

Ameliorative effects of fipronil toxicity in *Cirrhinus mrigala* and *Catla catla* via dietary carotenoids: effects on growth, antioxidant enzyme and immunity

Dureshahwar¹, Sajid Abdullah^{1*}, Khalid Abbas¹, Muhammad Shahbaz²

¹Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan

²Department of Botany, University of Agriculture, Faisalabad, Pakistan

*Corresponding author's email: drsajid@uaf.edu.pk

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Abstract

Widespread use of fipronil threatens non-target aquatic species while sustainable aquaculture is a significant solution for global food security. The present research investigated the protective effects of carotenoids on fish *Cirrhinus mrigala*, and *Catla catla*, during 1/3rd LC₅₀ of fipronil exposure. A three-month experiment was conducted with five dietary carotenoid treatment (Lycopene and Astaxanthin) i.e. (T₁, 100 mg/kg LYC), (T₂, 200 mg/kg LYC), (T₃, 100 mg/kg ASX), (T₄, 200 mg/kg ASX), (T₅ 100 mg/kg LYC + 100 mg/kg ASX) and (T₀, fipronil without carotenoids). All these six groups were exposed to 1/3rd LC₅₀ of fipronil insecticide with LC₅₀ values ranging from 5-8 µg/L⁻¹ for experimental fish species. Results revealed that the higher LYC (T₂) and ASX (T₄) doses improved growth and reduce FCR in both species). The combination of both carotenoids (LYC100 mg/kg + ASX100 mg/kg) in T₅ showed the highest weight gain, (138.4± 0.976) and (122.76±1.183) for Mori and Thaila respectively. The Lysozyme, IgM and WBCs were significantly increased (P<0.05) with LYC and ASX supplementation as compared to fipronil exposed group. Antioxidant enzyme activities (CAT, SOD, and POD) were highest in liver of both fish species among all other organs. Enzymes level were significantly reduced (P<0.05) in T₀ due to fipronil toxicity while carotenoid based diet showed improved antioxidant profile with highest (P<0.05) values in T₅ fed with combination of carotenoids. Therefore, these findings suggest that LYC and ASX supplementation particularly their combined use, alleviated fipronil toxicity in fish.

Keywords: Fipronil toxicity, Carotenoids, Astaxanthin, Lycopene, Growth, Antioxidant enzymes, Immunity, *Cirrhinus mrigala*, *Catla catla*

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Introduction

Aquaculture is involved in the financial industry, primarily in nations with low income (Dayani et al., 2024). Aquaculture provides almost 50% of the world's sea food and fish and is expected to provide 60% of food for Human consumption by 2030 (FAO, 2018). The gap between supply and need of sea food can be bridged by the efficient production of aquaculture. To increase the production of aquaculture environmentally sustainable food ingredients has become the focus of commercial applications and research (Dunham, 2011).

Pesticides and insecticide derivatives discarded from agriculture can contaminate drainage water used in raising aquatic animals. Bio-magnification of residual derivatives in the fish body usually has harmful effect on growth and overall health (Gewaily et al., 2021). Rearing water get these pollutants from drainage water which excreted from Agriculture sector (Fiorino et al., 2018; Stara et al., 2020). When these contaminated organisms consumed by human beings for feeding purposes can ultimately cause toxic effects and some serious health hazard (Abdel-Latif et al., 2020). These toxic substances which are accumulated in aquatic organisms suppress their oxidative state and immune system (Abdel-Tawwab et al., 2022).

Fipronil is an insecticide that is a member of the phenylpyrazole chemical class. Because it is less toxic than organochlorine and organophosphate, it is widely used in veterinary medicine, agriculture and residential and public hygiene to get rid of insects and other pests (Caboni et al., 2003). When xenobiotics like fipronil enter an aquatic environment, they change biologically, chemically and physically produce a variety of metabolites (Mamboungou et al., 2024). In addition to blocking chloride channels (GABA-gated), it disrupts the neural network and GluCl muscles of insects. The enzymatic antioxidant system by catalyzing the breakdown of reactive oxidative molecules these enzymes play a crucial role in protecting cells against oxidative damage caused by external stressor. (Li et al., 2022). High concentrations of these reactive oxygen species (ROS) have the potential to severely destroy important biomolecules and will negatively impact physiological processes (Juan et al., 2021). Due to the phenomena of bio-magnification these chemical pollutants have the disastrous effects on living organisms.

A variety of useful feed additives are added to the aqua feed to improve the feed conversion ratio, disease

resistance, antioxidant enzyme activity and immune response of aquatic animals (Abdel-Latif et al., 2020). The eco-friendly substance with antimicrobial and immunostimulant properties is progressively being examined as alternatives in feed (Buck et al., 2017 and Suphoronski et al., 2019). Innovations in the research and application of natural antioxidants because of their robust defense mechanisms that remove reactive oxygen species, plants and their extracts are useful for medical purposes and serve as potent antioxidants in the diets of many organisms (Hasanuzzaman et al., 2020).

In recent years, medicinal plants have been utilized for the cure of vast variety of xenobiotic, pharmacological and chemical toxicity (Ebrahimi et al., 2024). To enhance the feed conversion ratio, disease resistance, antioxidant enzyme activity and immunological response of aquatic animals, a variety of functional feed additives are added to the aqua feed (Dawood et al., 2018). More research is being done on natural substances with immunostimulant and antibacterial qualities as feed substitutes (Wang et al., 2024).

Carotenoids have attained a lot of interest and have shown financial advantages in the aquatic sector. Carotenoids are particular pigments found in the flowers, leaves and fruits of several plants (Caser et al., 2018). Additionally, carotenoids have been shown to enhance the physiological functions of organisms, including lipid metabolism, immune system function (Olatunde et al., 2020). Astaxanthin antioxidant properties are very strong (Ambati et al., 2014). It has been shown to have several significant processes, including immunomodulatory, anti-inflammatory and anti-cancer properties (Abd-El-Hack et al., 2019). Astaxanthin protect the oxidative damage by scavenging free radicals and extinguish single oxygen to terminate the chain reaction. It has beneficial impact on growth pigmentation and biochemical biomarkers. (Micah et al., 2022). Supplements with Astaxanthin may help to reduce the toxicity and also reduce the mortality and deformity rates (Dong et al., 2021). Lycopene (LYC) is another fat-soluble carotenoid present in fruits, vegetables, tomato extracts and their byproducts. Numerous research unequivocally demonstrate their contribution to enhancing fish development performance and antioxidant status (Souza et al., 2020). It is a powerful antioxidant and is 47 times more powerful than vitamin E. It can neutralize the single oxygen (O), oxidize unsaturated fatty acid, amino acid, or nucleic acid. By scavenging ROS, LYC included in diets helps humans, rodents

and fish avoid oxidative stress (Sharma and Goswami, 2011).

Fish in aquaculture experience stress and are exposed to many toxicants. LYC lessen the antioxidant damage that fish experience while exposed to dangerously high endosulfan levels (Zeng et al., 2021). Natural antioxidants are widely recognized for their exceptional antioxidative and antimicrobial capabilities, offering significant benefits in aquaculture and seafood preservation. Their inclusion in animal feed helps prevent lipid and protein oxidation as well as microbial spoilage, thereby enhancing the quality, safety and shelf life of the feed. These properties highlight the critical role of natural antioxidants in supporting sustainable aquaculture by improving both productivity and product quality (Beltran and Esteban, 2022).

The overuse of chemicals in agriculture to increase crop yields has put major carps in South-East Asian nations, including *Cirrhinus mrigala* and *Catla catla* in grave risk. Fipronil exposure was expected to induce oxidative and physiological alterations in fish. Therefore, this study aimed to evaluate the effects of LYC and ASX supplementation individually and combined on the growth, antioxidant enzyme activity and immune response of fish exposed to fipronil. These carotenoids in feed was expected to alleviate these effects and improve antioxidant, growth and immune responses.

Material and Methods

Experimental setup and fish

Healthy fingerlings of *Cirrhinus mrigala* (common name, Mori) and *Catla catla* (common name, Thaila)

were obtained from the Government Fish Seed Hatchery Faisalabad, Pakistan and transported to the Fish Toxicology Laboratory Fisheries Research Farms, University of Agriculture Faisalabad. The fishes with average body weight of 20.53 ± 0.15 g, were acclimatized for one week prior to experimentation. During the study, water quality parameters were maintained between the ranges: pH (6.8 -7.4), Temperature (30.1-30.8 °C), DO level (5.1-5.6 mg/L), Hardness (165- 176 mg/L), Alkalinity (275- 286 mg/L) for both fishes. Both fish species were placed in different aquaria with a water capacity of 100 liters. Fresh solution of fipronil were added to the aquaria and water was replaced every day to avoid deterioration of organic matter, Oxygen level were sustained using mechanical air pumps.

