Evaluation And Growth of *Gambusia Affinis* On *Panchagavya* Treated *Spirulina Platensis*

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ABSTRACT
In the present experiment panchagavya supplemented *Spirulina platensis* was used as supplementary feed for *Gambusia affinis*. After 90 days, *G. affinis* showed maximum growth performance and survival ability. To determine the impact of *S. platensis* supplemented with panchagavya and on growth and biochemical characteristics of mosquito fish *G. affinis.* Proximate body composition of *G. affinis*, results showed that there is a significant difference were observed in fish weight (g), protein (%), carbohydrate (%), lipid (%) and pigment (%). The highest protein, carbobydrate and lipid contents ($P < 0.05$) were found at fish maintained at panchagavya and other diets. It could be concluded that panchagavya treated *S. platensis* can be supplemented to *G. affinis* diet to improve growth performance without any adverse effect on feed efficiency or survival rate.

Key words: *S. platensis, G. affinis,* Panchagavya, growth and biochemistry.

INTRODUCTION

Nowadays, some algae like *Phormidium, Spirulina platensis, Chlorella* and *Scenedesmus* have been applied to cultivate in many wastewater treatment plants for improving the quality of waste water (Phang *et al.,* 2000; Travieso *et al.,* 2006; Jongkon *et al.,* 2008; Ungsethaphand *et al.,* 2009). The production of algae yields a biomass that should be valuable as an animal feed supplement. *S. platensis,* that grows fast as a valuable product (Cohen *et al.,* 1987; Henrikson, 1989). Moreover, the farmers are able to use it in their swine farms as a source of important protein substances thus helping to economize in terms of buying feed supplements, which are usually expensive while at the same time, increasing the quality of waste water micro alga for the production of biomass as health food and animal feed. It has been considered as an ideal food for astronauts by NASA. It represents the second most important commercial micro alga for the production of biomass as health food animal feed (Sasson, 1997)**.**

Aquaculture production is a major industry in many countries, and it will continue to grow as the demand for fisheries products increases and the supply from natural sources decreases. Of the global aquaculture production, Asia produces 91%, Europe 4.7%, North America 1.8%, China 65% and India 7 % of aquaculture food (Kutty, 1999; Sakthivel, 2001). India has been ranked second in global aquaculture production after China. *S. platensis* cultivation is wide spread in aquaculture applications particularly due to the use of their pigment as feed for tropical fish (Vonshak and Richmond, 1988). The application of this alga in aquaculture offers a very promising avenue for its use in fresh and processed form. Based on Feed Convertion Rate (FCR) and body weight gains, this algal diet was found to be superior to normal fish feed and even more superior to natural foods. It has substantial levels of Polyunsaturated fatty acid (PUFA) including gamma-linolenic acid, which is used in the synthesis of prostaglandins in fishes. *S. platensis* is preferred by Indian hatcheries for keeping as a standby because Spray dried powder is very expensive in local markets. So aquaculturists need to culture *S. platensis* for their extensive use (Jeeji Bai, 1998). In Thailand, Tapioca starch waste is used for *S. platensis* culture and the product is used as shrimp feed (Bunnag *et al.,* 1998). In the present study an attempt has been made to record the efficacy of *S. platensis* treated with panchagavya to *G.affinis* feed. The fish has traditionally been referred to as mosquito fish, and used in mosquito control programs.

Mosquito borne diseases continue to be a major problem in almost all tropical and subtropical countries. They are responsible for the transmission of the pathogens causing some of the most life threatening and debilitating diseases of man, like malaria, yellow fever, dengue fever, chikungunya, filariasis, encephalitis, etc. Use of fish in mosquito control has been well-known for more than 100 years. The present investigation deals with the study of feed supplementation of *S. platensis* to *G.affinis*.

