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REVIEW

Emergence of Plants in Fish Pigmentation

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Abstract Pigmentation plays a significant role in fish for consumer acceptability. Making and maintaining of aquarium containing pigmented ornamental fishes have evolved into a lucrative business in recent years. A number of pigments, primarily carotenoids are mainly responsible for the beautiful colouration of ornamental fishes. As fish cannot synthesize carotenoids de novo, there is a need of supplementation of carotenoids in the diet. Since natural sources of pigments are low-cost and more environmental friendly than the synthetic pigments, many studies have been carried out to test various plant-based natural carotenoids for enhancement of fish pigmentation. Forty-one plant species belonging to 28 families have been found widely used in imparting colouration to different species of fishes. Of these, 19 species were shown to be highly potential in fish pigmentation. Flowers, leaves, roots and whole plant are used in the study. Plant by-product like rice bran has also been used in fish pigmentation. The present review discusses about the potential plants used in fish pigmentation, their efficiency and the associative parameters along with the survival rate, growth and immune system promoter of the target species.

Keywords Bioactive compounds · Plant-based natural carotenoids · Ornamental fish · Sustainable aquaculture

Introduction

Fish trade has gained immense popularity in the recent years due to their aesthetic and high commercial value in the international market. Many food fishes are considered as ornamental fish due to their wide range of vibrant attractive colouration, body shapes, size and fin shapes. Of all these traits, body pigmentation along with its intensity and pattern plays a crucial role in determining the market value of ornamental fishes [26]. Hence, maintenance of the natural skin pigmentation is of utmost importance, as it is directly associated with its market demand and its acceptance or rejection by the aquarists [12].

Specialized cells in the skin of fish called chromatophores are primarily responsible for body pigmentation of fishes. These cells contain pigments which impart fishes its body colour. In fishes, carotenoids are the primary source of pigmentation. Carotenoids are a class of over 600 natural lipid soluble pigments found in plants, algae, photosynthetic, some non-photosynthetic bacteria and animals, which are required for healthy growth, metabolism, photosynthesis and reproduction, besides colour development [23]. In natural environment, the fishes meet the requirements for carotenoid by ingesting aquatic plants through their food chains [6]. However, ornamental fishes often show faded or degraded colouration especially when the fishes are kept under captivity for long duration and also in intensive culture condition. It is because like other animals, fish cannot synthesize carotenoids and they depend on dietary supply of these pigments to achieve their natural skin colouration [23, 37]. The dietary carotenoid source may be either synthetic or natural (plant and animal).

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Carotenoids derived from natural sources unlike their synthetic counterparts are less expensive and do not have any deteriorating effect on the environment. And thus several studies are being done on the use of natural carotenoids in pigmentation of fishes. Natural carotenoid source may be either plant based or animal based. Animal-based carotenoid sources, usually derived from by-products of crustacean such as shrimp meal, crab meal, are limited in supply as there is declining trend in catches of crustaceans from marine landing resources [21]. On the other hand, plant-based natural carotenoids are primarily derived from fruits, flowers, vegetables, etc. which are not only inexpensive, but also a potential source of mixed carotenoids [26]. Hence, plant-based carotenoids are the most preferred choice of natural pigmentation nowadays and there are many studies which have been carried out on the prospects and possibility of using plant as sources of fish pigments. In the present review article, an attempt has been made on the prospect of using various potential plants and their by-products in enhancing fish pigmentation as a path of sustainable aquaculture.

Plants Bioactive Compounds in Fish Pigmentation

Bioactive compounds refer to secondary metabolites or phytochemicals which influence different metabolic processes of the body. Plants are considered to be good source and reservoir of these bioactive compounds. The bioactive compound found in plants consists of member of heterogenous class which includes carotenoids, flavonoids, tocopherol, phytosterols and organosulfur compounds [15]. Bioactive compounds like carotenoids and flavonoids (anthocyanin) are predominantly used in fish pigmentation. These bioactive compounds help to impart different colouration or hues to fishes, thereby increasing their market value. Chemically, carotenoids are hydrophobic, polyunsaturated hydrocarbons consisting of 40 carbon atoms. Carotenoids consist of several conjugated double bond (the intensity of which helps in determining the colour) and have ringed structures in both their ends [16]. Carotenoids are divided into two types based on the availability of oxygen molecule –xanthophylls (oxygen-containing carotenoids) and carotenes (oxygen-lacking carotenoids). Apart from these, other carotenoids include cyclic carotenoids, epoxycarotenoids, hydroxycarotenoids, uncommon or species-specific carotenoids. Anthocyanins, on the other hand, are water soluble polyphenols constituting members of class flavonoids [49]. They help in imparting dark red to violet hue depending on the pH of the medium. Most common naturally occurring anthocyanins are cyanidin, peonidin, pelargonidin, delphinidin, malvidin and petunidin [49]. The present review found that more work has been done on carotenoids (as a potent colour influencer) to enhance fish pigmentation.

