**Effects of *Helianthus annuus* on Haematological and Biochemical Parameters in *Labeo rohita* (Hamilton, 1822) Fingerlings infected with *Aeromonas hydrophila***

**ABSTRACT**

In the present study, an attempt was made to appraise the curative potential of locally available plant *Helianthus annuus* leaf powder against *Aeromonas hydrophila,* a bacterial pathogen of *Labeo rohita.* The experimental feed was prepared using different concentrations of *H. annuus* leaf powder (0.5g, 1g, 1.5g, 2g and control) and the same were fed to *L. rohita* fingerlings infected with *A. hydrophila,* individual group wisefor 28 days. The effects of different concentrations of feed containing leaf powder of *H. annuus* on Haematological, Biochemical and Enzyme parameters in the blood of *L. rohita* fingerlings infected with *A. hydrophila* were studied. The results indicated that the parameters of the infected fish gradually improved from abnormal to normal levels at the cessation of the 28th day of experiment in the blood of fish. This change might be attributed to the intake of *H. annuus* leaf powder, containing feed. The results of the present investigation apparently suggested that the selected plant’s leaf powder had curative potential against *A. hydophila* infected *L. rohita* fingerlings. Therefore, it may be concluded that the *H. annuus* @ 2.0 g concentration in the diet of *L. rohita* fingerlings can be fed to cure the bacterial infection, particularly, *A. hydrophila.*

**Keywords:***Helianthus annuus, Labeo rohita, Aeromonas hydrophila,* Haematology.

**Introduction:**

The carps are considered as one of the most important aquaculture species worldwide in terms of production. They serve as a vital source of animal protein for billions of people globally. Both capture fisheries and aquaculture contribute to the livelihoods of more than 10% of the world's population (Anon. 2020). Fisheries and aquaculture are the fastest-growing industries in the World (Tacon, 2020). India is one of the major fish producing countries in the world employing over seven million persons in fishing and allied industries and contributing 60 crores annual1y to national income. They have been playing an important role in the economic development front on account of their contribution to food and nutritional security, national income, employment opportunities as well as generating livelihood options (Kumar and Shivani, 2014).

The average annual increase in global consumption of fish has outpaced population growth. Of the global animal protein consumption, 20% is met by fish suggesting the importance of fish in global food security and nutrition. India ranks second in global aquaculture production and Indian major carps (IMCs) contribute to more than 75% of its aquaculture economy (FAO, 2020). *Labeo rohita* (Rohu) is an IMC and among the top eleven finfish species produced in world aquaculture (FAO, 2020). It has high growth potential and is very popular regarding consumer preference. Hence, it is considered the most important freshwater species cultured in India. There are several species of aeromonads such as *Aeromonas hydrophila* that can infect fish and other aquatic animals and spread diseases to them (Abdella *et al*., 2017; Fernández-Bravo and Figueras, 2020).

Aquaculture industries are affected by certain major pathogens such as viruses (Gomez-Casado *et al*., 2011; Vega-Heredia *et al*., 2012), bacteria (Jacobs *et al*., 2009; Frans *et al*., 2011), fungi (Khoo, 2000; Ramaiah, 2006) parasites (Brooker *et al*., 2007; Guo and Woo, 2009) and other undiagnosed and emerging pathogens is now a primary constraint to the culture of many aquatic species, obstructing both economic and social development in many countries and a significant constraint on aquaculture production and trade (Smith, 2006). Disease outbreaks elevated the mortality rate and decrease production efficiency, causing high economic loss to the fish farmers (Madhuri *et al*., 2012; Verma and Gupta, 2015). Additionally, *A. hydrophila* is associated with Aeromonad red sore disease and epizootic ulcerative syndrome in carps and other fishes according to Barde (2023) underscoring its versatility as a pathogen in aquatic environments.

Most encountered health ailments of fishes in Assam include epizootic ulcerative syndrome, hemorrhagic septicemia, abdominal dropsy, fin and tail rot, and Popeye deformity (Saharia *et al*., 2021) caused mainly by *Aeromonas* spp., *Pseudomonas* spp., *Flavobacterium* spp., and *Edwardsiella* spp (Pękala-Safińska, 2018). In fish, the disease susceptible organs and tissues include skin, gills, fins, liver, kidney, spleen, and intestine (Wassif and Mohammed 2022). *Aeromonas* species are widely distributed throughout the world and one of the causative pathogens for a variety of fish, animal and humans in general and particularly aquaculture (Martínez-Murcia *et al*., 2005). *A. hydrophila* causes exophthalmia, fin rot, darkened and ulcerative lesions on the body and even severe bleeding (Hardi *et al*., 2016). Several *Aeromonas* sp., such as *A. hydrophila, A. sobria,* and *A. salmonicida,* are the causative agents of bacterial septicemia in aquaculture. The mortality rate caused by *Aeromonas* infection could be over 95 % (Zhan *et al*., 2004).

*Helianthus annuus*, commonly known as the sunflower, has shown significant antibacterial properties, especially in the essential oil derived from its receptacle (SEO). A recent study highlighted SEO's potent antimicrobial effects against a range of pathogens. The minimum inhibitory concentration (MIC) of SEO was determined to be 0.2 mg/mL for *Pseudomonas aeruginosa* and *Staphylococcus aureus*, 3.2 mg/mL for *Saccharomyces cerevisiae*, and 6.4 mg/mL for *Escherichia coli* and *Candida albicans* (Liu *et al*., 2020).

Although the earlier studies have been carried out on other parts of the sunflower for example, a study on sunflower bee pollen revealed antimicrobial activity against several bacterial strains, such as *Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella enterica,* and *Escherichia coli* (Fatrcová-Šramková *et al*., 2013). This indicates that various components of the sunflower plant might have antibacterial capabilities. The recent research has highlighted promising antibacterial activity in *H. annuus*, particularly in its essential oil and bee pollen, there is a scarcity of specific data on the antibacterial effects of sunflower leaf powder. The present investigation into leaf powder of *H. annuus* against *A. hydrophila* would be advantageous to fill this knowledge gap and offer more precise information for its potential uses. The increasing demand to minimize the use of synthetic antibiotics and chemotherapeutics in aquaculture has sparked a heightened interest in plant-based compounds as alternative methods to boost immune responses and enhance disease resistance in fish and shrimp (Chakraborty and Hancz, 2011; Naiel *et al*., 2023). Hence, the present study was aimed to evaluate the effects of dietary treatment of *H. annuus* leaf powder on Haematological, Biochemical and Enzyme parameters of *L. rohita* fingerlings challenged with *A. hydrophila* for the experimental period of 28 days.

**MATERIALS AND METHODS**

**Collection of Fish Fingerlings**

Freshwater fish fingerlings (12 ± 4 g) of *Labeo rohita* (Rohu) were collected from Goldfish Farms at Valamburi Street, Karanthai, Thanjavur District, Tamil Nadu. They were acclimatized to laboratory conditions for a month and later they were artificially infected with *A. hydrophila* pure culture obtained from K.A.P. Viswanathan Government Medical College, Tiruchirappalli, Tamil Nadu. The artificially infected fish fingerlings had six different types of disease symptoms and the same are depicted in Fig. 1. Then the feeding trials of feed containing leaf powder of *H. annuus* were started.

