Original Article
DOI: https://doi.org/10.70749/ijbr.v3i5.1303

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Protective Role of Tulsi (*Ocimum sanctum*) on Growth, Hematology, Liver Enzymes (ALP, ALT, and AST), and Immune System Against Lead-Induced Toxicity in Thaila (*Catla catla*)

Ayesha Noreen[1], Amara Akhtar[1], Amina Zafar[1], Ayesha Yaseen[1]

[1]Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Punjab, Pakistan.

ARTICLE INFO

Keywords: Lead Toxicity, Ocimum Sanctum, Catla Catla, Fish Hematology.

Correspondence to: Ayesha Noreen, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Punjab, Pakistan.
Email: ayeshanoreen0000@gmail.com

Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History
Received: 01-01-2025, Revised: 05-04-2025
Accepted: 23-04-2025, Published: 14-05-2025

ABSTRACT

Aquaculture is the biggest fish production sector that meets the demands of fish globally. Lead (Pb) pollution from various human activities poses a significant threat to aquatic life. The present study explored the adverse effects of lead-based toxicity in Catla catla (C. catla) and the ameliorative role of Ocimum sanctum (O. sanctum) supplementation. There were four treatments in which forty-eight fishes were equally distributed. T0 was the control group fed with a basal diet. T1 was fed with 7 mg/L lead. T2 was fed with O. sanctum (10 g/kg) and T3 was co-treated with lead (7 mg/L) and O. sanctum (10 g/kg). Lead and O. sanctum Group (T_3) showed improved growth (27.05±1.07) as compared to the lead group (21.11±1.38) which indicated that O. sanctum supplementation mitigated some of the adverse effects of lead toxicity. Length gain was maximum in T2 (12.23±0.58) as compared to other groups. RBC count (1.35±0.06), hematocrit (24.14 ±2.62) and hemoglobin (5.12±0.04) decreased in the lead group. WBC count (48.66±0.61) increased in lead-exposed group T1 as compared to other treatments. ALT (32.03 ±1.83), AST (57.16±1.56) and ALP Levels (27.93±4.25) increased in the lead group (T1) indicating significant liver damage due to lead toxicity. The T_3 group showed lower enzyme level ALP (23.58±2.07), ALT (28.26±1.86) and AST (53.34±0.98) compared to other treatments demonstrating the hepatoprotective effect of O. sanctum. Total serum protein (4.20±0.18 g/dL), albumin (2.12±0.10 g/dL) and globulin (2.08±0.08 g/dL) levels were altered in the lead group showing immune system dysfunction due to lead exposure. In contrast the T_3 group showed improved immune parameters with total serum protein (5.1±0.15 g/dL), albumin (1.46±0.17 g/dL) and globulin (2.13±0.26 g/dL), indicating the beneficial effects of O. sanctum supplementation in restoring immune function. Statistical analysis showed that the results are significant ($p<0.05$).

INTRODUCTION

Fish are an essential component of aquatic ecosystems and a major source of high-quality protein, omega-3 fatty acids and essential micronutrients for human consumption (Kumar *et al.*, 2022). They play a crucial role in global food security particularly in developing nations where fish provide a primary protein source (Balami *et al.*, 2019).Fish serve as critical bioindicators of aquatic ecosystem health due to their heightened sensitivity to environmental changes and pollutant exposure (Shah *et al.*, 2020).Their presence and condition reflect the overall water quality making them vital in ecotoxicological studies (Granek *et al.*, 2010) .

Aquaculture is one of the fastest-growing food production sectors contributing significantly to the global seafood supply while alleviating pressure on wild fish stocks (Younis *et al.*, 2018) .It accounts for approximately 53% of global fish production and plays a crucial role in economic development and employment generation (Desvignes *et al.*, 2010).However, the sustainability of aquaculture is increasingly threatened by environmental contaminants. Particularly, heavy metal pollution from industrial and agricultural activities (Khan *et al.*, 2011) .Effective management strategies are essential to mitigate these risks and maintain the long-term viability of aquaculture practices (FAO, 2020).

Heavy metals are highly persistent and toxic contaminants in aquatic ecosystems that threaten fish health and disrupt ecological balance (Javed and Usmani, 2019). Lead (Pb) is a particularly hazardous heavy metal that enters aquatic systems through industrial discharge, mining activities and agricultural runoff (Kumar *et al.*, 2022). It accumulates in water, sediments and fish tissues leading to bioaccumulation and biomagnification along the food chain, thereby threatening both aquatic life and human health (Garai *et al.*, 2021).