Potential Plants and Plant by-Products in Fish Pigmentation

Large numbers of experiments have been conducted on pigmentation of fishes by different plants and plant by-products by various scientists. Forty-one plant species belonging to 28 families have been found widely used in imparting colouration to different species of fishes. Of these, 19 species were shown to be highly potential in fish pigmentation (Table 1).

Marigold (*Tagetes erecta*) was found to be very good colour enhancer when incorporated in diet as marigold petal meal (15 g/100 g), marigold oleoresin (60, 120 and 180 ppm) and marigold flower meal (1.8%) in red sword tail (*Xiphophorus helleri*), rosy barb (*Puntius conchonius*), orange chromide (*Etroplus maculats*), koi carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss*) respectively [14, 24, 25, 47, 53]. Carotenoid content in gold fish (*Carassius auratus*) increased from 6.2 µg/g to 10.64–49.56 µg/g when fed with diet-containing marigold (200 mg/kg diet) [3]. Purity and brighter skin pigmentation were found in electric yellow chichlids (*Labidochromis caeruleus*) when they were experimentally fed with diets containing carotenoids extracted from marigold, nettle (*Urtica* sp.) and alfalfa (*Medicago sativa*) at a dose of 150 mg/kg of supplemented diet [54].

Marigold and alfalfa were found to be as effective as synthetic carotenoid sources e.g. apo aster in gold fish pigmentation [52]. Experiment on the enhancement of pigmentation of sword tail by feeding with diets containing 5% of each of dried marigold petal meal, shoe flower (*Hibiscus rosasiensis*) petal meal and ixora (*Ixora coccinea*) petal meal, highest carotenoid content in fish was recorded in marigold petal diet followed by ixora and hibiscus petal meal, respectively [19]. Another study conducted on gold fish using spirulina (*Arthospira* sp.), china rose (*Hibiscus rosa-sinensis*) petal, marigold petal and *Lactobacil* showed china rose petal (5 mg/kg) to be most efficient in pigmentation, better than the marigold diet (5 mg/kg) [45]. Incorporation of marigold (1.6%) and 4.4% red pepper (*Capsicum annum*) in diet can increase the pigmentation level of rainbow trout within 20 days [10]. Though the pigmentation by marigold and red pepper diet were found to be less effective than synthetic astaxanthin, Buyukcapar et al. [10] suggested that marigold and red pepper could be considered as an alternative to synthetic astaxanthin as a source of carotenoids for better colouration in fishes. In gold fish, red paprika, african tulip flower (*Spathodea campanulata*) and pomegranate (*Punica*

Table 1 Highly potential plants of different families with their effective doses used in pigmentation of different fish model