Determination of 96-hr LC₅₀ OF fipronil

The median lethal concentration (LC₅₀) for Fipronil over 96 hours was determined for Mori (Common name of *Cirrhinus mrigala*) and for Thaila (Common name of *Catla Catla*). Technical grade fipronil (97.2%) was used to prepare the stock-1 in analytical grade methanol (95%) at a 1 g/100 ml. stock-2 (working) was prepared by further dilution of stock-1 with deionized water which is used to obtain the required values. Fipronil concentration was started from 0.0 with the increase of $0.1 \mu\text{g L}^{-1}$. Dead fish were taken out of the aquarium every twelve hours, (12, 24, 36, 48....96) and the death rate was noted. Probit analysis with a 95% confidence interval was used to determine the 96-hour LC₅₀ of fipronil and one third of its sublethal concentration was selected for the experiment (Table 1).

Table-1. Lethal concentrations and 96-hr LC₅₀ for the fish exposed to Fipronil.

Pesticides	Fish species	LC ₅₀ ($\mu\text{g L}^{-1}$)	C.I (95%)	Lethal conc. ($\mu\text{g L}^{-1}$)	C.I (95%)	1/3 rd LC ₅₀
Fipronil ($\mu\text{g L}^{-1}$)	<i>Cirrhinus mrigala</i>	23.8866	21.6-25.9	36.5	33.4- 41.5	7.9622
	<i>Catla catla</i>	15.5084	14.2-16.7	22.8	20.8-26.0	5.1694

Fipronil= FIP, C.I.= Confidence Interval ($\mu\text{g L}^{-1}$); Lethal Concentrations ($\mu\text{g L}^{-1}$).

Experimental design

A 90 days' experiment was conducted at intensive culture system to check out the ameliorative effects of lycopene and Astaxanthin carotenoids for fipronil insecticides toxicity on *C. mrigala* and *C. catla*. This research was conducted with fipronil exposed control group T₀ in which water was treated with Fipronil and fish fed basal diet without carotenoids and five experimental groups in which T₁ contain (100 mg/kg LYC), T₂ (200 mg/kg LYC), T₃ (100 mg/kg ASX), T₄ (200 mg/kg ASX) T₅, (100 mg/kg LYC+100 mg/kg ASX) were used. Diluted solution of fipronil at a concentration of 1/3rd LC₅₀ of Fipronil (Determined previously) were added in all the treatments groups. The stocking density was 10 fish per aquarium with three replicates (10x3=30 and 06 treatments, 30x6=180 fish for Mori and 180 for Thaila, total number of fishes were 360 in this experiment). Growth metrics and Physiochemical parameters were observed on weekly basis throughout the experiment. The oxidative stress and immune response were measured at the end of trial.

Test chemical and feed ingredients

Fipronil MW 437.16; CAS 120068-37-3 is a comparatively recent addition to the arsenal of insecticides, classified as an N-phenylpyrazole. Technical grade of fipronil was purchased from Peart Agro chemicals Faisalabad Pakistan. Carotenoids including Lycopene (empirical formula: C₄₀H₅₆) and Astaxanthin (empirical formula: C₄₀H₅₂O₄) were bought from Sigma-Aldrich Nottingham, UK (NG9, 2JR). Different feed ingredients were purchased from Faisalabad market and basal diet were prepared using fishmeal, corn gluten, vitamins, mineral premix. All the ingredients including carotenoids were weighed and grounded and then mixed thoroughly to increase the palatability. To improve the digestibility oil was incorporated in the dried ingredients and mixed for 5-7 minutes. Five different diets were prepared by using different concentration of carotenoids T₁-T₅ and one control T₀ (fipronil+feed without carotenoids). The proximate composition and feed formulation are given in Table 2.

Table-2. Feed formulation and proximate composition of feed.

Ingredients	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Fishmeal (g)	24	24	24	24	24	24
Soybean Meal (g)	24	24	24	24	24	24
Wheat Flour (g)	12	12	12	12	12	12
Wheat Bran (g)	18	18	18	18	18	18
Rice Bran (g)	15	15	15	15	15	15
Fish Oil (g)	4	4	4	4	4	4
Vit. + Min.	2	2	2	2	2	2
Methionine	1	1	1	1	1	1
Total	100	100	100	100	100	100
LYC mg/kg	--	100	200	--	--	--
ASX mg/kg	--	--	--	100	200	--
LYC+ ASX mg/kg	--	--	--	--	--	100+100
Proximate composition (AOAC protocol)						
Ingredients	D.M.	C.P	E. E	Ash	C.F	
Fishmeal	92.2	51.8	10.6	15.5	1.4	
Soybean meal	91.8	43.7	4.5	6.8	6.5	
Rice bran	92.3	11.6	8.6	17.8	13.6	
Wheat flour	91.1	13.5	1.2	0.9	0.2	

DM= Dry mass, C.P= Crude protein, C.F= Crude Fiber, E.E= Ether extract, AOAC= Association of Official Analytical Chemists.

Evaluation of growth performance

Growth parameters

Different growth parameters in terms of weight gain (WG), length gain (LG), feed conversion ratio (FCR) and specific growth rate (SGR% day⁻¹) were measured weekly. The given formulas were used to determine these parameters.

Weight gain (WG g) = Final weight- Initial weight

Length gain (cm) = Final length – initial length

Feed Conversion Ratio:

$$FCR = \frac{\text{Total feed consumed by the fish}}{\text{Final weight-Initial weight}}$$

Specific Growth Rate % day⁻¹:

$$SGR = \frac{\ln(\text{Final weight}) - \ln(\text{Initial weight})}{\text{Number of days}} \times 100$$

Immune response

Blood sampling

At the end of experimental duration 5 fish per aquarium captured for blood sampling. Blood sampling was done for the conduction of hematological analysis. Blood was obtained from the fish caudal vein by using a 5ml plastic syringe. After collecting the blood sample, it was centrifuged for 10 minutes at 4000rpm. During centrifugation, serum was separated from blood to obtain the serum samples and this sample was stored at -20 OC.

Total white blood cell count

To calculate the white blood cells, a Neubaur hematocytometer was used. Turk's solutions were used to dilute the blood. Counting of WBCs was done in hematocytometer under the light microscope and was observed as 103 /microL. The following formula was used to calculate the WBCs.

$$\text{No of WBCs (103 /microL)} = \frac{\text{No.of WBCs} \times \text{diluion counted}}{\text{Area counted} \times \text{depth of fluid}}$$

Immunoglobulins

A two-site sandwich ELISA to quantitate IgM in Fish serum, plasma. A microplate had been pre-coated with an IgM antibody. The wells were pipetted with standards and samples, and any IgM present was bound by the immobilized antibody. Following the extraction of any unbound materials, the wells were

supplemented with an IgM-specific biotin-conjugated antibody. Following washing, the wells were filled with streptavidin-conjugated horseradish peroxidase (HRP). Addition of a substrate solution to the wells, after a wash to eliminate any unbound avidin-enzyme reagent, results in the development of colour according to the quantity of IgM bound in this initial stage. The colour development was halted, and the intensity of the colour was measured (Khandige et al., 2022).

Lysozymal activity

Serum lysozyme activity of fish were examined by turbidometric assays described by (Dawood et al., 2024). The wells of microplate were filled with 10µl of serum or mucus samples and then additional 190µl of substrate (0.2 mg Micrococcus lysodeikticus, lyophilized cell; Sigma-Aldrich) mL⁻¹ PBS, pH 7.4 were added. After five minutes their absorbance was measured and then incubated with gentle shaking at constant room temperature, at 450 nm with ImmunoMini NJ-2300 (Tokyo, Japan).

Antioxidant enzymes activity

To study antioxidant enzymes samples were obtained at the end of trial. Different levels of enzymes viz. SOD, CAT, and POD were measured from selected organs of fish. Organs (kidney, liver and gills) were removed from the sample and homogenized using a blender. Each organ was then separately placed in cold phosphate buffer (pH 6.5, 0.2 M) at a ratio of 1:4 (w/v). Following that, homogenates were centrifuged for 15 minutes at 4°C and 10,000 rpm. After that, clear supernatants were collected to measure the enzyme assay. (Giannopolitis and Ries, 1977) approach was used to measure the activity of SOD. The CAT enzyme lower the H₂O₂ concentration at 240 nm and were measure by the method of (Chance and Mehaly, 1995). The action of POD enzymes in different body organs of fish were measured to determine its potential to minimize the level of H₂O₂ at A₄₇₀ nm by (Civello et al., 1995).

Physico-chemical parameters

Water quality parameters were determined on a regular basis to maintain the fish health. Dissolved oxygen and temperature of water were recorded and measured by electronic meter (HANNA HI-9146) while pH was measured by a digital meter (HANNA HI-98107).

Statistical analysis

The statistical model of this study was Complete Randomized Design (CRD) and data was analyzed by One-way ANOVA and Tukey's test was used to compare the standard error means. Software statistics 8.1 and Origin lab used for statistical analysis. A significant criterion of $p < 0.05$ was agreed upon.