**Fig. 1: *L. rohita* fingerlings with various disease symptoms after *A. hydrophila* infection**

**a. Ulcer on the skin/scales**

**b. Exophthalmia and curved fins**

**d. Damaged Gill with change in colour**

**c. Epizootic Ulcerative Syndrome (Red spot Disease)**

**e. Ulcer on dorsal head with damaged eye *i.e.,* Exophthalmia**

**f. Fin rot /Tail rot Scale loss and damaged eye *i.e.,* Exophthalmia**



**Physiochemical parameters of water**

The methods suggested by APHA (1998) were adopted for the estimation of water quality characteristics and the results thereof are provided.

**Preparation of experimental diets**

The ingredients used for the preparation of experimental feeds were Fish meal, Rice bran, Maize, Soybeans, Groundnut oil cake, Wheat flour, Sunflower seed, Cassava flour, Salt, Vitamin and Minerals procured from the local market. The proportion of these ingredients in various concentrations of feed are provided in Table 1. The experimental groups were designated as T1, T2, T3, T4 and T5 and they were fed with prepared fish diet. The respective experimental fish fingerlings’ groups were fed with the feed prepared by incorporating *H. annuus* leaf powder at 0.5g (T2), 1g (T3), 1.5g (T4), 2g (T5) and T1 control (without leaf powder), individually. Diseased *L. rohita* fingerlings were distributed randomly into five groups each with ten fishes in each group. These fishes were fed with the feed prepared at four concentrations and observed at 7, 14, 21 and 28 days for any mortality or clinical signs or signs of recovery from infection. A control group was also maintained to make a comparison with the experimental fish groups. Further, all these experimental groups were compared with all these values obtained from normal (healthy fish fingerlings) animals.

**Table 1: The ingredients used for the preparation of experimental feed and their proportion**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ingredients** | **Treatment I**  **(Control)** | **Treatment II**  **(0.5 g)** | **Treatment III**  **(1.0 g)** | **Treatment**  **IV**  **(1.5 g)** | **Treatment V**  **(2.0 g)** |
| Fish meal | 30g | 30g | 30g | 30g | 30g |
| Rice bran | 12g | 11.5g | 11g | 10.5g | 10g |
| Mice | 15g | 15g | 15g | 15g | 15g |
| Soyabeans | 10g | 10g | 10g | 10g | 10g |
| Groundnut oil cake | 10g | 10g | 10g | 10g | 10g |
| Wheat flour | 10g | 10g | 10g | 10g | 10g |
| Sunflower seed | 5g | 5g | 5g | 5g | 5g |
| (Cassava flour) | 5g | 5g | 5g | 5g | 5g |
| Salt | 1g | 1g | 1g | 1g | 1g |
| Vitamin and Minarals | 2g | 2g | 2g | 2g | 2g |
| *Helianthus annuus* powder | - | 0.5g | 1.0g | 1.5g | 2.0g |
|  | 100g | 100g | 100g | 100g | 100g |

**Collection of Blood**

Blood was collected from the experimental fishes individually, immediately after their capture from tanks by severing the caudal peduncle as suggested by Michael *et al.,* (1994) once at weekly intervals *i.e.,* 7, 14, 21 and 28 days. A portion of blood collected was used to separate the serum for biochemical estimation of the same and the same was stored in the refrigerator for further analysis.

**Haematological parameters**

RBC and WBC counts were determined by the method of Armour *et al.* (1965). The differential Leucocytes count was enumerated by following the method of Sardar *et al.* (2001). The different leucocytes like neutrophils, basophils, eosinophils, lymphocytes and monocytes were identified, counted and expressed in percentage and they were tabulated. Dethloff *et al*. (1999) method was adopted for the estimation of Heamoglobin (g/dl) and the results were recorded.

**Serum Biochemical Analysis**

Biochemical parameters such as Total serum protein, serum albumin, serum globulin and they were quantified by adopting the Biuret method (Johnson *et al.,* 1999). Trinder (1969) method was adopted for Serum glucose estimation. Serum cholesterol was analyzed by the cholesterol oxidase – peroxidase (CHOD- POD) method suggested by Allian *et al.,* (1974). Serum triglycerides level was estimated by the method of glycerol phosphate oxidase – peroxidase (GPO - POD) as described by Trinder *et al.,* (1969). Serum uric acid of the experimental fishes was diagnosed by the method of urease/POD as prescribed by Newman (1999).

**Estimation of Enzymes**

The amount of Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were estimated by the modified AMP method of Thomas (1998) and Burtis and Ashwood (1999). All Biochemical estimations were performed in Star Plus Semi Auto Analyser. Haematological changes in the control and extract treated *L. rohita* fish were analyzed by Auto Hematology Analyzer (Mindray BC-2800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

**Statistical analysis**

The values obtained in the present investigation in triplicates were converted into Mean ± Standard Error values and the same are presented in Tables. The data collected on haematology, biochemistry and enzyme parameters were subjected to one-way Analysis of Variance (ANOVA) followed by SNK: Student -Newman Keuls post hoc test for comparing the impact of different concentrations of leaf powder of *H. annuus* and exposure days on the *L. rohita* fingerlings infected with *A. hydrophila* at 0.05 level. All these analyses were done by using SPSS (Statistical Package for Social Sciences) program version 16.0 for windows.

**RESULTS**

The results of physico-chemical parameters of fish tank water and tap water are provided in Table 2

**Table 2: Physico-chemical parameters of the fish tank water and tap water**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Parameters** | **Fish tank water**  **Mean ± S.E. (*n*=3)** | **Tap water**  **Mean ± S.E. (*n*=3)** |
| 1 | Temperature (oC) | 22.43±0.12 | 25.33±0.15 |
| 2 | pH | 6.46±0.07 | 6.77±0.07 |
| 3 | Total alkaloids (mg/l) | 623.33 ±14.53 | 224±2.31 |
| 4 | Total solids (mg/l) | 1953.9±0.76 | 517.6±0.89 |
| 5 | Total dissolved solids (mg/l) | 1277.7±0.49 | 876.33±0.73 |
| 6 | Calcium (mg/l) | 43.13±0.55 | 30.65±0.79 |
| 7 | Magnesium (mg/l) | 556.34±0.98 | 196.38±0.68 |
| 8 | Chloride (mg/l) | 0.02±0.00 | 0.02±0.00 |
| 9 | Carbonate (mg/l) | 16.10±0.06 | 18.20±0.12 |
| 10 | Bicarbonate (mg/l) | 629.94±0.71 | 389.33±0.63 |
| 11 | BOD (mg/l) | 8.14±0.02 | 6.24±0.14 |
| 12 | Dissolved oxygen | 6.5±0.12 | 6.7±0.15 |