S. no.	Family	Name of the plant		Effective dose	Model fish		References
		Scientific name	Common name		Scientific name	Common name	
1	Amaranthaceae	*Beta vulgaris*	Beet	10% of diet	*Pseudotropheus lombardoi*	Blue morph	[13]
		Spinacia oleracea	Spinach	1.33 g/100 g of diet	*Xiphophorus helleri*	Sword tail	[50]
2	Apiaceae	*Daucus carota*	Carrot	2.5% of diet along with 7.5% of pumpkin flour	*Carassius auratus*	Gold fish	[2]
3	Araceae	*Wolffia arrhiza*	Spotless water meal	150 g/kg of diet along with 25 g/kg spirulina	*Botia dario*	Queen loach	[17]
4	Asteraceae	*Calenduleae officinalis*	Pot marigold	10 g marigold petal powder/100 g dry weight feed	*Colisa lalia*	Dwarf gourami	[7]
		Tagetes erecta	Marigold	200 mg carotenoid content/kg of feed	*Carassius auratus* L.	Gold fish	[3]
				180 ppm marigold oleoresin diet	*Cyprinus carpio* L.	Koi carp	[47]
				Diet with 60 ppm marigold oleoresin	*Etroplus maculatus*	Orange chromide	[25]
				1.6% of marigold flower	*Oncorhynchus mykiss*	Rainbow trout	[10]
				Diet with 120 ppm of marigold oleoresin	*Puntius conchonius*	Rosy barb	[24]
				Marigold petal meal with 15 g/100 g feed of dry weight	*Xiphophorus helleri*	Sword tail	[14]
				5% of marigold petal meal	*Xiphophorus helleri*	Sword tail	[19]
5	Cactaceae	*Hylocereus polyrhizus*	Dragon fruit	10% of red dragon fruit peel meal	*Cyprinus carpio*	Koi carp	[33]
6	Caricaceae	*Carica papaya*	Papaya	Papaya meal in the diet with 6.82 ± 0.10 μg/g wet weights in case of male and 5.92 ± 0.09 μg/g wet weights in case of female	*Trichogaster fasciata*	Banded gourami	[12]
7	Convolvulaceae	*Ipomoea batatas* L.	Red yam	Diet with red yam flour of 9%	*Carrasius auratus*	Gold fish	[41]
8	Fabaceae	*Clitoria ternatea*	Butterfly pea	6% of butterfly leaf meal	*Xiphophorus helleri*	Sword tail	[42]
		Medicago sativa	Alfalfa	Diet with 15% of alfalfa	*Carassius auratus*	Gold fish	[52]
		Mimosa pigra	Giant mimosa	Dry giant mimosa leave feed of 100 g/kg of basal diet	*Carassius auratus* L.	Gold fish	[46]
				Dry giant mimosa leave feed of 100 g/kg of basal diet	*Cyprinus carpio* L.	Koi carp	[46]
9	Malvaceae	*Hibiscus rosasiensis*	Shoe flower	5% of hibiscus powder petal meal	*Xiphophorus hellerei*	Sword tail	[19]
		Hibiscus sabdariffa	Roselle calyx	160 mg of anthocyanin from calyx/kg diet	*Carassius auratus*	Gold fish	[39]
10	Rosaceae	*Pyrus malus*	Apple	5–10% of apple peel meal	*Schizothorax richardsonii*	Snow trout	[28]

Table 1 continued

S. no.	Family	Name of the plant		Effective dose	Model fish		References
		Scientific name	Common name		Scientific name	Common name	
11	Rubiaceae	*Ixora coccinea*	Ixora	5% of ixora petal meal	*Xiphophorus hellerei*	Sword tail	[19]
12	Rutaceae	*Citrus* sp.	Orange	Ground citrus peels of 600 g/1000 g of basal diet	*Carassius auratus*	Gold fish	[1]
13	Solanaceae	*Capsicum annum*	Pepper	5% red paprika feed	*Carassius auratus*	Gold fish	[32]
				Diet containing 50 g/kg red pepper	*Labidochromis caeruleus*	Blue streak hap	[55]
				Paprika oleoresin of 60 mg/kg diet	*Microgeophagus ramirezi*	Dwarf chichlid	[22]
				4.4% red pepper meal	*Oncorhynchus mykiss*	Rainbow trout	[10]
				3 g of red paprika per 1000 g of feed	*Sparus aurata*	Gilthead seabram	[51]
14	Verbenaceae	*Lantana camara*	Lantana	100 mg/1000 g of lantana feed	*Xiphophorus hellerei*	Sword tail	[57]

granatum) peel may be used as carotenoid sources. The dietary supplementation of 5% paprika showed significant pigmentation in fish [32]. Results from the experiment of Pham et al. [40] reported that inclusion of paprika extract (100 mg/kg of diet) could increase pigmentation of olive flounder (*Paralichthys olivacus*) and has similar potential as that of commercial synthetic astaxanthin.

