidant enzymes in fish responsible for protecting cells from hydrogen peroxide molecules and superoxide[9]. Aquaculture has experienced significant growth in recent years and is now one of the most rapidly expanding areas in food production and agriculture. This industry has the potential to offer sustainable seafood sources. The intensification of fish farming to meet global demands has placed additional stress on fish, resulting in stunted growth, weakened immune systems, and lower-quality flesh[10]. The common carp, scientifically known as Cyprinus carpio L., is a suitable, cost-effective, environmentally friendly, and feasible source of protein from animal origin. This particular fish species possesses diverse attributes that render it an excellent selection for aquaculture. Organism exhibits rapid proliferation, favorable flavor, heightened immunity against ailments pressure, and the capacity to effectively convert diverse natural and synthetic food sources into protein of superior quality [11]. Research indicates that food additives might enhance the antioxidant system in fish and reduce oxidative stress[12]. Various research studies have suggested that the use of plant materials and extracts can improve the growth and development of fish, increase their antioxidant capacity and immune responses, and reduce stress levels [10]. *Quercus Species* are small trees found in temperate, seasonally dry forests in the Northern Hemisphere. They are commonly found in adequately drained upland regions and frequently in the mountainous areas spanning Asia, Europe, North Africa, Central, North, and South America[13]. *Quercus aegilops*, a type of oak tree, makes up around 70% of the oak forests developed in the Region of Kurdistan. These forests create an uninterrupted mountainous expanse stretching from Iraq to Turkey and Iran, as stated by[14]. The oak is utilized as a source of livestock feed due to its low cost and widespread availability. The purpose of this is to protect the animals from oxidative damage and preserve their sensory and qualitative attributes[15]. Researchers[16-18] Utilized acorn materials/extracts as herbal supplements in fish diets to enhance growth, well-being, immunity, and innate immune responses. *Quercus aegilops* exhibits elevated amounts of total phenols, fatty acids, calcium, sodium, potassium, and phosphorus, along with enhanced antioxidant activity[19]. Virkon-S was initially created by Antec International Limited, based in Sudbury, Suffolk, UK, and introduced in 1986 for application in agricultural and animal industries. It is considered to be one of the most sophisticated agrarian disinfectants. It was among the initial oxidizing disinfectants employed in agriculture and remains at the forefront of livestock production and farm biosecurity. It has successfully fought against 500 pathogens that cause diseases, including viruses, bacteria, and fungi that are accountable for conditions like foot and mouth disease, avian influenza, Campylobacter, and Salmonella[20]. Virkon-S is globally utilized by the United Nations Food and Agriculture Organization and many governments to maintain biosafety and enhance emergency disease management plans, owing to its extensive antibacterial properties and high level of safety. Virkon-S, a disinfectant, was recognized as a quasi-drug for animals in Korea. It was utilized in 2016 to sanitize aquaculture facilities. It is authorized as a quasi-drug in Korea for disinfecting aquaculture facilities and equipment. While additional research on the impact of Virkon-S on humans is required, it shows promise as a viable solution for managing saprolegniasis, a condition that leads to significant financial losses in the aquaculture sector[21]. This study aimed to assess the impact of oak (*Quercus aegilops*) acorn seed extract into the diet on the performance and stress reduction ability of common carp (*Cyprinus carpio)*.

2. Materials and methods

2.1 Identification and isolation of Saprolegnia spp. from water, media, and infected fish

The baiting method was employed to isolate aquatic fungi from water samples collected from Khurmal ponds in Halabja province. "To obtain pure cultures from the environmental samples," a sterile petri plate with Chloramphenicol was used to pump a volume of 15-20 ml of pond water. Subsequently, sesame seeds (5-7 seeds per petri dish) were introduced, and the containers were kept in a controlled environment at 20°C for seven days. Daily examinations were conducted to observe the hyphal growth of aquatic fungus (**Figure 1:** A, B, C).