Microalgae or phytoplankton comprises the base of the food chain in the aquatic ecosystem (Kerswell, 2006). Among the microalgae, diatoms and nannoplanktons form the prime food source for the early critical stages of the life of the fish larvae crustaceans and molluscs. Generally fish meal based feed induce good growth. However, owing to the scarcity escalating cost of fish meal, alternative protein sources is gaining importance (Shetty and Nandeesha, 1988). Over 30 % of the current world algal production is sold for animal feed and over 50 % of the world production of *S. platensis* is used as feed supplements. In 1999, the production of microalgae for use in aquaculture reached 1000 tones (Spolaore, 2006*)*. The raw *S. platensis* fed tilapia show good flesh quality, and obtain slightly better sensory evaluations for color, texture and fattiness than commercial diet (CD)-fed fish (Lu and Takeuchi, 2002; Lu *et al.*, 2003). Nowadays, *S. platensis* can be used to establish immune-potentiating functions in carp (Watanuki *et al.*, 2006). Studies on the effect of *S. platensis* on reproduction in animals mainly focused upon rat. The study evaluating *S. platensis* as the sole dietary source of protein in sexually maturing rats pointed out that *S. platensis* as the sole protein source prove to be adequate nutrition to support normal growth and sexual maturation in rats (Contreras *et al.,* 1979). The effects of *S. platensis* supplement (0-20 %) have also been assessed for chick’s growth, egg production, egg quality, fertility, hatchability, and growth. The yolk color increased with each succeeding level of *S. platensis* and fertility was higher for all *S. platensis* treatments versus the control (Ross and Dominy, 1990).

To date, there is an amount of data that could be used to improve the growth of *G. affinis*. However, there has been no clear data to indicate whether the effects of *S. platensis* additives for nutrient utilization can be beneficial for growth and whether there is an accumulation of carotenoid pigment in *G. affinis* that have been grown with feed containing various ratios of *S. platensis* in flesh color. So the study was carried out to study the effect of Panchagavya supplemented *S. platensis* on the growth characteristics of *G. affinis.*

MATERIALS AND METHODS

Animal used for the experimental studies

Gambusia affinis Baird and Girard was taken for the present study. It is a small fish native to the fresh waters of the eastern and southern United States. It has become a pest in many water ways around the world following initial introductions early last century as a biological control agent for mosquitoes. *G. affinis* was described in 1853 from two locations in Texas (Baird and Girard, 1853). In some experiments using *G. affinis* collected in Spain (Fraile *et al.,* 1994) reported that temperature, photoperiod and reproductive state are all important in determining (in a proximal sense) reproduction in *G. affinis*.

The females are usually about 2 1/2 inches long and the males are about 1 1/2 inches long. A stout little fish, the back a little arched in front of the dorsal fin and the belly deep in front of the anal. The females are significantly larger than the males. The eyes are very large relative to the body. In mature females there is also a black patch above and somewhat forward of the vent. The eyes are greyish to olive, the dorsal fin has small black spots, and the caudal fin has several indistinct cross rows of small black spots. Males grow to 40 mm in length, while females reach 70 mm long (Fish Base, 2003).The mosquito fish reproduces rapidly, about 21 - 28 days. It does do not lay eggs; It bears live young and produce moderate numbers of young, Brood size is usually around 60 young, but large females can carry more than 300. Each female can produce three to four broods in her lifetime and each pregnancy can deliver 40 to 100 young. The females are significantly larger than the males. *G. affinis* fish, called Mosquito fish. Each fish eats up to 300 mosquito larvae per day. They eat mosquito larvae as soon as they hatch from the eggs laid by mosquitoes, thus used as live food for carnivorous aquarium fishes and also used as mosquito control (Fish Base, 2003). Mosquito fish have been proven to be environmentally friendly and extremely effective controlling mosquitoes.

The *G. affinis* was obtained from the fish collectors, Municipal office, Nagercoil, Kannyakumari District, Tamilnadu. In a total of 20 fishes, the average weight of each fish was determined before the experiment.

Experimental diets

The fish was feed with different combinations of diet with *S. platensis* as given below

Diet 1 The feed with100 % fishmeal (control)
Diet 2 The combination of fish feed-stuff supplemented with 75% dried *S. platensis* powder (T1)
Diet 3 The combination of fish feed-stuff supplemented with 50% dried *S. platensis* powder (T2)
Diet 4 The combination of fish feed with 25% dried *S. platensis* powder (T3)
Diet 5 The feed with100% dried *S. platensis* powder (T4)

Experimental set up

Five small (5 litre quantity) fish tank were used, for this a clean beaker was used and an average depth of 1 meter water level was maintained, healthy individuals of *G. affinis* having 0.50g, 0.50 g, 0.53 g, 0.52 g and 0.53 g in weight were introduced after acclimated for a fortnight feeding on the experimental diet in to the tank 1, tank 2, tank 3, tank 4 and tank 5 respectively. Hydrilla plant was introduced in to the tank for the fish to hide. The average weight of each fish was determined before the experiment. Each diet in triplicate was maintained in fish tank with 5L water. One group served as control.

