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Biofertilizer effect of free living nitrogen fixing bacterium on the growth and water quality properties of *Penaeid* prawn culture system

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Abstract: The role of phytoplankton is significant in improving the yield of prawn culture. The growth of phytoplankton will be effected various factors viz. dissolved oxygen, production of natural food to shrimp larvae in control of ammonia. The growth of phytoplankton can be enhanced by application of nitrogeneous fertilizers to improve water quality and growth performance of *Penaeus indicus*. The present research work is focussed on a free-living nitrogen fixing bacterium *Azotobacter chroococcum* supplemented by nitrogeneous fertilizers to increase the content of proteins, carbohydrates and wet weight of *P. indicus*. Ten animals of juveniles of *P. indicus* were collected and grown in controlled conditions for fifteen days. The liquid suspension of free living nitrogen fixing bacteria *A. chroococcum has been* inoculated for experimental treatment purpose. The inoculum has been introduced to four treatments and for findings of water quality analysis and phytoplankton growth analysis by Strickland and Parsons method and biochemical analysis by Raymont *et al.,* method. The research findings revealed that *A. chroococcum* supplemented with fertilizers will affect the growth of phytoplankton which is vital in prawn culture and enhance the growth of hormones and dry weight for improvement of the yield.

Key words: Free living nitrogen fixing bacterium; Water quality; *Azotobacter chroococcum;* wet weight and dry weight; Biochemical analysis.

Introduction

The role of Phytoplankton is significant in prawn culture. It is very useful in producing oxygen during the day, controlling the turbidity of pond water, preventing the growth of filamentous algae on the pond bottom and also in serving as the ammonia absorber to control the water quality. In a well-prepared pond, phytoplankton and zooplankton are rich in abundance. They contribute the food energy required for prawn larvae. (Shen *et al.,* 1992). Literature survey revealed that phytoplankton and growth rate of the shrimp (*Penaeus chinensis*) had a close relationship (Jiao, 1993).

Phytoplankton can easily be enriched in the prawn pond by applying inorganic fertilizers. Hepner (1962) revived data on the fertilizers applications in carp ponds at Israel and concluded that fertilizations with nitrogen and phosphorus, gave higher fish yields than fertilization with phosphorous alone. In brackish waters, nitrogen is an important limiting factor compared to phosphorus. Hence, nitrogen fertilizers are to be supplemented to the water for culture purpose. But, nitrogen biofertilizer like Azotobacters in brackish water pond system has not been reported to our knowledge, hence this study has been proposed to assess the phytoplankton productivity, water quality and growth performance of prawn species, *Penaeus indicus* as influenced by free living, nitrogen fixing bacterium *Azotobacter chroococcum.*

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Materials and Methods

Collection and acclimatization of test animals

Juveniles of *Penaeus indicus* (4±1cm length) was collected from the Koringa estuary (Mangroove forest) near Kakinada situated16.9891° N, 82.2475° E in southern coast of India and acclimatized in estuarine water for 3 days. Ten animals were used for treatment for 15 days in standing water (10 liters) with aeration, 25± 2 $g1^{-1}$ salinity, pH 7.5 and temperature 25±2°C. For each treatment, triplicates were maintained.

Preparation of bacterial inoculums

Two loopful inoculum of free living, nitrogen fixing *Azotobacter chroococcum* were inoculated into liquid Winogradsky's medium and were kept at 28±2° C for 3 days with continuous shaking. After three days, cells were washed by centrifugation (12,000 rpm) for 15 minutes. Liquid suspension (10^8 cells ml^{-1}) of *A. chroococcum* was taken for the present study.

Experimental design

Four treatments were maintained for each prawn species under 28±2°C for this treatment. They are as follows:

Treatment 1 (T_1): 1/2 strength urea, 1/2 strength superphosphate, *A. chroococcum*

Treatment 2 (T_2): 1/2 strength urea, 1/2 strength superphosphate (No *A. chroococcum*)

Treatment 3 (T_3): (No urea), 1/2 strength superphosphate, *A. chroococcum*

Treatment 4 (T_4): (No urea, no superphosphate and no *A. chroococcum*).

Treatment 4 was maintained as the control. The strength of urea and superphosphate used was as suggested in prawn farming manual of Thapar water base Ltd. (1993).

