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# ORGANIC ACIDS AND PROBIOTICS AS GROWTH ENHANCERS IN THE CULTURE OF *LITOPENAEUS VANNAMEI*

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## ABSTRACT

The Organic acids and probiotics play a vital role in the culture of many terrestrial and aquatic organism, these have gained prominence as alternative biomedicine to antibiotics. produced by the probiotic bacteria and are produced by leading manufacturers these have a number of applications in many fields such as Aquaculture, food preparations, poultry, sewage water treatment, animal feed production, human consumption. The effect of dietary supplementations of citric acid, formic acid, lactic acid or their salts improved the growth of *Litopenaeus vannamei* was studied. All the organic acids and probiotics studied enhanced the activity of the gut and pond ecosystem when compared with the control. The percentage of decrease in total *vibrio* count (TVC) when the feed mixed with (3p/kg and 5g/kg organic acid) and (2kg/ha and 3kg/ha of Probiotics) applied in Experimental pond A and experimental pond B respectively. The results have been promising with the variance clearing measured the number of colonies formed in the cultures obtained from Control pond(with as low as $0.24\times102 \pm0.27$ to $40.21 \times10^2 \pm 0.13$ in the pond water) (with as low as $0.15\times102 \pm0.13$ to$39.23 \times 10^2 \pm 0.85$ in the gut), similarly the observations in both the Experimental pond A and experimental pond B have been tabulated.

**KEYWORDS:** *Litopenaeus vannamei*, Organic acids, Probiotics, Bacillus, Total Vibrio Count (TVC).

## INTRODUCTION

The study was conducted at Vaadachepurupalli, Visakhapatnam district in Andhra Pradesh. Organic acids act as growth promoters and enhance the antimicrobial activity of the organism; they also enhance the nutrient digestibility, survival and maintain a static endogenous micro floral quantity and composition. As organic acid is very essential and useful it's called "A gut environment modifier (GEM) designed to improve feed quality and an alternative to antibiotics". It improves digestibility of the aquaculture feeds, increases the feed intake of aquatic species, has a strong antimicrobial effect and acts against gram negative bacteria leads to prevention of the diseases, it acts as a feed hygiene and feed quality regulator (anti-mould), it reduces buffering capacity of the feed, It helps in the decrease of pH in the feed which prevents the ammonia formation in the faecal matter, It also helps in the faster acidification of stomach content towards optimal pH for pepsin digestion, acidification of the hepatopancreas, gut acidification, stimulation of enzyme secretion, improves protein digestion and also increases amino acid digestibility. In rearing ponds of *Litopenaeus vannamei*, the use of commercial probiotics has shown beneficial effects by improving survival, feed conversion, growth rate and keeping the parameters of water quality at optimum levels (Shariff *et al.*, 2001; Wang *et al.*, 2005). Studies of organic acids and probiotics to improve growth or survival in crustacean larvae are very scanty. The application of probiotics in the aquaculture ecosystems and in the feeds to the animal is one of the most promising areas where sustainable culture can be established and the practice of application of probiotics is reported by many aqua-culturists. Knowledge of probiotics has increased, currently it is know that these microorganisms have an antimicrobial effect through modifying the intestinal microbiota, secreting antibacterial substances like bacteriocins and organic acids (Myers., 2007). Organic acids and their salts are generally regarded as safe compounds and those with one or more carboxyl group (-COOH) in their structure are often used as antimicrobials in the livestock feed industry. In shrimp culture there are different bacterial strains used as probiotics and the popular probiotic bacteria belong to *Nitrosomonas* spp., *Cellulomonas* spp., *Bacillus* spp., *Pediococcus* spp., *Nitrobacter* spp., *Rhodococus* spp., *Rhodobacter* spp., *Enterobacter* spp., *Lactobacillus* spp., *Actinomycetes* spp., *Pseudomonas* spp., *Saccharomyces* spp., Denitrifying bacteria, *Bifidobacterium, Carnobacterium, Alteronomonas* spp., *Streptococcus* spp.,

