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Effect of carrot (*Daucus carota*) flour on pigmentation and blood parameters of *Catla catla* and *Cyprinus carpio*

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Abstract
The primary aim of this study was to determine the effect of carrot flour (*Daucus carota*) on pigmentation and blood parameters of *Catla catla* (Thaila) and *Cyprinus carpio* (Common Carp). Pigmentation is often a key indicator for fish in terms of consumer acceptability. Thaila has immense cultural and economic importance in South Asia because of its flesh quality and large size. Common carp is also an important edible fish and the third most frequently introduced species all over the world due to its ornamental colors. Carrot flour, rich in vitamin A, is a natural vegetable feed source as a pigmentation enhancer because it contains carotenoids in large amounts. High amounts of beta-carotene also act as an antioxidant and have a beneficial effect on fish health. For this experiment, an 8-week trial was conducted with two groups (one experimental and one control) in triplicate, having an equal number of fish (n = 20 each). After completion of the trial, blood and skin samples from both fish species were taken and subjected to a hemocytometer and a spectrophotometer, respectively. Statistical inference using the t-test was done to get the final results of the trial. The carrot flour supplementation led to increased red blood cell counts in both species, with significant changes in other hematological parameters as well. Additionally, both species exhibited enhanced pigmentation in dorsal and ventral scales when fed carrot flour according to 2.5% of the body weight of the fish. These findings suggest potential benefits of the carrot flour in improving hematological parameters as well as pigmentation in fish, leading to better consumer acceptability and potential economic benefits.

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Introduction

Aquaculture has an important role in fulfilling the demands and needs of humans for fish. All other aquatic animals are a huge contributor to the fisheries sector. Aquatic sources that are derived from the aquaculture industry have high nutritional values with quality protein, minerals, vitamins, and omega-3 fatty acids (Arshad et al., 2022). To increase fish production, aquaculture has not been limited to extensive culture, but it has moved towards semi-intensive and intensive culture systems as well. Furthermore, in aquaculture systems, nutrition has a significant role to play (Tiwari and Kaur, 2022).

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With respect to the nutritional value of food products, fish is a major source of nutrients for the nutritionally vulnerable human population. Fish is the main source of animal protein, but it also contains essential micronutrients like vitamin B and D, minerals like phosphorus, zinc, iodine, selenium, and iron, and also long-chain polyunsaturated fatty acids (Noreen et al., 2025). All these compounds are extremely beneficial for the adult fish's health and growth. These compounds are not readily available in all diets, but are present in fish (Béné et al., 2016; Awuchi et al., 2022). Around the globe, people get a maximum share of dietary protein and run their small-scale recreation business from freshwater fisheries, especially in those areas where other employment and nutrition sources are hard to find (McIntyre et al., 2015; Boyd et al., 2022).

Thaila (*Catla catla*) basically feeds on zooplankton and is a surface feeder. Along with other carps, *C. catla* made a contribution of millions of tons to the total production of aquaculture (Sharma et al., 2017). It is an extensively cultured and very popular freshwater edible fish. However, common carp (*Cyprinus carpio*) is also one of the important fish in the aquaculture industry, especially in European and many Asian countries (Öz and Ucak, 2023). Nutrient availability in water and aerobic decomposition of organic matter are highly affected by *C. carpio* because it feeds on benthic organisms. If common carp is not in enough density in a water body, nutrient availability will increase, and this will enhance the photosynthesis and plankton production (Rahman, 2015; Hammadi et al., 2024).

Common carp farming is among the largest in the world, ranking third among farmed freshwater fish. Its omnivorous nature and ability to utilize various fiber-rich feed components make it suitable for sustainable animal nutrition (Eljasik et al., 2022). Carp farming holds potential for incorporating waste into feed and energy strategies within a circular economy framework (Kowalska et al., 2022).

The evaluation of hematological parameters has been proven to be very important to study the health status of fish (Witeska et al., 2022). These parameters provide us with extrinsic and intrinsic factors that influence these values. These parameters are studied worldwide to evaluate the fish health (Reshi et al., 2023). These values show that changes in the internal environment of fish, along with extrinsic and intrinsic factors, cause variability of blood parameters in fish. The blood parameters are important in evaluating a fish's health status (Ahmed et al., 2020; Witeska et al., 2022).