The experimental groups were fed with the respective concentration of FM replaced with *S. platensis* inclusion level of 25 %, 50 %, 75 % and 100 % incorporated diets. The feeding was adjusted to two times a day (6:00 am and 6:00 pm). The feeding experiment was prolonged for one month; mild aeration was given continuously in order to maintain the optimal oxygen level, temperature and pH were measured. Normal fish feed and fresh *S. platensis* feed was supplied as feed, in different ratio ie. 100 % fish feed, 25:75 *S. platensis* and fish feed, 50:50 *S. platensis* and fish feed, 75:25 *S. platensis* and fish feed and 100 % *S. platensis* were used no other supplementary feed was given. Before collecting the fish for sampling, the aquatic plants were particularly removed from the beaker so that the netting could be easily carried out. Sample fish were collected once in five days with the help nylon net. The collected fish were measured and weighed and then re-introduced in to the respective tank (Plate1).

Determination of food indices

After the feeding trial, the food indices parameters such as Survival Rate (SR), Weight Gain (WG), Specific Growth Rate (SGR), and Condition Factor (CF) were individually determined by following equations (Takinay and Davis, 2001).

Weight Gain (WG) = Final weight (g) – Initial weight (g)

100 % fish feed

25% *Spirulina* + 75% fish feed

50% *Spirulina* + 50% fish feed

75% *Spirulina* + 25% fish feed

100% *Spirulina*

Plate: 1 Growth performance of *G. affinis* treated with *S. platensis*

Biochemical contents of the *G. affinis*

Estimation of total Protein

Concentration of total protein was estimated (Lowry *et al.*, 1951) using ethanolic precipitated sample. The blue colour was the result of biuret reaction of protein with copper ions in alkali solution and reduction of the phosphomolybdic -

phosphotungstic of Folin-Ciocalteau's reagent by the tyrosine and tryphtophan present in the treated protein. This colour intensity was measured at 660 nm against a blank which was devoid of protein sample. Bovine Serum Albumin (BSA) was used as the standard

Estimation of Carbohydrate
The carbohydrate was estimated by the method of Roe (1955) using trichloro acetic acid (TCA) extracted sample. Carbohydrates are hydrolysed into simple sugars by diluted HCl in hot acidic medium. Glucose is dehydrated into hydroxyl-methyl furfural. This compound reacts with anthrone and produce green colored product, which was measured at 630 nm against a blank. Glucose was used as the standard.

Estimation of total lipid
Total lipid was extracted with chloroform-methanol mixture, following the method of Barnes and Black-Stock (1973) and estimated by the method of Folch *et al.*, (1957). Lipid reacts with vanillin in a medium of H_2SO_4 and phosphoric acid to form a pink coloured chromogen, which is proportional to the lipid content of the sample, measured at 540 nm against the reagent blank. Olive oil was used as the standard.

RESULTS AND DISCUSSION
Growth performance of *G. affinis* fed with *S. platensis* (30 days)
The growth rates of *G. affinis* fed with fish meal replaced experimental diets are given in Table 1 - 3. The duration of the experiment was 30 days. Four different levels of experimental diets (25, 50, 75 and 100 % of *S. platensis*) were used. The water temperature varied between 25 and 29 ºC during the experiment. Statistically proven differences in temperature between both experimental variant were not found. The values received for pH were slightly alkaline and they were similar for the tanks from the control and experimental groups. Results showed that final body weight, weight gain and specific growth rate (SGR) increased significantly ($P < 0.05$) with increasing *S. platensis* level in fish diet up to 100 %. Best significant ($P < 0.05$) values of Feed Conversion Ratio (FCR) were observed in fish fed diet with 100 % *S. platensis* (1.01 g) followed by diets with 75, 50 and 25 % *S. platensis* (0.92, 0.83 and 0.77 g, respectively).
Survival rate did not differ significantly among treatments. The results of the present study have shown that Final Body Weight (FBW), Weight Gain (WG), Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR %/day) of *G. affinis* were affected significantly ($P < 0.05$) by different levels of *S. platensis* in the diet (Table 1). The highest significant ($P < 0.05$) values of FBW, WG and SGR%/day were obtained with the fish maintained at 100 % *S. platensis* level, they were found to be 1.01 g, 0.48 g and 1.6 g, respectively, followed by fish maintained at 75, 50 and 25 % *S. platensis* level. However, the least values ($P < 0.05$) of FBW, WG and SGR %/day were recorded with fish maintained at 25 % *S. platensis* level with the value of 0.77 g, 0.26 g and 0.86 g, respectively (Figures 1, 2 and 3).

