International Journal of Fisheries and Aquatic Studies 2019; 7(2): 52-55

E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(2): 52-55

www.fisheriesjournal.com
Received: 23-01-2019
Accepted: 26-02-2019

Ponce Granillo Carol Vianey
Laboratorio de Análisis Químico del Alimento Vivo. Universidad Autónoma Metropolitana-Unidad Xochimilco, Mexico, North America, Mexico

Monroy Dosta María del Carmen
Laboratorio de Análisis Químico del Alimento Vivo. Universidad Autónoma Metropolitana-Unidad Xochimilco, Mexico, North America, Mexico

Castro Mejía Jorge
Laboratorio de Análisis Químico del Alimento Vivo. Universidad Autónoma Metropolitana-Unidad Xochimilco, Mexico, North America, Mexico

Correspondence
Monroy Dosta María del Carmen
Laboratorio de Análisis Químico del Alimento Vivo. Universidad Autónoma Metropolitana-Unidad Xochimilco, Mexico, North America, Mexico

Use of *Tagetes erecta, Capsicum annuum* and probiotic *Rhodococcus* sp. for growth and coloration increase in *Pterophyllum scalare*

Ponce Granillo Carol Vianey, Monroy Dosta María del Carmen and Castro Mejía Jorge

Abstract
The aim of this investigation was to evaluate and compare the effect of *Tagetes erecta*, *Capsicum annuum* and probiotic *Rhodococcus* sp. in growth, survival and coloration of fish *Pterophyllum scalare*. *T. erecta* buttons and chili were dehydrated and grinded to obtain the extracts following [1]. Bacteria *Rhodococcus* was cultured in BHI medium until obtaining a concentration of $2x10^7$ufc/mL. Extracts and probiotic bacteria were used to enrich the administrated food to fish *P. scalare*. Every fifteen days biometrics were made to evaluate the growth and survival. By last, the increase in coloration in fish was evaluated [2], through measurement of pigments in fish tissue by spectrophotometry at a wave length of 500nm. Treatments where *T. erecta*, *Capsicum annuum* and *Rhodococcus* sp. pigments were added, obtained higher survival percentage than control treatment. Treatment with better results was *Capsicum* with 93.33% survival, weight gain 2.16 g, and total length gain of 19.01 mm. Lowest values were obtained in control treatment 1.02 g in weight gain and 8.73 mm of total length gain. Regarding to coloration of *P. scalare*, the best results were obtained in chili treatment, followed by *Rhodococcus* sp. Variance analysis in carotenoids content in tissue presented significant differences between treatments with a P=0.01.

Keywords: *Capsicum annuum*, carotenoids, growth, *Pterophyllum scalare, Tagetes erecta*, *Rhodococcus* sp.

1. Introduction
Aquarism in Mexico is an activity that has significant increase, and it also contributes to job creation, recreation, commerce and economic wellness of many rural communities in our country. In Mexico approximately 50 million ornamental fish with a wide diversity of shapes and colors, are commercialized [3, 4]. By being an activity that handles large amounts of organisms, problems arise like water quality, stress and diseases that impact the production by obtaining organisms of small size, little colorful, or sick. Generally, to maintain healthy organisms and help the growth, additives or complements are used, that include hormones, antibiotics and some salts, nevertheless, and their inappropriate use can cause adverse damage to fish and the increase in production costs [5].

Alternately commercial probiotics have been used to satisfy the demand of friendly environmental practices for the development of sustainable aquaculture, higher growth rates, and increase the immunologic response which gives them higher resistance to diseases. Also are some probiotics of genus *Rhodococcus* that produce carotenoids pigments source of antioxidants [6, 7]. Considering that in aquaculture focused in ornamental fish, the coloration is one of the main points for its commercialization and aquatic species are unable to *de novo* synthesize carotenoids, the astaxanthin or appropriate precursors as well as pigment producer probiotic strains, must be supplied in the diet or in the culture system [8]. Mainly because carotenoid pigments have a physiological importance in fish, that imply antioxidant properties, hormonal precursors that intervene in reproduction processes [9].