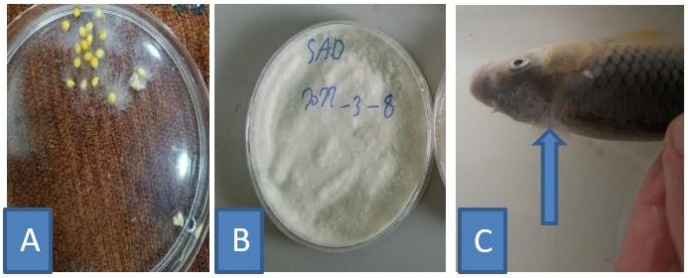

Figure 1: A- Wet culture of developing Saprolegnia spp. on sesame seeds. **B**- Displays Saprolegnia spp. After 3-4 days of being cultured on Sabouraud Dextrose Agar (SDA) at 20 **°C**, long hairs with a whitish cottony color emerged. **C** - The apparent lesion found in common carp can be attributed to the presence of hyphal mats that resemble cotton wool and ulcerations on the body. Common carp deliberately infected with saprolegniasis exhibited clinical indications of Saprolegnia parasitica on their bodies, observed ten days after exposure to the fungal zoospores.

2.2 Isolating and identifying Saprolegnia species

The identification of growth disengages was achieved by observing the presence of elongated non-septate hyphae, along with masses of varying length and width, which had a simple and intact cell membrane. The sporangia contained many spores that were separated from the basal significant hyphae through a process known as Saprolegnoid (**Figure 2:** A and B). Identifying the strain in our sample as *Saprolegnia spp* was based on physical characteristics such as the presence of asexual phases (zoosporangium, zoospores, and pimple), coenocytic hyphae, and the absence of oogonia[22].

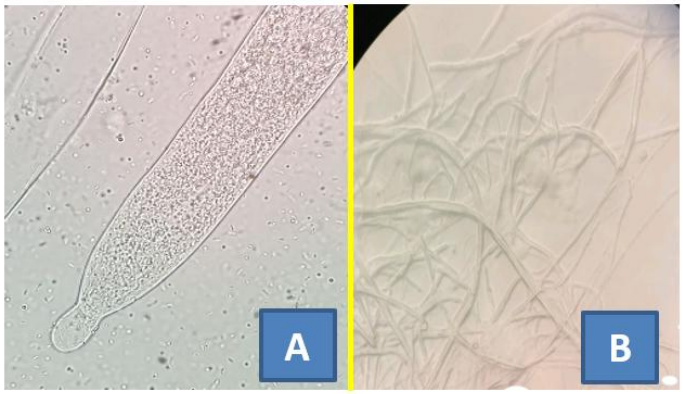

Figure 2: A and B Microscopic investigation confirmed the presence of Saprolegnia spp. Zoosporangia in these individuals exhibit either a cylindrical or spherical shape and house a significant quantity of spores. This trait is consistently noted in Saproleginoid (Figure 2: A). The objects displayed abranched non-septet hyphae morphology (Figure 2: B), were transparent, and possessed a cellular membrane. All members of the Saproleginiaceae family[23] have this characteristic.

2.3 Experimental design of artificial infection

Young common carp (*Cyprinus carpio*) were sourced from a nearby fish farm in Baqubah, Baqubah is the capital of Iraq's Diyala Governorate in from Iraq. Fish were then acclimatized for three weeks under laboratory conditions at the University of Sulaymania's College of Veterinary Medicine before the start of the study. Fish were haphazardly distributed among plastic aquaria, with a density of 9 fish per 70-L tank. The fish were nourished with a commercial feed containing 28% crude protein. Subsequently, fish weighing 60g, 70g, and 80g were allocated into eight distinct groups. Fungal isolates were put into the fish habitat at 2 X 10^4 zoospores per liter. Upon observing the presence of cotton wool on the fish (as shown in **Figure 1:** C), Different treatment concentrations were introduced into the tanks, and the fish were then exposed to the subsequent treatments. The experiment consisted of eight groups: one control group without treatment (no exposure to *Saprolegnia* zoospores) and seven other groups challenged with *Saprolegnia* zoospores. These groups included: F Infection, Infection+OAK 5% Oak seed extract, Infection+OAK 10%, Infection+OAK 15%, Infection+OAK 5%+Vercon 1g/l Virkon antifungal, Infection+OAK 10%+Vercon 1g/l, and Infection+OAK 15%+Verkon 1g/l. The duration of the trial was 14 days. Compressed air was supplied to each tank using air stones linked to an air pump. The water in all the tanks was replenished daily with well-oxygenated well water. At the end of the research, fish from each aquarium were gathered, enumerated, and subsequently weighed as separate groups.

