E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2025; 13(3): 61-67

www.fisheriesjournal.com
Received: 11-03-2025
Accepted: 13-04-2025

Maricris B Asuncion
Provincial Institute of Fisheries, Isabela State University-Roxas Campus, Roxas, Isabela, Philippines

Isagani P Angeles JR
Institute of Fisheries, Freshwater Fisheries Center, Isabela State University, Echague, Isabela, Philippines

Marites R Castro
Provincial Institute of Fisheries, Isabela State University-Roxas Campus, Roxas, Isabela, Philippines

Mary Joy L Asprec
Institute of Fisheries, Freshwater Fisheries Center, Isabela State University, Echague, Isabela, Philippines

Laila G Castro
Institute of Fisheries, Freshwater Fisheries Center, Isabela State University, Echague, Isabela, Philippines

Corresponding Author:
Maricris B Asuncion
Provincial Institute of Fisheries, Isabela State University-Roxas Campus, Roxas, Isabela, Philippines

Survival and hematological profile of Nile tilapia *Oreochromis niloticus* fed diet with oregano *Oreganum vulgare* L. leaf extract at different concentrations challenged with *Pseudomonas aeruginosa*

Maricris B Asuncion, Isagani P Angeles JR, Marites R Castro, Mary Joy L Asprec and Laila G Castro

DOI: https://www.doi.org/10.22271/fish.2025.v13.i3a.3098

Abstract
This study assessed the survival and hematological profile of Nile Tilapia *Oreochromis niloticus* fed with different concentrations of oregano leaf extract (OLE) after 24 hours of bacterial stress challenge. The fish were fed with oregano leaf extract at 1 ml, 2 ml, 3 ml, and 0 ml, served as the control. Fish blood samples were collected 6 weeks after rearing and 24 hours after the bacterial stress challenge. The fish were injected with 0.2 ml of *Pseudomonas aeruginosa* intraperitoneally. As a result, *Pseudomonas aeruginosa* had a significant effect on the blood parameters of the fish after the experimental challenge. RBC counts of Nile tilapia fed with a commercial diet decreased while the fish fed with OLE were maintained. This result showed that OLE protected the fish from infection during the bacterial stress challenge. Thus, it can be concluded that at 3ml/kg, oregano leaf extract acts as an immunostimulant for *Oreochromis niloticus* against *Pseudomonas aeruginosa*. Further studies on the different fish species challenge are recommended.

Keywords: Oregano, *Oreochromis niloticus*, *Pseudomonas aeruginosa*, immunostimulant, Hematological profile, *Oreganum vulgare* L.

1. Introduction
Aquaculture is a crucial global industry that provides essential food for the world's population and a valuable source of animal protein. (Soliman *et al*., 2016) [1]. Tilapias are a prime aquaculture commodity marketed globally in the Philippines, among the top tilapia-producing countries (Miao *et al*., 2020) [2]. The aquaculture industry has recently expanded with the critical need to find sustainable and prosperous animal protein sources for the increasing population (FAO, 2020) [3]. In 2020, production from the Tilapia industry contributed 263,871.29 MT or 11.4% to the total national fisheries aquaculture production of 2,324,000 MT. About 93% of Tilapia production came from freshwater; the remaining 7% was from brackish and marine water (PSA, 2021) [4].
Nile tilapia (*O. niloticus)* is a candidate fish species for intensive and super-intensive farming systems due to their high growth rate and tolerance against stressful conditions (Dawood *et al*., 2020) [5]. Pond culture (freshwater and brackish water), cage culture (dam reservoirs, lakes, and seawater), mono-sex male tilapia culture, saltwater culture, rice-fish (tilapia) culture, and aquaponics are among the most preferred culturing techniques (Romana-Eguia *et al*., 2020) [6]. However, intensive systems weaken the immune system and decrease the animal's resistance to environmental stressors and pathogens (Dawood *et al*., 2019) [7]. Despite being somewhat hardy fish (Romana-Eguia *et al*., 2020) [6], tilapias are still susceptible to diseases such as bacterial, parasitic, fungal, and viral diseases (Amal *et al*., 2011 [8]; El-Sayed, 2019 [9]; Romana-Eguia *et al*., 2020) [6].

2. Methodology

2.1 Hematological Analysis

Fish blood samples were collected 6 weeks after rearing and 24 hours after the bacterial stress challenge. After the feeding experiment, three healthy fish from each treatment were randomly selected and immersed in MS-222 (tricaine methane sulfonate at 1g/500 mL water) for the collection of fish blood samples. A 0.1 ml of blood was collected through caudal vasculature and stored in tubes containing di-potassium Ethylene Diaminetetraactic (EDTA k2) with a 1 cc syringe. Blood samples were analyzed right after the blood collection at Roxas Diagnostic Laboratory, Roxas Isabela.