Results

Growth performance of *Cirrhinus mrigala* and *Catla catla*

A statistically significant difference in growth metrics were observed between the fipronil exposed control and experimental groups. Weekly assessment of growth parameters throughout the trial showed improved weight gain, increased specific growth rate (SGR % day⁻¹), and reduced feed conversion ratio (FCR) in the groups treated with carotenoid supplemented for both fish species. In contrast, exposure to fipronil and basal diet without carotenoid supplements in the fipronil exposed control group (T₀) exhibited growth suppression for both species,

highlighting the detrimental alterations of fipronil toxicity on growth performance.

The results demonstrated that the highest weight gain (138.4±0.976) and (122.76±1.183) was measured in T₅ (100mg/kgLYC +100mg/kgASX) treatment for *C. mrigala* and *C. catla* respectively as compared to control (fipronil exposed) and other experimental groups. The outcomes revealed that addition of carotenoids improved the growth performance such as weight gain, specific growth rate and decrease the feed conversion ratio. The average weight gain for Mori with 200 mg/kg carotenoids was 122.8±0.783 (T₂) with LYC and 118.86±0.952 (T₄) with ASX and for Thaila 117.2±0.871 (T₂) with LYC and 112.26±1.386 (T₄) with ASX. It was noted that the specific growth rate was also highest in treatment T₅ which was 2.5±0.084 and 2.6±0.115 for *C. mrigala* and *C. catla* respectively. The addition of LYC and ASX significantly ($p \leq 0.05$) enhanced the feed utilization and growth indices (Table 3). While the addition of 100 mg/kg of both carotenoids in fish diet also improved the growth values of targeted fish species as compared to control group which was treated only with toxicant fipronil, and fed with basal diet with no carotenoid.

Table-3. Growth performance (WG, LG, SGR% day⁻¹, FCR) of Fish Mori and Thaila after 3-months exposure of induced Fipronil toxicity in control and treated groups with the supplementation of carotenoids.

variables	T0	T1	T2	T3	T4	T5	P values
Mori							
I.W (g)	20.53±0.145a	20.5±0.088a	20.7±0.120a	20.6±0.185a	20.9±0.176a	20.7±0.066a	0.3525
F.W (g)	103.9±1.013e	124.7±0.976c	143.56±0.751b	118.5±1.026d	139.8±0.814b	159.2±1.026a	0.0000
W.G (g)	82.5±1.097 e	104.2±1.058c	122.8±0.783 b	97.86±1.189d	118.86±0.952b	138.4±0.976a	0.0000
I.L (c)	13.5±0.202a	13.8±0.145a	13.5±0.202a	13.6±0.173a	14.2±0.176a	13.6±0.120a	0.0916
F.L (c)	21.8±0.317c	22.9±0.185abc	23.3±0.264ab	22.2±0.491bc	23.5±0.233ab	23.9±0.202a	0.0032
L.G (c)	8.3±0.120 c	9.1±0.264 abc	9.7±0.392 ab	8.6±0.333 bc	9.3±0.264 ab	10.2±0.296 a	0.0055
FCR	2.4±0.066 a	2.0±0.088 b	1.4±0.057 c	2.2±0.057 ab	1.6±0.057 c	1.4±0.066 c	0.0000
SGR %	1.6±0.064 d	1.8±0.057 cd	2.2±0.088 ab	1.8±0.066 cd	2.1±0.057 bc	2.5±0.084 a	0.0000
Thaila							
I.W (g)	20.53±0.145ab	20.63±0.088ab	20.53±0.088ab	20.33±0.088b	21.06±0.202a	20.66±0.088ab	0.0261
F.W (g)	96.5±1.069d	118.43±1.098c	137.73±0.895b	114.1±1.249c	133.3±1.183b	143.43±1.270a	0.0000
W.G (g)	75.96±1.166d	97.8±1.135 c	117.2±0.871 b	93.76±1.160c	112.26±1.386b	122.76±1.183a	0.0000
I.L (c)	12.4±0.176a	12.4±0.202a	12.5±0.120a	12.9±0.218a	13.2±0.208a	12.6±0.202a	0.0793
F.L (c)	20.1±0.290c	20.7±0.233bc	21.9±0.202ab	20.5±0.290 c	21.0±0.296abc	22.1±0.288 a	0.0013
L.G (c)	7.7±0.2 c	8.3±0.208 abc	9.4±0.251 ab	7.6±0.493 c	7.8±0.166 bc	9.4±0.491 a	0.0037

FCR	2.6±0.033 a	2.2±0.057 c	1.6±0.066 d	2.5±0.057 ab	2.2±0.088 bc	1.6±0.057 d	0.0000
SGR %	1.6±0.115 c	1.8±0.088 bc	2.3±0.088 a	1.7±0.057 c	2.2±0.115 ab	2.6±0.115 a	0.0001

The results are presented as mean value ±SE= Standard Error (n = 3 replicate aquaria per treatment). Significant differences are denoted by different superscripted letters ($P < 0.05$). I.W= Initial weight, F.W= Final weight, W.G= weight gain, I.L= Initial length, F.L= Final length, L.G= Length gain, FCR= Feed conversion ratio, SGR= specific growth rate.

Antioxidant enzyme activity

At the end of experiment samples from each aquarium were taken (n=3) dissected and different organs were isolated to determine the antioxidant enzyme activity of both species. The results of antioxidant enzyme activities, including Catalase, Superoxide Dismutase, and Peroxidase, are detailed in Tables 4, 5 and 6 for Liver, Kidney and Gills. Initially, groups T₁ (100 mg/kg LYC) and T₃ (100 mg/kg ASX) with Fipronil exposure showed no significant improvement as compared to the fipronil exposed control group (T₀), which fed basal diet (0% ASX, LYC) which experienced a decline in enzyme levels. However, higher doses of LYC and ASX (T₂: 200 mg/kg LYC; T₄: 200 mg/kg ASX) demonstrated better results than T₁, and T₃, (100 mg/kg LYC, 100 mg/kg ASX) sustaining higher enzyme concentrations even in a pesticide-contaminated environment.

The enzyme activity in different organs showed different results as the liver is detoxifying organ the

highest level of enzymes was observed in it. In liver different levels of enzymes SOD, 142.21±0.954 CAT, 184.8±0.964 and POD 3.64±0.023 were observed for Mori, and different values of CAT, 176.83±1.128, SOD 140.31±0.722, POD 3.15±0.020 for Thaila were observed. Action of these enzymes were higher in kidney as compared to gills in both species. Particularly, the combination of 100mg/kg LYC and 100mg/kg ASX in T₅ resulted in the highest enzyme levels, indicating that this combination of both carotenoids effectively mitigated damage caused by fipronil and enhanced enzyme activity in fish. This pattern persisted throughout the trial, with T₅ consistently showing superior results compared to all other treatments. While T₂ and T₄ showed slightly better results than T₁ and T₃, the combination in T₅ remained the most effective, these findings underscore the protective role of carotenoids against pesticide-induced oxidative stress, with combined treatments offering enhanced benefit.

Table-4. Measurement of Liver antioxidant enzymes (CAT, SOD, POD) in *Mori* and *Thaila* following 3-month fipronil exposure and different levels of dietary carotenoids (LYC, ASX).

variables	T0	T1	T2	T3	T4	T5	P values
Mori							
CAT							0.000
U/mg	107.71±0.848d	145.72±0.939c	182.66±1.225a	143.33±1.068c	152.48±0.736b	184.8±0.964a	
SOD							0.000
U/mg	74.97±0.938 d	101.62±1.082c	139.3±1.126 a	98.7±1.081 c	113.73±1.146b	142.21±0.954a	
POD							0.000
U/mg	2.35±0.020 e	2.94±0.020 d	3.42±0.026 b	2.94±0.020 d	3.28±0.012 c	3.64±0.023 a	
Thaila							
CAT							0.000
U/mg	115.29±0.276e	138.57±0.176c	169.68±0.174b	122.36±0.294d	140.75±0.296c	176.83±1.128a	
SOD							0.000
U/mg	69.67±0.912e	95.96±0.913 d	134.21±0.954b	94.2±1.081 d	110.64±0.879c	140.31±0.722a	
POD							0.000
U/mg	2.22±0.017d	2.61±0.020 c	2.73±0.011b	2.62±0.014c	2.79±0.008b	3.15±0.020a	

The results are presented as mean value ±SE.= Standard Error (n = 3 replicate aquaria per treatment). Significant differences are denoted by different superscripted letters ($p < 0.05$). CAT = Catalase, SOD= Super oxidase Dismutase, POD=Peroxidase.