The impact of different concentrations of feed consisting of leaf powder of *H. annuus* on total RBC, WBC and Haemoglobin content of *L. rohita* fingerlings infected with *A. hydrophila* is presented in Table 3. It is obvious from the results that the control group (Treatment I) had significantly lowest Total RBC, Total WBC and Haemoglobin during all through the four weeks period when compared to other experimental groups. However, in the treatment groups (II-V) a significant increase was observed in the Total RBC, Total WBC and Haemoglobin count as compared to Control (ANOVA; p<0.05) and this might be due to the intake of *H. annuus* leaf powder containing feed (Table 3). There was a significant difference between all the experimental groups in respect of total RBC, total WBC and Haemoglobin count except Treatment V and Treatment I (SNK Test, p<0.05) (Table 3). The blood extracted from the *L. rohita* fed with the diet consisting of 0.5g of *H. annuus* leaf powder on the 7th day of exposure had the lowest RBC (2.33±0.09106/mm3), WBC (13.76±0.20 103/mm3) and Haemoglobin (5.23±0.12g/dl), respectively. On the other hand, the blood obtained from the fish fed with a diet containing 2.0g of *H. annuus* leaf powder on the 28th day of exposure had the highest number of RBC *i.e.,* 3.27±0.09(106/mm3), WBC *i.e.,* 20.65±0.19 (103/mm3) and Haemoglobin (9.17±0.09g/dl) and it is

almost similar to normal (healthy fish values) total RBC, total WBC and Haemoglobin count (Table 3).

**Table 3: Blood parameters of *L. rohita* (infected with *A.hydrophila* – Treatments I - V) fed with various concentrations of diet containing leaf powder of *H. annuus***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Blood parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment IV (1.5 g)** | **Treatment V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | |
| **RBC 106/mm3** | 7 Days | 3.06±0.01a | 2.20±0.12abcd | 2.33±0.09abc | 2.33±0.07a | 2.37±0.03a | 2.43±0.03ab |
| 14 Days | 3.14±0.02bc | 2.20±0.06abcd | 2.37±0.07abc | 2.43±0.03bc | 2.63±0.09bc | 2.67±0.09ab |
| 21 Days | 3.17±0.01bc | 2.23±0.09abcd | 2.50±0.06abc | 2.57±0.09bc | 2.80±0.06bc | 3.03±0.07cd |
| 28 Days | 3.29±0.04d | 2.50±0.06abcd | 2.70±0.06cd | 2.83±0.07d | 3±0.06cd | 3.27±0.09cd |
| **WBC 103/mm3** | 7 Days | 21.62±0.19a | 12.70±0.13a | 13.76±0.20a | 14.93±0.20a | 15.65±0.18ab | 16.60±0.20a |
| 14 Days | 22.41±0.15bc | 13.94±0.14b | 14.67±0.13b | 15.69±0.17b | 17.67±0.19ab | 18.67±0.15b |
| 21 Days | 23.61±0.18bc | 15.79±0.09c | 16.79±0.11c | 17.76±0.15cd | 18.58±0.19c | 19.64±0.20c |
| 28 Days | 22.62±0.17d | 16.69±0.20d | 17.70±0.14d | 18.66±0.11cd | 19.74±0.13d | 20.65±0.19d |
| **HB g/dl** | 7 Days | 9.04±0.01a | 4.9±0.05abc | 5.23±0.12a | 5.44±0.07a | 5.80±0.06a | 6.07±0.09a |
| 14 Days | 9.11±0.01bc | 5.4±0.15abcd | 5.43±0.67b | 5.87±0.88b | 6.67±0.88b | 7.67±0.88b |
| 21 Days | 9.12±0.02bc | 5.53±0.88abcd | 5.77±0.88c | 6.50±0.58c | 7.63±0.88c | 8.37±0.88c |
| 28 Days | 9.18±0.01d | 5.83±0.03abcd | 7.53±0.88d | 8.03±0.03d | 8.50±0.06d | 9.17±0.09d |

**RBC - Red Blood Cell, WBC- White Blood Cell, HB- Haemoglobin**

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05)**

The effect of different concentrations of feed consisting of leaf powder of *H. annuus* on different leucocytes of *L. rohita* fingerlings infected with *A. hydrophila* is given in Table 4. The various leucocytes count was found to be lower in the blood extracted from the control group *i.e.,* Treatment I throughout the experimental period than the other Treatment groups (II-V).It is evident from the results, statistically, a significant increase was observed in basophils, eosinophils, lymphocytes, monocyte and neutrophils count of *L. rohita* fingerlings collected from Treatment V (ANOVA, p<0.05; vide Table 4). The observed increase in basophils, eosinophils, lymphocytes, monocyte and neutrophils count was statistically different between Treatment I and Treatment IV (SNK Test, p<0.05; vide Table 4). The basophils, eosinophils, lymphocytes, monocyte and neutrophils count ranged from 2.33±0.33%,1.33±0.33%, 17.33±0.33%, 4±0.00% and 2.67±0.58% (on the 7th day of exposure at 0.5g concentration level) to 4.33±0.33%, 4.33±0.33%, 26.33±1.20%, 9.33±0.33% and 6.33±0.33% (on the 28th day of exposure at 2.0g of concentration), respectively*.* The basophils, eosinophils, lymphocytes, monocyte and neutrophils count in the present investigation was gradually increased during different exposure days and at treatment groups and almost reached normal (close to healthy fish) values in the blood of *L. rohita* fingerlings (fed with the diet consisting of 2.0g of *H. annuus* leaf powder) at the end of the 28th day.