2.2 Preparation of Bacteria Culture

The fish underwent bacterial stress challenge after 6 weeks of feeding experiment. *Pseudomonas aeruginosa* bacteria was used to challenge the stock and evaluate the effect of the diet with oregano leaf extract (OLE) on the blood parameters of the fish. The *Pseudomonas aeruginosa* isolates were obtained from the PHilMech Collection of Microorganisms (PHilMec), BioResource Laboratory Section (BRLS)-Laboratory Service Division (LSD), Science City of Muñoz, Nueva Ecija and was sub-cultured at Freshwater Fisheries Center Isabela State University compound Echague Isabela.

A pure colony of *Pseudomonas aeruginosa* was sub-cultured using nutrient agar at 25˚C for 24 hours (Hossain *et al*., 2006) [10]. Following the method of Rahmawati *et al*. (2021) [11] with slight modification, 33 grams of nutrient agar powder were dissolved in 1000 mL of distilled water, adhering to the dosage recommended on the product label. The mixture was then sterilized in an autoclave at 121°C for 15 minutes. Once sterilized, the nutrient agar (NA) medium was inoculated with bacteria from pure culture isolates. The inoculated plates were subsequently incubated at 37°C for 24 hours. *Pseudomonas aeruginosa* bacterial growth on the NA media was centrifuged at 3000 rpm for 20 minutes (Hossain *et al*., 2006) [10]. The supernatant was then removed and added with a physiological NaCl solution of 0.9% until the volume corresponded to the initial volume, then underwent divortex. This process was done twice. After rinsing with 0.9% physiological NaCl, the bacterial density was calculated using a colony counter (Uppal *et al*., 2012) [12]. The density of *P. aeruginosa* that was used in this study is 0.2 ml (Hossain *et al*., 2006) [10].

2.3 Artificial Infection of *Pseudomonas aeruginosa*

Tilapia underwent fasting before the bacterial stress challenge. Ten fish from each treatment weighing 29.9 ± 11.42 were immersed in MS-222 (tricaine methane sulfonate) at 1g/500ml water and injected with *Pseudomonas aeruginosa* with a density of 0.2 ml/fish (Hossain *et al*., 2006) [10]. A 0.2 ml *Pseudomonas aeruginosa* suspension was injected intraperitoneally with a 1 ml syringe into the tilapia. The eyes of the tilapia were covered with a clean, wet cloth to prevent the fish from becoming stressed after the injection (Hardi *et al*., 2014) [13]. Injected tilapia was then transferred into a separate experimental drum for further observation. Mortality was monitored to ascertain the clinical signs and survival rate of the experimental fish.

This study upholds the ethical principles of animal welfare, scientific honesty, and environmental responsibility. By adhering to established guidelines, the research ensures both the reliability of findings and the humane treatment of experimental animals, supporting ethical aquaculture development.

2.4 Observation of Mortalities after Artificial Infection

Mortalities of the test fish were observed and recorded after 11 and 12 hours followed by 26, 29, 43, 45, 52, 53, 61, and 68 hours after artificial infection. Deceased fish were promptly removed and disposed of in designated areas to prevent contamination that could impact the remaining test fish.

2.5 Statistical Analysis

Blood profile data were analyzed using one-way ANOVA, followed by Tukey's HSD test for multiple comparisons ($p < 0.05$). An independent t-test was used to assess changes in the hematological profile after the bacterial stress challenge. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics Version 22.

3. Results and Discussion

3.1 Hematological Parameters

3.2 White Blood Cells

A significant decrease (P=0.009) in WBC was observed in 2 ml OLE after the 24-hour bacterial stress challenge (Table 2). The same result was observed in *C. carpio* due to extensive hemorrhage in the skin, eye, hepatopancreas, and swim bladder after oral administration of high doses of microcystins (Navratil *et al*., 1998) [14]. A decrease in WBC count or leukopeniais attributed to a toxicity-induced general stress response or specific toxic action affecting articulating leukocytes or leucopoiesis resulting in immunosuppression (Ligina *et al*., 2022) [15]. Hematologic abnormalities involving erythrocytes of fish include anemia and abnormal morphology, and fish leukograms are affected by disease, inflammatory processes, nutrition, stress, and physiologic and environmental factors (Campbell *et al*., 2007) [16].

Table 2: White Blood Cell (WBC) Counts of Nile Tilapia Fed with Oregano Leaf Extracts Before and After Bacterial Stress Challenge

	WBC before Bacteria Stress Challenge	WBC after 24hrs Bacteria Stress Challenge	T-stat	P(1-tailed)
Control/0 ml OLE	138.7	108.9	1.72	0.09 ^{ns}
1 ml OLE	143.1	109.9	1.79	0.07 ^{ns}
2 ml OLE	155.6	123.2	3.84	0.009 **
3 ml OLE	146.83E+10	122.03E+10	2.73	0.06 ^{ns}

Note: Ns-not significant; *-significant; **-highly significant

Treatment means within the same column of different letters are significantly different at (P≤0.05). WBC (White Blood Cells).