Lead toxicity severely impacts fish physiology, metabolism and overall survival by interfering with enzymatic functions and disrupting normal biochemical pathways (Kim and Kang, 2015). It primarily accumulates in vital organs such as the liver, kidneys, gills and muscles where it induces oxidative stress and cellular damage (Fazio, 2019). Prolonged lead exposure weakens immune responses alters growth performance and increases mortality rates making it a critical concern for aquaculture (Sharma *et al.*, 2021).

Medicinal plants are increasingly being explored as natural alternatives to synthetic treatments for enhancing fish health and mitigating toxicant exposure (Al-Jawasreh, 2020) .These plants contain bioactive compounds with antioxidant, antimicrobial and immunostimulatory properties making them effective in mitigating heavy metal-induced stress (Yuan *et al.*, 2007). Their application in aquaculture supports sustainable fish farming by reducing dependency on chemical treatments and antibiotics (Sikotariya and Yusufzai, 2019).

Ocimum sanctum, commonly known as Tulsi. It is a widely recognized medicinal plant with hepatoprotective, immunomodulatory and antioxidant effects (Cohen, 2014). It contains bioactive compounds such as eugenol and flavonoids that enhance immune function improve metabolic processes and provide protection against environmental stressors (Yuan *et al.*, 2007). Studies suggest that incorporating *O. sanctum* into the diet can mitigate lead toxicity while enhancing growth performance and survival rates in fish (Shewita & Taha, 2011).

Hematological parameters serve as critical indicators of fish health and are widely used to assess the physiological impact of toxicants (Blaxhall, 1972) . Lead exposure disrupts hematological balance by reducing hemoglobin levels, red blood cell (RBC) count and hematocrit values leading to anemia and impaired oxygen transport (Fazio, 2019). However, supplementation with *O. sanctum* has been shown to restore these parameters improving blood profile and overall fish health (Mondal *et al.*, 2009).

The liver is the primary detoxification organ in fish and plays a key role in metabolizing toxic substances including heavy metals (Van der Oost *et al.*, 2008). Lead exposure significantly alters liver enzyme activity, increasing levels of alanine transaminase (ALT) and aspartate transaminase (AST) which indicate hepatocellular damage (Shahsavani *et al.*, 2010). Studies suggest that *O. sanctum* supplementation stabilizes liver function, reducing oxidative stress and promoting hepatic recovery (Shewita & Taha, 2011).

The immune system is essential for protecting fish against infections and environmental stressors, including heavy metal toxicity (Silva-Gomes *et al.*, 2015). Lead exposure suppresses immune responses making fish more susceptible to diseases and mortality (Rombout *et al.*, 2005). *O. sanctum* known for its immunostimulatory properties enhances both innate and adaptive immune mechanisms counteracting the immunosuppressive effects of lead toxicity (Yuan *et al.*, 2007).

This study aimed to evaluate the ameliorative potential of *O. sanctum* in mitigating lead-induced toxicity in *Catla catla*. By examining its effects on growth, hematological parameters, liver function and immune response, this research provided valuable insights into sustainable strategies for improving fish health in polluted aquatic environments. Thus, its use could contribute significantly to enhancing aquaculture sustainability and reducing the reliance on chemical treatments.

MATERIAL AND METHOD

Heavy metals are excessively present in our environment and affect every single living organism all over the world. Fish are aquatic organisms that have direct exposure to heavy metals through water. This research was performed to investigate the possible curative effects of *O. sanctum* against lead nitrate.

Experimental Animals

Forty-eight fishes of normal weight and normal size of *C. catla* kept at the Department of Zoology, Wildlife and Fisheries in the University of Agriculture Faisalabad, (PARS) laboratory. They were purchased from Punjab Fish Hatchery, Satyana Road, Faisalabad. The fingerlings were acclimatized in a lab environment for one week.

Chemical Used

O. sanctum leaf extract (10 g/day) and lead (7 mg/L) were used in the experiment.

Experimental Design

Four treatment groups were established:

Table 1

Different treatments applied in experimental beds

Treatments	Diet composition	Lead nitrate	O. Sanctum
$T_{0(control)}$	Basal diet	None	None
T_1	Basal diet	7mg/L	None
T_2	Basal diet	None	10g/kg
T_3	Basal diet	7mg/L	10g/kg

After 28 days of trial, fish were dissected and samples were collected from all treatments of fish for analysis.