Table 1 - Specific growth of *G. affinis* fed with fish meal replaced experimental diets

Diet %	Initial weight (g)	Final weight (g)	Weight gain (g)	Specific growth rate (%)	Survival rate (%)
Control	0.50±0.12	0.74±0.16	0.24±0.04	0.8	100
25	0.51±0.18	0.77±0.22	0.27±0.04	0.86	100
50	0.53±0.22	0.83±0.25	0.30±0.03	1.0	100
75	0.52±0.19	0.92±0.23	0.35±0.04	1.16	100
100	0.53±0.18	1.01±0.25	0.48±0.07	1.6	100

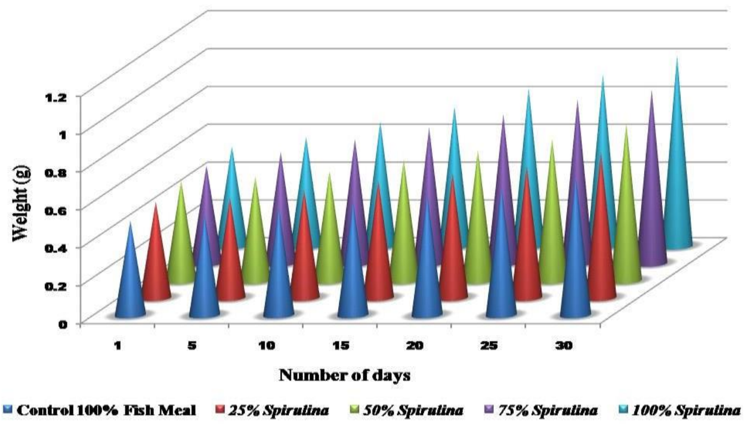

Figure 1. Biomass of *G. affinis* fed with fish meal and *S. platensis*

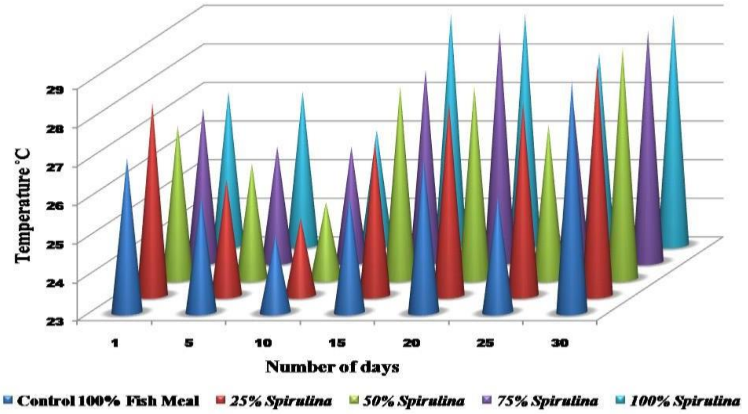

Figure 2. Effect of temperature on growth of *G. affinis* fed with fish meal and *S. platensis*

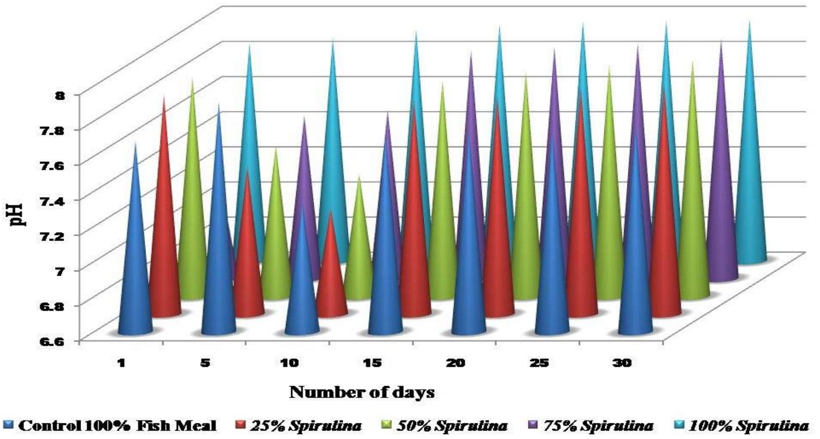

Figure 3. Effect of pH on growth of *G. affinis* fed with fish meal and *S. platensis*