Synthetic astaxanthin and canthaxanthin have been widely used in aquaculture, nevertheless, its costs are very high. This has promoted the search of new pigment sources of lower cost [10]. Among which are bacteria of *Rhodococcus* genus that are widely distributed in the environment, particularly in soil and aquatic environment [11], there are pigmented colonies of cream, red, or orange color, being ideal candidates for their use in bioprocesses, both in industrial and environmental applications [11]. On the other hand, *Tagetes erecta* is characterized by presenting colors ranging from yellow to red [12], medicinal, pesticide properties are attributed and in Mexico it is used to stain clothes [13]. Members of *Capsicum* genus also have been reported as natural sources of colorant, capsaicin and substances as ascorbic acid. The two main components in chilis are: capsaicinoids that are alkaloids that provide chilis their pungency feature and carotenoids that provide a high nutritional value and color [14]. Therefore, the aim of this study is to evaluate and compare the effect of *Tagetes erecta*, *Capsicuum annuum* and *Rhodococcus* sp. in growth, survival and coloration of fish *Pterophyllum scalare*.

2. Materials and Methods

2.1 Obtention of *Tagetes erecta*, *Capsicum annuum* and *Rhodococcus* sp.

One kilogram of *T. erecta* buttons was obtained, which were pressed, dehydrated and grinded with a mortar; also, 500g of *C. annuum*, were deveined, cut and placed in tin paper at room temperature for its dehydration and after that were grinded.
Rhodococcus sp. bacteria was previously isolated in the intestinal tract of rainbow trout and its probiotic capacity in fish was tested [15]. The strain was cultured in BHI medium, until reaching a concentration of $2x10^7$ cfu/mL for its addition to fish food.

2.2 Diets preparation

With *T. erecta* and *C. annuum* already dehydrated and grinded, the pigment extraction process was made Ten grams of the obtained flour was mixed with 20 mL of ethanol at 80%, then heated at 80°C in an electric blanket for four hours, to then filtered it in Whatman paper (#1), and the recovered extract was gauge at 5 mL by adding ethanol at 80%, the process was repeated until obtaining the necessary volume [1]. Later to prepare the food 10g of flake (Wardley) were weighed and 20 mL of extract was added. With respect probiotic bacteria, 2 mL were added to same flake proportion. Impregnated food was placed in a low temperature stove during 24 hours at 30°C until completely dried and was administrated to fish. Food quantity was adjusted as the growth of the fish advanced considering the 10% of total biomass.

2.3 Experimental design

The 120 fish of the specie *P. scalare*, with an initial mean weight of 2.25 g and mean length of 50.1 mm were distributed randomly in twelve fish 40 L tanks capacity, at 21°C, constant aeration and a photoperiod of 12:12 hrs. The experimental diets were: a) *T. erecta* extract (three tanks), b) *C. annuum* extract (three tanks), c) *Rhodococcus* sp. at $2x10^7$ cfu/mL (three tanks), and d) Control group (three tanks), only with commercial food. Feeding period took place for 60 days. Solid wastes (feces and food) were removed with 0.3 mm mesh light nets and with the aim of a siphon. Every 15 days was monitored the pH, dissolved oxygen (O_2), ammonium (NH_4), nitrites (NO_2) and nitrates (NO_3) to maintain water quality.

2.4 Biometric parameters obtention

Daily the survival record was carried out and every 15 days, fish were measured using a digital Vernier (Cienceware) considering total length. Also, were weighed with a digital balance (Adventure Pro Ohaus).

2.5 Fish coloration

To evaluate the increase in fish coloration it was used the method for pigment extraction in tissue [2]. For which 1g of corporal tissue of fish was taken (except from the head and digestive tract), and was placed in 10 mL vial, where 2.5g of anhydrous sodium sulfate were added. The sample was pressed against the vial walls with a glass stirrer, 5 mL of chloroform were added and was left during two days at 0°C. When chloroform formed a clear layer of one to two centimeters over the residual, the optic density lecture was taken at 500 nm in a spectrophotometer, for which aliquots of 0.03 mL from the prepared samples were taken and were contrasted to control and blank. The total content of carotenoids was calculated as µg per tissue humid weight with the next formula:

$$\text{Total carotenoid content} = \left[\frac{\text{Absortion in maximun wave length}}{0.25 \text{ x sample weight (g)}}\right] * 10$$

Where
10= dilution factor
0.25= extinction coefficient

2.6 Statistical Analysis

For generated data it was made a data base in Excel to make descriptive statistic, a variance analysis (ANOVA) to determine significant differences between treatments. When differences were founded, a multiple means comparison analysis was made, using Tukey test.