Determination of Growth Performance and Feed Utilization

Growth performance was estimated through weekly gross weights of fingerlings from each experimental group. Feed utilization and growth performance were analyzed in terms of absolute weight gain (WG), weight gain percentage, specific growth rate (SGR), survival rate (SR)and feed conversion ratio (FCR).

Weight Gain (%)

$$WG\% = \frac{Final\ weight - Initial\ weight}{\text{Initial weight}} \times 100$$

Absolute Weight Gain

The following formula were applied for the estimation of AWG in grams.

Absolute weight gain (AWG)= Final weight(g) - Initial weight(g)

Specific Growth Rate (SGR)

$$SGR = \frac{\text{Initial weight(g)} - \text{Final weight(g)}}{\text{experimental duration}} \times 100$$

Survival Rate (%)

$$Survival\ rate\ (\%) = \frac{\text{Final number of fingerlings}}{\text{Inital number of fingerlings}} \times 100$$

Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Total dry feed intake(g)}}{\text{weight gain(g)}} \times 100$$

Hematological Evaluation

The evaluation of hematological parameters was carried out with the help of an automated hematology analyzer.

Biochemical Analysis

The levels of the liver enzymes ALT, ALP and AST were determined using ELISA kits.

Immunity Analysis

Total protein, albumin and globulin were measured spectrophotometrically using commercial kits.

Statistical Analysis

Results of this study were displayed in the form of Mean ± SEM and it were computed by one-way ANOVA through SPSS software. The level of significance would be set at $p < 0.05$ (Inkielewicz-Stepniak *et al.*, 2012).

RESULTS

The experiment was conducted to assess the effects of *Ocimum sanctum* on growth performance, liver enzyme activity, hematological parameters, and immune response in *Catla catla*. A total of 48 fish were acclimatized for one week before being randomly assigned to four treatments (T_0, T_1, T_2, and T_3) with 12 fish per aquarium. All fish were fed commercial pellets at 3% of body weight twice daily. T_0 served as the control group, while T_1, T_2, and T_3 were exposed to lead, *O. sanctum*, and a combination of both for four weeks. Growth performance was assessed weekly, while hematological, biochemical analyses and immune system were evaluated at the end of the trial.

Estimation of Growth Performance

Growth rate was determined in terms of weight gain and total length gain on weekly basis. The assessment of growth in fish is a key aspect of aquaculture and biological research. This study aimed to assess fish growth performance through weekly measurements providing valuable insights into their development and guiding best practices for sustainable aquaculture and management.

Estimation of Hematological Parameters

The hematological parameters that is red blood cells, white blood cells, platelets, hematocrit and hemoglobin concentrations were evaluated using the CBS test. These measurements were associated to established reference ranges for healthy fish. Any deviations from established reference ranges were examined to assess stress responses and physiological imbalances.

Estimation of Liver Enzymes

ALP, ALT and AST of liver were analyzed through biochemical analysis. Elevated levels of ALT and AST are indicative of liver cell damage or increased permeability of liver cell membranes. Enzyme activity within normal ranges suggested that liver function was not significantly compromised under the experimental conditions.

Estimation of Immune System

The immune system in fish is assessed through key parameters such as total serum protein, albumin and globulin levels. Higher serum protein, albumin and globulin levels reflect enhanced immune activity, while reduced levels may signal compromised immunity or nutritional deficiencies. These parameters provide critical insights into fish health and resilience to environmental stressors.

Table 2

Growth rate in different treatments of C. catla.

Groups	Weight gain (g)	Length gain (cm)
T_0	27.11±0.77	10.5±0.54
T_1	21.1±1.38	6.89±0.62
T_2	28.63±1.03	12.23±0.58
T_3	27.5±1.07	11.14±0.69

This table indicated that weight gain and length gain were minimum in lead exposed group (T1) as compared to other treatments T0, T2, T3.

Table 3

Comparison of different treatments on liver enzymes in C. catla

Liver enzymes	T_0	T_1	T_2	T_3
ALP	23.72±1.85	27.93±4.25	24.79±3.38	23.58 ±2.07
ALT	25.56±0.63	32.03±1.83	26.26±1.60	28.26±1.86
AST	53.36±1.07	57.16±1.56	54.43±1.15	53.3±0.98

This table indicated that there was an elevated levels of ALP, ALT and AST in lead exposed group as compared to other treatments

Table 4
Hematological parameters of C. catla exposed to different treatments for four weeks.