Normal value for WBC at 108.67 to 238 x 10^3/mL (Kefas *et al*., 2015) [17].

In addition, a decrease in WBCs may also indicate a release of smaller immature erythrocytes due to splenic contraction, and the spleen is a major storage of organs for blood cells (Ruane *et al*., 2000) [18]. Erythrocytes are produced in the spleen and kidneys and low erythrocyte levels indicate anemia (Yanuhar *et al*., 2021) [19]. In general, WBC may change concerning various environmental parameters or stimuli such as infection (Romano *et al*., 2017) [20]. WBC plays a major role in the immune defense system of fish (Magnadottir 2006) [21].

3.3 Red Blood Cells

All Nile tilapia fed with different concentrations of OLE maintain the RBC count after 24 hours of bacterial stress challenge compared to fishes fed with a commercial diet that decreases significantly (P=0.02) (Table 3). A significant decrease was also observed in freshwater fish exposed to heavy metals (Narayanan 2009) [22]. Gram-negative bacteria, such as *Aeromonas spp* and *Pseudomonas spp,* are common causes of septicemia and hemorrhagic anemia in freshwater fish (Groff *et al*., 1999). [23]

In addition, enhanced RBC counts were also recorded when Nile tilapia was fed with oregano essential oil, which showed improved HGB, RBCs, and WBCs respectively (Mohammadi *et al*., 2020) [24] thus, enhanced RBC was described as a strategy to improve blood capacity to carry oxygen under the high energy demand condition (Eslamloo *et al*., 2014) [25]. In addition, RBC has an important role in the immunity of fish (Yanuhar *et al*., 2012 [26]; Yuwanita *et al*., 2013) [27]. This signifies that OLE can protect erythrocyte levels from destruction.

Table 3: Red Blood Cell (RBC) counts of Nile Tilapia Fed with Oregano Leaf Extracts Before and After Bacterial Stress Challenge

	RBC before Bacteria Stress Challenge	RBC after 24hrs Bacteria Stress Challenge	T-stat	P(1-tailed)
Control/0 ml OLE	2.34E+12	1.75E+12	3.75	0.02 *
1 ml OLE	2.3	1.73	1.26	0.14 ns
2 ml OLE	2.35	1.92E+12	1.95	0.06 ns
3 ml OLE	2.3	2.20E+12	0.28	0.4 ns

Note: Ns-not significant; *-significant; **-highly significant
Treatment means within the same column of different letters are significantly different at (P≤0.05). RBC (Red Blood Cells)
Normal values for RBC at 0.05 TO 4.2 x 10^{12}/L (Witeska 2013) [28]

Carvacrol is mostly present in oregano and is synthesized by chemical methods (Yadav *et al*., 2009) [29]. Carvacrol possesses a wide range of biological activities, including antibacterial and antifungal (Chavan *et al*., 2014) [30], antiviral (Sánchez *et al*., 2015) [31] antioxidant, modulating of immunity response (Khazdair *et al*., 2018) [32], anti-inflammatory (Fitsiou *et al*., 2016) [33] and anticarcinogenic properties (Özkan *et al*., 2011) [34]. Oregano was also found to contain that exhibits antioxidant activity. Flavonoids found in oregano are a class of phytochemicals that possess antibacterial activity against a wide range of microbial species, including pathogenic and other undesirable bacteria present in the digestive tract (Srividya *et al*., 2010) [35].

In addition, a recent study showed that oregano has antimicrobial activity against *Enterococcus hirae* and clinical pathogens and ampicillin-resistant *Escherichia coli* strains (Moghrovyan *et al*., 2019) [36]. The carvacrol present in oregano can prevent free radicals and hazardous compounds from interacting with cellular DNA (Alagawany *et al*., 2021) [37].

3.4 Hemoglobin and Hematocrit

The HGB and HCT decreased significantly in Nile tilapia fed with 1 ml OLE (P=0.03; P=0.05) and 2 ml OLE (P=0.01; P=0.05) (Tables 4 & 5). On the contrary, Nile tilapia fed with a commercial diet, and 3 ml OLE maintained the HGB and HCT post-stress challenge. Similar results were also observed in the HGB and HCT in chum salmon *Oncorhynchus keta* infected with *Vibrio anguillarum*, in rainbow trout *Oncorhynchus mykiss* infected with *Aeromonas/Streptococcus*, and in cichlid fish with the epizootic ulcerative syndrome were previously reported (Harbell *et al*., 1979 [38]; Barham *et al*., 1980 [39]; Pathiratne *et al*., 1998) [40]. Similar observations were also made by Silveira-Coffigny *et al*., 2004 [41] in *Oreochromis aureus* exposed to nitrite which indicates shrinkage of erythrocytes but no hemolytic effect. The decrease in hemoglobin value indicates abnormalities in fish health (Yanuhar *et al*., 2021) [18] and if the fish is affected by disease, the blood hematocrit value is low, resulting in a low erythrocyte count (Rahmaningsi *et al*., 2019) [42]. A decrease in hematocrit indicates that erythrocytes are being affected or destroyed by the infection (Harikrishnan *et al*., 2003) [43].