2.4 An ethanol extract of oak seeds (Quercus aegilops)

An ethanol extract of oak seeds (*Quercus aegilops*) was made following the method described by[24]. The newly harvested acorns were gathered from the local region of Sulaimani, Iraq. Specimens were rinsed with distilled water on three occasions and subsequently air-dried using a fan at a temperature of 25 °C for 48 hours. Subsequently, the desiccated seeds were ground into a fine powder, and 50 g of this powder was combined with 500 mL of 80% ethanol. The solution was allowed to stand at ambient temperature for three days, after which it underwent filtration using a 500 µm mesh. The obtained solution was condensed in an oven at a temperature of 40 °C for 48 hours. Following the ethanol evaporation, the solution was moved to a freeze-dryer and maintained at -50 °C for 72 hours. The desiccated substances were gathered and utilized for the formulation of the diet.

2.5 The feeding regimens and care of the fish

The study involved the development of diets that included oak seed extracts in addition to control diets. These were created by combining the feedstuffs with 0.0% (control), 5%, 10%, or 15% oak (as shown in **Table 1:** Subsequently, (100 mL) of water was incorporated into each kilogram of the mixture to create cohesive dough. The dough was pressed through a food processor sieve with a mesh size of 3 mm, and the resulting strands were left to air-dry at a temperature of 25°C for 24 hours.

Table 1: Displays the ingredients and proximate chemical makeup (expressed as a percentage of dry matter) of diets that include varying amounts of oak (Quercus aegilops) acorn seed extract levels (g/kg diet).

Ingredients	oak 5%	oak 10%	oak 15%
Ground corn	110	55	55
Barley	170	85	85
Wheat meal	180	90	90
Soya	340	170	170
Oak powder extract	5	10	15
Crude protein	200	100	100

Table 2: Antifungal drug Virkon (100g), Water System Disinfection, Dilution Rate

Composition	Active ingredient
Potassium peroxymonosulfuate	21.50 %
Sodium chloride	1.50 %
Sulphamic acid	5.0 %
Malic acid	10.0 %
Another ingredient, up to	100.0 %
Water System Disinfection	Dilution Rate
Terminal disinfection	1:200 – 1:100 (10g of Virkon to every one L of water)
Continuous disinfection	1:1000

2.6 Sampling;

At the end of the research period, six fish were haphazardly selected from each aquarium and subjected to anesthesia using buffered (tricaine methane sulfonate 30 mg/L). Subsequently, the weight of each fish was measured. Subsequently, the fish underwent dissection, and the liver was removed and weighed.

2.7 Extraction of Antioxidant Enzymes

The crude extract was obtained by homogenizing a frozen liver sample weighing 1 gram in a phosphate buffer. A sample is maintained at a temperature of -20°C until it is used to extract enzymes in the liver. The specimen was rinsed with distilled water on two occasions. The sample weighing 1.0 g was rapidly sliced into tiny pieces and mixed thoroughly in a solution of (50 mL) of 100 mM sodium sulfate buffer (pH 7.0), one mM ascorbic

acid, and 0.5% (w/v) polyvinylpyrrolidone. The homogenization process took place for 5 minutes at a temperature of 4 °C. Homogenate was filtered using three layers of cheesecloth. Then the remaining liquid was subjected to centrifugation at a force of 5,000 times the acceleration due to gravity for 15 minutes. Sediment material was gathered, while the remaining residue from the samples was mixed with (1.0 ml) of high-performance liquid chromatography (HPLC) grade methanol by vigorously shaking. The resulting mixture was then passed through a disposable filter with a pore size of 2.5 micrometers and stored at four °C for further analysis. Finally, 20 microliters of the prepared sample were injected into the HPLC system under optimal conditions[25].