**Table 4: Differential leucocytes count of *L. rohita* (infected with *A.hydrophila* – Treatments I - V) fed with various concentrations of diet containing leaf powder of *H. annuus.***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Blood parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment**  **II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment IV (1.5 g)** | **Treatment**  **V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| **Basophils %** | 7 Days | 3.67±0.33ab | 2±0.00abc | 2.33±0.33abcd | 2.67±0.33abcd | 3±0.00abc | 3.33±0.33ab |
| 14 Days | 4±0.00abc | 2.33±0.33abc | 2.67±0.33abcd | 3±0.00abcd | 3.33±0.33abcd | 3.67±0.33abcd |
| 21 Days | 4.67±0.33bcd | 2.67±0.33abc | 3±0.00abcd | 3.33±0.33abcd | 3.67±0.33abc | 4±0.00bcd |
| 28 Days | 5±0.00cd | 3±0.00d | 3.33±0.33abcd | 3.67±0.33abcd | 4±0.00bcd | 4.33±0.33bcd |
| **Eosinophils %** | 7 Days | 4±0.00ab | 1.33±0.67abc | 1.33±0.33abcd | 1.67±0.33abc | 2±0.00a | 2.33±0.33a |
| 14 Days | 4.67±0.33abcd | 1.67±0.33abc | 2±0.00abcd | 2.33±0.33abcd | 2.67±0.33bcd | 3.33±0.33bc |
| 21 Days | 5±0.00bcd | 2±0.00abcd | 2.33±0.33abcd | 2.67±0.33abcd | 3.33±0.33bcd | 3.67±0.33bc |
| 28 Days | 5.33±0.33bcd | 2.33±0.33cd | 2.67±0.33abcd | 3.33±0.33bcd | 3.67±0.33bcd | 4.33±0.33d |
| **Lymphocytes %** | 7 Days | 26.45±0.17a | 15.67±0.33abc | 17.33±0.33abcd | 19.67±0.33a | 22±1.00a | 22.33±0.88 a |
| 14 Days | 27.62±0.15b | 16.33±0.67abc | 18.33±0.88abcd | 21±1.16bcd | 24.67±1.20b | 24.67±1.76bcd |
| 21 Days | 27.44±0.07c | 15.33±0.33abc | 17±0.58abcd | 20.33±0.88bcd | 23±1.16cd | 25±0.58bcd |
| 28 Days | 28.05±0.09d | 15.33±0.88d | 19.33±0.88abcd | 23.33±1.20bcd | 24.67±0.88cd | 26.33±1.20bcd |
| **Monocytes %** | 7 Days | 9.29±0.12a | 3.67±0.33abc | 4±0.00ab | 4.33±0.33a | 4.67±0.33a | 5.67±0.33ab |
| 14 Days | 8.90±0.03bc | 4±0.00abc | 4.33±0.33ab | 5.33±0.33bc | 6±0.00bc | 6.67±0.33ab |
| 21 Days | 9.61±0.10bc | 4.67±0.33abc | 5.33±0.33c | 6±0.00bc | 6.67±0.33bc | 7.67±0.33bc |
| 28 Days | 9.61±0.10cd | 5.67±0.33d | 7.33±0.33d | 7.67±0.33d | 8.33±0.33d | 9.33±0.33d |
| **Neutrophils %** | 7 Days | 6.50±0.06a | 2.33±0.33abcd | 2.67±0.58abcd | 3±0.58acb | 3.33±0.33abcd | 3.67±0.33a |
| 14 Days | 6.53±0.23bc | 2.67±0.33abcd | 3.33±0.33abcd | 3.67±0.33acb | 4.33±0.88abcd | 4.67±0.33bc |
| 21 Days | 6.90±0.06bc | 3.33±0.33abcd | 3.67±0.33abcd | 4±0.58acb | 4.67±0.33abcd | 5.67±0.33bc |
| 28 Days | 7.47±0.07d | 3.67±0.33abcd | 4±0.58abcd | 4.33±0.33cbd | 5.33±0.33abcd | 6.33±0.33cd |

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05).**

The results of the present study revealed that the biochemical parameters (Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid) in the Treatment I (Control Group) was comparatively lower than that of the other treatment groups *i.e.,* II-V. A significant increase could be observed in the Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid content in the blood of *L. rohita* fingerlings of all other Treatment Groups as compared to the Treatment I (Control Group) (ANOVA, p<0.05; vide Tables 5-6). The difference in Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid content between control and experimental groups was found to be statistically significant (at four different concentrations and exposure days) except Treatment III and Treatment IV (SNK Test, p<0.05; vide Tables 5-6). The blood extracted from *L. rohita* fingerlings fed with diet containing 2.0g of *H. annuus* leaf powder (on the 28th day of the experiment) had the highest amount of Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid content 2.29±0.06g/dl, 2.12±0.04mg/dl, 2.39±0.02g/dl, 106.81±0.03g/dl, 129.65±0.53mg/dl, 236.73±1.55mg/dl and 0.98±0.01mg/dl, respectively. On the other hand, the blood obtained from *L. rohita* fingerlings fed with a diet containing 0.5g of *H. annuus* leaf powder (on the 7th day of exposure) had the lowest amount of Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid content 1.67±0.01g/dl, 1.36±0.03mg/dl, 0.99±0.00g/dl, 79.88±0.01g/dl, 74.58±0.02mg/dl, 198.21±0.92mg/dl and 0.24±0.02mg/dl, respectively (Tables 5-6)*.* It is apparent from the results of the present investigation, at the cessation of the 28th day of an experiment, the biochemical parameters almost reached to normal levels where the *L. rohita* fingerlings fed were with diet consisting of 2.0g of *H. annuus* leaf powder and it indicated that the fingerlings recovered from *A. hydrophila* infection.

**Table 5: Biochemical parameters of *L. rohita* fingerlings (infected with *A. hydrophila*) fed with various concentrations of diet containing leaf powder of *H. annuus***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Biochemical**  **Parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment IV (1.5 g)** | **Treatment**  **V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| **Total Protein g/dl** | 7 Days | 2.56±0.05abcd | 1.56±0.01a | 1.67±0.01ab | 1.75±0.02a | 1.86±0.02a | 1.94±0.02abc |
| 14 Days | 2.74±0.03abcd | 1.63±0.03bc | 1.75±0.02ab | 1.85±0.02b | 1.94±0.01b | 1.99±0.01abc |
| 21 Days | 2.64±0.04abcd | 1.67±0.01bc | 1.83±0.02c | 1.97±0.01cd | 1.98±0.01cd | 2.07±0.01abc |
| 28 Days | 2.75±0.06abcd | 1.79±0.00d | 1.93±0.01d | 1.97±0.01cd | 2.08±0.01cd | 2.29±0.06d |
| **Albumin mg/dl** | 7 Days | 2.20±0.04ab | 1.34±0.03abcd | 1.36±0.03a | 1.46±0.01a | 1.55±0.02a | 1.56±0.02a |
| 14 Days | 2.27±0.02ab | 1.37±0.01abcd | 1.47±0.01b | 1.53±0.01b | 1.71±0.01b | 1.77±0.01b |
| 21 Days | 2.37±0.01cd | 1.43±0.00abcd | 1.70±0.01c | 1.77±0.15c | 1.89±0.04dc | 1.96±0.01c |
| 28 Days | 2.42±0.02cd | 1.43±0.02abcd | 1.79±0.04d | 1.88±0.17d | 1.94±0.17dc | 2.12±0.04d |
| **Globulin g/dl** | 7 Days | 2.14±0.01a | 0.88±0.01a | 0.99±0.00a | 1.30±0.04a | 1.68±0.01a | 1.78±0.01a |
| 14 Days | 2.19±0.01b | 0.97±0.02b | 1.46±0.01b | 1.99±0.00c | 1.79±0.00b | 1.97±0.01bc |
| 21 Days | 2.28±0.01c | 1.14±0.03cd | 1.65±0.02c | 1.90±0.01b | 2.11±0.03c | 1.98±0.04bc |
| 28 Days | 2.42±0.01d | 1.18±0.01cd | 1.98±0.01d | 2.10±0.03d | 2.25±0.05d | 2.39±0.02d |
| **Glucose g/dl** | 7 Days | 116.39±0.2a | 77.69±0.00a | 79.88±0.01a | 81.17±0.02c | 83.70±0.03b | 85.36±0.02a |
| 14 Days | 116.46±0.1bcd | 88.97±0.01b | 90.32±0.03b | 93.39±0.08a | 95.26±0.10a | 99.87±0.01b |
| 21 Days | 116.48±0.13bcd | 92.82±0.01c | 95.77±0.01c | 99.31±0.04d | 102.68±0.11c | 106.50±0.24cd |
| 28 Days | 116.93±0.02bcd | 94.96±0.02d | 96.99±0.00d | 99.80±0.03b | 104.29±0.05d | 106.81±0.03cd |