Table 4: Hemoglobin (HGB) (mg/L) of Nile Tilapia Fed with Oregano Leaf Extracts Before and After Bacterial Stress Challenge

	HGB before Bacteria Stress Challenge	HGB after 24hrs Bacteria Stress Challenge	T-stat	P(1-tailed)
Control/0 ml OLE	104.3	85.3	1.5	0.1 ns
1 ml OLE	107.33E+10	72.66E+11	2.48	0.03 *
2 ml OLE	118.33E+10	90.33E+11	3.39	0.01 *
3 ml OLE	116	93.33E+11	1.91	0.06 ns

Note: Ns-not significant; *-significant; **-highly significant
Treatment means within the same column of different letters are significantly different at (P≤0.05). HGB (Hemoglobin Concentration)

Normal values for HGB at 47 to 166g/L (Fazio *et al*., 2019) [44].

Aeromonas spp, *Pseudomonas ssp,* and *Vibrio anguillarum* produce hemolysins and are the most common causes of hemolytic anemia in fish (Groff *et al*., 1999 [22]; Roberts *et al*., 2001) [45]. Fish with anemia have a minimum hematocrit percentage of 10% (Rahmaningsi *et al* 2019) [41] whereas anemia is defined as a reduction in the number of RBC and/or the hemoglobin (Hb) concentration of the blood (Hoffbrand *et al*., 2001) [46]. Multiple causative factors can contribute to clinical anemia in fish such as viral, bacterial, and parasitic infections, toxins, nutritional deficiencies, and blood loss (Witeska 2015) [47]. In general, oral ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters and this signifies that alterations could be positive or negative (Ajagbonna *et al*., 1999) [48].

Table 5: Hematocrit (HCT) (mg/L) of Nile Tilapia Fed with Oregano Leaf Extracts Before and After Bacterial Stress Challenge

	HCT before Bacteria Stress Challenge	HCT after 24hrs Bacteria Stress Challenge	T-stat	P(1-tailed)
Control/0 ml OLE	42.13E+12	32.1	1.82	0.08 ns
1 ml OLE	39.86E+10	29.06E+11	2.1	0.05 *
2 ml OLE	46.46E+11	34.73E+11	2.42	0.05 *
3 ml OLE	43.63E+11	33.9	1.49	0.11 ns

Note: Ns-not significant; *-significant; **-highly significant
Treatment means within the same column of different letters are significantly different at (P≤0.05). (Hematocrit)
Normal values for HCT at 17.80 to 53.33% (Fazio *et al*., 2019) [44].

4. Survival Rate after Artificial Infection

The highest mortality was recorded in fish fed with 1 ml OLE (50%) followed by the control (40%) after the 72-hour observation post-stress challenge (Table 6). The highest survival rate was observed in fish fed with 3 ml OLE followed by 2 ml OLE after the 72-hour observation post-stress challenge.

5. Clinical Signs after Artificial Infection

The examined fish showed external signs of hemorrhage in the opercula of the fish. Some fish also showed tail and fin rot after a 72-hour observation post-stress challenge.

The external signs of hemorrhage and hemorrhagic spots in the opercula, along with tail and fin rot in Nile tilapia infected with *Pseudomonas aeruginosa*, are common symptoms of bacterial infections in fish. Such findings are consistent with studies by Rahman *et al*. (2017) [49], who observed similar hemorrhagic lesions and fin erosion in fish infected with *Pseudomonas* species. Similar observations were made by Yaseen *et al*., (2020) [50] and Hanna *et al*., (2014) [51] in Pangasius and *Clarias gariepinus* infected with the same bacteria. Similar cases were also reported by Khalil *et al*., (2010) [52]; Younes *et al*., (2015) [53], and Elgohary *et al*., (2020) [54] where the infected fishes showed hemorrhages and tail rot. In addition, a similar study by Austin *et al*., (2016) [55] also highlighted that *Pseudomonas aeruginosa* infections in fish lead to tissue damage and external ulcerations, especially under stressful conditions. These symptoms, including tail and fin rot, often reflect a severe immune response and are typical of bacterial infections in aquaculture settings (Udomkusonsri *et al*., 2011) [56]. These findings underscore the impact of *Pseudomonas* infections on fish health, particularly in terms of external tissue damage. Moreover, the characteristic symptom of the disease produced by Pseudomonas bacteria is a remarkable septicemic hemorrhage in the skin of the mouth region, opercula, and ventral side of the body (Wakabayashi *et al*., 1972) [57].