Growth performance of *G. affinis* fed with commercial fish meal (90 days)

The present investigation has been conducted to understand the effect of Panchagavya treated *S. platensis* on growth performance of *G. affinis* for a period of 90 days. Two different experimental diets *ie* 100 % of *S. platensis* and 100 % of Panchagavya treated *S. platensis* were used. The influence of commercial fish meal fed *S. platensis* on growth performance of *G. affinis* during the 90 days of experiment the mean weight of *G. affinis* on the first day of stocking in the control (100 % fish meal treated) was 0.89 g (Table 2). At the end of the experiment, the mean weight of the control fishes was found to be 1.71 g. The water temperature of the experiment varied between 26 °C to 29 °C. The pH fluctuated from 7.55 to 7.78 (Figures 3). Hence coming to conclusion that using Panchagavya supplemented *S. platensis* in the fish can raise its growth performance and survival ability.

Growth characteristics of *G.affinis* in Panchagavya supplemented *S. platensis*

The present investigation has been conducted to understand the effect of *S. platensis* on growth performance of *G. affinis* for a period of 90 days. After 90 days (Table 2), groups that received *S. wightii* showed increase in body weight (1.77 g) in comparison with 100 % fish meal treated group. The temperature showed variation from 26 °C to 29 °C. The pH varied from 7.54 to 7.81.

Influence of *panchagavya* supplemented *S. platensis* on *G. affinis*

The effects of *S. platensis* on the growth performance of *G. affinis* were studied. *Panchagavya* supplemented *S. platensis* was used as supplementary feed. One experimental diet consisting of *S. platensis* meal was used in the trial and compared to a control diet, a basal diet (100 % Fish meal) with 0 % algae. The initial size of *G. affinis* from the control group was 0.8 g, whereas the group fed with experimental feed was at 0.82 g. The duration of the trial was 90 days (Table 2). The weight gain, condition factor and average daily growths of *G. affinis* fed with experimental diet were higher (1.77 g) than those from the group fed with feed without algae supplement (1.71 g).

Table 2- Influence of panchagavya supplemented *S. platensis* on *G. affinis*

Days	Fish meal (g)	Temp °C	pH	Panchagavya (g)	Temp °C	pH	% over control
1	0.89±0.15	27	7.55	0.82±0.12	28	7.54	0.0202
6	0.82±0.18	26	7.58	0.87±0.19	25	7.56	0.0504
12	0.84±0.22	26	7.59	0.94±0.51	26	7.59	0.1008
18	0.92±0.11	26	7.6	0.99±0.26	26	7.6	0.0706
24	0.99±0.16	26	7.62	1.05±0.22	25	7.64	0.0606
30	1.03±0.26	26	7.67	1.11±0.21	26	7.64	0.0808
36	1.09±0.28	28	7.65	1.18±0.13	28	7.7	0.091
42	1.15±0.29	26	7.67	1.25±0.47	27	7.72	0.1012
48	1.25±0.31	27	7.7	1.33±0.37	28	7.73	0.081
54	1.32±0.41	27	7.75	1.41±0.51	28	7.74	0.0912
60	1.4±0.39	26	7.74	1.45±0.11	27	7.75	0.0507
66	1.45±0.28	26	7.74	1.51±0.16	28	7.77	0.0609
72	1.52±0.48	26	7.75	1.58±0.14	28	7.79	0.0609
78	1.59±0.37	27	7.76	1.66±0.55	28	7.8	0.0711
84	1.67±0.51	27	7.77	1.71±0.17	29	7.81	0.0407
90	1.71±0.49	29	7.78	1.77±0.31	29	7.81	0.061

Proximate body composition of *G. affinis* experimental feed (*S. platensis* and *panchagavya*)

Proximate body composition of *G. affinis*, results in Table 3 showed that there is a significant difference were observed in fish weight (g), protein (%), carbohydrate (%), lipid (%) and pigment (%). Proximate body composition of *G. affinis* results are showed in Table 3. The highest protein contents ($P < 0.05$) were found at fish maintained at Panchagavya in the diet with the values of 34.89 over the control (33.87 %). Fish fed diet containing Panchagavya get the highest significant ($P < 0.05$) carbohydrate contents were recorded with fish maintained at panchagavya diet with the value of 3.6 % compared to 100 % fish meal diet (3.4 %). Lipid content in the fish body showed significant differences among treatments (100 % fish meal, *S. wightii* and panchagavya ($P < 0.05$). Fish maintained at *S. wightii* in the diet were significantly the highest ($P < 0.05$) in body lipid content than other treatments, with the value of 1.82 %, followed by fish maintained at panchagavya over the control (100 % fish meal 1.34 %). The result clearly showed that the maximum pigment level was present in the fish fed with Panchagavya (2.86 %) when compared to control (2.69 %), respectively. With the obtained results, it could be concluded that seaweeds (*S. wightii)* can be supplemented to *G. affinis* diet to improve growth performance without any adverse effect on feed efficiency or survival rate.