Table-5. Antioxidant enzyme activity (CAT, SOD, POD) in Kidney of Mori and Thaila after 3-months exposure to Fipronil insecticide and different levels of carotenoids Lycopene and Astaxanthin.

variables	T0	T1	T2	T3	T4	T5	P values
Mori							
CAT							0.000
U/mg	84.48±0.610e	99.54±0.927d	125.23±0.642b	98.56±0.433d	108.88±0.389c	142.87±0.955a	
SOD							0.000
U/mg	58.72±0.560e	101.46±0.522c	105.55±0.538b	79.243±0.433d	103.49±0.595bc	117.75±0.867a	
POD							0.000
U/mg	1.15±0.006 c	2.81±0.017 b	2.88±0.348 ab	2.86±0.018 ab	3.18±0.012 ab	3.53±0.086 a	
Thaila							
CAT	82.79±0.641						0.000
U/mg	d	104.56±0.554b	141.03±0.808a	90.24±0.332 c	105.40±0.308 b	142.47±0.783a	
SOD	51.67±0.552						0.000
U/mg	e	96.17±0.583 c	110.32±0.600b	69.63±0.490 d	109.83±0.808 b	122.41±0.671a	
POD							0.000
U/mg	1.11±0.025 e	2.57±0.012 bc	2.63±0.011 b	2.52±0.016 cd	2.46±0.020 d	3.08±0.032 a	

The results are presented as mean value ±SE=Standard Error (n = 3 replicate aquaria per treatment). Significant differences are denoted by different superscripted letters ($P < 0.05$). CAT = Catalase, SOD= Super oxidase Dismutase, POD=Peroxidase.

Table-6. Antioxidant enzyme activity (CAT, SOD, POD) in Gills of Mori and Thaila after 3-months exposure to Fipronil insecticide and feed with different levels of carotenoids Lycopene and Astaxanthin.

variables	T0	T1	T2	T3	T4	T5	P values
Mori							
CAT							0.000
U/mg	116.9±0.814f	126.5±0.665d	136.43±0.497b	123.26±0.664e	130.36±0.371c	139.6±0.435a	
SOD							0.000
U/mg	69.4±0.642e	89.46±0.554d	109.96±0.895b	88.3±0.642d	103.80±0.577c	117.09±0.232a	
POD							0.000
U/mg	1.60±0.017 c	2.78±0.020 b	2.89±0.291 b	2.75±0.014 b	3.16±0.020 ab	3.48±0.038 a	
Thaila							
CAT							0.000
U/mg	73.09±0.726d	94.86±0.913c	105.4±0.550b	93.7±0.945c	105.46±0.611b	111.23±0.635a	
SOD							0.000
U/mg	67.02±0.353d	86.86±0.783c	115.43±0.638b	84.46±0.664c	114.63±0.231b	120.21±0.412a	
POD		2.48±0.014					
U/mg	1.33±0.011 e	bc	2.54±0.026 b	2.35±0.014 d	2.41±0.023 cd	2.95±0.023 a	

The results are presented as mean value ±SE.= Standard Error (n = 3 replicate aquaria per treatment). Significant differences are denoted by different superscripted letters ($P < 0.05$). CAT = Catalase, SOD= Super oxidase Dismutase, POD=Peroxidase.

Immune efficiency

At the end of 90-day dietary trial samples were taken and investigated the immunomodulatory effects of carotenoid-enriched (LYC and ASX) diets. Results of the several immune parameters showed significant improvements for both fish *C. mrigala* and *C. catla*.

The addition of carotenoids to the diet positively influenced key immune markers, including immunoglobulin (IgM), lysozyme activity, and WBCs as compared to the fipronil exposed control group which was given basal diet without carotenoids. Treatment T₅ with combined supplementation (100

mg/kg LYC + 100 mg/kg ASX) elicited the strongest immune response and showed the highest IgM level in *C. mrigala*, followed by the T5 the next best response was seen in T₂ and T₄ in which 200 mg/kg LYC and ASX were given as compared to T₁ and T₃ in which 100 mg/kg of these two carotenoid supplements were added in diets. From T₁-T₅ elevated lysozyme level were observed as compared to T₀ in both fish. A significant reduced level of white blood cells was observed in only fipronil induced group (T₀). Furthermore, in *C. catla* marked increase in IgM and Lysozyme were seen in T₅ where the combination of

both carotenoids were given surpassing the single carotenoid diet (T₁, T₂, T₃, T₄). Increasing the carotenoid concentration to 200 mg/kg in T₂, and T₄ resulted better response of immunological markers as compared to 100 mg/kg supplementation in T₁, T₃ groups. Lycopene alone was more effective than Astaxanthin alone in enhancing immune parameters. These findings showed that dietary supplementation of carotenoids boosted overall immunity of fish of *C. mrigala* and *C. catla*. The Figure 1, 2, 3 showed the variations in results for immune efficiency of Mori (a) and Thaila (b).

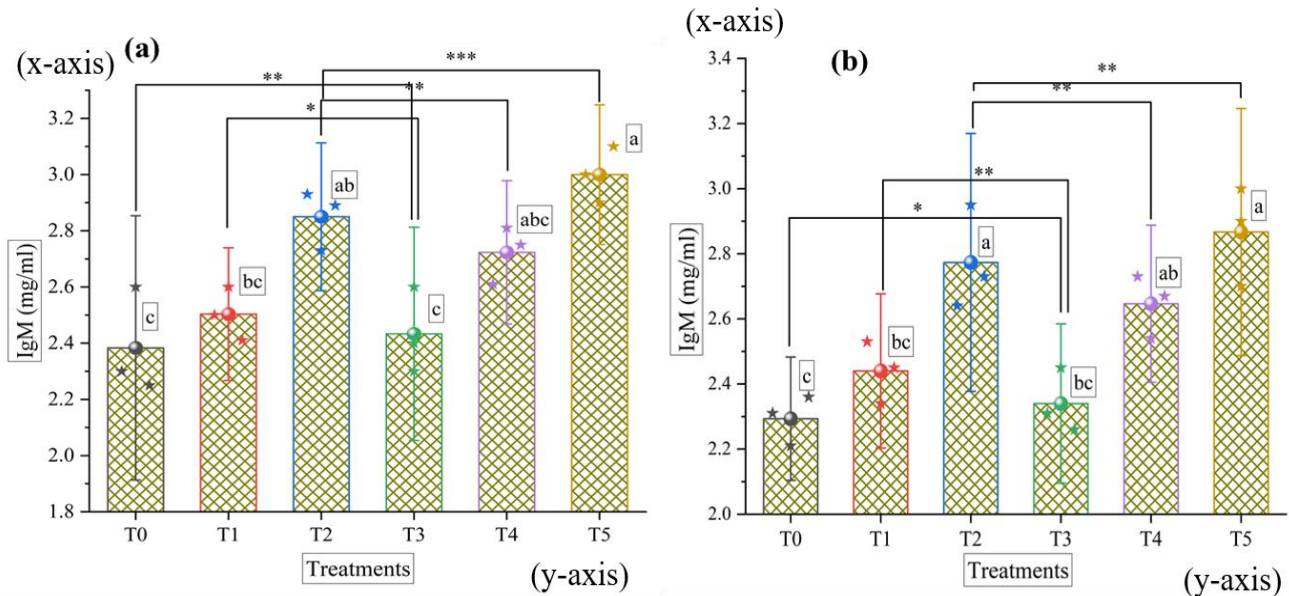


Figure-1. Expression of Immune parameters (IgM) of fish at the end of trial (a) *Cirrhinus mrigala* and (b) *Catla catla* after 3-months fed with dietary Carotenoids supplemented (LYC, ASX) diet during Fipronil exposure. The results are presented as mean value $\pm$ SE. = Standard Error (n = 3 replicate aquaria per treatment). Significant differences are denoted by different superscripted letters ($p < 0.05$). Error bars shows standard errors between means.