Test animals of *Penaeus indicus* were fed with Aquastar feed (commercial) at 6.00 hrs every day at a rate of 10% of body weight. The faeces and left-over feed were collected every day morning from each treatment and dry weights were noted. The amount of feed consumed and dry weights of the animal were also recorded.

Water quality analysis
The dissolved oxygen and ammonia were analyzed by Strickland and Parsons (1972) method.

Phytoplankton growth analysis
The phytoplankton growth production was estimated based mainly on the estimation of the green pigments (Chlorophyll-a) of the phytoplanktonic levels. (Strickland and Parsons 1972) method has been followed in the present study.

Prawn growth analysis
Total length was taken by measuring with the help of a normal graduated scale. Fresh weight was taken by weighing the animal in live condition in an electronic balance. Food conversion efficiency (FCR) was determined on dry weight basis following the procedure described by Crisp (1971).

Biochemical analysis in Prawns
Protein was estimated by following the method of Raymont *et al.,* (1964) and carbohydrates by Dubois *et al.,* (1956).

Results

Effect of *A. chroococcum* on phytoplankton growth in *P. indicus* cultured water.
The content of chlorophyll–a increased between 5th and 15th days of culture period (Fig. 1). This increase in growth was by 113.42% in culture medium supplemented with urea, superphosphate and *A. chroococcum*. This was followed by 84.11% in the culture medium mixed with urea and superphosphate, by 31.29% in the culture medium treated with super phosphate and *A. chroococcum* and by 8.0% in the culture medium without any supplementation. The level of chlorophyll-a was statistically significant among the treatments and days of culture.

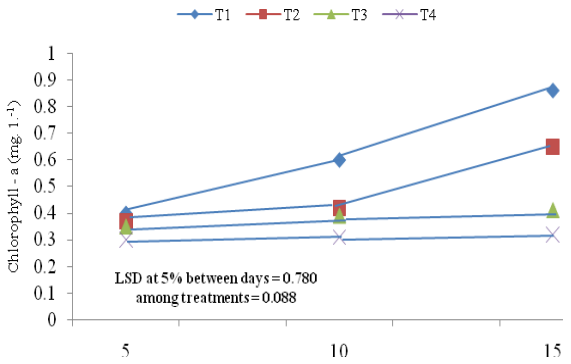

Fig. 1: Level of chlorophyll-a in *P. indicus* culture water

Effect of *A. chroococcum* on water quality in *P. indicus* cultured water
The content of ammonia decreased between 5th and 15th days of Prawn culture (Fig.2). This decrease was by 5.56% in the culture medium supplemented with urea, superphosphate and *A. chroococcum*. This was followed by 3.83% in the culture medium admixed with urea and superphosphate, by 4.53% in the culture medium treated with superphosphate and *A. chroococcum* and by 4.89% in the culture medium without any supplementation. The level of ammonia was statistically significant among the treatments and the days of culture.

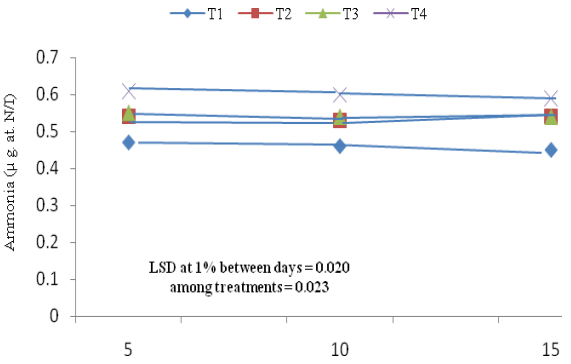

Fig. 2: Level of ammonia in *P. indicus* culture water

The levels of dissolved oxygen increased with increasing days of culture period (Fig.3). This increase was by 129.64% in the culture medium treated with urea, superphosphate and *A. chroococcum*. This was followed by 104.82% in the culture medium treated with urea and superphosphate, by 86.60% in the culture medium admixed with superphosphate and *A. chroococcum*, by 78.35% in the culture medium without any supplementation. The level of oxygen was statistically significant among the treatments and the days of culture.