2.8 Liver tissue is homogenous;

The liver was separated and washed with phosphate buffer saline. To prepare liver homogenate, 1 g of liver tissue is homogenous in 9 ml of cold phosphate-buffered saline PBS (0.064 mol/L) with a pH of 7.4. The homogenization procedure was conducted on ice utilizing an electric tissue homogenizer[26]. They centrifuged the homogenate at 5000 RPM for 5 minutes at four degrees °C. The homogenate supernatant was carefully transferred into Eppendorf tubes and stored in a deep freeze (-80 °C) until the levels of malondialdehyde (MDA) were measured[27].

2.9 MDA estimation;

The concentration of MDA in the liver was measured using spectrophotometry with a TBA solution. The following substances were added to a liver homogenate of 150µl: Combine 1 milliliter of trichloroacetic acid (TCA) at a concentration of 17.5% with 1 milliliter of thiobarbituric acid (TBA) at a concentration of 0.66%. Thoroughly mix the solution using a vortex. Place the mixture in boiling water and incubate it for 15 minutes. Afterward, allow the solution to cool. Next, introduce 1 milliliter of 70% trichloroacetic acid (TCA) into the mixture and allow it to remain at room temperature for 20 minutes. Afterward, place the mixture in a centrifuge and spin it at a speed of 2000 revolutions per minute (rpm) for 15 minutes. Ultimately, retrieve the liquid portion for additional examination using scanning spectrophotometry, following the procedure outlined by[28].

The concentration of MDA is determined using the following calculation:

MDA (µmol/L) = Absorbance at 532 nm x D/ L x Eo Where

L: Light bath (1cm)

Eo: Extinction coefficient 1.56 x 105 M -1. CM-1

D: Dilution factor = 1 ml Vol. used in ref./ 0.15 =6.7

2.10 Statistical analysis;

The statistical analysis used the GraphPad Prism program (version 9.0). All data were depicted as the mean ± standard error of the mean (SEM) and subsequently analyzed via one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests for analysis. The significance degree of $P < 0.05$ was considered statistically significant.

3. Results

C		F	FO 5%	FO 10%	FO 15%	FVO 5%	FVO 10%	FVO 15%
CAT	47.57 ± 0.26	37.19 ± 0.56	23.15 ± 0.77	25.76 ± 0.25	28.99 ± 0.45	40.20 ± 0.36	42.18 ± 0.081	44.10 ± 0.36
SOD	84.90 ± 0.30	56.29 ± 0.42	44.86 ± 1.00	46.60 ± 0.51	48.28 ± 0.54	82.82 ± 0.52	88.10 ± 0.37	88.70 ± 0.50
GTP	62.46 ± 0.68	31.22 ± 0.56	20.08 ± 0.40	21.55 ± 0.52	22.68 ± 0.42	35.93 ± 0.42	38.15 ± 0.42	38.70 ± 0.36
G6PD	134.2 ± 1.10	72.34 ± 0.38	57.21 ± 0.40	57.12 ± 0.36	59.19 ± 0.36	72.94 ± 0.21	74.42 ± 0.56	78.41 ± 0.41
GTST	62.77 ± 0.43	31.82 ± 0.27	32.83 ± 0.77	35.76 ± 0.22	36.00 ± 0.69	40.72 ± 0.54	44.06 ± 1.16	49.07 ± 0.63
MDA	1.562 ± 0.008	2.233 ± 0.003	3.010 ± 0.001	3.174 ± 0.004	3.228 ± 0.002	4.346 ± 0.147	3.402 ± 0.156	3.927 ± 0.033

These groups included: C: Control, F Infection, FO: Infection + OAK 5% Oak seed extract, infection; I+ OAK 10%, infection; I+ OAK 15%, FVO: Infection + OAK 5%+ Vercon 1g/l Virkon antifungal, Infection + OAK 10% + Vercon 1g/l, and infection; I+ OAK 15% + Verkon 1g/l.