Parameters	Treatments			
	T_0	T_1	T_2	T_3
Red blood cells	2.07±0.03	1.35±0.06	2.09±0.02	2.08±0.02
Hemoglobin	7.33± 0.008	5.12±0.04	8.2±0.09	7.36±0.005
Hematocrit	32.14±0.56	24.14±2.62	30.94±0.54	31.57±0.45
White blood cells	42.87±0.16	48.66±0.61	42.77±0.14	42.99±0.05
Platelets	314.92±0.32	291.58±0.48	313.42±0.46	310.97±0.15

This table indicating that the mean comparison of different treatments in which red blood cells, platelets, hemoglobin and hematocrit decreased while white blood cells increased in T_1 as compared to other treatments.

Table 5
Immune parameters of C. catla exposed to different treatments for four weeks.

Parameters	Treatments			
	T_0	T_1	T_2	T_3
Total protein	5.1±0.15	2.93±0.08	5.43±0.29	5.26±0.29
Albumin	2.16±0.88	1.46±0.17	2.26±0.89	2.1±0.57
Globulin	2.66±0.12	2.13±0.26	2.83±0.06	2.76±0.18

This table indicating that the mean comparison of different treatments in which Total protein, Albumin and Globulin decrease in T_1 as compared to other treatments.

DISCUSSION

The present study evaluated the protective effects of *Ocimum sanctum* supplementation against lead-induced toxicity in *Catla catla* focusing on growth performance, liver function, hematological parameters and immune response. The results demonstrated that lead exposure significantly impaired fish health, while *O. sanctum* supplementation at 10 g/kg exhibited protective effects, enhancing physiological and biochemical responses.

Growth performance was significantly affected by lead exposure, with weight gain reduced to (21.1±1.38 g) in the lead-exposed group (T1) compared to the control (T0). However, fish fed *O. sanctum*-supplemented diets (T2) exhibited a higher growth rate (28.63±1.03 g), supporting the role of eugenol in promoting metabolism and nutrient absorption. These findings align with previous studies reporting improved growth performance in *Oreochromis niloticus* and *Cyprinus carpio* with *O. sanctum* supplementation (Mondal *et al.*, 2009); (Al-Dubakel *et al.*, 2011)). The protective effects of *O. sanctum* may be attributed to its bioactive compounds, which enhance protein metabolism, improve digestion and boost overall growth performance.

Liver enzyme analysis revealed that lead exposure significantly elevated ALT (32.03±1.83 U/L) and ALP (27.93±4.25 U/L), indicating hepatic stress and metabolic disruption. The increase in ALT and ALP levels suggested liver cell damage and increased permeability of hepatocyte membranes due to oxidative stress induced by lead toxicity. However, fish receiving *O. sanctum* supplementation exhibited reduced ALT (26.26±1.60 U/L) and ALP (23.58±2.07 U/L), suggesting hepatoprotective effects, likely due to its antioxidant properties (Cohen, 2014). The hepatoprotective role of *O. sanctum* has been linked to its ability to enhance liver enzyme function, reduce oxidative damage and support liver regeneration.

Hematological parameters further reflected the toxic impact of lead, with RBC (1.35±0.06 10^6/µL) and hemoglobin (5.12±0.04 g/dL) significantly reduced, likely due to lead interference in heme synthesis. Lead exposure affects erythropoiesis by inhibiting key enzymes in the heme biosynthesis pathway, leading to anemia and reduced oxygen transport capacity. However, *O. sanctum*-treated fish (T2) showed improved RBC (2.07±0.03 10^6/µL) and hemoglobin (8.2±0.09 g/dL), indicating its role in promoting erythropoiesis and oxygen transport (Maske and Satyanarayan, 2012). Conversely, WBC levels increased in the lead-exposed group (T1) (48.66±0.61 10^3/µL) due to immune activation under stress, while *O. sanctum* supplementation maintained balanced WBC levels (42.87±0.16 10^3/µL), reflecting its immunomodulatory potential (Alak et al., 2018; Rauta et al., 2022).

