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Impact of fungal pigments (Carotenoids) in feed for *Cyprinus carpio* fish (KOI KARP)

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Abstract
The bright colours of ornamental fish are not just attractive, but have antioxidant and photoprotective functions. These colours are due to carotenoids present in the fish skin. However the fish cannot synthesize these pigments and thus depend on exogenous sources. Apart from rendering bright colours, these pigments have antioxidant, antimicrobial and photoprotective function. Pigmentation in ornamental fish increases their market value. In this study, carotenoid pigments were isolated from *Penicillium purpurogenum* and *Talaromyces purpurogenum*, used to make top coated fish feed and fed to Golden Koi (*Cyprinus carpio)* fish to check for enhancement in colour and other properties. The study indicated that the pigments were non - toxic to the fish, increased the fish weight and protein content and showed an enhancement in the skin color. High resolution liquid chromatography mass spectrometry data showed the presence of an additional carotenoid astaxanthin in the test fish. Thus the fungal pigment fed to the fish enhanced its colour and other properties.

Keywords: Fungi, carotenoids, fish feed, *Cyprinus carpio*, HRLCMS

1. Introduction
Since early civilization, products have been made attractive in presentation by addition of colours. Colorants are the most sensitive part of any commodity to enhance consumer acceptability [1]. Pigments can be classified based on their origin as natural, synthetic (lab made) and inorganic. Natural dyes are derived from naturally occurring sources such as plants (e.g., indigo and saffron); insects (e.g., cochineal beetles and lac scale insects); animals (e.g., some species of mollusks or shellfish); and minerals (e.g., ferrous sulfate, ochre, and clay) without any chemical treatment [2]. Natural colours, extracted from fruits, vegetables, seeds, roots and microorganisms are called “bio colours” and have proven to be safe colouring agents. Synthetic pigments are undesirable and harmful, but can cause adverse effects to the environment. Colours derived from minerals (lead chromate, copper sulphate) may cause serious health problems. Strong consumer demand for natural products has prompted many researchers to look for alternatives to synthetic pigments [3].Synthetic colours are being substituted by natural colour additives, and they have a market estimated in US$ 600 million and steadily growing at around 2 % annually. Natural colours now make up 31 per cent of the colourings market, compared with 40 per cent for synthetics, according to LFI [4].
The advantages of pigment production from microorganisms include easy and fast growth in cheap culture medium, independence from weather conditions, colours of different shades and antimicrobial activity [5]. According to a study the global market for carotenoid was $ 766 million in 2007 and is expected to increase to $919 million by 2015 with a compound annual growth rate (CAGR) of 2.3% and beta - carotene alone shared the market value at $247 million in 2007 [6]. Microbial pigments are suitable for mass production, but these are inherently less stable than synthetic ones, a problem that explains the limited palette of commercial microbial colour additives [7].
Feed colorants are substances which are added in trace amounts to a diet or feed mixture to facilitate its ingestion (through improved visibility of feed particles) or to impart a desired colouration within the carcass of the cultured fish or shrimp. Along with imparting bright colours it can also serve a good source of vitamins [8]. Indian waters possess a rich diversity of ornamental fish, with over 100 indigenous varieties, in addition to a similar number of exotic species that are bred in captivity [9]. India has recorded at least 150 commercially important fish species and trade mainly indigenous freshwater species collected from rivers [10].

Ornamental fish keeping and its propagation has been an interesting activity and which can provide aesthetic pleasure and financial openings. It has been estimated that the world trade on ornamental fishes is around US $ 4.5 billion, of which freshwater ornamental fish trade forms about 85% [11]. Keeping in mind the above points, the present study focuses on the use and impact of fungal pigments in making top coated fish feed for *Cyprinus carpio* (Karp) ornamental fish variety. When compared with the reported literature, algae and plant extracts have been used as fish feed preparation for colour enhancement.

2. Methods

2.1 The experimental fish: Golden Koi (*Cyprinus carpio)* fish were obtained from Aquaculture fishes, Kurla, Mumbai, India. Prior to the start of the experiments, the fish were acclimatised to the commercial diet for one week and were fed twice daily to apparent satiation. At the beginning of the experiment, the fish were starved for one day, weighed and the fish with similar weights (5.00 gm - 6.50 gm) were allotted randomly to separate tanks (three fish to each tank). For all the experiments the fish were maintained in circular tanks (20 cm diameter and 40 cm height with lid and equipped with air pump). Water quality parameters were monitored. The pH was maintained at 7.5 to 8.0, dissolved oxygen (monitored by dissolved oxygen probe) was 5 mg/L and temperature was 28 to 32 ^{0}C. The fish were fed with pigment coated feed twice daily with 0.13 gm of the feed. A three week study (at 7 day interval) was done with the top coated feed. The experiment was done on control and test fish (three fish each) and the same treatment was followed in all the tanks. The tanks were cleaned daily and two thirds of the water replaced before feeding [12].