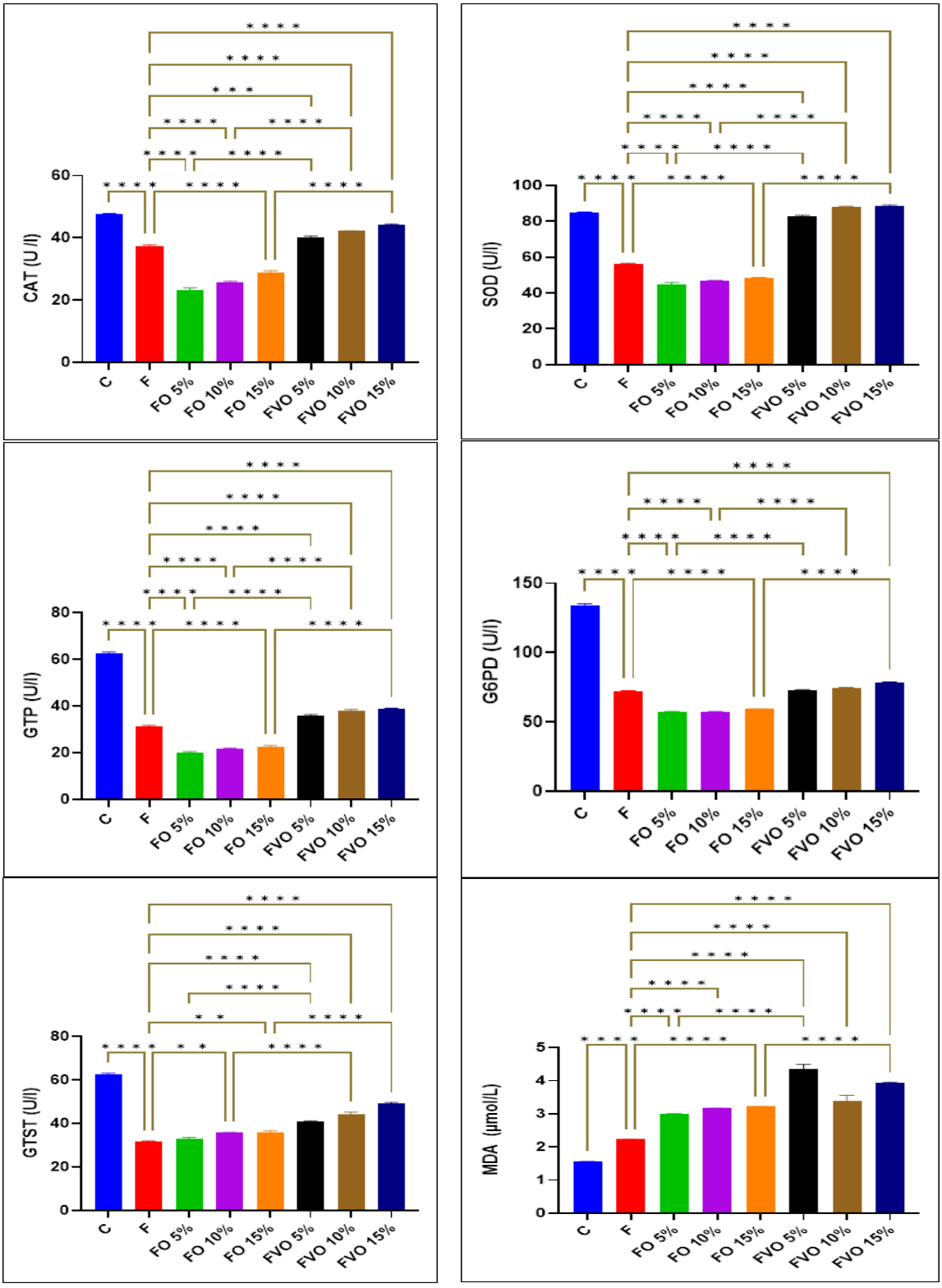

Figure 2: The bar chart shows a significant difference among groups of liver SOD, CAT, GTP, G6PD, GTST (U/I), and MDA (µmol/L) in the fish aquarium groups.