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05)**

**Table 6: Biochemical parameters of *L. rohita* fingerlings (infected with *A. hydrophila*) fed with various concentrations of diet containing leaf powder of *H. annuus***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Biochemical**  **Parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment**  **IV (1.5 g)** | **Treatment**  **V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| **Cholesterol mg/dl** | 7 Days | 133.33±0.13a | 72.27±0.03a | 74.58±0.02a | 78.50±0.16a | 80.40±0.04ba | 85.20±0.04a |
| 14 Days | 133.74±0.04bcd | 75.76±0.12b | 76.76±0.02b | 78.36±0.02b | 86.27±0.01ba | 90.72±0.08cb |
| 21 Days | 133.85±0.02bcd | 86.89±0.02c | 88.59±0.15c | 90.72±0.08c | 95.64±0.09c | 100.46±0.02cb |
| 28 Days | 134.28±0.32bcd | 90.15±0.17d | 96.81±0.22d | 108.32±1.20d | 114.88±2.14d | 129.65±0.53d |
| **Triglycerides mg/dl** | 7 Days | 240.67±0.22cabd | 193.80±0.74ab | 198.21±0.92a | 201.72±1.86a | 219.36±0.94bac | 226.55±2.55cab |
| 14 Days | 242.71±0.20cabd | 197.23±0.99ab | 201.47±0.75b | 210.63±1.19bc | 217.43±1.27bac | 228.47±0.90cab |
| 21 Days | 244.57±0.17cabd | 198.54±1.33bcd | 209.79±0.96c | 214.07±2.82bc | 218.20±1.59bac | 231.67±1.99cab |
| 28 Days | 245.65±0.15cabd | 203.05±1.99bcd | 220.87±1.33d | 225.12±2.81d | 233.47±1.86d | 236.73±1.55bd |
| **Uric acid mg/dl** | 7 Days | 1.05±0.02abcd | 0.23±0.01a | 0.24±0.02ab | 0.28±0.01a | 0.31±0.02a | 0.47±0.01a |
| 14 Days | 1.09±0.02abcd | 0.26±0.01bc | 0.29±0.01ab | 0.37±0.01b | 0.49±0.00b | 0.68±0.01b |
| 21 Days | 1.09±0.02abcd | 0.31±0.02bc | 0.49±0.10c | 0.58±0.00c | 0.85±0.03dc | 0.77±0.01c |
| 28 Days | 1.11±0.02abcd | 0.38±0.01d | 0.78±0.01d | 0.84±0.02d | 0.96±0.02dc | 0.98±0.01d |

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05).**

It is understood from the results that the effect of different concentrations of feed consisting of leaf powder of *H. annuus* on various enzymatic parameters *viz.,* Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) of blood collected from the control group (Treatment I) had comparatively lower amount than the other treatment groups (Treatments II-V). The quantity of AST, ALT, ACP and ALP was found to be significantly higher in the blood extracted from *L. rohita* fingerlings of all treatment groups than the Treatment I (Control group) (ANOVA, p<0.05; vide Table 7). The difference in Alkaline Phosphatase between all experimental groups was found to be statistically significant (at four different concentrations and exposure days) (SNK test, p<0.05; vide Table 7)*.* The blood extracted from the *L. rohita* fed with the diet consisting of 0.5g of *H. annuus* leaf powder on the 7th day of exposure had the lowest amount of AST, ALT, ACP and ALP (19.67±0.11 IU/L, 27.89±0.02 IU/L, 0.38±0.06 IU/L and 18.74±0.04 IU/L, respectively).Contrary to this, the blood obtained from the fish fed with diet containing 2.0g of *H. annuus* leaf powder on the 28th day of exposure had the highest amount of AST, ALT, ACP and ALP (29.98±0.01 IU/L 39.47±0.15 IU/L, 1.07±0.04 IU/L and 33.36±0.12 IU/L, respectively). It is obvious from the results that at the cessation of the 28th day of the experiment, all the four enzyme parameters studied in the blood of *L. rohita* fingerlings infected with *A. hydrophila* gradually increased and attained normal values and this might be due to the intake of *H. annuus* leaf powder containing feed (Table 7).

**Table 7: Enzymatic parameters of *L. rohita* (infected with *A. hydrophila* – Treatments I - V) fed with various concentrations of diet containing leaf powder of *H. annuus***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Blood parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment IV (1.5 g)** | **Treatment**  **V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| AST (IU/L) | 7 Days | 35.57±0.24 b | 18.43±0.06a | 19.67±0.11a | 20.64±0.18a | 22.59±0.09a | 23.52±0.07a |
| 14 Days | 33.53±0.19c | 19.40±0.11b | 21.27±0.09bc | 23.78±0.01b | 25.63±0.11b | 26.85±0.04cb |
| 21 Days | 34.67±0.10 a | 19.92±0.03c | 21.63±0.04bc | 24.92±0.03c | 26.85±0.05d | 28.53±012cb |
| 28 Days | 36.64±0.24d | 20.17±0.05d | 22.66±0.33d | 26.69±0.29d | 27.83±0.07c | 29.98±0.01d |
| ALT (IU/L) | 7 Days | 47.27±0.58c | 26.41±0.00acb | 27.89±0.02a | 29.33±0.09a | 31.37±0.09a | 32.83±0.05a |
| 14 Days | 46.67±0.16bad | 26.93±0.47acb | 28.84±0.07b | 30.85±0.05b | 32.69±0.15b | 33.66±0.10bc |
| 21 Days | 44.83±0.06bad | 26.92±0.02acb | 29.79±0.16c | 31.85±0.04d | 33.78±0.10c | 34.84±0.03bc |
| 28 Days | 47.57±0.14bad | 27.96±0.01d | 30.68±0.09d | 32.39±0.09c | 35.72±0.11d | 39.47±0.15d |
| ACP (IU/L) | 7 Days | 1.47±0.01abcd | 0.27±0.01a | 0.38±0.06a | 0.47±0.01a | 0.59± 0.01a | 0.65±0.02a |
| 14 Days | 1.47±0.01abcd | 0.35±0.01b | 0.62±0.02b | 0.66±0.02b | 0.75±0.01b | 0.88±0.01b |
| 21 Days | 1.48±0.00abcd | 0.49±0.01c | 0.76±0.01c | 0.80±0.01c | 0.87±0.02cd | 1.01±0.03c |
| 28 Days | 1.5±0.01abcd | 0.56±0.02d | 0.94±0.02d | 0.97±0.01d | 1.04±0.16cd | 1.07±0.04d |
| ALP (IU/L) | 7 Days | 33.89±0.03cb | 17.57±0.17a | 18.74±0.04a | 19.60±0.01a | 23.37±0.28a | 21.62±0.13a |
| 14 Days | 32.74±0.13cb | 18.58±0.34b | 20.85±0.03b | 23.30±0.12b | 25.69±0.0b | 29.78±0.02b |
| 21 Days | 32.71±0.09da | 19.55±0.08c | 24.74±0.06c | 27.52±0.14c | 29.52±0.16c | 30.78±0.04c |
| 28 Days | 33.57±0.16da | 20.54±0.18d | 28.52±0.07d | 29.60±0.22d | 32.37±0.12d | 33.36±0.12d |

**AST - Aspartate Aminotransferase, ALT- Alanine Aminotransferase,** [**ACP- Acid phosphatase, ALP- Alkaline Phosphatase**](https://labtestsonline.org/tests/alkaline-phosphatase-alp)

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05).**

**Discussion**

Water quality is directly related to fish production, and it is essential to a healthy, balanced, and functioning of aquaculture system (DeLong *et al.,* 2009 and Bryan *et al.,* 2011).  Bryan *et al.* (2011) reported that an ideal pH range for freshwater aquaculture should range between 6.5 and 7.0, though a pH range of 6.1 to 8.0 is also considered satisfactory for the survival and reproduction of fish. Ngugi *et al.* (2007) gave a range from 20°C to 35°C as ideal for tilapia culture. According to Riche and Garling (2003), the preferred DO for optimum growth of tilapia is above 5 mg/L. The results of the present work are in conformity with these earlier observations.