## MATERIAL AND METHODS

The application of probiotics in the experimental ponds was followed uniformly in all the farms. The probiotic used for present study is SUPER BIOTIC (Plate 5 Fig B) which is a composition of water probiotics having the strength of 10 million colony forming units (CFU) i.e. 109 cfu/g of the probiotic was used. The probiotic strains in this SUPER BIOTIC were *Bacillus spp.* like *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megatirium* and *Bacillus polymixa*. The probiotic was applied at the rate of 2kgha-1 in the experimental pond A and 3kgha-1 in the experimental pond B at the frequency of every 15 days during the three years of the study starting from 45 DOC (Table 1(b)). The probiotics application followed the same procedure in all the experimental ponds during the study period. The application of probiotics were followed by soaking the probiotics material in 4 liters of water overnight for leaching in non-contaminated fresh water and later applied uniformly all over the ponds. For the studies on the role of organic acids, the commercial organic acid used in the present study is BAYERS' BAYMIX LATIBON (Plate 5, Fig. A) which is composed of formic, lactic, benzoic and propionic acids. The organic acid BAYMIX LATIBON is applied at the rate of 3 gkg-1 feed in the experimental pond A and 5 gkg-1 feed (Table 1(a)) in the experimental pond B in both summer and winter crops during the study period starting from 30 DOC based on the requirement. Application of organic acids was stopped two days prior to the application of SUPER BIOTIC and was started two days after the application of SUPER BIOTIC. The Organic acid is mixed in the feed with commercially available feed binder "Gell it" which was applied 10 ml/kg feed. In the process of top dressing in the feed the organic acid were weighed with simple balance of sensitivity 10 gm and mixed thoroughly with the binder "Gell It". The feed was broadcasted in the ponds after drying of feed pellets for 20 to 30 minutes in shade. The feed for the experimental study ponds for every feeding time was freshly mixed with organic acid. Later the feed was dried in the shade and broadcasted in every feeding time. The application of the organic acid was followed every feeding time. The application of the organic acid was followed every day starting from 45 DOC during the culture in the entire crop period of both season's summer and winter in the three years of study period 2011 to 2013.

### Statistical applications

The statistical package used for interpreting the available data was GRAPHPAD PRISM 6.0 Scientific Software for evaluation of the total *Vibrio* colony (TVC) counts. Histograms were used to interpret the growth data of the results obtained. The pie charts were used to represent

the total percentage production of shrimp *Litopenaeus vannamei* in Andhra Pradesh during the year 2011 and 2013. The pie charts were applied for representing the data of the survival rate and growth during the study period in all the ponds of the eight work stations. The results of the immunological indices were tabulated and represented by using the statistical tool 2-D line charts.

## RESULT

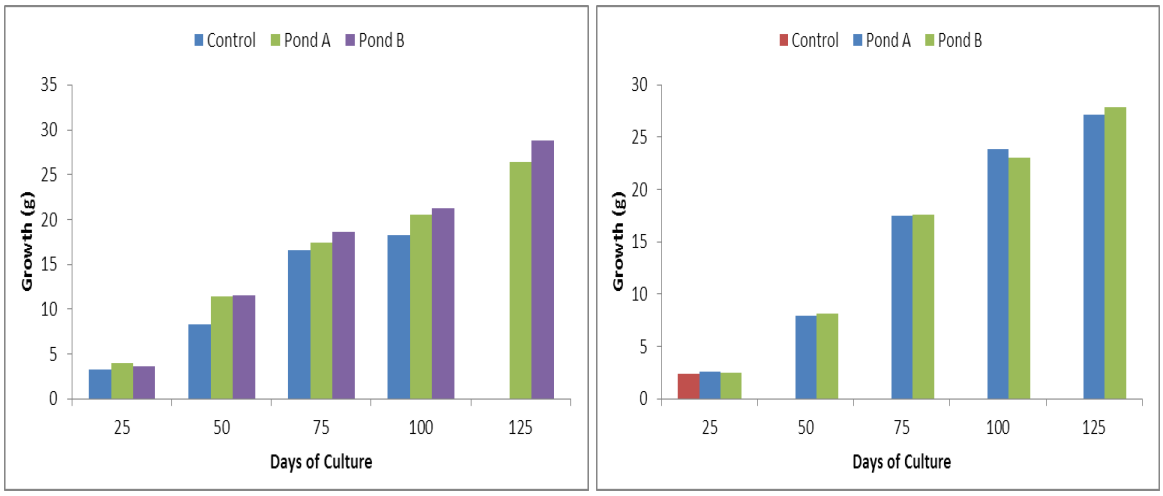
**Table 12: Growth of *Litopenaeus vannamei* (grams) at Vadacheepurupalli during the year 2011**