2.2 Preparation of fungi: The fungi producing red pigments were isolated from spoilt onion and identified by morphological characterisation and 18S rRNA studies. They were identified as *Penicillium purpurogenum* (SR2) and *Talaromyces purpurogenus* (SR4). The fungal isolates were grown on crude, cheap substrate like onion peel and whey based medium. The pigments were purified by using acidified butyl acetate (pH 2) by solvent- solvent extraction process. They were dried in vacuum evaporator (Make: Superfit) and sterilised by membrane filteration (0.45 micron, Himedia, Mumbai). They were characterised by UV- Visible spectroscopy, HPTLC and HRLCMS. These studies indicated that the pigments contained mixture of natural stereoisomers of carotenoids.

Table 2.1: Mixture of carotenoids present in the pigments SR2 and SR4

Carotenoids present in Pigment	
SR2	**SR4**
Fucoxanthin Violaxanthin Peridinin Neurosporaxanthine Ketolutein D Diaponeurosporene Spheroidenone Leprotene	Neurosporaxanthine Ketolutein D Erythroxanthin sulphate Diaponeurosporene Bacteriorubixanthinol

2.3 Antioxidant potential of the pigments: The total antioxidant potential of the pigment was done by the phosphomolybdenum [13, 14], and Ferric reducing antioxidant power (FRAP) methods [15] using Ascorbic acid as a standard in both the methods.

2.4 Toxicity tests on fish: The toxic effects of the pigments were checked on the fish. For each pigment three fish were injected with the respective pigments SR2 and SR4. The fish were injected intraperitoneally in the abdominal cavity posterior to the pelvic girdle with 50 µl and 100 µl of SR2 and SR4 pigments. Un-injected control fish (3 in number) were maintained at the same conditions. Water quality parameters were monitored as mentioned above. The fish were fed with normal diet twice daily (0.13 gm) during this study. The effect of the pigment on the survival and changes in the appearance of fish was noted after 72 hours [12].

2.5 Preparation of the feed: The processed pigments were used to top coat the commercial fish feed. The commercial feed was procured from Kijaro Basic – floating type-Aquasystem - Malaysia) (table 2.2)

Table 2.2: Components of the commercial fish feed.

Fish meal	Calcium
Wheat flour	Crude fat 4%
Ricebran	Moisture 10%
Yeast	Crude ash 12%
Vitamin	

After calculating the amount of the respective pigment 96 mg/kg of pigment for SR2 and 85mg/kg of pigment for SR4 were added to market fish feed and dried at 55 ^{0}C. 1 gm of dried fish pellet was used to re extract the pigment in acetone by vortexing for 5 minutes, centrifugation at 10000 rpm for 10 minutes. The UV-Vis scan was done to check for the presence of the characteristic peaks of the pigments in the fish feed [16].

2.6 Impact of the feeding: The colour assessment was done to check the effect of the pigments in enhancement of fish skin colour by the following methods.

2.6.1 During feeding (Visual observation of the fish): The experimental fish were observed at 7 day intervals and the observation was noted. There were 10 panellists who noted down their observation by comparing the test fish with the control fish visually. They graded the colour and any other changes in the fish appearance after every 7 days on a scale of 1 to 10; where 1 was no colour change and 10 was maximum colour change [17].

2.6.2 Post feeding: After the three week period, the fish were weighed, anesthetized with 0.1% w/v lignocaine gel and sampled. The fish skin was peeled (1 gm) and suspended in acetone (5 ml) and vortexed for 10 minutes to extract maximum colour. The extract was centrifuged at 10000 rpm for 10 minutes and used for the analysis [18].

2.6.2.1 Visible spectrophotometry: The extract was scanned at 400-700 nm on Visible spectrophotometer to check for the characteristic peak. The measurement of the colour was then done at 465 nm and the colour was compared to the control.