4. Discussion

Due to economic considerations and the ongoing risk of disease outbreaks in fish farms, fish producers are highly motivated to explore methods that can improve fish development and well-being. To achieve these objectives effectively, one can employ feed supplements that have the potential to enhance fish development performance and strengthen antioxidant and non-specific immune systems[29]. Aquaculture poses a continuous risk of free radicals to fish due to factors including high fish handling and stocking density. Consequently, fish must possess robust antioxidant defense mechanisms to resist these detrimental impacts[30]. Oaks, officially classified as Quercus species,

encompass a diverse group of plant species that are distributed globally[31]. They are utilized globally as a source of human sustenance and as a medicinal plant due to their advantageous qualities, including antioxidant and antibacterial characteristics[32].The addition of oak plant seed extract significantly improved the performance of common carp in this investigation. Furthermore, food intake increased due to the heightened need for nutrients during fish development or due to sensory stimulation and subsequent enhancement of hunger caused by the inclusion of oak plant seed extract in the diet. Its mix of phenolic chemicals, minerals, vitamins, essential oils, aromatic substances, amino acids, lipids, carbohydrates, proteins, and different sterols is responsible for the growth-promoting effects of oak plant seed extract[13]. These components possess stimulatory and digestive properties[19]. Acorns of Quercus contain a diverse array of phenolic compounds, spanning from simple molecules like phenolic acids to more intricate polyphenols like flavonoids, polymers, and derived stilbenes[33]. These compounds have some advantageous qualities, such as antimicrobial, antioxidant, anti-inflammatory, and anticarcinogenic activities[13]. Chemicals exhibited a positive effect on the overall performance and immune response of fish, resulting in an improvement in overall health and productivity[6]. The study demonstrates that fish aquarium groups that were exposed to *Saprolegnia* zoospores have notable alterations in all hepatic oxidative and antioxidant enzymes. Nevertheless, an overproduction of ROS can inhibit the antioxidant defense system by inhibiting several enzymes crucial for antioxidant activity, including glutathione peroxidase (GPx), superoxide dismutase (SOD), and glutathione S-transferase (GST). Disruption of the antioxidant defense system is recognized as a factor in the progression of infectious illnesses in fish[34]. This study demonstrates that the concentrations of liver enzymes, including (CAT), (SOD), (GTP), (G6PD), and (GTST) (U/I), in the groups treated with oak extract at levels of 5%, 10%, and 15% showed a more pronounced reduction evaluated to the control groups. In contrast, the groups that received oak extract at doses of 5%, 10%, and 15% in addition to verkon exhibited a notable reduction compared to the control group. Nevertheless, the concentrations of liver malondialdehyde (MDA) (µmol/L) in the groups that received 5%, 10%, and 15% oak extract showed a notable increase in comparison to the control group. Furthermore, the groups of fish that received both oak extract and verkon antifungal experienced a more significant elevation in MDA levels compared to the other three groups of fish that were solely given oak extract. The research was carried out by[35]. This suggests that the reduction in enzyme activities could be attributed to the defensive impact of the extract. The group of fish in the aquarium that were fed with oak extract 5% + verkon, oak extract 10% + verkon, and oak extract 15% + verkon showed more modulation in liver enzymes compared to the other three groups of aquarium fish that were given oak extract 5%, oak extract 10%, and oak extract 15%. On the other hand, the group of infections that did not receive plant extract and Verkon antifungal experienced a significant decrease in liver enzymes such as (CAT), (SOD), (GTP), (G6PD), and (GTST) (U/I) in contrast to the control group, subsequent mycelial growth**.** However, the level of liver malondialdehyde (MDA) (µmol/L) showed a more significant rise in all group infections compared to the control group. In general, the liver enzymes catalase (CAT), (SOD), (GTP), (G6PD), and (GTST) (U/I) show a significant decrease in their levels in infected groups compared to the level of liver malondialdehyde (MDA) (µmol/L). This decrease is more noticeable in the infected groups than in the control group. This study intended to evaluate the antifungal efficacy of Virkon-S against *Saprolegnia parasitica*, the primary pathogen responsible for saprolegniasis. The results of this analysis propose that Virkon-S can be utilized to control saprolegniasis without inducing any adverse effects on both cultured fish cells and fish in tanks[21].