Study on pigmentation in blue streak hap (*L. caeruleus*) observed red pepper as a good source of natural carotenoid that can be used as an alternative of synthetic carotenoids in fish pigmentation [55]. According to Wassef et al. [51], gilthead seabram (*Sparus aurata*) when fed with red pepper supplemented diet (3 g/1000 g of feed) showed more red pigmentation than diet supplemented with carrot (*Daucus carota*). Diet with carrot (2.5%) and of pumpkin (7.5%) (*Cucurbita* sp.) flour potentially increased the colour intensity and brightness of the skin in gold fish [2]. Studies have shown that diet-containing carrot and beetroot (*Beta vulgaris*) can cause appearance of orange pigmentation in head and fins of climbing perch (*Anabus testudineus*) [20]. In an experiment on pigmentation of sword tail with diets of carrot and spinach (*Spinacia oleracea*), it was found that fish fed with carrot developed better red colouration whereas fish fed with spinach showed more yellow pigmentation [50]. However, incorporation of spinach (1.33 g/100 g) and carrot (1.82 g/100 g) in the diet was effective to enhance the orange pigmentation in the fish [50]. Unlike carrot, curry leaves (*Murraya koenigii*) and mango (*Mangifera indica*) peel, higher dosage of beet root incorporated feed resulted in decreased carotenoid content in the fish blue morph (*Pseudotropheus lombardoi*) [13]. Orange (*Citrus* sp.) peel carotenoid was found to be highly effective and more preferable than synthetic astaxanthin. A study was carried out in sword tail where 40–80 mg/kg of orange peel carotenoid was used in diet for eight weeks and the pigmentation showed no difference with fishes fed with synthetic astaxanthin and their customer preferences were also significantly increased [31]. Sweetbriar rose (*Rosa rubiginosa*) was observed to have great effectiveness in the enhancement of pigmentation in sword tail when carotenoid extract from its petal was incorporated in the diet (2000 mg/kg) [6]. According to Zutshi and Madiyappa [57], diet-containing flower of lantana (*Lantana camara*) showed remarkable improvement in orange-red colouration of sword tail. In an experiment on pigmentation of sword tail with diets containing china rose, cyme rose (*Rosa indica*), ixora and firecracker flower (*Crossandra infundibuliformiss*), Joseph et al. [29] found the highest carotenoid content in fish fed with firecracker flower diet followed by china rose, ixora and cyme rose, respectively. Diet containing 6% butterfly pea (*Clitoria ternatea*) leaf meal enhanced red colouration in the head of sword tail [42].

Red yam flour (*Ipomoea batatas* L.), orange peel diet showed significant potential in pigmentation of goldfish [1, 41]. According to Perez-Escalente et al. [39], gold fish fed with diet-containing anthocyanin extract from flour of roselle calyx (*Hibiscus sabdariffa*) showed higher chromatophore cells in dermal tissue than the control specimens which indicated the enhancement of coloration. Good pigmentation can be obtained in gold fish and koi carp or fancy carp by feeding the fishes with feed containing 100 g of dry giant mimosa (*Mimosa pigra*) leaves [46]. Rose petal meal showed positive effect on enhancement of pigmentation in koi carp, rosy barb and dwarf gourami (*Colisa lalia*) [26, 38]. Studies observed that feeding of banded gourami (*Trichogaster fasciata*) with diet-containing papaya (*Carica papaya*), showed positive correlation between increase in dietary carotenoid levels and increase in muscle and skin carotenoid concentration in both female and male fishes [12].

Dananjaya et al. [11] found that diet-containing bixine (0.20 g/kg), an extract of achiote or annatto (*Bixa orellena*) to ensure strong pigmentation, acceptable growth and feed utilisation in gold fish. Safari and Atash [44] recorded that blood carotenoid content of rainbow trout increased significantly when fed with achiote seed meal diet (200–250 mg/kg) [11]. Examination on fantail guppy (*Poecilia reticulata*) fed with turmeric (*Curcuma longa*) powder-containing diet (45 mg/50 g) showed very good pigmentation in muscle and caudal fin of the fish [35]. Tan et al. [48] reported that hawthorn (*Crataegus monogyna*) extract supplemented diet at the dose of 1.0 g/kg and 0.5 g/kg were found to be useful both in intestinal health and skin pigmentation of golden pompano (*Trachinotus ovatus*). Spotless watermeal (*Wolffia arrhiza*) was found to be very effective in the enhancement of pigmentation in queen loach (*Botia dario*) when incorporated at the dose of 150 g/kg of diet along with 25 g/kg of spirulina [17]. According to Kim et al. [30], rice (*Oryza sativa*) bran mineral induce pigmentation in zebra fish (*Danio rerio*) through ERK signalling pathway and ERK phosphorylation. Investigation proved that supplementation of diet with 5–10% of apple (*Pyrus malus*) peel meal provides great pigmentation in snow trout (*Schizothorax richardsonii*) [28]. Incorporation of tea (*Camellia sinensis*) leaves (25 mg/kg) in the diet of fancy carp was found to be effective in providing good colouration [56]. Fancy carps treated with diet-containing raw mulberry (*Morus* sp.) and cassava (*Manihot esculenta*) leaves also showed higher concentration of serum astaxanthin. Gokoglu and Kolak [18] used tomato (*Solanum lycopersicum*) as a natural carotenoid source in pigmentation of gold fish and found no satisfactory result. Biyikli et al. [9] also observed no significant differences on the colouration of yellow tail cichlid (*Pseudotropheus acei*) when fed with diet-containing woad (*Isatis tinctoria*) extract. There was no major change in the level of astaxanthin muscle of rainbow trout by the addition of corn (*Zea* sp.) gluten meal in the diet [43].