Carotenoids are functional units of pigmentation in fish and are obtained easily from natural vegetable plant sources (Sathyaruban et al., 2021). Carotenoids give red, orange, and yellow colors because their color ranges from yellow to red. The term 'carotene' is related to the compounds having the formula $C_{40}H_{56}$ (Kim, 2016). We can find carotenoids in plant chloroplasts. In photosynthesis, carotenoids act as a catalyst. β-carotene especially gives the bright orange color to carrot tubers (Sihaloho and Zaidar, 2023). Carotenoids improve a number of physiological processes, including growth, survival, and disease resistance, along with their contribution to the red to yellow hue of fish organs and skin (Sathyaruban et al., 2021). Like many other higher vertebrates, fish need to consume a diet rich in carotenoid supplements since they are unable to produce carotenoids internally (Judan Cruz et al., 2021). Carotenoids are abundant in plants with vibrant colors, like beets, carrots, and tomatoes. A prior study has indicated the significance of these veggies in augmenting the color and growth of fish when incorporated into their diet (Ashokkumar et al., 2023). Fish diets must contain carotenoids for the fish to be healthy and vibrant overall, for an enhanced acceptance rate in the industry. Fish coloration and health outcomes can be greatly impacted by carotene-rich diet supplementation (Biswas et al., 2024). In order to maximize aquaculture techniques and guarantee fish health, it is imperative to comprehend the function of carotenoids in fish nutrition.

Different types of carotenoid sources have been widely studied previously by researchers. The effect of carrot flour on *C. catla* and *C. carpio* fingerlings' pigmentation and blood profile was thoroughly studied in the current investigation, adding to the literature of fish acceptance by consumers and also the producers of the aquaculture sector.

Materials and Methods

The current research study was done in the Fisheries Research Farm located at the Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. *Cyprinus carpio* (Common carp) and *Catla catla* (Thaila) were used in this study.

Experimental trial

Fingerlings (120) of *C. catla* and *C. carpio* each were taken from the Fisheries Research Farm, University of Agriculture, Faisalabad. Fingerlings of both fish species, each with an average weight of 2.96 ± 0.025 g, were kept in glass aquariums using randomized placement and acclimatized for 48 h.

Two different groups (one control and the other experimental group) were made with 20 specimens in each group of fish. Feed under investigation (carrot flour) was given to the specimens in the experimental group, and specimens in the control group were supplied only with commercial feed (100%). Trials were continued for 8 weeks. The glass aquariums were provided with full aeration, keeping optimum physico-chemical parameters of water quality.

Experimental feed preparation

Carrot flour was taken as experimental feed to the experimental group of fish according to 2.5% of the body weight of the fish, twice daily. Carrot flour was prepared using fresh carrots. The carrots were firstly washed with clean water, cut into thin slices, and then subjected to oven-drying at 60 °C, followed by grinding into fine powder. The powder was then stored to be used as an experimental diet for fish under investigation in an air-tight container.

$$\text{Amount of } \frac{\text{feed}}{\text{day}} \text{ (g) for T2} = \frac{\text{total weight of fish stocked} \times \text{feeding rate at 2.5\% body weight}}{100} = 3.75$$

Blood sampling

First of all, 10 fish from each experimental group were subjected to euthanasia, and the samples were collected using a clean syringe from the fish's caudal vein. The blood samples were stored in EDTA vacutainers and stored to be used in a haematocytometer as per the protocol followed by Oluyemi et al. (2008).

Haematological parameters

A hemocytometer was used to measure white blood cells (WBC) and red blood cells (RBC) counts. There were two parts of the sample; the first portion of the sample was treated with 10% EDTA to determine several haematological parameters like WBCs, RBCs, and hemoglobin (HB), etc. A blood sample (each 100 mL) was incubated at 37 ºC for one hour in flat-bottomed microplates. This step was necessary to make the cell adhesion process easier. For washing, a saline buffer was used. Washing was continued for 3 minutes for a precise count. The smears were stained to determine each cell count (differential staining of leukocytes).

Spectrophotometer analyses

For spectrophotometer analyses, the protocol described by Maiti et al. (2017) was followed. The samples were collected from the dorsal and ventral scales of randomly selected fish from each treatment group, with 5 fish taken from each group for carotenoid analysis. The samples were transferred to pre-weighed glass tubes and were ground in acetone with about 1.5 g of Na_2SO_4 and homogenized with a homogenizer. Extraction from the sample was made at 450 nm. The extracted samples were stored in a refrigerator at 4 ºC for 3 days. The samples were extracted three to four times until no more color could be obtained. The solution was firstly centrifuged at 5000 rpm, and then absorbance values were noted at 471 nm using a spectrophotometer.