3. Results

3.1 Survival and growth of *P. scalare*

Survival in all treatments were above 70%. *C. annuum* show better results with 77. 77% and lowest values with 72.22% in *T. erecta* and Control treatments. *Rhodococcus* sp. treatment show 75% survival.
In Table 1 were shows the mean values of organism's length trough 60 experimental days with enriched treatments.

Table 1: Mean values (±S.D.) of total length of *P. scalare* at experimental enriched treatments

Sample day	Experimental treatment			
	T. erecta	***C. annuum***	***Rhodococcus sp.***	**Control**
0	53.353	51.342	53.410	48.779
S.D.	3.385	2.727	3.371	4.252
15	55.800	57.434	53.541	53.519
S.D.	4.218	3.965	3.365	4.408
30	56.808	60.645	55.994	57.017
S.D.	3.820	4.031	3.003	4.454
45	57.049	62.840	56.843	57.479
S.D.	5.067	6.050	3.088	3.727
60	64.258[a]	69.707	61.329[a]	58.079[a]
S.D.,	7.748	7.959	7.015	5.431
Length gain	10.905	18.365	7.919	9.300

Organisms with *C. annuum* treatment show highest length gain with 18.365 mm, and lowest value was recorded with *Rhodococcus* sp. treatment (7.919 mm). *Rhodococcus* sp., organisms did not show significant differences after 60 experimental days with respect their total length (*Rhodococcus* sp. /Control P=0.895; *Rhodococcus* sp./*T. erecta* P=0.066).
Figure 1 show growth tendency curve of *P. scalare* organisms with respect their total length at 60 experimental days.

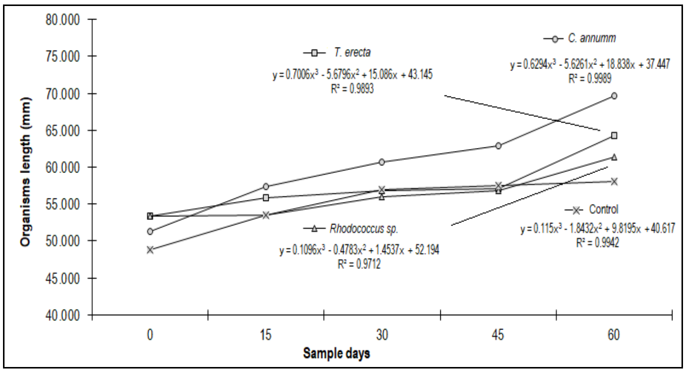

Fig 1: Tendency curve of total length of *P. scalare* organism at four experimental enriched treatments

With respect weight, Table 2 show mean values at four experimental enriched treatments.

Table 2: Mean values (±S.D.) of *P. scalare* weight at four experimental enriched treatments

Simple Day	Experimental treatment			
	T. erecta	*C. annuum*	*Rhodococcus sp.*	Control
0	2.244	2.415	2.447	2.503
S.D.	0.260	0.183	0.229	0.212
15	3.198	3.579	2.968	2.736
S.D.	0.348	0.357	0.445	0.253
30	3.270	3.645	3.165	2.962
S.D.	0.560	0.806	0.317	0.313
45	3.431	3.896	3.598	3.266
S.D.	0.273	0.310	0.372	0.293
60	4.012	4.570	3.657[a]	3.524[a]
S.D.	0.462	0.513	0.397	0.254
Weigth Gain	1.768	2.155	1.210	1.021

The highest value was shown in *C. annuum* treatment with 2.155 g, and lowest values in Control treatment with 1.021 g. Only *Rhodococcus* sp. and Control treatments did not shown significant differences at 60 experimental days (P=1.000).
At Figure 2, tendency curves were shown about weight gain.