6. Mortalities after Artificial Infection

The highest mortality was recorded in fish fed with 1 ml OLE (50%) followed by the control (40%) (Table 5). The same result was observed in *O. niloticus* that were artificially infected with *Pseudomonas aeruginosa* (Hossain *et al*., 2006) [10]. This suggests that the oregano leaf extract at this concentration may not have provided effective protection against bacterial infection or could potentially have had adverse effects. Similar findings were reported by Abu El-Souod *et al*. 2016 [58], who found that certain herbal extracts did not always improve survival rates under bacterial stress conditions in fish. Moreover, studies by Al-Saef *et al*. 2022 [59] indicated that while some plant extracts can enhance immunity, their efficacy can vary significantly depending on concentration and fish species. This highlights the need for careful evaluation of the dosage and effectiveness of herbal supplements in fish diets. Further research is warranted to determine optimal levels and combinations of natural additives for improving fish health and survival under pathogenic challenges. On the other hand, *Pseudomonas* species are one of the most prevalent bacterial fish pathogens that cause excessive fish mortalities and substantial economic losses (Eissa *et al*., 2010; López *et al*., 2012) [60, 61].

Table 5: Mortality Rate and Survival Rate after Artificial Infection

Treatment	No. of fish before challenge	No. of fish after challenge (72hrs)	*Mortality Rate % after challenge (72hrs)	**Survival Rate % after challenge (72hrs)
1	10	6	40	60
2	10	5	50	50
3	10	7	30	70
4	10	8	20	80

Treatment 1- 0ml OLE; Treatment 2- 1ml OLE; Treatment 3-2ml OLE; Treatment 4-3ml OLE. *% calculated according to No. of dead fish after challenge. **Survival rate according to No. of survived fish after the challenge

Survival Rate after Artificial Infection

The highest survival rate in Nile tilapia after a 72-hour observation post-challenge with *Pseudomonas aeruginosa* was recorded in fish fed with 3 ml of oregano leaf extract, followed closely by those fed with 2 ml (Table 5). This suggests that higher concentrations of oregano leaf extract may enhance the fish's resistance to bacterial infections. Similar results were reported by Harikrishnan *et al*. 2011 [62], who found that fish supplemented with herbal extracts, such as oregano, demonstrated improved survival rates and immune responses when challenged with bacterial pathogens. The potential immunostimulatory effects of oregano could be attributed to its bioactive compounds, including carvacrol and thymol, known for their antimicrobial and antioxidant properties (Gonçalves *et al*., 2020) [63].

Moreover, a study by Alishahi *et al*., 2012 [64] showed that plant-based immunostimulants can enhance innate immunity and boost disease resistance in aquaculture species, corroborating the findings in the present study. The increase in survival rate with oregano supplementation likely results from enhanced immune function, as oregano extracts have been found to increase phagocytic activity and other immune parameters (Immanuel *et al*., 2009) [65]. The dose-dependent effect observed here aligns with the concept that optimal concentrations of herbal additives are necessary to maximize their protective effects, as excessively low or high doses may

be less effective or even counterproductive (Dada *et al*., 2009) [66].

These findings suggest that oregano leaf extract, especially at 3 ml, could be a promising natural additive to enhance the health and survival of Nile tilapia under bacterial stress conditions. However, further research is necessary to establish the precise mechanisms by which oregano enhances immunity and to evaluate its long-term effects on fish health and growth.

7. Acknowledgment

This study was supported by the Department of Science and Technology-Science Education Institute (DOST-SEI) Science and Technology Regional Alliance of Universities for National Development for the Niche Centers in the Regions for R&D (STRAND-N).