Serum biochemical parameters indicated that lead exposure resulted in a decline in total protein (2.93±0.08 g/dL), albumin (1.46±0.17 g/dL) and globulin (2.13±0.26 g/dL), signifying impaired liver function and suppressed immunity. The reduction in total protein and albumin suggests metabolic stress and reduced protein synthesis, while lower globulin levels indicate compromised immune responses. However, *O. sanctum* supplementation significantly restored protein (5.43±0.29 g/dL), albumin (2.26±0.89 g/dL) and globulin (2.83±0.06 g/dL), further highlighting its role in improving immune function and metabolic stability (Dubey and Pandey, 2018); (Chhaba *et al.*, 2020). These findings supported the immunostimulatory effects of *O. sanctum*, which enhances both innate and adaptive immune responses, making fish more resilient to environmental stressors.

The study concluded that lead exposure significantly impairs the growth, hematological parameters, liver function and immune system of *Catla catla*, highlighting the detrimental effects of environmental pollutants on aquaculture. However, supplementation with *O. sanctum* at 10 g/kg in the diet provides substantial protective effects by enhancing growth performance, stabilizing hematological parameters, reducing liver enzyme levels and strengthening immune response, thereby mitigating lead-induced toxicity. These findings indicated the promising potential of *O. sanctum* as a dietary additive in aquaculture to alleviate heavy metal stress.

CONCLUSION

This study confirmed that lead exposure has detrimental effects on the growth, hematology, liver function and immune response of *Catla catla*. Fish subjected to lead toxicity exhibited reduced growth, anemia, elevated liver enzymes and weakened immunity. These outcomes suggested that *O. sanctum* served as a beneficial dietary supplement in aquaculture by mitigating the harmful effects of environmental pollutants like lead and promoting improved fish health and performance.

REFERENCES

Al-Hamadany, Q., Al-Dubakel, A., & Jabir, A. (2011). Growth performance and implication of a thermal-unit growth coefficient of grass carp Ctenopharyngodon idella and silver carp Hypophthalmichthys Molitrix larvae reared in Recirculation system. *Journal of King Abdulaziz University-Marine Sciences*, *22*(2), 33-43. https://doi.org/10.4197/mar.22-2.3

Al-Jawasreh, R. I. M. (2020). Analytical and biological studies on the immunomodulatory potential of flavonoids in fish aquaculture. https://iris.unive.it/handle/10579/17843

Balami, S., Sharma, A., & Karn, R. (2019). Significance of nutritional value of fish for human health. *Malaysian Journal of Halal Research*, *2*(2), 32-34.

Blaxhall, P. C. (1972). The haematological assessment of the health of freshwater fish. *Journal of Fish Biology*, *4*(4), 593-604. https://doi.org/10.1111/j.1095-8649.1972.tb05704.x

Chhaba, B., Goud, E. A., Das, S. A., Karmakar, S., Rout, S. K., & Kachave, V. C. The effects of Tulsi and marigold as dietary supplement on growth, haematological and biochemical parameters in Labeo rohita (Hamilton). https://doi.org/10.33545/26174693.2024.v8.i4b.971

Cohen, M. (2014). Tulsi - Ocimum sanctum: A herb for all reasons. *Journal of Ayurveda and Integrative Medicine*, *5*(4), 251. https://doi.org/10.4103/0975-9476.146554

Desvignes, J. F., Laroche, J., Durand, J. D., & Bouvet, Y. (2001). Genetic variability in reared stocks of common carp (Cyprinus Carpio L.) based on allozymes and microsatellites. *Aquaculture*, *194*(3-4), 291-301. https://doi.org/10.1016/s0044-8486(00)00534-2

Dubey, R., & Pandey, S. K. (2018). Medicinally important constituents of tulsi (Ocimum spp.). *Synthesis of Medicinal Agents from Plants*, 151-176. https://doi.org/10.1016/b978-0-08-102071-5.00007-6

FAO, F. 2020. Agriculture Organization of the United Nations 2018 The state of world fisheries and aquaculture 2018—Meeting the sustainable development goals. CC BYNC-SA 3

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*, *500*, 237-242. https://doi.org/10.1016/j.aquaculture.2018.10.030

Garai, P., Banerjee, P., Mondal, P., & Saha, N. C. (2021). Effect of heavy metals on fishes: Toxicity and bioaccumulation. *J Clin Toxicol. S*, *18*(001).