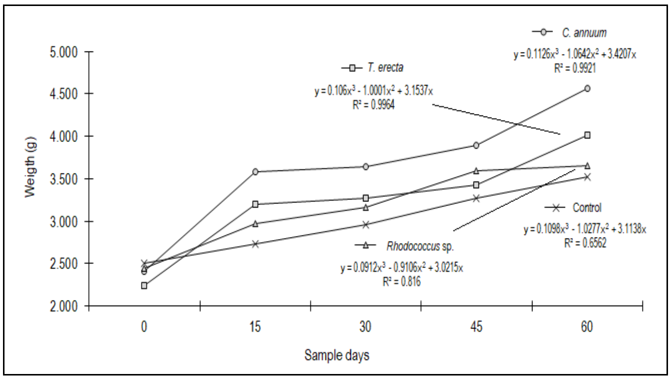

Fig 2: Tendency curves of *P. scalare* weight at four experimental enriched treatments

3.2 *Pterophyllum scalare* pigmentation

Table 3 shows the optical density and carotenoids values at different experimental treatments. Highest values of optical density were shown at *C. annuum* treatment (0.228-0.271). Lowest values were shown in control treatment (0.052-0.081). With respect carotenoids, highest values were shown in *C. annuum* (5.700-6.775) and lowest in Control treatment (1.300-2.025). ANOVA test show significant differences between all experimental treatments (P=0.01).

Table 3: Optical density, carotenoids density of different treatments

Treatment	Optical Density (500 NM)	Carotenoids
T. erecta	0.189	4.725
T. erecta	0.140	3.500
T. erecta	0.184	4.600
C. annuum	0.263	6.575
C. annuum	0.228	5.700
C. annuum	0.271	6.775
Rhodococcus	0.176	4.400
Rhodococcus	0.191	4.775
Rhodococcus	0.192	4.800
Control	0.077	1.925
Control	0.052	1.300
Control	0.081	2.025

4. Discussion

The results in this investigation showed a higher survival (< 90%) of *Pterophyllum scalare* by using *Capsicum annus*, *Tagetetes erecta* and *Rhodococcus* sp. pigments. These results are similar other study [16], using *Tagetes erecta* for rainbow trout pigmentation, with 86% of survival. These can be due to effect of pigments in fish physiology by reducing oxidative stress, it also has been said that carotenoid pigments are excellent precursors of hormones and necessary for growth and reproduction of fish [17]. On the other hand, it has been very documented that the use of probiotic microorganisms improves the survival of fish [18]. In this study, was obtained a survival of 100% using *Rhodococcus* sp. bacteria. This because the probiotics stimulate the immune system, which allows them to resist better to environmental variations and illness [19, 15].
Related to higher weight gain (2.16 g) and total length gain (19.01mm) obtained in chili treatment, Other Autor [10] mentioned a value of 2.13 g of weight gain in rainbow trout. Some studies have shown that pigments can make a positive effect on fish growth [20], which worked of Perez *et al*, 2012, with anthocyanin extracted from Jamaica (*Hibiscus sabdariffa*) and observed that growth rate, gain weight and survival, increased as the anthocyanin dose in diet increased too, showing significant differences regarding to control test.
With respect fish culture, a fundamental acceptation criterion is the visual impact given by the coloration red-pink, red-orange of the flesh, because consumers prefer those tonalities than with salmonids characteristics. Also, in ornamental fish, skin pigmentation alongside body and fins shape are the most important quality criterion in the market. Fish as other animals are unable to synthesize carotenoids and depend on pigment supplement in diet [21], even though there are different synthetic pigments in market they are not accessible to everyone because they are very expensive, so the use of pigments obtained from natural sources at low cost and wide distribution are very important. In this investigation the treatment than obtained better results in tissue pigmentation in *Pterophyllum scalare* was chili treatment, followed by *Rhodococcus* sp., observing a good acceptance to food enriched with carotenoids obtained from chili. These results are contrary to that reported in other study [22], which did not obtain significant differences with the use of *Capsicum annuum* in food of *Carassius auratus* comparing to control group.
Variations between studies can be caused to the relation between pigmentation intensity and quantity of pigment retained in muscle, which varies according to physiological factors such as fish weight, growth rate, maturation, genetic factors and own characteristics of each specie [23]. The result

in this study are encouraging because processes to obtain synthetic carotenoids implies the use of chemicals, which causes environmental damage by residuals. Also, synthetic carotenoids are expensive and have a use limitation in the food formulation depending on each specie, also if it is use in excess, can be harmful [24].

5. Conclusion

In conclusion, the use of pigments obtained from a natural product of low cost and of worldwide distribution such as chili, is a good option to increase survival, growth and pigmentation of *P. scalare*, which was an ornamental fish that occupy the third place of commercial demand in Mexico.

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