Statistical analyses

The data obtained from the experiment were subjected to statistical interference. The *t*-test was used to compare both treatments for final interception.

Results

Hematological parameters

Catla catla

To assess the changes in blood parameters of *C. catla*, a hematological analysis was performed (**Table 1**, **Figure 1-3**). The minimum and the maximum values of RBCs in the control group were 1.22 × 10^5 µL and 1.53 × 10^5 µL, respectively, and in the experimental group were 1.51 × 10^5 µL and 2.1 × 10^5 µL. Likewise, for HB,7.1 g/dL and 2.9 g/dL were the maximum and the minimum, respectively, in the control group, and 11.8 g/dL and 5.9 g/dL as the maximum and the minimum in the experimental group. The HCT values in the control group were found to be 7.5% and 20.8%, and in the experimental group, 17.2% and 28.2%, representing the minimum and maximum, respectively.

Table 1: Hematological parameters of *Catla catla*

Indicators	T_o	T_1	*P*-value

RBCs (µL)	1.37 ± 0.12	1.68 ± 0.238	0.0159*
Hb (g/dL)	5.1 ± 1.935	9.04 ± 2.129	0.0078*
HCT (%)	13.4 ± 5.578	22.52 ± 5.578	0.0104*
WBC (10^3 µL)	136.87 ± 6.678	155.36 ± 7.745	0.0019*
Lymphocytes (%)	47.2 ± 7.006	74.58 ± 7.523	0.0002*
Platelets (10^3 µL)	134.6 ± 14.046	160.4 ± 17.714	0.017*
MCH (pg)	26.39 ± 11.081	24.95 ± 9.706	0.4166
MCHC (g/dL)	37.98 ± 7.412	43.138 ± 8.978	1.8595

The WBC values in the control group were 130.01 × 10^3 µL and 143.01 × 10^3 µL, and in the experimental group, 146.5 × 10^3 µL and 163.8 × 10^3 µL as the minimum and the maximum values. The lymphocyte count values were 41.2% and 55.3% in T_0 and 65.8% and 85.6% in T_1, respectively, as the minimum and the maximum values. The platelet count was 114 × 10^3 µL and 156 × 10^3 µL in To and 137 × 10^3 µL and 168 × 10^3 µL in T_1 as the minimum and the maximum values, respectively. The MCH values in the control group were 14.52 pg and 38.6 pg, whereas in the experimental group, they were 16.7 pg and 39.87 pg, respectively (**Table 1, Figure 2**). The MCHC values in the control group were 28.9 g/dL and 49.4 g/dL as the minimum and the maximum values; on the other hand, in the experimental group, 30.2 g/dL and 53.92 g/dL, respectively, were the minimum and maximum values.

The normal range for white blood cells (WBC) in fish ranges from 100-200 × 10^5 µL, but we got slightly elevated values, which is attributed to the immune-boosting effect of carotenoids in carrot flour (**Table 1, Figure 3**).