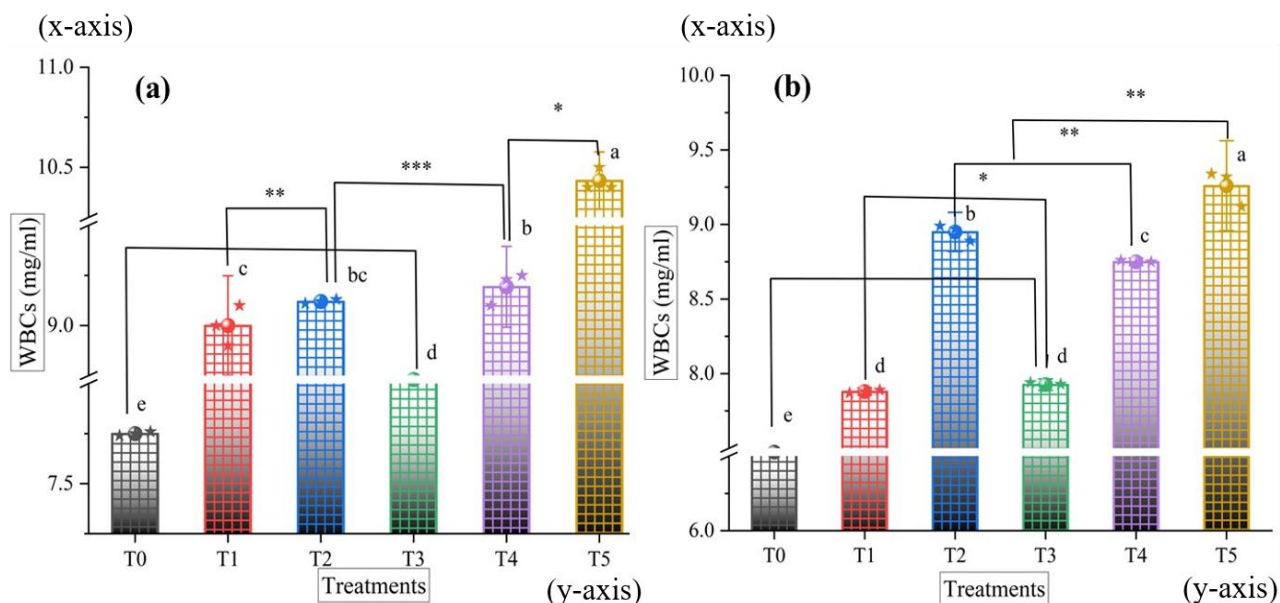


Figure-2. Calculations of (WBCs) of fish after completion of trial (a) *Cirrhinus mrigala* and (b) *Catla catla* after 3-months fed Carotenoids (LYC, ASX) supplemented diet during Fipronil exposure.

The results are presented as mean value $\pm$ SE. = Standard Error (n = 3 replicate aquaria per treatment). Significant differences are denoted by different superscripted letters ($p < 0.05$). Error bars shows standard errors between means.

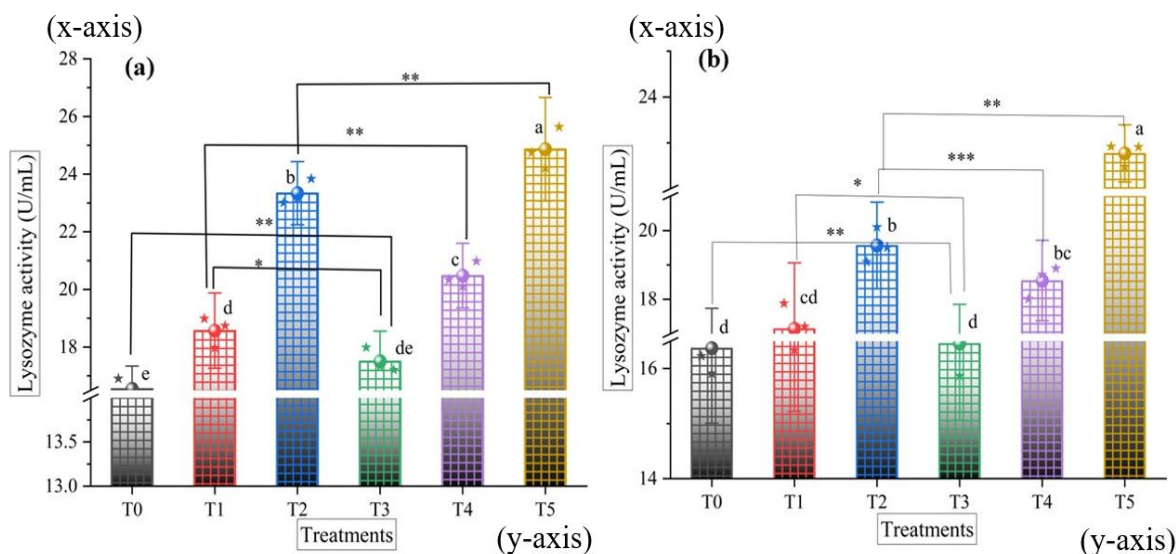


Figure-3. Measurement of Lysozymal activity of fish (a) *Cirrhinus mrigala* and (b) *Catla catla* after 3-months fed Carotenoids (LYC, ASX) supplemented diet during Fipronil exposure.

The results are presented as mean value $\pm$ SE. = Standard Error (n = 3 replicate aquaria per treatment). Significant differences are denoted by different superscripted letters ($p < 0.05$). Standard bars shows standard errors between means.

Physicochemical parameters

The physicochemical parameters were monitored on weekly basis to maintain a suitable environment for *C. mrigala* and *C. catla* during the 3-month dietary trial.

Regular maintaining and monitoring is essential for reliability of trial. The mean values for all the parameters for both species were remained constant. The recorded range for pH 6.8-7.4, Temperature 30.1-

30.8°C, dissolved oxygen 5.1-5.6 mg/L, Hardness: 165-176 mg/L, Alkalinity: 275-286 mg/L as presented in Table 7. These consistent and stable water quality

parameters throughout the experimental period ensure the stable environment and minimal environmental stress for fish and increase the accuracy of the results.

Table-7. Water quality parameters during 3-month exposure of fipronil and carotenoids based diet for *Cirrhinus mrigala* and *Catla catla*, T= Temperature, DO= Dissolved Oxygen, ALK= Alkalinity, HRD= Hardness.

Parameters	Treatments					
	T0	T1	T2	T3	T4	T5
<i>Catla catla</i>						
pH	7.26±0.145	7.03±0.088	6.86±0.120	7.1 ±0.115	7.23±0.088	7.4±0.057
T	30.2±0.611	30.7±0.723	30.5±0.523	30.5±0.698	30.7±0.698	30.3±0.617
DO	5.43±0.328	5.06±0.218	5.2±0.208	5.36±0.233	5.1±0.230	5.5±0.233
ALK	283.46±1.762	282.43±1.877	283.33±1.876	280.86±1.361	279.13±1.348	276.9±1.682
HRD	180.7±1.493	183.8±1.562	176.61±1.204	176.3±1.193	176.1±1.682	168.83±1.297
<i>Cirrhinus mrigala</i>						
pH	7.26±0.145	7.03±0.088	6.86±0.120	7.1 ±0.115	7.23±0.088	7.4±0.057
T	30.2±0.611	30.7±0.723	30.5±0.523	30.5±0.698	30.7±0.698	30.3±0.617
DO	5.43±0.328	5.06±0.218	5.2±0.208	5.36±0.233	5.1±0.230	5.5±0.233
ALK	283.46±1.762	282.43±1.877	283.33±1.876	280.86±1.361	279.13±1.348	276.9±1.682
HRD	180.7±1.493	183.8±1.562	176.61±1.204	176.3±1.193	176.1±1.682	168.83±1.297

T= Temperature, DO= Dissolved Oxygen, ALK= Alkalinity, HRD= Hardness. The results are presented as mean value ±SE.= Standard Error (n = 3 replicate aquaria per treatment).

Correlation heatmap and polar heat map

The relationships between growth performance, biochemical indices, and water-quality indicators in Mori and Thaila were investigated using Pearson correlation analysis as an exploratory method (Figure 4). Red and blue, respectively, indicate positive and negative correlations in the heatmaps. Fish weight, POD, SOD, IgM, WBCs, lysozyme activity, and dissolved oxygen (DO) showed positive relationships with a number of measured parameters for Mori (Figure 4a), while FCR, hardness, and alkalinity showed negative correlations with a number of growth and biochemical indicators. Temperature and pH showed different patterns of connection with the

variables that were measured. Similar to this, fish weight and enzymatic indices generally showed positive correlations in Thaila (Figure 4b), whereas FCR and hardness have shown negative correlations with a number of investigated parameters. A number of growth and metabolic markers showed unfavourable relationships with alkalinity and temperature. Because some factors may be connected and water-quality measurements may have shown little change, these results should be regarded cautiously as they indicate exploratory connections. Further research is necessary to determine the biological implications of the discovered correlations since they do not prove causal links.