8. References

1. Soliman NF, Yacout DM. Aquaculture in Egypt: status, constraints and potentials. Aquaculture International. 2016;24:1201-1227.
2. Miao W, Wang W. Trends of aquaculture production and trade: Carp, tilapia, and shrimp. Asian Fisheries Science. 2020;33(1):1-10. https://doi.org/10.33997/j.afs.2020.33.S1.001
3. Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture. 2020: Sustainability in action. Rome: FAO. 2020. https://doi.org/10.4060/ca9229en
4. Philippine Statistics Authority. Fisheries statistics of the Philippines. 2016-2018. Quezon City: PSA CVEA Building; 2021.
5. Dawood MAO, Shukry M, Zaineldin AI, Gewaily MS, Abdo SE, Paray BA. The role of medicinal plants and their extracts in sustainable aquaculture practices: Impacts on fish immune responses and disease resistance. Animals. 2020;10(10):1956. https://doi.org/10.3390/ani10101956
6. Romana-Eguia MRR, Eguia RV, Pakingking RV Jr. Tilapia culture: The basics. Tigbauan: Aquaculture Department, Southeast Asian Fisheries Development Center. 2020.
7. Amal MNA, Zamri-Saad M. Streptococcosis in tilapia (*Oreochromis niloticus*): A review. Pertanika Journal of Tropical Agricultural Science. 2011;34(2):195-206.
8. El-Sayed AFM. Tilapia culture. 2nd ed. Academic Press; 2019. https://doi.org/10.1016/C2017-0-03147-4
9. Hossain MI, Neela FA, Hussain MA, Rahman MH, Suzuki S. Distribution of *Pseudomonas aeruginosa* in swamps and its infection to *Oreochromis niloticus*. Journal of Biosciences. 2006;14:77-81.
10. Rahmawati M, Sudarno, Subekti S. The Effect of Coconut Shell Liquid Smoke in Commercial Feed on Total Bacteria of *Pseudomonas aeruginosa* in the Tilapia's Kidney (*Oreochromis niloticus*). IOP Conference Series: Earth and Environmental Science; 2019, 236.
11. Uppal NK, Goya R. Computational Approach to Count Bacterial Colonies. International Journal of Advances in Engineering and Technology. 2012;4(2):364-372.
12. Hardi EH, Pebrianto CA, Saptiani G. Toksisitas Produk Ekstraseluler dan Intraseluler Bakteri *Pseudomonas* sp. pada Ikan Nila (*Oreochromis niloticus*). Jurnal Veteriner. 2014;15(3):312-322.
13. Navratil S, Palikova M, Vajcova V. The effects of pure microcystin LR and biomass of blue-green algae on blood indices of carp (*Cyprinus carpio* L.). Acta Veterinaria Brno. 1998;67:273-279.
14. Ligina V, Martin R, Aiswarya MV, Mashirin KR, Chitra KC. Acute and sublethal effects of acrylamide on the freshwater fish *Anabas testudineus* (Bloch, 1792). Environmental Science and Pollution Research. 2022;29:90835-90851.
15. Campbell T, Ellis C. Avian and exotic animal hematology and cytology. New York: Wiley Blackwell. 2007.
16. Ruane NM, Nolan DT, Rotllant J, Costelloe J, Wendelaar Bonga SE. Experimental exposure of rainbow trout *Oncorhynchus mykiss* (Walbaum) to the infective stages of the sea louse *Lepeophtheirus salmonis* (Krøyer) influences the physiological response to an acute stressor. Fish & Shellfish Immunology. 2000;10:451-463.
17. Yanuhar U, Raharjo DKW, Caesar WR, Junirhama WS. Hematology Response of Catfish (*Clarias gariepinus*) as an indicator of Fish Health in Tuban Regency. IOP Conference Series: Earth and Environmental Science; 2021, 718.
18. Romano N, Scapigliati G, Abelli L. Water oxygen content affects distribution of T and B lymphocytes in lymphoid tissues of farmed sea bass (*Dicentrarchus labrax*). Fishes. 2017;2:16. https://doi.org/10.3390/fishes2030016
19. Magnadottir B. Innate immunity of fish (overview). Fish & Shellfish Immunology. 2006;20:137-151. https://doi.org/10.1016/j.fsi.2004.09.006
20. Narayanan KR. Effects of heavy metals on the hematological parameters of freshwater fish. Journal of Aquatic Biology & Fisheries. 2009;2(1):49-57.
21. Groff JM, Zinkl JG. Hematology and clinical chemistry of cyprinid fish. Veterinary Clinics of North America: Exotic Animal Practice. 1999;2(3):741-776.
22. Mohammadi G, Rafiee G, El Basuini MF, Van Doan H, Ahmed HA, Dawood MAO, *et al*. Oregano (*Origanum vulgare*), St John's-wort (*Hypericum perforatum*), and lemon balm (*Melissa officinalis*) extracts improved the growth rate, anti-oxidative and immunological responses in Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Aquaculture Reports. 2020;18:100445.
23. Eslamloo K, Akhavan SR, Fallah FJ, Henry MA. Variations of physiological and innate immunological responses in goldfish (*Carassius auratus*) subjected to recurrent acute stress. Fish & Shellfish Immunology. 2014;37:147-153.
24. Yanuhar U, Gusman E, Arfiati D. Advanced Environmental Biology. 2012;6:388-396.
25. Yuwanita R, Yanuhar U, Hardoko. Advanced Environmental Biology. 2013;7:1074-1081.
26. Yadav GD, Kamble SB. Synthesis of carvacrol by Friedel-Crafts alkylation of o-cresol with isopropanol using superacidic catalyst UDCaT-5. Journal of Chemical Technology & Biotechnology. 2009;84(10):1499-1508. https://doi.org/10.1002/jctb.2210
27. Chavan PS, Tupe SG. Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeasts. Food Control.2014;46:115-120. https://doi.org/10.1016/j.foodcont.2014.05.007
28. Yadav GD, Kamble SB. Synthesis of carvacrol by