2.6.2.2 HRLCMS analysis: The skin colour extracted was subjected to High Resolution Liquid Chromatography Mass Spectroscopy (HRLCMS) (Agilent Technologies - IIT-

Bombay) to check for the presence of the difference in the metabolites that may be giving an enhancement of colour. The column was Zorbax SB C18 RRHD, 2 x 1.5mm, 1.8 micron, Agilent Technologies. The Mobile phase used was: Solvent A-100% milliq water + 0.1% formic acid; Solvent B- 100% ACN +0.1% formic acid [19, 20].

2.6.2.3 Protein content of fish muscle: The fish muscle was collected in phosphate buffered saline (pH 7.0), macerated and used to analyse the protein content by the Folin Ciocalteau method using Bovine serum albumin (BSA) as a standard [21].

3. Results

3.1 Antioxidant potential of the pigments: As seen from the table 3.1, the SR2 and SR4 in comparison to a known antioxidant ascorbic acid showed 152 µg/ml and 196 µg/ml of antioxidant activity respectively. The FRAP method gave results similar to the phosphomolybdenum method in terms of Ascorbic acid standard. The FRAP values of SR2 and SR4 were 7.29 and 33.49 µm respectively (FRAP value of Ascorbic acid is 2).Thus both the pigments have good antioxidant potential and hence could be tested for further applications

Table 3.1: Antioxidant potential of pigments by Phosphomolybdenum and FRAP assay (in terms of Ascorbic acid).

Test	Phosphomolybdenum method ; Antioxidant potential (µg/ml)	FRAP assay (FRAP value of Ascorbic acid is 2)
Unknown SR2	152	7.29
Unknown SR4	196	33.49

3.2 Toxicity test of the pigment on fish: As seen from the fig 3.1, it was noted that the fish survived the injection treatment. This study plays a very important role in the use of fungal fish feed and ensures that the pigments extracted from the fungus do not affect the fish and can be used in preparation of feed. It was also observed incidentally that the fish had showed the capacity to regenerate the fins, thus showing normal growth and regeneration. The visible spectra of the pigment in the feed was similar to that of the original pigment when measured at 510 nm.

Fig.3.1: Injection of the fish with the pigments to check the toxic effect

3.3 Impact of the feeding

3.3.1 During feeding (Visual observation of the fish): The fish were fed and the results were noted as compared with the control fish. The table 3.2, shows the results as noted by the 10 panellists on a scale of 1 to 10 at an interval of 7 days for three weeks. The results show that at the start of the study, the colour of the fish was yellowish orange (scale 0-3) but as the top coated feed was fed, the colour enhancement occurred (scale 8-10).

Table 3.2: Summary for visual identification of fish skin colour enhancement (scale 1- no colour change and 10 colour change)

Scale	Control		SR2		SR4	
	% 0 d	% 21 d	% 0d	% 21d	%0d	% 21 d
1	100	30	100	20	100	0
2	0	10	0	0	0	10
3	0	40	0	0	0	0
4	0	20	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	40	0	20
9	0	0	0	20	0	20
10	0	0	0	20	0	50

All the fish survived the study period without any changes in the appearance and behaviour as noted by the observers. The panellists results show that at the 0 day all the fish (Control, SR2 and SR4) showed the same colour. As the feeding period progressed (7 day, 14 day and 21 day), the SR2 and SR4 colour enhancement was noted by the panellists. However a colour change was not noted for the control at the end of 21 day.