Recent research has shown that haematological parameters in fish are strongly linked to their reactions to environmental changes. These parameters are important indicators of the health of fish and can signal alterations in their surroundings (Cazenave *et al*., 2005; Parrino *et al*., 2018). Over the past few years, infectious diseases caused by *A. hydrophila* have become a major problem in fish culture causing heavy economic losses because of high mortalities. Plant-based extracts have been proven to enhance survival and immunocompetence in cultured fish. Several plant extracts that have active ingredients and various biological activities have been reported as suitable for use as supplements in aquaculture (Citarasu 2010, Madhuri *et al*., 2013, Chakraborty *et al*., 2014, Sivasankar *et al*., 2015, Syahidah *et al*., 2015).

It is apparent from the results of the present investigation that the selected plant had curative potential against *A. hydophila* infected *L. rohita* fingerlings. The present findings agree with the earlier results of Abutbul *et al*., (2004) in Tilapia fish fed with a diet containing ethyl acetate extract of *Rosmarinus officinalis* leaf powder. Rao *et al*., (2006) reported the disease resistance against *A. hydrophila* was enhanced in *L. rohita* fed with 0.5% of *A. aspera*.

The haematological values such as RBC and WBC count and haemoglobin obtained in the present study almost in agreement with earlier findings of Sahu *et al.* (2007). According to the results, *H. annuus* leaf powder could increase hemoglobin content, WBC and RBC levels in experimental groups compared to control group. In accordance with the present findings, Sahu *et al.,* (2007)reported that WBC and RBC counts were higher in *L.* *rohita* fingerlings fed with *Mangifera indica* kernel when compared to control. The hematological parameters in the present investigation such as RBC, WBC, hemoglobin and differential leukocytes counts increased significantly during every week after feeding with *H. annuus* leaf powder when compared to control group.

Nya and Austin (2009) observed and reported that increased levels of total erythrocytes, total leucocytes, lymphocytes, monocytes, serum total protein, globulin, phagocytic activity, superoxide anion production (respiratory burst activity) serum lysozyme activity, serum bactericidal activity, serum alternate complement pathway activity in *L.* *rohita* infected with *A. hydrophila* after being fed with dietary *Zingiber officinale* for 14 days. The result of the present study corroborates with the results of Nya and Austin (2009).

Similarly, enhanced serum total protein, albumin and globulin values could be recorded in the experimental groups of *L. rohita* fingerlings fed with *A. aspera* seed and *M. indica* kernel (Rao *et al.,* 2006 and Sahu *et al.,* 2007). The results of the present study obviously demonstrate that the total protein, albumin and total globulin levels increased significantly in fish following feeding of medicinal plant mixed diet. The glucose, triglyceride, ALT, AST and ALP of *L.* *rohita* were not influenced by different levels of *H. annuus* leaf powder. The herbal plants may be used as a potential and promising sources of pharmaceutical agents against fish pathogens in organic aquaculture according to Abdul and Haniffa, (2011) and Turker *et al*., (2009**)**.

**Conclusions**

The results of our findings suggest that the haematological and biochemical parameters of the *L. rohita* fingerlings infected with *A. hydrophila* were significantly recovered after being fed with different concentrations of *H. annuus* leaf powder employed in this study. Plants are undoubtedly considered as important sources of potentially useful chemical compounds for the development of new therapeutic agents against fish pathogens. It is apparent from the results of the present investigation that the leaves of *H. annuus* had curative potential against *A. hydophila* infected *L. rohita* fingerlings. Undoubtedly, this plant’s leaves must possess many phyto-compounds which can act against the target bacteria in the *L. rohita* fingerlings and can recover them from infection when they were fed consistently for 28 days @ 2.0g *H. annuus* leaf powder containing feed. Therefore, it may be concluded that the leaf powder of *H. annuus* @ 2.0 g concentration in the diet of *L. rohita* fingerlings can cure the bacterial infection, particularly, *A. hydrophila.* Further, this plant’s leaves curative potential against other bacterial and fungal pathogens of fishes may also be studied and reported in future.