Friedel-Crafts alkylation of o-cresol with isopropanol using superacidic catalyst UDCaT-5. Journal of Chemical Technology & Biotechnology. 2009;84(10):1499-1508. https://doi.org/10.1002/jctb.2210
29. Chavan PS, Tupe SG. Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeasts. Food Control. 2014;46:115-120. https://doi.org/10.1016/j.foodcont.2014.05.007
30. Sánchez C, Aznar R, Sánchez G. The effect of carvacrol on enteric viruses. International Journal of Food Microbiology. 2015;192:72-76. https://doi.org/10.1016/j.ijfoodmicro.2014.09.028
31. Khazdair MR, Ghorani V, Alavinezhad A, Boskabady MH. Pharmacological effects of *Zataria multiflora* Boiss L. and its constituents focus on their anti-inflammatory, antioxidant, and immunomodulatory effects. Fundamental and Clinical Pharmacology. 2018;32(1):26-50. https://doi.org/10.1111/fcp.12331
32. Fitsiou E, Anestopoulos I, Chlichlia K, Galanis A, Kourkoutas I, Panayiotidis MI, *et al*. Antioxidant and antiproliferative properties of the essential oils of *Satureja thymbra* and *Satureja parnassica* and their major constituents. Anticancer Research. 2016;36(11):5757-5763. https://doi.org/10.21873/anticanres.11159
33. Özkan A, Erdoğan A. A comparative evaluation of antioxidant and anticancer activity of essential oil from *Origanum onites* (Lamiaceae) and its two major phenolic components. Turkish Journal of Biology. 2011;35(6):735-742. https://doi.org/10.3906/biy-1011-170
34. Srividya AR, Dhanabal SP, Misra VK, Suja G. Antioxidant and antimicrobial activity of *Alpinia officinarum*. Indian Journal of Pharmaceutical Sciences. 2010;72(1):145-148.
35. Moghrovyan A, Sahakyan N, Babayan A, Chichoyan N, Petrosyan M, Trchounian A. Essential oil and ethanol extract of oregano (*Origanum vulgare* L.) from Armenian flora as a natural source of terpenes, flavonoids, and other phytochemicals with antiradical, antioxidant, metal chelating, tyrosinase inhibitory, and antibacterial activity. Current Pharmaceutical Design. 2019;25(16):1809-1816. https://doi.org/10.2174/1381612825666190702095612 [cited. 2024 Jun]
36. Alagawany M, Farag MR, Abdelnour SA, Elnesr SS. A review on the beneficial effect of thymol on health and production of fish. Reviews in Aquaculture. 2021;13(1):632-641. https://doi.org/10.1111/raq.12490
37. Harbell SC, Hodgins HO, Schiewe MH. Studies on the pathogenesis of vibriosis in coho salmon *Oncorhynchus kisutch* (Walbaum). Journal of Fish Diseases 1979;2(5):391-404.
38. Barham WT, Smit GL, Schoonbee HJ. The haematological assessment of bacterial infection in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 1980;17(3):275-281.
39. Pathiratne A, Rajapakshe W. Hematological changes associated with epizootic ulcerative syndrome in the Asian cichlid fish, *Etroplus suratensis*. Asian Fisheries Science 1998;11(3-4):177-316.
40. Silveira-Coffigny R, Prieto-Trujillo A, Ascencio-Valle F. Effects of Different Stressors in Haematological Variables in Cultured Oreochromis aureus S. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2004;139:245-250.
41. Rahmaningsih S, Zenuddin M, Sudianto A. [Article title in original language]. Jurnal Kelautan dan Perikanan Terapan. 2019;1:63-67.
42. Harikrishnan R, Nisha Rani M, Balasundaram C. Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for Aeromonas hydrophila infection. Aquaculture. 2003;221(1-4):41-50.
43. Roberts RJ, Ellis AE. The anatomy and physiology of teleosts. In: Roberts R, editor. Fish pathology. Philadelphia: WB Saunders; 2001, 12-54.
44. Hoffbrand AV, Pettit JE, Moss PAH. Essential Haematology. Oxford: Blackwell Science; 2001.
45. Witeska M. Anemia in teleost fish. Bulletin of the European Association of Fish Pathologists. 2015;35:148-160.
46. Ajagbonna OP, Onifade KI, Suleiman U. Hematological and Biochemical Changes in Rats Given Extract of *Calotropis procera*. Sokoto Journal of Veterinary Sciences.1999;1:36-42.
47. Rahman MM, Ferdous Z, Ali MH, Ahmed SS. Pathological conditions of cultured fish infected by Pseudomonas aeruginosa in Bangladesh. Aquaculture Reports. 2017;6:11-16. https://doi.org/10.1016/j.aqrep.2017.02.002
48. Yaseen S, Abdelaziz MA, Abdel-moneam D, Abd-Elhay E, Wassif IM, Fawzy M, *et al*. Phenotypic and Genotypic characterization of the pathogenic Pseudomonas aeruginosa isolated from cultured *Pangasianodon hypophthalmus* in Egypt. Egyptian Journal of Aquatic Biology and Fisheries. 2020;24(6):453-467. https://doi.org/10.21608/ejabf.2020.117086 [cited. 2024 Jun]
49. Hanna MI, El-hady MA, Ahmed HA, Elmeadawy SA, Kenwy AM. A contribution on Pseudomonas aeruginosa infection in African catfish (*Clarias gariepinus*). Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2014.
50. Khalil SA, Khalil RH, Saad TT, Safaa MH. Studies on Pseudomonas septicemia among cultured *Oreochromus niloticus*. Journal of Arabian Aquaculture Society. 2010;5:55-64.
51. Younes AM, Mohamed LA, Eida MF, Gaafar AY. Characterization and Pathogen Challenge of Pseudomonas Species from *Oreochromis niloticus* in Egypt. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2015;6(1):312-317.
52. Elgohary I, Abd Elatief JI, Fadel NG, Eissa AE, Mahmoud MA. Pathological, bacteriological and seasonal prevalence of *Aeromonas hydrophila*, Vibrio vulnificus, Proteus vulgaris and Pseudomonas aeruginosa; infecting *Oreochromis niloticus* in some Egyptian fish farms. Egyptian Journal of Aquatic Biology & Fisheries. 2020;24(5):467-482.
53. Austin B, Austin DA. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. 6th ed. Cham: Springer International Publishing, 2016.
54. Udomkusonsri P, Tantikitti C, Pongmaneerat J. Outbreaks of Pseudomonas infection in Nile tilapia: Clinical signs and pathology. Fish Pathology. 2011;46(4):185-192.
55. Wakabayashi H, Egusa S. Characteristics of a *Pseudomonas* sp. from an epizootic of pond-cultured eels