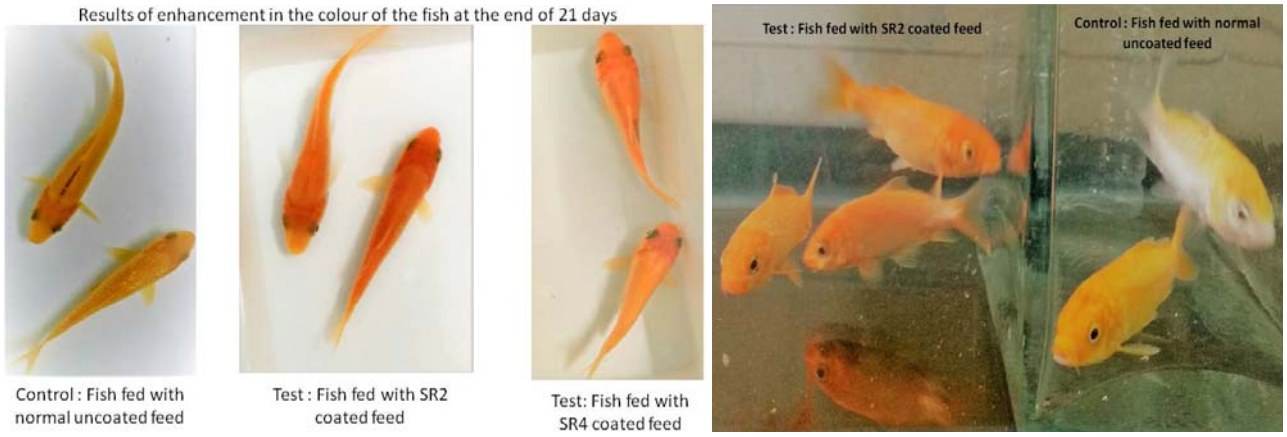

Fig. 3.2: Enhancement of fish skin colour during the feeding period with SR2 and SR4 top- coated feed.

3.3.2 Post feeding-Testing of fish metabolites-The fish were sampled and checked for fish metabolites in the skin, muscle protein content and weight of the fish analysed. The results from the fig. 3.3, show that the fish showed an enhancement in the skin colour at the end of 21 days.

Fig 3.3: Enhancement in the fish skin colour fed with the top coated diet

3.3.2.1 Visible spectrometry absorbance measurements of fish skin colour: As seen from table 3.3, there is a marked increase in the absorbance of the extracted fish skin colour when comparison of control and test fish samples is done. Thus the fish showed an enhancement in the skin colour.

Table 3.3: Absorbance of the control and test fish skin colour

Fish skin colour	% increase in colour
Control	---
SR2 fed	89.75
SR4 fed	74.50

3.3.2.2 High resolution liquid chromatography mass spectroscopy (HRLCMS): The High resolution liquid chromatography mass spectroscopy (HRLCMS) results as seen from table 3.4,showed the presence of carotenoid Astaxanthin in the fish fed with SR2 and SR4 top coated feed. This carotenoid was not present in the control fish.

Table 3.4: HRLCMS results showing a comparison between control, SR2 and SR4 top coated fed fish. (√ indicates presence, X indicates absence)

Carotenoid detected in fish skin	Formula	Mol. mass	m/z	Control	SR2 fed fish	SR4 fed fish
Taraxanthin	$C_{40}H_{56}O_3$	567.42	584.43c	√	X	√
Lutein	$C_{40}H_{56}O$	551.43	550.42	√	√	√
Tunaxanthin	$C_{40}H_{56}O_2$	551.42	568.42	√	√	√
Echinenone	$C_{40}H_{54}O$	551.43	550.42	√	√	√
Canthaxanthin	$C_{40}H_{52}O_2$	547.39	564.40	√	X	√
Astaxanthin	$C_{40}H_{52}O4$	597.39	596.38	X	√	√

As seen from the table 3.4, the HRLCMS of the fish metabolites showed the presence of Astaxanthin in the fish fed with the carotenoid SR2 and SR4 while the control fish did not show the presence of this carotenoid. This could be the reason for the increase in the colour intensity of the fish which were fed the diet as compared to the unfed control fish.

3.3.2.3 Estimation of protein content of fish muscle protein

Table 3.5: Weight of the fish at 21 day and Protein content of fish muscle.

Sample	Protein content per gm of sample (mg/gm)	% increase in protein content	Weight of the fish at 0 day (gm)	Weight of the fish at 21 day (gm)	% increase in weight of the fish
Control fish	9.67 ± 0.09	--	5.84±0.07	5.85±0.01	---
SR2 fed fish	16.08 ± 0.1	66.28	6.45±0.3	7.47±0.32	15.81
SR4 fed fish	14.89 ± 0.05	53.98	6.41±0.16	7.09±0.1	10.61

As reported from the table 3.5, the control fish did not show an increase in the weight but the fish fed with SR2 and SR4 top coated feed did show an increase in the weight. Similarly there was a marked difference in the muscle protein content of the test fish as compared to the controls Both the results indicate that the SR2 and SR4 top coated feed supports the fish growth.

4. Discussion

Reports have been made on the antioxidant potential of microbial pigments. These pigments are studied since they may serve a protective role against the occurrence of free radicals and thus serving as antioxidants [22]. The extracted fungal pigments when injected did not have an effect on the appearance and survival of the fish and thus were safely used in making feeds. Gabriel *et al.* [23] state that this is a common method to see the diet effect of pigments, antibiotics and feed supplements in fish.