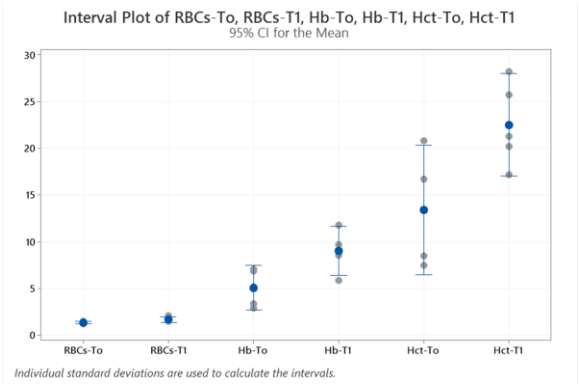

Figure 1: Graphical representation of RBCs, HB, and HCT in *Catla catla*

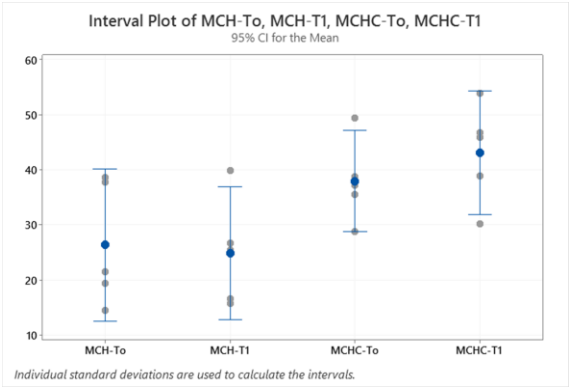

Figure 2: Graphical representation of MCH and MC in *Catla catla*

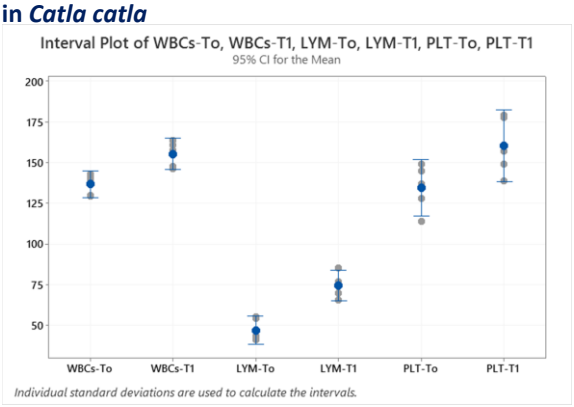

Figure 3: Graphical representation of WBC, LYM, and PLT in *Catla catla*

Cyprinus carpio

The minimum and the maximum values of RBCs in the control group were 1.29 × 10^5 µL and 1.58 × 10^5 µL, and in the experimental group, 1.76 × 10^5 µL and 3.89 × 10^5 µL, respectively. Likewise, for the HB values, the control group had 8.1 g/dL and 3.6 g/dL, as maximum and minimum values, respectively, whereas in the experimental group, 9.5 g/dL and 15.9 g/dL were the minimum and the maximum values (**Table 2, Figure 4**). The HCT values in the control group were found to be 7.9% and 26.1%, and in the experimental group, 18.7% and 30.2 %, as the minimum and the maximum values, respectively. The WBC values in the control group were 133.3 × 10^3 µL and 160.01 × 10^3 µL, and in the experimental group, 148.5 × 10^3 µL and 168.8 × 10^3 µL as the minimum and the maximum values, respectively (**Table 2, Figure 3**). This elevation in the range of white blood cells could have been due to carrot flour enriched with carotenoids (**Table 2, Figure 6**). The lymphocyte count values were 65.5% and 78.8% in To, and 80.4% and 88.6% in T_1 as the minimum and maximum, respectively.

Table 2: Hematological parameters of *Cyprinus carpio*

Indicators	**T_o**	**T_1**	***P*-value**
RBCs (µL)	1.382 ± 0.116	3.08 ± 0.826	0.0051*

Hb (g/dL)	6.04 ± 2.071	11.96 ± 2.457	0.0017*
HCT (%)	14.32 ± 7.372	23.98 ± 5.438	0.0231*
WBC (10^3 µL)	142.96 ± 7.244	161.48 ± 12.42	0.0103*
Lymphocytes (%)	71.32 ± 5.276	84.96 ± 3.693	0.0007*
Platelets (10^3 µL)	117.6 ± 5.224	151.6 ± 16.501	0.0035*
MCH (pg)	16.40 ± 1.529	15.92 ± 2.433	0.3576
MCHC (g/dL)	15.08 ± 1.311	14.57 ± 1.709	0.3068

We found the minimum and the maximum MCH values as 14.62 pg and 18.76 pg as the minimum and the maximum, in the control group, and 13.44 pg and 18.97 pg, as the minimum and maximum values, respectively, in the experimental group.

The MCHC values in the control group were 13.42 g/dL and 16.94 g/dL, representing the minimum and maximum values, respectively (**Table 2, Figure 5**). In contrast, the values in the experimental group were 12.76 g/dL and 16.87 g/dL, as the minimum and the maximum, respectively.

Pigmentation

Catla catla

For pigmentation analysis, the absorbance (optical density values) from a spectrophotometer at 471 nm was used (**Table 3, Figure 7**). In the dorsal scales, the pigmentation values were found as 0.230 and 0.478, whereas in the experimental group, 0.672 and 1.675, the minimum and the maximum values, respectively, were found. Whereas in the ventral scales of *C. catla*, the minimum and the maximum values in the control group were 0.215 and 0.453, and in the experimental group, the values were 0.354 and 1.167, respectively.