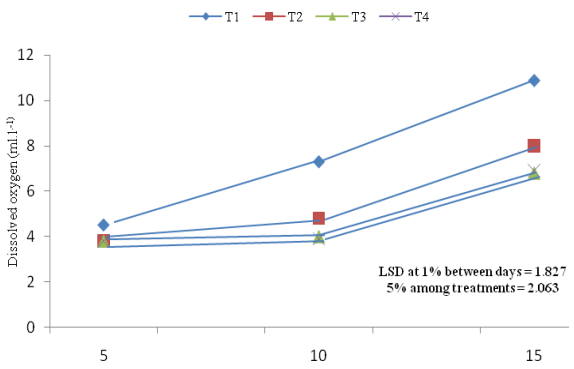

Fig. 3: Level of dissolved oxygen in *P. indicus* culture water

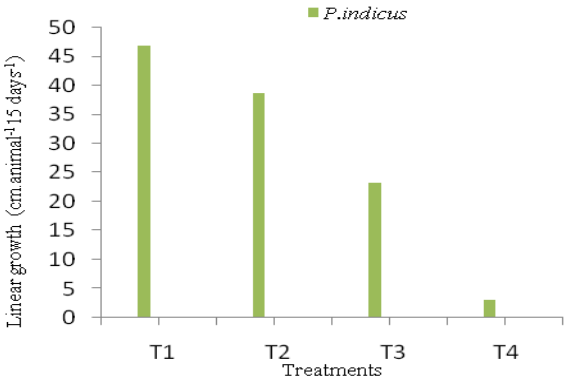

Fig.4: Linear growth of *P. indicus*

Effect of *A. chroococcum* on prawn growth of *P. indicus*

The linear growth of the prawn was higher by 46.9% in prawns grown in the culture medium supplemented with urea, superphosphate and *A. chroococcum* as compared to the culture medium without any supplementation (Fig.4). The wet biomass increased by 23.81% in prawns grown in culture medium supplemented with urea, superphosphate and *A.chroococcum* as compared to the culture medium without any supplementation (Fig.5).

The production, consumption, assimilation, metabolism, assimilation efficiency, gross growth efficiency, net growth efficiency were maximum in prawns grown in the culture medium admixed with urea, super phosphate and *A. chroococcum* and found minimum in prawns grown in the culture medium without any supplementation of urea, superphosphate and *A.chroococcum* (Table 1). The food conversion ratio (FCR) was minimum (1.8) in prawns grown in the culture medium supplemented with urea, superphosphate and *A.chroococcum* and found maximum (2.7) in prawns grown in the culture medium without any supplementation (Table 1).

Table 1: Budget giving dry weight gain of *Penaeus indicus* after 15 days of growth under treatments with or without urea, super phospate and *A. chroococcum*

Treatment	Initial wt	Final wt	Weighted Mean	Production	Consumption	Faecal Output	Relative growth rate	Assimilation	Metabolism	Assimilation efficiency	Gross growth efficiency	Net growth efficiency	Dry food consumed Wet weight gain
	W_1	W_2	W	W_1-W_2	C	F	P/W 20	A C-F	R C-(P+F)	A/C%	P/C%	K_2 or P/A%	FCR
T_1	0.057	0.1299	0.0935	0.0729	0.469	0.045	0.0779	0.424	0.351	90.41	15.54	17.19	1.8
T_2	0.086	0.1511	0.1186	0.0651	0.474	0.062	0.0549	0.412	0.347	86.92	13.73	15.80	2.0
T_3	0.124	0.1823	0.1532	0.0583	0.472	0.075	0.0381	0.397	0.338	84.11	12.35	14.69	2.2
T_4	0.124	0.1840	0.1540	0.0600	0.464	0.087	0.0389	0.377	0.317	81.25	12.93	15.92	2.7

T_1 Urea, superphosphate and *A. chroococcum*; T_2 Urea and superphosphate; T_3 *A. chroococcum* alone; T_4 without anything