In the other experiments carried out the ornamental fish were fed with a cocktail of algae containing natural stereoisomers of ß – carotene, zeaxanthin, canthaxanthin, lutein and astaxanthin. The fish metabolise the pigments and deposit them in the natural receptors and this depends on the type of the carotenoid and is also fish species specific. Similar type of work can be seen in a commercial fish feed, NatuRose which is a microalgal pigment preparation of *Haematococcus pluvialis*, has excellent stability and has proven to be a superior source of astaxanthin for Koi fish and other fresh water and marine ornamentals. [24] Thus other sources of carotenoids need to be studied with respect to colour enhancement in the fish. Taufik *et al.* [25] have studied the effect of zeaxanthin from red paprika on *Kohaku koi* fish. They have determined the effect of feeds enriched with different doses (0, 4, 8, 12 mg / kg of feed) of red pepper in *Kohaku Koi* fish for 60 day. They suggested from their study that the best concentration of zeaxanthin for these fish species should be 12 mg / kg. Of the feed.

Liu *et al.* [19] have studied three principal carotenoids, namely Zeaxanthin, Alloxanthin and Lutein by UV – vis absorption patterns in extraction of channel catfish fillet and compared them with the standards.

Studies show that fish contain a large variety of carotenoid pigments which are species specific. But culturing of the fish leads to loss/fading of the colour. The pigmentation can be enhanced by supplementation of the feed with carotenoid pigments obtained from microbial/ plant/animal sources. Selvakumar and Kandasamy [18] have reported the use of carotenoids from plant sources like Chinese rose and marigold petal in ornamental fish variety. The principal sources of Astaxanthin from animal sources is shrimp, krill, crab and yeast *Phaffia rhodozyma* or chemically synthesized astaxanthin. Astaxanthin esters are more superior to free astaxanthin for deposition and colour enhancement [26].

Lorenz *et al.* [26] have studied the effect of astaxanthin on Sea bream (*Chrysophrys major, Pagrus major*, Tai, Red Snapper) which is a highly prized fish for its red pigmentation. Astaxanthin cannot be synthesized by fish and it has to be supplemented in their diet exogeneously. Other carotenoids like lutein, canthaxanthin and zeaxanthin produce an undesirable colour. Carotenoids may serve as fertilization hormone precursors, prevent lipid peroxidation, stress protectant and photooxidative damage.

The most common carotenoids observed in fish are tunaxanthin, lutein, beta carotene, doradexanthin, zeaxanthins, canthaxanthins, beta carotene, astaxanthin and eichinenone. Fish do not have the capacity to synthesize pigment, but they can metabolise and convert one pigment to another. In red type karp, lutein is converted to astaxanthin [27].

Bixin, an apo carotenoid from the seed coat (pericarp) of plant achiote (*Bixa orellana*) was used by Dananjaya *et al* [21], to enhance the pigmentation in gold fish *Carassius auratus* and they observed an effect on the fish growth performance. There was a total gain in the fish weight by 7.06 to 8.31 gm in the fish which were fed 0.2 gm/kg of bixin. They also conclude from their results that bixin could serve as a multifunctional fish feed supplement along with colour enhancer in ornamental fish.

We thus conclude that fungal pigments could be used and further explored for the enhancement of colour in ornamental fish varieties.

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6. References

1. Clydesdale FM. Color as a factor in food choice. Critical Reviews in Food Science and Nutrition, 1966; 33:83-101.
2. Chengaiah B, Mallikarjuna Rao K, Mahesh Kumar K, Alagusundaram M, Madhusudhana Chetty M. Medicinal Importance of Natural Dyes, International Journal of Pharmtech Research Coden (USA), 2010; 2(1):144-154.
3. Ryu BH, Park BG, Chi YE, Lee JH. Production of Purplish red pigment in mixed culture of Streptomyces propurpuratus ATCC 21630 and Bacillus sp. R-89, Korean Journal of Applied Microbiology and Bioengineering. 1989; 17(4):327-333.
4. Mapari SAS, Thrane U, Meyer AS. Fungal polyketide azaphilone pigments as future natural food colorants, Trends in Biotechnology, 2010; 28(6):300-307.
5. FDA/IFIC. Regulation of color additives, Food and Drug Administration.1993, FDA/IFIC Brochure.
6. Kushwaha SC, Kramer JK, Kates M. Isolation and characterization of C50-carotenoid pigments and other polar isoprenoids from Halobacterium cutirubrum, Biochim Biophys Acta, 2014; 398(2):303-14.
7. De Carvalho JC, Ghiggi V, Woiciechowski AL, Vandenberghe LP, de Soccol CR, Brar *et al*. (eds.), Biotransformation of Waste Biomass into High Value