Table 3: Pigmentation in *Catla catla*

	T_o	T_1	*P* value
DS	0.38 ± 0.099	1.19 ± 0.388	0.001**
VS	0.34 ± 0.095	0.76 ± 0.349	0.016*

DS: In the dorsal scale; VS: In the ventral scale

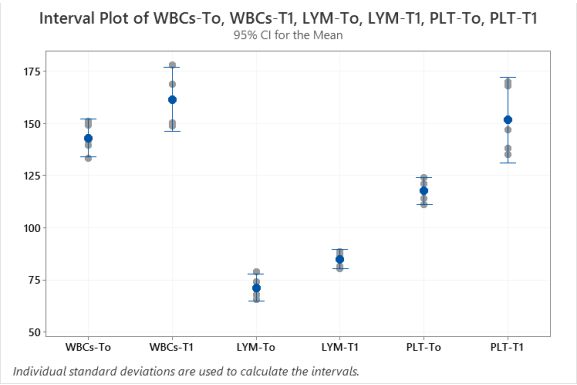

Figure 6: Graphical representation of WBC, LYM, and PLT in carp

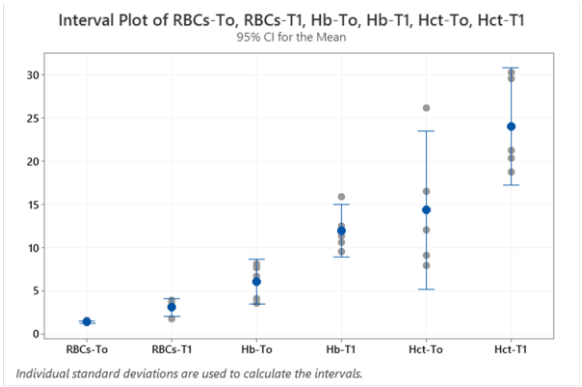

Figure 4: Graphical representation of RBC, HB, and HCT in carp

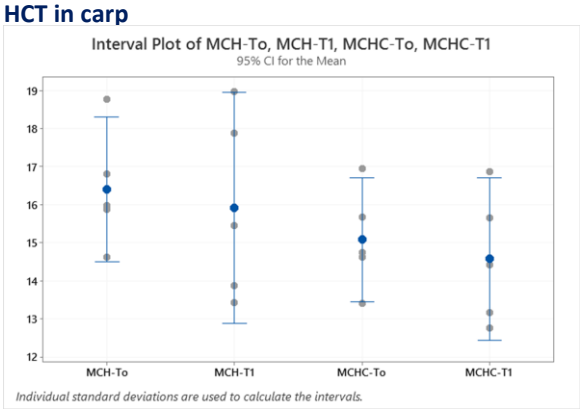

Figure 5: Graphical representation of MCH, and MCHC in carp

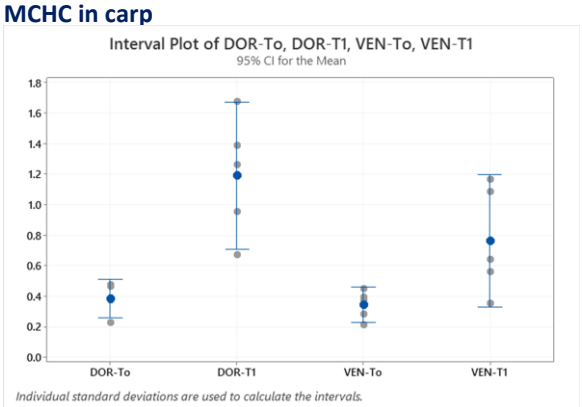

Figure 7: Graphical representation of pigmentation in *Catla catla*

Cyprinus carpio

In the dorsal scales, the values of the control group (T_0) were 0.346 and 0.879, and in T_1, 1.269 and 1.945 were recorded as the minimum and the maximum values, respectively (**Table 4, Figure 8**). On the other hand, in the ventral scale, the minimum and the maximum values in T_0 were 0.516 and 0.867, while in T_1, the values were 0.983 and 1.893, respectively.