Conclusions

Research was conducted to examine the effect of oak seed extract (Quercus aegilops) on the oxidative stress of common carp. The results suggested that the oak seed extract may enhance the antioxidant activity of liver enzymes in common carp. The groups that were fed oak with 5% + virkon, oak with 10% + virkon, and oak with 15% + virkon exhibited better performance compared to other concentrations, such as oak with 5%, oak with 10%, and oak with 15%. This has the potential to enhance aquaculture productivity and enhance fish resilience against unfavorable environmental conditions. Virkon-S is endorsed as a quasi-drug for sterilizing aquaculture facilities and equipment in the Kurdistan region. The study's findings show that Virkon-S can effectively treat saprolegnia without having any adverse side effects on cultivated fish cells or fish in tanks. Virkon-S is a suitable option for managing saprolegniasis, which leads to significant financial losses in the aquaculture sector.

Conflict of interests

None.

Author's contribution

As evidence of the collaborative atmosphere, each author contributed equally vital ideas. The researchers carefully crafted and executed the research framework, after which they conducted an extensive analysis of the data and incorporated their findings into a unified manuscript. Their seamless collaboration and collective knowledge fuelled each stage of this endeavor, establishing this work as a veritable testament to our shared commitment.

Funding

This article's authorship and publication followed an independent investigation that received no external financial assistance.

References

1. aM. M. Hussein, K. Hatai, Fisheries science 2002, 68, 1067-1072; bA. Pérez-Jiménez, H. Peres, V. C. Rubio, A. Oliva-Teles, British Journal of Nutrition 2012, 108, 1202-1209.
2. P. Van West, Mycologist 2006, 20, 99-104.
3. M. D. Baldissera, C. F. Souza, C. C. Zeppenfeld, M. C. Velho, B. Klein, L. B. Abbad, A. F. Ourique, R. Wagner, A. S. Da Silva, B. Baldisserotto, Aquaculture 2020, 516, 734635.
4. H. Sies, Current Opinion in Toxicology 2018, 7, 122-126.
5. R. Pamplona, D. Costantini, American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 2011, 301, R843-R863.
6. E. Ahmadifar, N. Sheikhzadeh, K. Roshanaei, N. Dargahi, C. Faggio, Aquaculture 2019, 507, 341-348.