Biochemicals, DOI 10.1007/978, 2014. Springer Science+Business Media New York.
8. The nutrition and feeding of farmed fish and shrimp; a trainingmanual. http://www.fao.org/fishery/topic/13611/en. 12 Mar, 2013.
9. Babu Raja TD, Nammalwar P, Jacob C. Thomson, Dinesh K. P. B. Potential of ornamental fish culture and marketing strategies for future prospects in India, International Journal of Biosciences and Nanosciences. 2014; 1(5):119-125.
10. Rani P, Immanuel S, Ranjan Kumar N. Ornamental Fish Exports from India: Performance, Competitiveness and Determinants, International Journal of Fisheries and Aquatic Studies. 2014; 1(4):85-92.
11. Murti VS. Marine ornamental fishes of India, eprints.cmfri.org.in/7786/1/678-Mur.pdf
12. Zhou C, Ge Xianping, Lin Heizhao, Niu Jin. Effect of dietary carbohydrate on non –specific immune response, hepatic antioxidative abilities and disease resistance of juvenile golden pompano (Trachinotus ovatus), Fish and Shellfish Immunology, 2014; 41:183-190.
13. Madduluri Suresh, Sitaram B. *In vitro* evaluation of anti oxidant activity of methanolic and ethanolic leaf extracts of five indigenous plants in south India, Der Pharma Chemica, 2013; 5(6):246-249.
14. Amalya JT, Sumathy JH. Antioxidant activity of Carotenoid Extract of the leaves and flowers of Peltophorum pterocarpum, International Journal of Medicine and Pharmaceutical Research. 2015; 3(1):902-910.
15. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay,Anal Biochem. 1996; 239(1):70-76.
16. Jintasataporn Orapint, Yuangsoi Bundit. Stability of Carotenoid Diets during Feed Processing and Under Different Storage Conditions, Molecules. 2012; 17:5651-5660.
17. Ako H, Tamaru CS, Asano L, Yuen B, Yamamoto M. Achieving natural colouration of fish under culture, Proceedings of the twenty eighth US Japan Nutural resource aquaculture panel UJNR Technical Report No. 28, Honolulu, USA, 1999.
18. Selvakumar D, Dhevendaran K. Application of Microbial Carotenoids as a Source of Colouration and Growth of Ornamental Fish Xiphophorus helleri, World Journal of Fish and Marine Sciences. 2011; 3(2):137-144.
19. Liu Shaoyang, Lia Yilin, Wuc Yonnie, Bell Leonard N, Davis Donald, Wanga Yifen. Extraction, identification and quantitation of carotenoids in discoloured channel catfish (Ictalurus punctatus) fillets, Journal of Food Composition and Analysis, 2012; 26:154-159.
20. High resolution liquid chromatography mass spectroscopy, www.rsic.iitb.ac.in/hr-lcms.html
21. Danajaya SHS, Munasinghe DMS, Ariyaratne JL, De Zoysa M. atural bixin as a potential carotenoid for enhancing pigmentation and colour in goldfish (Carassius auratus), Aquaculture Nutrition, 2015; 23(2):255-263.
22. Mohana DC, Thippeswamy S, Abhishek RU. Antioxidant, antibacterial, and ultraviolet -protective properties of carotenoids isolated from Micrococcus spp. Radiat Prot Environ. 2013; 36:168-174.
23. Gabriel Ndakalimwe Naftal, Qiang Jun. Dietary Aloe vera improves plasma lipid profile, antioxidant, and hepatoprotective enzyme activities in GIFT-tilapia (Oreochromis niloticus) after Streptococcus iniae challenge, Fish Physiol Biochem, 2015; 41:1321-1332.
24. Commercial fish feed NAtu Rose, www.ruscom.com/cyan/web02/pdfs/naturose/axbul54.pdf
25. Taufik DTW, Zaelani K, Andayani S. Effects of zeaxanthin extract of red paprika (Capsicum annum. L.) to the red skin color in kohaku koi fish (Cyprinus carpio) International Journal of Biosciences, 2015; 6(6):78-84.
26. Lorenz RT. A Review of Astaxanthin as a Carotenoid and Vitamin Source for Sea Bream, Natu Rose Technical Bulletin no. 052; 1998.
27. Gupta SK, Jha AK, Pal AK, Venkateshwarlu G. Use of natural carotenoids for pigmentation in fishes, Natural Product Radiance. 2007; 6(1):46-49.