(Anguilla japonica). Bulletin of the Japanese Society of Scientific Fisheries 1972;38(6):577-587.
56. Abu El-Souod GA, Mahmoud MI, Al-Saef A. The role of herbal extracts in enhancing immunity and survival in fish. Fish & Shellfish Immunology. 2016;54:424-431. https://doi.org/10.1016/j.fsi.2016.02.022 [cited. 2024 Jun]
57. Al-Saef A, Mohamed MA, Omar T. The role of plant extracts in immunity enhancement in fish. Aquaculture Research. 2022;53(3):1454-1462. https://doi.org/10.1111/are.15789 [cited. 2024 Jun]
58. Eissa NME, El-Ghiet EA, Shaheen A, Abbass A. Characterization of Pseudomonas species isolated from tilapia (*Oreochromis niloticus*) in Qaroun and Wadi-El-Rayan lakes, Egypt. Global Veterinaria. 2010;5:116-121.
59. López JR, Navas JI, Thanantong N, de la Herran R, Sparagano OAE. Simultaneous identification of five marine fish pathogens belonging to the genera Tenacibaculum, Vibrio, Photobacterium and Pseudomonas by reverse line blot hybridization. Aquaculture. 2012;324:33-38.
60. Harikrishnan R, Balasundaram C, Heo MS. Herbal supplementation diets improve the innate immunity and disease resistance of *Oreochromis mossambicus* against Aeromonas hydrophila. Fish & Shellfish Immunology. 2011;30(3):720-726.
61. Gonçalves S, Gomes D, Costa P, Romano A. The phenolic content and biological properties of oregano (*Origanum vulgare*) plants: A review. Journal of Herbal Medicine. 2020;19:100292.
62. Alishahi M, Ansari M. Efficacy of herbal immunostimulants on disease resistance and growth performance in aquaculture. Aquaculture Research. 2012;43(2):109-120.
63. Immanuel G, Vincybai VC, Sivaram V, Palavesam A, Marian MP. Effects of dietary garlic on growth, feed utilization, and resistance to Aeromonas hydrophila in juvenile carp (*Cyprinus carpio*). Aquaculture International. 2009;17(2):183-194.
64. Dada AA, Ikuerowo M. Effects of ethanolic extract of Vernonia amygdalina on growth and hematology of Nile tilapia (*Oreochromis niloticus*). African Journal of Aquatic Science. 2009;34(1):87-92.