7. S. M. Billiard, J. N. Meyer, D. M. Wassenberg, P. V. Hodson, R. T. Di Giulio, Toxicological Sciences 2008, 105, 5-23.
8. M. L. Kelly, J. R. Berry, D. A. Dwyer, J. Griinari, P. Y. Chouinard, M. E. Van Amburgh, D. E. Bauman, The Journal of Nutrition 1998, 128, 881-885.
9. M. Yousefi, S. M. Hosseini, Y. A. Vatnikov, E. V. Kulikov, S. G. Drukovsky, Aquaculture 2019, 505, 473-480.
10. Z. Fazelan, Y. A. Vatnikov, E. V. Kulikov, V. G. Plushikov, M. Yousefi, Aquaculture 2020, 518, 734833.
11. M. N. Khan, K. Shahzad, A. Chatta, M. Sohail, M. Piria, T. Treer, Croatian Journal of Fisheries: Ribarstvo 2016, 74, 71-80.
12. S. Yılmaz, S. Ergun, E. Şanver Çelik, M. Yigit, C. Bayizit, Aquaculture nutrition 2019, 25, 1207-1217.
13. E. Burlacu, A. Nisca, C. Tanase, Forests 2020, 11, 904.
14. N. R. Khwarahm, Ecological Processes 2020, 9, 1-16.
15. V. C. Ferreira, D. Morcuende, S. H. Hérnandez-López, M. S. Madruga, F. A. Silva, M. Estévez, Journal of Food Science 2017, 82, 622-631.
16. S. Bohlouli, G. Ghaedi, M. Heydari, A. Rahmani, E. Sadeghi, Aquaculture nutrition 2016, 22, 745-751.
17. B. A. Paray, S. M. Hoseini, S. H. Hoseinifar, H. Van Doan, Aquaculture 2020, 524, 735276.
18. G. Rashidian, S. Bahrami Gorji, M. N. Farsani, M. D. Prokić, C. Faggio, Natural Product Research 2020, 34, 2413-2423.
19. V. T. Papoti, N. Kizaki, A. Skaltsi, P. D. Karayannakidis, M. Papageorgiou, Food science and biotechnology 2018, 27, 819-828.
20. V. Marchetti, F. Mancianti, G. Cardini, E. Luchetti, Veterinary research communications 2006, 30, 255-261.
21. H. S. Rahman, T.-J. Choi, PeerJ 2018, 6, e5706.
22. F. de la Cruz Hernández-Hernández, F. G. G. de Muñoz, A. Rojas-Martínez, S. Hernández-Martínez, H. Lanz-Mendoza, Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America 2003, 54, 37-45.
23. W. C. Coker, The Saprolegniaceae: With notes on other water molds, Vol. 20, University of North Carolina Press, 1923.
24. B. Sadeghi-Nejad, F. Shiravi, S. Ghanbari, M. Alinejadi, M. Zarrin, Jundishapur Journal of Microbiology 2010, 3, 36-40.
25. E. H. Alici, G. Arabaci, Annual Research & Review in Biology 2016, 1-7.
26. E. Icel, A. Icel, T. Uçak, Y. Karakurt, B. Elpeze, F. Keskin Çimen, H. Süleyman, Cutaneous and ocular toxicology 2019, 38, 88-92.
27. S. El-Sheikh, M. Khairy, H. Abdel Fadil, A. Abo-Elmaaty, Zagazig Veterinary Journal 2016, 44, 244-250.
28. T. Weinstein, A. Chagnac, A. Korzets, M. Boaz, Y. Ori, M. Herman, T. Malachi, U. Gafter, Nephrology Dialysis Transplantation 2000, 15, 883-887.
29. H. M. Abdel-Latif, M. Abdel-Tawwab, A. F. Khafaga, M. A. Dawood, Aquaculture 2020, 526, 735432.
30. M. Abdel-Tawwab, I. Adeshina, A. Jenyo-Oni, E. K. Ajani, B. O. Emikpe, Fish & shellfish immunology 2018, 78, 346-354.
31. W. Almeida, G. Santana, W. Vieira, I. Wanderley, E. Freire, A. Vasconcellos, Brazilian Journal of Biology 2008, 68, 427-431.
32. H. Nourafcan, M. Nasrollahpour, I. Bajalan, International Journal of Farming and Allied Sciences 2013, 2, 1153-1155.
33. V. Cheynier, Phytochemistry reviews 2012, 11, 153-177.
34. E. M. de Souza, R. C. de Souza, J. F. Melo, M. M. da Costa, A. M. de Souza, C. E. Copatti, Aquaculture 2019, 504, 7-12.
35. T. Babalola, M. Adebayo, D. Apata, J. Omotosho, Tropical Animal Health and Production 2009, 41, 371-377.