GRANEK, E. F., POLASKY, S., KAPPEL, C. V., REED, D. J., STOMS, D. M., KOCH, E. W., KENNEDY, C. J., CRAMER, L. A., HACKER, S. D., BARBIER, E. B., ASWANI, S., RUCKELSHAUS, M., PERILLO, G. M., SILLIMAN, B. R., MUTHIGA, N., BAEL, D., & WOLANSKI, E. (2010). Ecosystem services as a common language for coastal ecosystem-based management. *Conservation Biology*, *24*(1), 207-216. https://doi.org/10.1111/j.1523-1739.2009.01355.x

Inkielewicz-Stepniak, I., Radomski, M. W., & Wozniak, M. (2012). Fisetin prevents fluoride- and dexamethasone-induced oxidative damage in osteoblast and hippocampal cells. *Food and Chemical Toxicology*, *50*(3-4), 583-589. https://doi.org/10.1016/j.fct.2011.12.015

Javed, M., & Usmani, N. (2017). An overview of the adverse effects of heavy metal contamination on fish health. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *89*(2), 389-403. https://doi.org/10.1007/s40011-017-0875-7

Khan, M. A., Khan, S., & Miyan, K. (2011). Aquaculture as a food production system: a review. *Biology and medicine*, *3*(2), 291-302.

Kim, J., & Kang, J. (2015). The lead accumulation and hematological findings in juvenile rock fish Sebastes schlegelii exposed to the dietary lead (II) concentrations. *Ecotoxicology and Environmental Safety*, *115*, 33-39. https://doi.org/10.1016/j.ecoenv.2015.02.009

Kumar, N., Chandan, N. K., Gupta, S. K., Bhushan, S., & Patole, P. B. (2022). Omega-3 fatty acids effectively modulate growth performance, immune response, and disease resistance in fish against multiple stresses. *Aquaculture*, *547*, 737506. https://doi.org/10.1016/j.aquaculture.2021.737506

Maske, N. S., & Satyanarayan, S. (2012). Effect of special fish feed prepared using potato peels on fresh water fish Labeo rohita. *J. Indus. Pollut. Control*, *29*, 33-38.

Mondal, S., Mirdha, B. R., & Mahapatra, S. C. (2009). The science behind sacredness of Tulsi (Ocimum sanctum Linn.). *Indian J Physiol Pharmacol*, *53*(4), 291-306.

ROMBOUT, J., HUTTENHUIS, H., PICCHIETTI, S., & SCAPIGLIATI, G. (2005). Phylogeny and ontogeny of fish leucocytes. *Fish & Shellfish Immunology*, *19*(5), 441-455. https://doi.org/10.1016/j.fsi.2005.03.007

Shah, N., Khisroon, M., & Shah, S. S. (2020). Assessment of copper, chromium, and lead toxicity in fish (Ctenopharyngodon idella Valenciennes, 1844) through hematological biomarkers. *Environmental Science and Pollution Research*, *27*(26), 33259-33269. https://doi.org/10.1007/s11356-020-09598-z

Sharma, P., Pandey, A. K., Udayan, A., & Kumar, S. (2021). Role of microbial community and metal-binding proteins in phytoremediation of heavy metals from industrial wastewater. *Bioresource Technology*, *326*, 124750. https://doi.org/10.1016/j.biortech.2021.124750

Sikotariya, S., & Yusufzai, S. I. (2019). Effect of Ocimum sanctum (Tulsi) powder on the growth and survival in Cirrhinus mrigala fingerlings. *Journal of Entomology and Zoology Studies*, *7*(4), 239-244.

Silva-Gomes, S., Decout, A., & Nigou, J. (2014). Pathogen-associated molecular patterns (PAMPs). *Encyclopedia of Inflammatory Diseases*, 1-16. https://doi.org/10.1007/978-3-0348-0620-6_35-1

Van der Oost, R., Beyer, J., & Vermeulen, N. P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology*, *13*(2), 57-149. https://doi.org/10.1016/s1382-6689(02)00126-6

Younis, A. M., Soliman, Y. A., Elkady, E. M., & El-Naggar, M. H. (2018). Assessment of polycyclic aromatic hydrocarbons in surface sediments and some fish species from the Gulf of Suez, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, *22*(4), 49-59. https://doi.org/10.21608/ejabf.2018.12472

Yuan, S., Chen, D., Zhang, K., & Yu, B. (2007). Effects of oxidative stress on growth performance, nutrient Digestibilities and activities of Antioxidative enzymes of weanling pigs. *Asian-Australasian Journal of Animal Sciences*, *20*(10), 1600-1605. https://doi.org/10.5713/ajas.2007.1600