Table 3 - Proximate composition of *G. affinis* fed with experimental diet

Sample	Fish control	Panchagavya	% over control
Fish weight(g)	1.71±0.49	1.77±0.31	0.0712
Protein%	33.87±0.02	34.89±0.01	1.5424
Carbohydrate%	3.4±0.03	3.6±0.01	0.2070
Lipid%	1.34±0.02	1.82±0.02	0.4865
Pigments%	2.86±0.02	2.69±0.01	0.1750

The type of feed ingested and their nutritional quality is known to be one of the main factors affecting fish body composition (Meyers, 1999). Superior performance of fish with the experimental diet (100% panchagavya and 100% fish meal) reflects on the ability of *G. affinis* to utilize the supplementary ingredients effectively. Ingredients of the experimental diet have shown to improve the nutrient contents. Fishes are known to utilize high levels of carbohydrates (Manjappa *et al.,* 2009). The carbohydrates as spared proteins for growth as revealed from the results of this study. This could have been due to the changes in the formulated experimental diet. Further, carbohydrates improve the pelleting quality and nutrient value of the diet (Lovell, 1989).

Fiber content of the diet affects feed digestibility and food retention in the gut thereby influencing absorption of nutrients. Nutritive value of edible flesh revealed that experimental diet affected protein and fat, both being lowest in control and highest in experimental diet fed groups. However, there was no difference in moisture level in control and experimental diet fed groups. This is indicative of protein accretion and true growth involving an increase in the structural tissue such as muscle and various organs (Fafioye *et al.,* 2005; Lovel, 2002). It has been reported that fish growth would be better when fed with higher protein containing feed especially animal protein (Rai and Bista, 2001). Dietary lipid level is known to influence muscle lipid positively (Guler *et al.,* 2008). A significant increase in fat level in fish receiving the experimental diet indicates enhanced lipid production which can be related to the fat and NFE levels of the diet. Influence of nutrients on body composition has also been reported in major carp rohu (Umer *et al.,* 2011) and also on other major carps (Khan *et al.,* 2012). These reports justify the present results.

Several studies were conducted to evaluate the replacement of fish meal by plant ingredients in diets of fish (Gouveia and Davies, 2000; Kaushik *et al.,* 2004) data about the potential use of seaweeds in fish diets is scarce (Nagler *et al.,* 2003;

Buschmann *et al*., 2001). Apart of their potential nutritional value as protein substitutes, algae may also give an important contribute in fish diets as lipid sources (Nakagawa *et al.*, 1987), and binding (Hashim and Mat Saat, 1992) or colouring agents (Gouveia *et al.,* 2003). These results coincided with the earlier findings of the potential use of seaweed in fish diet. Supplementations of macroalgae meals in diets enhance the growth, lipid metabolism, physiological activity, stress response, disease resistance and carcass quality of many fishes (Ergun *et al*., 2009; Guroy *et al*., 2011 & 2013).

In contrast, data obtained by El-Sayed (1994) demonstrated that the use of a microalgae meal, *S. platensis*, as protein source for silver sea bream (*Rhabdo sargussarba*) fingerlings could successfully substitute up to 75 % of the fish meal protein without any adverse effects on growth performance and feed utilization efficiency. These results revealed that the attraction of fish to algae diets seems to be species-specific and might be indicative of a general beneficial effect of low level supplementation of seaweeds in fish diets (Davies *et al*., 1997) but Valente et al. (2006) reported that the relative low nutritive value of seaweeds could explain their depression effect on overall growth performance. The results on present study indicated the beneficial role of selected algae *S. platensis* supplemented with panchagavya as the growth promoters for mosquito fish *G. affinis.* The overall study shows that panchagavya supplemented feed increased the growth of the fishes when compared to control. So these studies concluded the possibilities of using panchagavya as growth promoters to *G. affinis* in future at farm level.

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