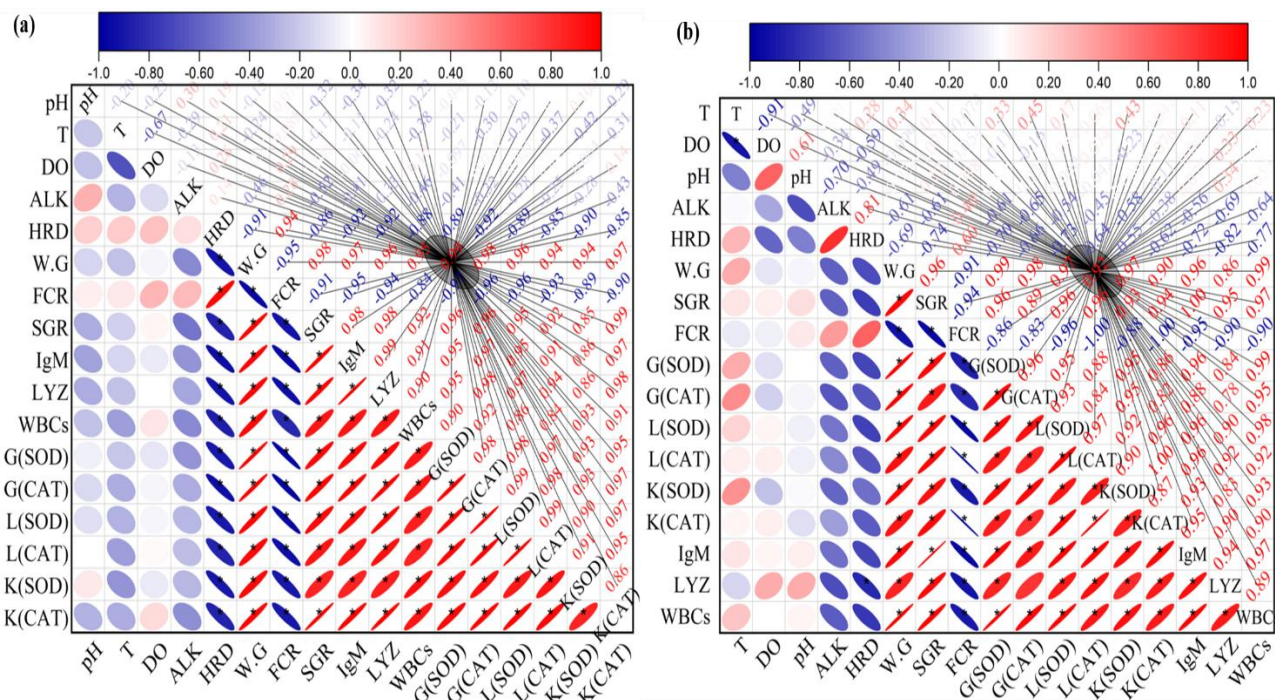


Figure-4. Heatmap of *Cirrhinus mrigala* (a) and *Catla catla*, (b) T= Temperature, Do= dissolved oxygen, ALK= Alkalinity, HRD= Hardness, W.G= weight gain, FCR= Feed conversion ratio, SGR= specific growth rate, IgM= Immunoglobulins, LYZ= Lysozymal activity, WBCs= white blood cells, SOD (G)= Super oxidase dismutase for Gills, CAT (G) = Catalase for Gills, POD (G)= Peroxidase for gills, SOD (L)= Super oxidase dismutase for Liver, CAT (L) = Catalase for Liver, POD (L)= Peroxidase for Liver, SOD (K)= Super oxidase dismutase for Kidney, CAT (K) = Catalase for Kidney, POD (K)= Peroxidase for Kidney.

Discussion

The use of innovative eco-friendly feed additives to promote fish development, immunological responses and disease resistance has been the focus of study in recent decades (Dawood et al., 2020; Van Doan et al., 2020). Pesticide-induced water contamination is known to negatively affect the survival, reproduction and productivity of aquatic life, including fish, (Shellfish and Finfish species) algae and aquatic plants (Jabeen et al., 2015; Albano et al., 2021). One of the most often used pesticides is Fipronil, which raises questions regarding its effects on the environment (Moreira et al., 2021). The aim of this study was to mitigate the toxic effects of fipronil by using carotenoids (LYC and ASX) to improve the overall health of fish in terms of growth, oxidative stress and immune efficiency.

Addition of Fipronil (pesticide) in present experiment caused serious health hazards in fish it produces free radicals which leads to oxidative stress which showed

by the fipronil exposed T₀ treatment. Similar to this study, Arslan and Gunal (2023) observed at the sublethal and acute effects of Fipronil in *Unio delticatus*, an aquatic non-target species, using histopathological observations, physical effects and antioxidant mechanisms. Because they prevent oxidative stress, the antioxidant enzymes CAT and SOD are frequently employed to monitor the dangers of pesticides and herbicides (Jin et al., 2010). Similarly, these enzymes were taken as parameter to observe the disturbed antioxidant activity during fipronil exposure in current research in which fipronil induced group T₀ shows a considerable decline in activities of SOD, CAT and POD as compared to other treatments.

In present study fipronil exerted significant toxic effects on vital organs like kidney, gills and particularly in liver due to its detoxification activity. Antioxidant enzymes CAT, SOD, and POD were decreased across all treatments specially in T₀, the fipronil exposed carotenoid free group. Similarly,

Ramesh et al. (2023) determined the toxicity in *C. carpio* of the pesticide pyrethroid alpha-cypermethrin for 35 days and concluded that oxidative stress biomarkers were affected in the gill, liver, and muscle tissues.

Carotenoids are also called as immunostimulant that can improve the immune function and antioxidant capacity of organisms (Babin et al., 2015). Lycopene's strong antioxidant scavenging effect makes it a chemo protectant agent. Furthermore, it is regarded as the carotenoid that works best in contrast to biological ROS (Sahin et al., 2014). Results of current study also showed the same pattern as in T₂ and T₄ LYC containing diet improve that antioxidant activity of fish and reduce the toxic effects by depleting the ROS caused by pesticide. Abd-El-Gawad et al. (2019) studied a substantial increase of the antioxidant state of Yellow Perch fed a Lycopene-incorporated diet for 2 months. They reported that the liver SOD and CAT activity had significantly increased. As a potent antioxidant, LYC has been proposed in a number of studies to reduce the oxidative stress responses in fish species exposed to pesticides by reducing free radicals ROS (Dawood et al., 2020).

Pesticide-induced oxidative stress arises from direct lipid membrane interactions or reactive oxygen species (ROS) generation (Tahir et al., 2024), leading to pathological conditions, heightened disease susceptibility, and growth retardation. Similar effects were evident in the T₀ (fipronil exposed) treatment of this study, where fish experienced direct fipronil exposure. To counteract fipronil toxicity, the current study evaluated LYC and ASX as dietary supplements to enhance fish health, including growth performance, enzymatic antioxidants, and immune response.

Abd-El-Hakim et al. (2019) attempted reducing the immune-toxic and antioxidant impacts of exposure to fipronil (FP) on *Oreochromis niloticus* using the β -glucan (β G) natural or synthetic substances that can be used to improve immunity in fish and gave fish protection against infection by increasing the production of antibodies. These results are compatible with the results of the present study. Immunity parameters (IgM, Lysozyme and WBCs) proved to be adversely affected by Fipronil toxicity; showing the lowest performance in T₀ (Fipronil induced) and were improved significantly by addition of synthetic carotenoid in diet in all other treatments (T₁- T₅).

In the present study enhanced growth performance and restored liver antioxidant enzyme activities (SOD, CAT and POD) were observed in T₂ and T₄ where 100

and 200mg/kg Astaxanthin fed to the fish as feed additives. These findings counteracted the pesticide-induced suppression, aligning with the findings of Chien et al., 2003 which have shown that Astaxanthin effectively improves the immunity of aquatic animals (*Penaeus monodon*) and increases their resistance to pathogen infection and enhance its antioxidant defense capability stress which support present research results. Kaleshtari et al. (2019), investigated effect of substituting synthetic ASX to check notable improvements in growth performance in Rainbow Trout. A 12-week study was conducted by Kalinowski et al. (2023) on Rainbow Trout found that ASX affected oxidative pathway and was good for the trout's liver. In present study same results. In these two treatment ASX improve the growth rate and antioxidant enzyme activity of liver which was reduced due to toxic effects of pesticide.

The present study shows the values obtained from samples for antioxidant enzymes viz. Catalase, Superoxide dismutase and Peroxidase showed variations among the examined tissues, with numerically higher activities generally observed in the liver, followed by kidney and gills. The protective role of lycopene and vitamin E against toxicity induced by diazinon (DZN insecticide) was observed by Ibrahim (2015). Hussain et al. (2022) conducted an experiment to determine the effects of lycopene supplemented diet on growth performance, nutrient digestibility and antioxidant status of *Catla catla* fingerlings. Lycopene supplemented diet optimally improved growth parameters, nutrient utilization and antioxidant status of fish at 40mgkg⁻¹. (Yonar et al., 2020) conducted a study to investigate the ameliorating effects of lycopene on TCF-induced toxicity in carp by evaluating hematological parameters and oxidant/antioxidant status. Notable increases in the tissue MDA levels were observed in the TCF-treated groups. Results of present study shows the dietary supplementation of LYC and ASX with Fipronil exposed groups (T₁-T₅) succeeded in improving examined parameters (Growth, Enzymatic indices immunity) as compared with the (T₀) which received fipronil without carotenoid supplementation. Thomaz et al. (2009) reported significant decreases of the SOD and CAT activities in *Oreochromis niloticus* after TCF exposure.

Conclusion

In conclusion, the present study shows that carotenoids significantly improve the fish health, growth performance and oxidative capacity in *C. mrigala* and *C. catla*. They improve the immunity in terms of IgM and lysozyme activity when fed with carotenoids containing diet. The study demonstrated that exposure to fipronil 1/3rd LC₅₀ induced significant changes in the growth performance and oxidant/antioxidant status in terms of SOD, CAT and POD of *C. mrigala* and *C. catla*. Carotenoids (LYC and ASX) supplementation in feed seemed to enhance the antioxidant enzymes and mitigate the toxicity triggered by Fipronil in the fish. This study may enhance the knowledge on the prooxidant effects of these carotenoids for sustainable aquaculture, as safe additives for fish health, productivity and consumer satisfaction.