**References**

1. Abdella, B., El-Wazzan, E. and El-Sersy, N. A. 2017. Pathogenicity and antibiotic susceptibility of two bacterial pathogens associated with the clam Tapes decussates in some Egyptian fisheries. *Ege J Fish Aquat Sci*., 34:383–389.
2. Abdul, K. M. and Haniffa, M. A. 2011. Evaluation of antibacterial activity of medicinal plants on fish pathogen *Aeromonas hydrophila*. *J Res Biol.*, *1*, 1-5.
3. Abutbul, S., Golan-Goldhirsh, A., Barazani, O. and Zilberg, D. 2004. Use of Rosmarinus officinalis as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis sp.*). *Aquaculture.,* 238:97–105.
4. Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W. F. P. C. and Fu, P. C. 1974. Enzymatic determination of total serum cholesterol. *Clinical chemistry.*, *20*(4), 470-475.
5. Aneja, K.R. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International Publishers, Fourth Edition., 245-275.
6. Anonymous 2020. National Fisheries Development Board (NFDB), Department of Fisheries, Ministry of Fisheries, Animal Husbandry & Dairying, Government of India.
7. APHA, A. 1998. Wef. *Standard methods for the examination of water and wastewater.*, *21*, 1378.
8. Aquaticcommunity.com; 2011.
9. Armour, F.E., Blood, F. R. and Belden, D. A. 1965. *Manual for Laboratory Work in Mammalian Physiology: By Fred ED'Amour, Frank R. Blood, and Don Belden, Jr*. U. of Chicago.
10. Austin, B. and Austin, D.A. 1999. “Chapter 2: Characteristics of the diseases.” In Bacterial Pathogens: Diseases of Farmed and Wild Fish. Third ed. Springer-Praxis, Praxis Publishing, Ltd. Chichester., 13–15.
11. Barde, R. D. (2023). Exploring the Disease-Causing Potential of *Aeromonas hydrophila* obtained from Freshwater Carp-*Cirrhinus mrigala*. *J. Surv. Fish. Sci*, *10*, 1725-1732.
12. Bhatnagar, A. 2008. Productivity and fish biodiversity of selected ponds of Haryana. *Project report submitted to Department of Fisheries, Government of Haryana*.
13. Brooker, A. J., Shinn, A. P. and Bron, J. E. 2007. A review of the biology of the parasitic copepod *Lernaeocera branchialis* (L., 1767) (*Copepoda: Pennellidae*). *Advances in Parasitology.*, *65*, 297-341.
14. Bryan, R., Soderberg, W., Blanchet, H. and Sharpe, W. E. 2011. Management of Fish Ponds in Pennsylvania,p. 1-32.
15. Burtis, C. A. and Ashwood, E. R. 1999. Tietz text book of clinical chemistry 3rd ed. *Philadelphia, London Pub. Saunders WB & CO*.
16. Campbell, R. A. 2004. CPUE Standardisation and the construction of indices of stock abundance in a spatially varying fishery using general linear models. *Fisheries Research.*, *70*(2&3), 209-227.
17. Cazenave, J., Wunderlin, D. A., Hued, A. C., & Bistoni, M. D. L. A. (2005). Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. *Hydrobiologia*, *537*, 25-33.
18. Chakraborty, S. B., & Hancz, C. (2011). Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture*, *3*(3), 103-119.
19. Chakraborty, S.B., Horn P. and Hancz C. 2014. Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. *Rev. Aquacult*., 6: 1-19.
20. Choudhury, D., Pal, A. K., Sahu, N. P., Kumar, S., Das, S. S. and Mukherjee, S. C. 2005. Dietary yeast RNA supplementation reduces mortality by *Aeromonas hydrophila* in rohu (*Labeo rohita L.*) juveniles. *Fish & Shellfish Immunology.*, *19*(3), 281-291.
21. Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry – *Aquacult. Int*., 18: 403-414.
22. Das, B. K., Pradhan, J. and Sahu, S. 2009. The effect of *Euglena* viridis on immune response of rohu, *Labeo rohita* (Ham.). *Fish & shellfish immunology.*, *26*(6), 871-876.
23. DeLong, D. P., Losordo, T. M. and Rakocy, J. E. 2009. Tank Culture of Tilapia, South Regional Aquaculture center. *SRAC Publication.*, p. 282.
24. Dethloff, G. M., Schlenk, D., Khan, S. and Bailey, H. C. 1999. The effects of copper on blood and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). *Archives of Environmental Contamination and Toxicology.*, *36*(4), 415-423.
25. Dhasarathan, P., Palaniappan, R. and Singh, A. R. 2000. Effect of endosulfan and butachlor on the digestive enzyme and proximate composition of the fish *Cyprinus carpio*. *Indian Journal of Environment & Ecoplanning.*, *3*(3), 611-614.
26. Fatrcová-Šramková, K., Nôžková, J., Kačániová, M., Máriássyová, M., Rovná, K., & Stričík, M. (2013). Antioxidant and antimicrobial properties of monofloral bee pollen. *Journal of Environmental Science and Health, Part B*, *48*(2), 133-138.
27. FAO 2001. State of the World’s Forests.
28. FAO 2020 “Sustainability in action.” State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy.
29. Fernández-Bravo, A. and Figueras, M.J. 2020. An update on the genus aeromonas: taxonomy, epidemiology, and pathogenicity. *Microorganisms*., 8(1):129.
30. Frans, I., Michiels, C. W., Bossier, P., Willems, K. A., Lievens, B. and Rediers, H. 2011. *Vibrio anguillarum* as a fish pathogen: virulence factors, diagnosis and prevention. *Journal of fish diseases.*, *34*(9), 643-661.
31. Garg, S. K., Jana, S. N. and Bhatnagar, A. 2003. Effect of inland groundwater salinity on digestibility and other aspects of nutrition physiology in *Mugil cephalus* and *Chanos chanos* (Forsskal). In *Proceedings of 3rd Interaction Workshop Fish production using brackishwater in arid-ecosystem*., pp. 17-18.
32. Gomez-Casado, E., Estepa, A. and Coll, J. M. 2011. A comparative review on European-farmed finfish RNA viruses and their vaccines. *Vaccine.*, *29*(15), 2657-2671.
33. Guo, F. C. and Woo, P. T. K. 2009. Selected parasitosis in cultured and wild fish. *Veterinary Parasitology.*, *163*(3), 207-216.
34. Hardi, E. H. and Pebrianto, C. A. 2012. Isolation and postulat koch test *Aeromonas* sp. and *Pseudomonas* sp. in Tilapia (*Oreochromis niloticus*) in Loa Kulu aquaculture Kutai Kartanegara. *Jurnal Ilmu Perikanan Tropis.*, *16*(2), 35-39.
35. Hardi, E. H., Kusuma, I. W., Suwinarti, W. and Agustina, N. R. 2016. Short communication: Antibacterial activity of *Boesenbergia pandurata*, *Zingiber zerumbetand* *Solanum ferox* extracts against *Aeromonas hydrophila* and *Pseudomonas sp*. *Nusantara Bioscience.*, *8*, 18-21.
36. Jacobs, J. M., Stine, C. B., Baya, A. M. and Kent, M. L. 2009. A review of mycobacteriosis in marine fish. *Journal of fish diseases.*, *32*(2), 119-130.
37. Johnson, A.M., Rohlfs, E.M. and Silverman, L.M. 1999. Proteins. In: Burtis, C.A., Ashwood, E.R., editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company, p. 477-540.
38. Khoo, L. 2000. Fungal diseases in fish. In *Seminars in Avian and exotic pet medicine*., 9(2), 102-111.
39. Kiran, B. R. 2010. Physico-chemical characteristics of fishponds of Bhadra project at Karnataka. *RASĀYAN J Chem.*, *3*(4), 671-676.
40. Kumar, S. T. and Shivani, P. 2014. Marine fisheries; its status, sustainable management and socio-economic status of the marine fishers of Odisha, through Indian Marine Policy: A case study. *Research Journal of Animal, Veterinary and Fishery Sciences.*, *2*(7), 10-19.