Table 4: Pigmentation in *Cyprinus carpio*

	T_o	T_1	*P* value
DS	0.59 ± 0.202	1.59 ± 0.266	0.0001**
VS	0.64 ± 0.153	1.31 ± 0.352	0.0057*

DS: In the dorsal scale; VS: In the ventral scale

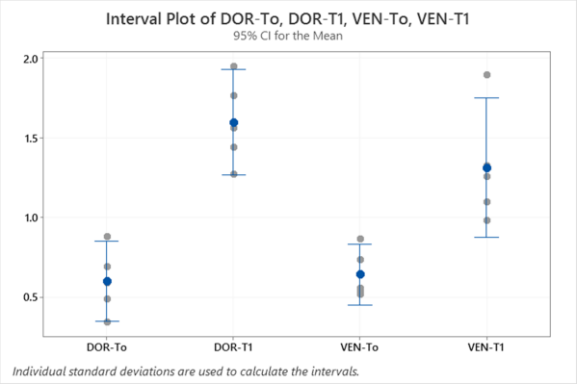

Figure 8: Graphical representation of pigmentation in *Cyprinus carpio*

Discussion

Carrot flour was supplied as a feed to two different fish species, *Catla catla* and *Cyprinus carpio* fingerlings, to determine its effect on blood parameters and pigmentation of skin. Blood parameters included checking of red blood cells, white blood cells, hemoglobin, hematocrit, platelets, lymphocytes, MCH, and MCHC. Carotenoids, especially β-carotene, which is used as a natural pigment enhancer in aquaculture (Gupta et al., 2007), were abundant in carrot flour made from carrots (*Daucus carota*). In the present study, the results showed that when fed with carrot feed, changes could be clearly seen in the blood parameters of fish hemoglobin and lymphocytes. Similar results have been found by Altinterim and Onder (2019), showing that carrot feed supplementation stimulated the erythropoiesis density at a high level. Fish fed with β-carotene through carrot-based feed showed higher indices in skin colour and hematological parameters in fish, as already reported by Alishahi et al. (2014). Carrot flour feed showed higher immunological indices, and an increase in hematological markers like red blood cells, white blood cells, and platelets might have a significant role in boosting the immune system.

Research on fish that were fed with vegetable feed source (carrot feed) showed their skin color brightened and showed increased color intensity, i.e., mostly intensity increased to bright red and orange colour (Wagde et al., 2018). This report also supports our findings. Carrot feed is also used to brighten up the dull colours of fish, for example, in *C. catla*, to increase its market value (Weerakkody and Cumaranatunga, 2016).

For ornamental fish to have color on their skin and food fish to have muscular pigmentation, carotenoids are essential (Gupta et al., 2007). Fish need nutritional supplements since they are unable to produce carotenoids from scratch (Nakano and Wiegertjes, 2020). Further, being an excellent source of vitamin A, the carrot diet has a strong impact on the hematological and immunological profile of the fish as indicated by Guimarães et al. (2016).

Carrot flour, being rich in beta-carotene and vitamin A, is vital for maintaining fish health, especially the blood profile. Moreover, it had a tremendous effect on the color intensity of fish, ultimately increasing their market value, being more effective in the case of ornamental fish (Darsiani et al., 2025). Overall, the findings suggest that carrot flour supplementation at 2.5% of the body weight of fish positively influences pigmentation in both common carp and Thaila fingerlings, as evidenced by the significant differences in pigmentation between the control and the experimental groups in both species. However, further research is required to ascertain how carrot flour affects fish, assess feed durability, and determine the most effective administration strategies for aquaculture species.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors
Conceptualization and designing of the study: IM, SP, FR, Arooj. Conduction of experiments: IM, SP, Arooj, AA, TF, NM. Data collection, visualization, and interpretation: IM, SP, FR, A, TF. Formal statisti-

cal analysis: IM, SP, A, AA, TF. Writing of first draft: IM, SP, UZ, NM, Arooj. Proofreading and approval of the final version: IM, SP, FR, Arooj, AA, US, TF, NM, Arooba.

Permissions and ethical compliance
This work was approved by the Institutional Ethical Review Board/Committee (IERB/C) of the University of Agriculture, Faisalabad, Pakistan (Approval number 3181-84 dated 16-02-2024).

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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Declaration of generative AI and AI-assisted technologies in the writing process
It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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