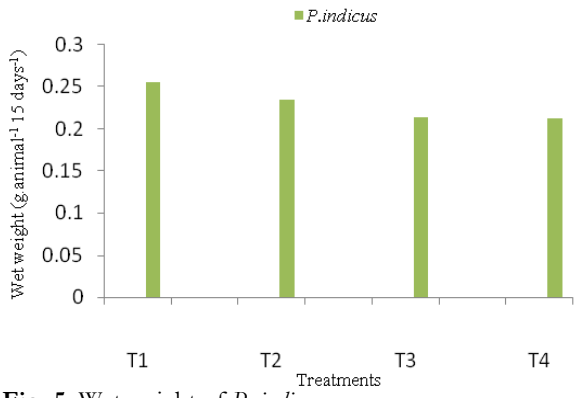

Fig. 5: Wet weight of *P. indicus*

Similarly, the percentage of protein content was high by 22.04% in prawns grown in the culture medium supplemented with urea, superphosphate and *A.chroococcum* as compared to the culture medium without any supplementation (Fig.6). In the same way, carbohydrate content was higher (62.0%) in prawns grown in the culture medium supplemented with urea, superphosphate and *A.chroococcum* than that without any supplementation (Fig.7).

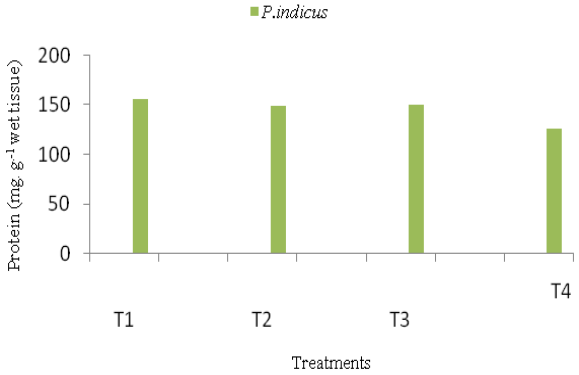

Fig. 6: Level of protein in *P. indicus*

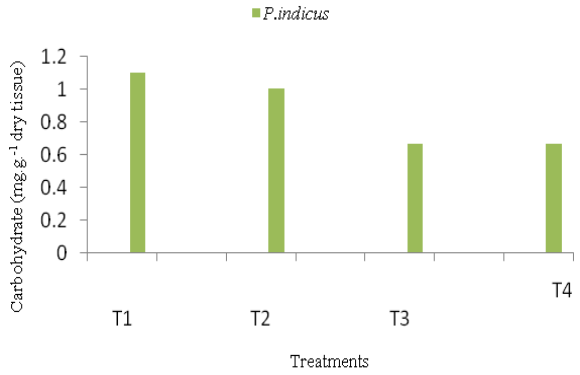

Fig. 7: Level of carbohydrate in *P. indicus*

Discussion

Growth of the prawn species was improved with the incoculation of *A.chroococcum* supplemented with half strength of urea and superphosphate as was evident by increase in the content of proteins (Fig.6), carbohydrate (Fig.7), gross growth efficiency, net growth efficiency, assimilation efficiency, (Table 1) wet weight, (Fig.5) linear growth (Fig.4) of the prawn species, *P.indicus*. This improvement can be attributed to the better growth of the phytoplankton (Fig.1). This phytoplanktonic growth improved the water quality by the removing the level of ammonia (Fig.2) and by enhancing the level of oxygen in the culture medium (Figs.3). These effects were higher in the treatment supplemented with urea, superphosphate and *A.chroococcum* than other nutrients. Boyd (1976) also reported that nitrogen fixation alone was apparently not sufficient to maintain as much algal production as occurred in ponds. The addition of urea with *A.chroococcum* provides nitrogen for the growth of phytoplankton as the nitrogen is vital for that photosynthetic organism (Neori and Krom, 1991). Besides nitrogen availability, the stimulation of phytoplanktonic growth may also be attributed to the growth hormones that are produced by Azotobacter (Fig.1). To support this, Dhar *et al.,* (1990) reported that the increase in concentration of phytohormone (Indole-acetic acid) directly promoted the chlorophyll content in phytoplanktonic cyanobacteria.

Conclusion

The research results revealed that the usage nitrogeneous fertilizer, urea and phosphatic fertilizer superphosphate composite in 1:1 ratio along with *A.chroococum* bacterial species has shown potential in enhancing the growth of phytoplankton due to improvement of water quality and in turn enhance the tissue growth adequately and increase the dry weight of the body of the prawn and finally results in the high yield of the prawns.

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