Transport, Absorption and Deposition of Carotenoids in Fishes

Carotenoids, being hydrophobic in nature, are not easily soluble in the chemical environment of the gastrointestinal tract and thus their transport and absorption in fishes are related to plasma lipoprotein. High-density lipoprotein plays an important role in carotenoid transportation in peripheral tissues in fishes [5, 36]. Absorption of carotenoids in fishes is a time-consuming process. In the course of absorption, carotenoids first get mixed with lipids, thereby forming an emulsion which then gets solubilised in the bile salt and are then carried to the intestinal brush border, from where they are absorbed. Fishes are able to reduce as well as oxidize dietary carotenoids. Reduction and oxidation of carotenoids help in their transformation from one form to another, thereby providing different colouration. Studies on gold fish and koi carp have shown that they are able to oxidize zeaxanthin and lutein to astaxanthin [34]. Yellowtail, on the other hand, is capable of producing tunaxanthin (carotenoid which provides yellow colouration), thereby reducing astaxanthin [16]. Deposition of carotenoids in different fish species takes place in different areas such as integument, gonads and muscles.

Associative Parameters

Along with pigmentation, some plants and plant by-products were found to exhibit their effects on other associative parameters such as survival rate, growth, phagocytic activity and even in social interactions. For sustainable maintenance, survival rate of fishes is an important associative parameter which needs to be considered primarily with the rate of pigmentation. Most of the time, the survival rate of fishes differs according to nature and dose of feeds. Golandaj et al. [19] found different survival rates of sword tail fed with diet-containing marigold (96.66%), ixora (86.66%) and shoe flower (96.66%). Orange chromide fed with 80 ppm, 120 ppm and 180 ppm of marigold oleoresin showed survival rate of 82.14%, 67.85% and 71.42%, respectively [25] and the fish, rosy barb fed with 80 ppm, 120 ppm and 180 ppm marigold oleoresin, recorded survival rate of 89.99%, 96.66% and 83.33%, respectively. In some studies, there are reports of plants with less or no pigmentation effect but high survival rate. Jebaraja et al. [27] showed that gold fish when fed with amaranth (*Amaranthus* sp.) and drumstick (*Moringa* sp.) leaf meal showed less pigmentation but 100% survival rate.

Incorporation of plant parts and by-products as a source of natural carotenoid for pigmentation has an impact on the growth of fishes also. Decreased feed conversion ratio (FCR) and improved growth rate of snow trout was noticed with diet containing larger amount of carotenoids in the form of both apple peel and spirulina [28]. Although some research recorded positive relationship between intake of carotenoid, growth and survival rate of fishes. Quite a few studies showed no effect or relationship between pigmentation, survival and growth rate of fishes. Harpaz and Padowicz [22] reported that, dietary carotenoids had no effect on survival and growth rate on the post larval or mature stage of dwarf chichlid (*Microgeophagus ramirezi*). Studies also observed the retardation of growth rate in carnivorous fishes when fed with diets containing plant materials. Similar result was also recorded in diets with 6.6% red pepper or higher than that and diet with 2.4% marigold or higher than that which observed negative effect in the growth of rainbow trout [10].

Studies have shown that zebrafish embryo when treated with extract of rice bran ash stimulates and enhances melanin biosynthesis by positively affecting the transcription factors. Melanin production directly effects hypopigmentation, forage free radicals, blocks the entry of UV radiation and thereby prevent skin damage as well as skin cancer [30]. When oliver flounder was fed with diet-containing paprika extract, increased radical foraging activities were observed in the muscles, plasma and liver [40]. Koi carp when fed with carotenoid supplemented diet showed higher level of phagocytic activity [56]. Gupta et al. [21] reported that fishes having notable level of carotenoids show more resistivity against bacterial and fungal diseases. Various study showed that carotenoids such as astaxanthin have great potential in improving physical condition of fishes. Fishes administered with natural astaxanthin showed reduce mortality rate when infected with *Aeromonas salmonicida* [4]. Pigmentation may also affect the associative interaction between male and female fishes. Female gourami prefer male with high pigmentation for interaction [8].

Conclusions

Plant and plant by-products based natural colouring agents are highly potential commercial bioresource which can improve the pigment enhancing strategy in the ornamental fish farming. There are other positive associative effects in survival rate, growth, social interaction and healthiness of the fish observed from the natural carotenoids. More popularisation and inclusive studies on the usage of potential plant-based carotenoids will help in developing a sustainable aquaculture in near future.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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