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Contribution of Authors

Dureshahwar & Abdullah S: Conceptualized the study, designed research methodology, supervised experiments and edited the manuscript.

Dureshahwar, Abdullah S & Abbas k: Conducted feeding experiments, collected data and laboratory analysis.

Dureshahwar, Abdullah & Shahbaz M: data analysis, interpretation of results and manuscript editing.

Dureshahwar, Abdullah S, Abbas k & Shahbaz M: Contributed to data interpretation, scientific discussion and critical revision of the manuscript.

All authors have read and approved the final draft of the manuscript.

References

Abd-El-Gawad EA, Wangand HP and Yao H, 2019. Diet supplemented with synthetic carotenoids: Effects on growth performance and biochemical and immunological parameters

of Yellow Perch (*Perca flavescens*). Front. Physiol. 10:1-13.

<https://doi.org/10.3389/fphys.2019.01056>

Abd-El-Hack ME, Abdelnour S, Alagawany, Abdo M, Sakr MA, Khafaga AF, Mahgoub SA, Elnesr and Gebriel MG, 2019. Microalgae in modern\ cancer therapy: Current knowledge. Biomed. Pharm. 111:42-50
<https://doi.org/10.1016/j.biopha.2018.12.069>

Abd-El-Hakim Y, Neamat-Allah AN, Baeshen M and Ali HA, 2019. Immune-protective, antioxidant and relative genes expression impacts of β -glucan against fipronil toxicity in Nile Tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 94:427-433.
<https://doi.org/10.1016/j.fsi.2019.09.033>

Abdel-Tawwab M, Mousa MAA and Mamoon A, 2022. Dietary Chlorella vulgaris modulates the performance, antioxidant capacity, innate immunity and disease resistance capability of Nile Tilapia fingerlings fed on plant-based diets. Anim. Feed Sci. Technol. 283:1-11.
<https://doi.org/10.1016/j.anifeedsci.2021.115181>

Abdel-Latif HM, Dawood MAO, Ledouble SM and El-Matbouli M, 2020. The nature and consequences of co-infections in tilapia: A review. J. Fish Dis. 43:651-664. <https://doi.org/10.1111/jfd.13164>

Albano M, Panarello G, Di-Paola D, Capparucci F, Crupi R, Gugliandolo E and Savoca S, 2021. The influence of polystyrene microspheres abundance on development and feeding behavior of Artemia salina (Linnaeus, 1758). App. Sci. 11:1-17.
<https://doi.org/10.3390/app11083352>

Ambati RR, Phang SM, Raviand S and Aswathanarayana RG, 2014. Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications. A review. Marine Drugs, 12:128-152.
<https://doi.org/10.3390/md12010128>

Arslan PA and Gunal AC, 2023. Does fipronil effect on aquatic organisms? Physiological, biochemical and histopathological alterations of non-target freshwater mussel species. Water, 15:1-14.
<https://doi.org/10.3390/w15020334>

Babin A, Saciat C, Teixeira M, Troussard JP, Motreuil S, Moreauand J and Moret Y, 2015. Limiting immunopathology: Interaction between

- carotenoids and enzymatic antioxidant defences. *Develop. Comp. Immunol.* 49:278-281.
<https://doi.org/10.1016/j.dci.2014.12.007>
- Buck EL, Mizubuti IY, Alfieri AA, Otonel RAA, Buck LY, Souza FP, Prado-Calixto OP, Poveda-Parra AR, Alexandre Filho L and Lopera-Barrero NM, 2017. Effect of propolis ethanol extract on myostatin gene expression and muscle morphometry of Nile tilapia in net cages. *Genet. Mol. Res.* 16:1-13. DOI <https://doi.org/10.4238/gmr16019404>
- Beltran JMG and Esteban MA, 2022. Nature-identical compounds as feed additives in aquaculture. *Fish Shellfish Immunol.* 123:409-416. <https://doi.org/10.1016/j.fsi.2022.03.010>
- Caboni P, Sammelson RE and Casida JE, 2003. Phenylpyrazole insecticide photochemistry, metabolism and gabaergic action: Ethiprole compared with fipronil. *J. Agric. food chem.* 51:7055-7061. <https://doi.org/10.1021/jf030439l>
- Caser M, Angiolillo FD, Chitarra W, Lovisolo C, Ruffoni B, Pistelli L, Pistelliand L and Scariot V, 2018. Ecophysiological and phytochemical responses of *Salvia sinaloensis* fern to drought stress. *Plant Growth Regulation*, 84:383-394. <https://dx.doi.org/10.1007/s10725-017-0349-1>
- Chance M and Mehaly AC, 1995. Assay of catalase and peroxidase. *Methods in Enzymology* 2:764-817
<https://doi.org/10.1002/9780470110171.ch14>
- Chien YH, Panand CH and Hunter B, 2003. The resistance to physical stresses by *Penaeus monodon* juveniles fed diets supplemented with astaxanthin. *Aquaculture*, 216:177-191. [https://doi.org/10.1016/S0044-8486\(02\)00056-X](https://doi.org/10.1016/S0044-8486(02)00056-X)
- Civello MP, Martinez GA, Chaves AR and Anon MC, 1995. Peroxidase from strawberry fruit (*Fragaria ananassa* Duch.): Partial purification and determination of some properties. *J. Agri. food chem.* 43: p. 2601. <https://doi.org/10.1021/jf00058a008>
- Dawood MA, Abdo S, El-Kassas E, El-Naggar S, Moustafa EM and Abou Asa S, 2024. Chicken egg lysozyme enhanced the growth performance, feed utilization, upregulated immune-related genes, and mitigated the impacts of *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.* 146, 109377 <https://doi.org/10.1016/j.fsi.2024.109377>
- Dawood MA, Abdel-Tawwab M and Abdel-Latif HM, 2020. Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish. *Reviews in Aquaculture*, 12(4): 2511-2526. <https://doi.org/10.1111/raq.12455>
- Dawood MAS, Koshio MA and Esteban, 2018. Beneficial roles of feed additives as immunostimulants in aquaculture: A review. *Rev. Aquac.* 10:950-974. <https://doi.org/10.1111/raq.12209>
- Dunham RA, 2011. *Aquaculture and fisheries biotechnology: Genetic approaches*: 2nd Ed, Cabi, South Asia <https://doi.org/10.1079/9781789243468.0000>
- Dayani S, Jafari V, Safari R and Hoseinifar SH, 2024. Effects of using different levels of dietary chitosan on some immune indices and antioxidant defense system in Vannamei Shrimp (*Litopenaeus vannamei*). *Aquat. Anim. Nut.* 10:43-55. doi. 10.22124/janb.2024.27503.1244
- Dong J, Li C, Dai D, Zhang M, Gao Y, Li X, Li M, Zhang J, Wang X and Zhou C, 2021. Protective effects of astaxanthin from *Haematococcus pluvialis* on the survival and oxidative stress of Zebrafish embryos induced by microcystin-LR. *J. App. Phycol.* 33:2261-2271. <https://doi.org/10.1007/s10811-021-02448-6>
- Ebrahimi Y, Abdalkareem JS, Mohammed BA, Salman AN, Jabbar AM, Hameed MN, Goudarzi MA and Parsaei P, 2024. Determination of antioxidant properties of *Mentha longifolia*, *Pistacia khinjuk* and *Eucalyptus globulus*. *Casp. J. Environ. Sci.*, 22:601-606. 10.22124/CJES.2022.6065
- Fiorino E, Sehonova P, Plhalova L, Blahova J, Svobodova Z and Faggio C, 2018. Effects of glyphosate on early life stages: Comparison between *Cyprinus carpio* and *Danio rerio*. *Environ. Sci. Poll. Res.* 25:8542-8549. <https://doi.org/10.1007/s11356-017-1141-5>
- FAO, 2018. part 1-World review. *The state of world Fisheries and Aquaculture*. Rome, Italy
- Gewaily MS, Shukry M, Abdel-Kader MF, Alkafafy M, Farrag FA, Moustafa EM, Doan HV, Abd-Elghany MF, Abdelhamid AF, Eltanahy A