41. Liu, X. S., Gao, B., Li, X. L., Li, W. N., Qiao, Z. A., & Han, L. (2020). Chemical composition and antimicrobial and antioxidant activities of essential oil of sunflower (*Helianthus annuus* L.) receptacle. *Molecules*, *25*(22), 5244.
42. Madhuri, S., Mandloi, A. K., Govind, P. and Sahni, Y. P. 2012. Antimicrobial activity of some medicinal plants against fish pathogens., 3(4), 28-30
43. Madhuri, S., Mandloi, A.K., Govind P. and Sahni Y.P. 2013. Antimicrobial activity of some medicinal plants against fish pathogen. IRJP 3: 28-30.
44. Maji, U. J., Mohanty, S., Pradhan, A. and Maiti, N. K. 2017. Immune modulation, disease resistance and growth performance of Indian farmed carp, *Labeo rohita* (Hamilton), in response to dietary consortium of putative lactic acid bacteria. *Aquaculture international.*, *25*(4), 1391-1407.
45. Martinez-Murcia, A. J., Soler, L., Saavedra, M. J., Chacón, M. R., Guarro, J., Stackebrandt, E. and Figueras, M. J. 2005. Phenotypic, genotypic, and phylogenetic discrepancies to differentiate *Aeromonas salmonicida* from *Aeromonas bestiarum*. *International Microbiology.*, *8*(4), 259-269.
46. Michael, D. R., Srinivas, S. D., Sailendri, K. and Muthukkaruppan, V. R. 1994. A rapid method for repetitive bleeding in fish. *Indian Journal of Experimental Biology.*, *32*, 838-838.
47. Naiel, M. A., El-Kholy, A. I., Negm, S. S., Ghazanfar, S., Shukry, M., Zhang, Z., ... & Abdel-Latif, H. M. (2023). A mini-review on plant-derived phenolic compounds with particular emphasis on their possible applications and beneficial uses in aquaculture. *Annals of Animal Science*, *23*(4), 971-977.
48. Newman, D. J. 1999. Renal function and nitrogen metabolites. *Tietz textbook of clinical chemistry.*, 1204-1270.
49. Ngugi, C. C., Bowman, J. R. and Omolo, B. 2007. A new guide to fish farming in Kenya.
50. Nya, E. J. and Austin, B. 2009. Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of fish diseases.*, *32*(11), 971-977.
51. Pandey, J., Pandey, U. and Tyagi, H. R. 2000. Nutrient status and cyanobacterial diversity of a tropical freshwater lake. *Journal of Environmental Biology* *21*(2), 133-138.
52. Parrino, V., Cappello, T., Costa, G., Cannavà, C., Sanfilippo, M., Fazio, F., & Fasulo, S. (2018). Comparative study of haematology of two teleost fish (Mugil cephalus and *Carassius auratus*) from different environments and feeding habits. *The European Zoological Journal*, *85*(1), 193–199.
53. Patil, D. B. and Tijare, R. V. 2001. Investigation of Pollution Mystery of Suspected Carcinogen Cr (VI) and it's Control. *Journal of Industrial Pollution Control.*, *17*(1), 43-47.
54. Pękala-Safińska, A. (2018). Contemporary threats of bacterial infections in freshwater fish. *Journal of veterinary research*, *62*(3), 261.
55. Ramaiah, N. 2006. A review on fungal diseases of algae, marine fishes, shrimps and corals. *Indian Journal of Marine Sciences.*, *35*(4), 380-387.
56. Rao, Y. V., Das, B. K., Jyotyrmayee, P. and Chakrabarti, R. 2006. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish & Shellfish Immunology.*, *20*(3), 263-273.
57. Riche, M. and Garling, D. 2003. Feeding tilapia in intensive recirculating systems. *USDA North Central Regional Aquaculture Center Fact Sheet*, *114*.
58. Sadashivaiah, C. R. R. C., Ramakrishnaiah, C. R. and Ranganna, G. 2008. Hydrochemical analysis and evaluation of groundwater quality in Tumkur Taluk, Karnataka State, India. *International journal of environmental research and public health.*, *5*(3), 158-164.
59. Saharia, P. K., Hussain, I. A., Pokhrel, H., Kalita, B., Borah, G., & Yasmin, R. (2021). Prevalence of motile Aeromonas Septicaemia (MAS) in fish culture systems of the Central Brahmaputra Valley zone of Assam, India. *Aquaculture Research*, *52*(3), 1201-1214.
60. Sahu, S., Das, B. K., Pradhan, J., Mohapatra, B. C., Mishra, B. K. and Sarangi, N. 2007. Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. *Fish & shellfish immunology.*, *23*(1), 109-118.
61. Santhosh, B. and Singh, N. P. 2007. Guidelines for water quality management for fish culture in Tripura, ICAR Research Complex for NEH Region, Tripura Center, Lembucherra-799210, Tripura (west). P. 29, 2007.
62. Sarder, M. R. I., Thompson, K. D., Penman, D. J. and McAndrew, B. J. 2001. Immune responses of Nile tilapia (*Oreochromis niloticus* L.) clones: I. Non-specific responses. *Developmental & Comparative Immunology.*, *25*(1), 37-46.
63. Sikoki, F. D. and Veen, J. V. 2004. Aspects of water quality and the potential for fish production of shiroro reservoir Nigeria. *Liv. Sys. Sus. Dev.*, pp *2*- 7.
64. Sivasankar, P., Anix Vivek Santhiya, A. and Kanaga, V. 2015. A review on plants and herbal extracts against viral diseases in aquaculture. *J. Med. Plants*., 3: 75-79.
65. Smith, V. H. 2006. Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. *Limnology and oceanography.*, *51*(1&2), 377-384.
66. Sudheesh, P. S., Al-Ghabshi, A., Al-Mazrooei, N. and Al-Habsi, S. 2012. Comparative pathogenomics of bacteria causing infectious diseases in fish. *International journal of evolutionary biology.*, 1-16.
67. Syahidah, A., Saad, C.R., Daud, H.M. and Abdelhadi, Y.M. 2015. Status and potential of herbal applications in aquaculture: A review. *Iran. J. Fish*. Sci., 14: 27-44.
68. Tacon, A. G. 2020. Trends in global aquaculture and aqua feed production: 2000–2017. *Reviews in Fisheries Science & Aquaculture.*, *28*(1), 43-56.
69. Talwar, P. K. and Jhingran, A. G. 1991. *Inland fishes of India and adjacent countries* (2). CRC Press.
70. Thampuran De Long, N., Surendran, P. K., Mukundan, M. K. and Gopakumar, K. 1995. Bacteriological studies on fish affected by epizootic ulcerative syndrome (EUS) in Kerala, India., 8(2), 103- 111
71. Thomas, T. R. 1998. *Rough surfaces*. World Scientific.
72. Trinder, N., Woodhouse, C. D. and Renton, C. P. 1969. The effect of vitamin E and selenium on the incidence of retained placentae in dairy cows. *Veterinary Record.*, *85*, 550-553.
73. Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of clinical Biochemistry.*, *6*(1), 24-27.
74. Turker, H., Yildirim, A. B. and Karakaş, F. P. 2009. Sensitivity of bacteria isolated from fish to some medicinal plants. *Turkish Journal of Fisheries and Aquatic Sciences.*, *9*(2), 181-186.
75. Vega‐Heredia, S., Mendoza‐Cano, F. and Sanchez‐Paz, A. 2012. The infectious hypodermal and haematopoietic necrosis *Diseases.*, *59*(2), 95-105.
76. Verma, G. and Gupta, A. 2015. Probiotics application in aquaculture: improving nutrition and health. *J Anim Feed Sci Tech.*, *3*, 53-64.
77. Wassif, I., Mohammed, R. 2022. Use of thyme and thymol as immunostimulant agents to control experimental *Aeromonas hydrophyla* infection in Nile tilapia (*Oreochromis niloticus*). *Zagazig Vet J.,* 50:241–254.
78. Yesmin, S., Rahman, M. H., Hussain, M. A., Khan, A. R., Pervin, F. and Hossain, M. A. 2004. *Aeromonas hydrophila* infection in fish of swamps in Bangladesh., 7(3), 409-411.
79. Zhan, X. L., Clemens, J. C., Neves, G., Hattori, D., Flanagan, J. J., Hummel, T. and Zipursky, S. L. 2004. Analysis of Dscam diversity in regulating axon guidance in Drosophila mushroom bodies. *Neuron.*, *43*(5), 673-686.