- and Dawood MA, 2021. Dietary *Lactobacillus plantarum* relieves Nile Tilapia (*Oreochromis niloticus*) juvenile from oxidative stress, immunosuppression and inflammation induced by deltamethrin and *Aeromonas hydrophila*. *Front. Mar. Sci.* 8:1-15. <https://doi.org/10.3389/fmars.2021.621558>
- Giannopolitis CN and Ries SK, 1977. Superoxide dismutase occurrence in higher plants. *Plant Physiology* 59:309-314. DOI: 10.1104/pp.59.2.309
- Hasanuzzaman M, Bhuyan MB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M and Fotopoulos V, 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants* 9:1-52. <https://doi.org/10.3390/antiox9080681>
- Hussain S, Khalid M, Bashir F, Shahzad M and Hussain A, 2022. Impact of lycopene supplemented canola meal-based diet on growth performance, nutrient digestibility and antioxidant status of *Catla catla* fingerlings. *J. Anim. Plant. Sci.* 32:571-577. <https://doi.org/10.36899/JAPS.2022.2.0456>
- Ibrahim ATA, 2015. Protective role of lycopene and vitamin E against diazinon-induced biochemical changes in *Oreochromis niloticus*. *Afr. J. Environ. Sci. Technol.* 9:557-565. <https://doi.org/10.5897/AJEST2014.1853>
- Jabeen F, Chaudhry AS, Manzoor S and Shaheen T, 2015. Examining pyrethroids, carbamates and neonicotinoids in fish, water and sediments from the Indus River for potential health risks. *Env. Mon. Assess.* 187:1-11. <https://doi.org/10.1007/s10661-015-4273-4>
- Jin M, Li L, Xu C, Wen Y and Zhao M, 2010. Estrogenic activities of two synthetic pyrethroids and their metabolites. *J. Environ. Sci.* 22:290-296. [https://doi.org/10.1016/S1001-0742\(09\)60107-8](https://doi.org/10.1016/S1001-0742(09)60107-8)
- Juan CA, De-la-Lastra JMP, Plou FJ and Perez-Lebena E, 2021. The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int. J. Mol. Sci.* 22:1-21. <https://doi.org/10.3390/ijms22094642>
- Kaleshtari AB, Hosseini SV, Farhangi M and Rafiee G, 2019. Replacement of carrot powder with synthetic astaxanthin in the Rainbow Trout diet: Effect on the growth performance and blood parameters. *Aquat. Anim. Nut.* 5:59-70. 10.22124/janb.2019.13473.1059
- Kalinowski CT, Betancor MB, Torrecillas S, Sprague M, Larroquet L, Veron V, Panserat S, Izquierdo MS, Kaushik SJ and Dicharry SF, 2023. More than an antioxidant: Role of dietary astaxanthin on lipid and glucose metabolism in the liver of Rainbow Trout (*Oncorhynchus mykiss*). *Antioxidants* 12:1-14 <https://doi.org/10.3390/antiox12010136>
- Khandige PS, Babu KMPS, Sadananda V and Gowrish S, 2022. Isolation, Production, and Characterization of Serum Immunoglobulin M (IgM) of Indian Major Carp *Cirrhinus mrigala* (MRIGAL). *Adv. Pharmacol. Pharm. Sci.* 2022: 2339924. DOI: 10.1155/2022/2339924
- Li J, Ran X, Zhou M, Wang K, Wang H and Wang Y, 2022. Oxidative stress and antioxidant mechanisms of obligate anaerobes involved in biological waste treatment processes: A review. *Sci. Total Environ.* 838:1-15. <https://doi.org/10.1016/j.scitotenv.2022.156454>
- Mamboungou J, Fernandes EKK, Vieira LG and Rocha TL, 2024. Hazardous fipronil insecticide effects on aquatic animals health: Historical review and trends. *Sci. Total Environ.* 954:1-14. <https://doi.org/10.1016/j.scitotenv.2024.176334>
- Micah AD, Wen B, Wang Q, Zhang Y, Yusuf A, Thierry NNB, Tokpanou OS, Onimisi MM, Adeyemi SO and Gao JZ, 2022. Effect of dietary astaxanthin on growth, body color, biochemical parameters and transcriptome profiling of juvenile Blood Parrotfish (*Vieja melanurus* × *Amphilophus citrinellus*). *Aquac. Rep.* 24:1-15. <https://doi.org/10.1016/j.aqrep.2022.101142>
- Moreira SP, Ferreira S, Couto B, Machado-Sousa M, Fernandez M, Raposo-Lima C and Morgado P, 2021. Protective elements of mental health status during the COVID-19 outbreak in the Portuguese population. *Int. J. Environ. Res. Pub. Health.* 18:1-10. <https://doi.org/10.3390/ijerph18041910>

- Olatunde A, Tijjani H, Ishola AA, Egbuna C, Hassan S and Akram M 2020. Carotenoids as functional bioactive compounds. *Functional Foods and Nutraceuticals: Bioactive Components, Formulations and Innovations* pp 415-444. https://doi.org/10.1007/978-3-030-42319-3_20
- Ramesh M, Bindu CF, Mohanthi S, Hema T, Poopal RK, Ren Z and Li B, 2023. Efficiency of hematological, enzymological and oxidative stress biomarkers of *Cyprinus carpio* to an emerging organic compound (alphamethrin) toxicity. *Environ. Toxicol. Pharmacol.* 101:1-17 <https://doi.org/10.1016/j.etap.2023.104186>
- Sharma NA and Goswami UC, 2011. Functioning of lycopene in mammalian system: A review. *Proceed. Zool. Soci.* 64:1-7. <https://doi.org/10.1007/s12595-011-0005-0>
- Souza FPD, Lima ECS, Urrea-Rojas AM, Suphoronski SA, Facimoto CT, Bezerra-Junior JDS, Oliveira TES, Pereira UDP, Santis GWD and Oliveira CALD, 2020. Effects of dietary supplementation with a microalga (*Schizochytrium* sp.) on the hemato-immunological and intestinal histological parameters and gut microbiota of Nile Tilapia in net cages. *PLOS One* 15:1-19. DOI: 10.1371/journal.pone.0226977S
- Stara A, Pagano M, Capillo G, Fabrello J, Sandova M, Vazzana I, Zuskova E, Velisek J, Matozzo V and Faggio C, 2020. Assessing the effects of neonicotinoid insecticide on the Bivalve Mollusc (*Mytilus galloprovincialis*). *Sci. Total Environ.* 700:1-12. <https://doi.org/10.1016/j.scitotenv.2019.134914>
- Suphoronski SA, Chideroli RT, Facimoto CT, Mainardi RM, Souza FP, Lopera-Barrero NM, Jesus GFA, Martins ML, Di Santis GW, de Oliveira A, Gonçalves GS, Dari R, Frouel S and Pereira UP, 2019. Effects of a phytogenic, alone and associated with potassium diformate, on tilapia growth, immunity, gut microbiome and resistance against francisellosis. *Sci. Rep.* 9:1-14. <https://doi.org/10.1038/s41598-019-42480-8>
- Sahin K, Yazlak H, Orhan C, Tuzcu M, Akdemir F and Sahin N, 2014. The effect of lycopene on antioxidant status in Rainbow Trout (*Oncorhynchus mykiss*) reared under high stocking density. *Aquaculture* 418:132-138. <https://doi.org/10.1016/j.aquaculture.2013.10.009>
- Thomaz JM, Martins ND, Monteiro DA, Rantin FT and Kalinin AL, 2009. Cardio-respiratory function and oxidative stress biomarkers in Nile Tilapia exposed to the organophosphate insecticide trichlorfon. *Ecotoxicol. Environ. Safe.* 72:1413-1424. <https://doi.org/10.1016/j.ecoenv.2008.11.003>
- Tahir R, Afzal F, Ghaffar A, Liang J, Shrestha A, Habiba U and Yang S, 2024. Effectiveness of lycopene dietary enrichment for growth performance, digestive enzymes, blood health, immunity and antioxidant status of common carp (*Cyprinus carpio*) against chronic glyphosate toxicity. *Aquaculture* 590:741084. <https://doi.org/10.1016/j.aquaculture.2024.741084>
- Van Doan H, Hoseinifar, Hung SH, Lumsangkul TQ, Jaturasitha C, El-Haroun S and Paolucci EM, 2020. Dietary inclusion of chestnut (*Castanea sativa*) polyphenols to Nile tilapia reared in biofloc technology: Impacts on growth, immunity, and disease resistance against *Streptococcus agalactiae*. *Fish & Shellfish Immunology*, 105, 319-326. <https://doi.org/10.1016/j.fsi.2020.07.010>
- Wang J, Deng L, Chen M, Che Y, Li L, Zhu L, Chen G and Feng T, 2024. Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade. *Anim. Nut.* 17: 244-264. <https://doi.org/10.1016/j.aninu.2024.01.012>
- Yonar SM, Yonar ME, Pala A, Saglam N and Sakin F, 2020. Effect of trichlorfon on some haematological and biochemical changes in *Cyprinus carpio*: The ameliorative effect of lycopene. *Aquac. Rep.* 16:1-6. <https://doi.org/10.1016/j.aqrep.2019.100246>
- Zeng X, Du Z, Ding X and Jiang W, 2021. Protective effects of dietary flavonoids against pesticide-induced toxicity: A review. *Trends Food Sci. Technol.* 109:271-279 <https://doi.org/10.1016/j.tifs.2021.01.04>