**Summer - 2011**

| Days of culture | Control | Pond A | Pond B |
|---|---|---|---|
| 25 | 3.30 ± 0.37 | 3.97 ± 0.27 | 3.62 ± 0.69 |
| 50 | 8.32 ± 0.29 | 11.37 ± 0.80 | 11.50 ± 0.64 |
| 75 | 16.63 ± 0.71 | 17.47 ± 0.77 | 18.63 ± 0.85 |
| 100 | 18.26 ± 0.12 | 20.58 ± 1.02 | 21.28 ± 0.54 |
| 125 | - | 26.45 ± 0.23 | 28.85 ± 1.45 |

**Winter - 2011**

| Days of culture | Control | Pond A | Pond B |
|---|---|---|---|
| 25 | 2.43 ± 0.32 | 2.62 ± 0.46 | 2.51 ± 0.15 |
| 50 | - | 7.98 ± 0.44 | 8.15 ± 0.58 |
| 75 | - | 17.48 ± 0.55 | 17.62 ± 0.63 |
| 100 | - | 23.85 ± 0.96 | 23.02 ± 0.97 |
| 125 | - | 27.12 ± 0.84 | 27.92 ± 0.29 |




**Summer Crop** **Winter Crop**

**Fig 5: Growth of *Litopenaeus vannamei* at Vadacheepurupalli during the year 2011**

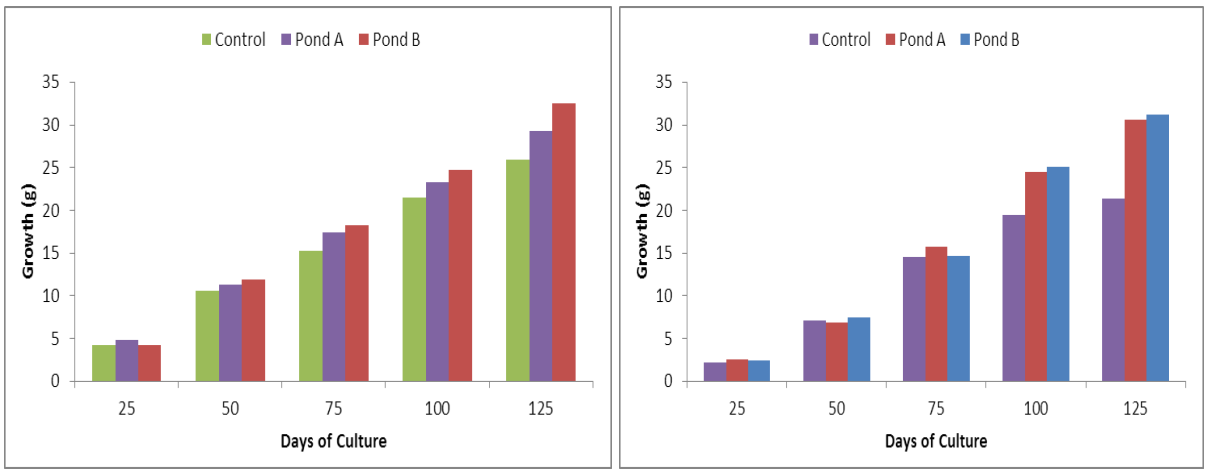
**Table 13: Growth of *Litopenaeus vannamei* (grams) at Vadacheepurupalli during the year2012**

**Summer - 2012**

| Days of culture | Control | Pond A | Pond B |
|---|---|---|---|
| 25 | 4.22 ± 0.28 | 4.82 ± 0.60 | 4.20 ± 0.73 |
| 50 | 10.57 ± 0.42 | 11.30 ± 0.57 | 11.87 ± 0.42 |
| 75 | 15.30 ± 0.52 | 17.38 ± 0.69 | 18.23 ± 0.51 |
| 100 | 21.47 ± 0.51 | 23.32 ± 0.69 | 24.76 ± 0.91 |
| 125 | 25.98 ± 0.45 | 29.33 ± 0.78 | 32.56 ± 1.52 |

**Winter - 2012**

| Days of culture | Control | Pond A | Pond B |
|---|---|---|---|
| 25 | 2.13 ± 0.24 | 2.50 ± 0.71 | 2.45 ± 0.36 |
| 50 | 7.12 ± 0.52 | 6.88 ± 0.66 | 7.45 ± 0.77 |
| 75 | 14.52 ± 0.79 | 15.70 ± 0.72 | 14.67 ± 0.94 |
| 100 | 19.42 ± 0.89 | 24.52 ± 0.72 | 25.10 ± 1.85 |
| 125 | 21.40 ± 0.73 | 30.55 ± 0.78 | 31.20 ± 0.44 |




**Summer Crop** **Winter Crop**

**Fig 6: Growth of *Litopenaeus vannamei* at Vadacheepurupalli during the year 2012**

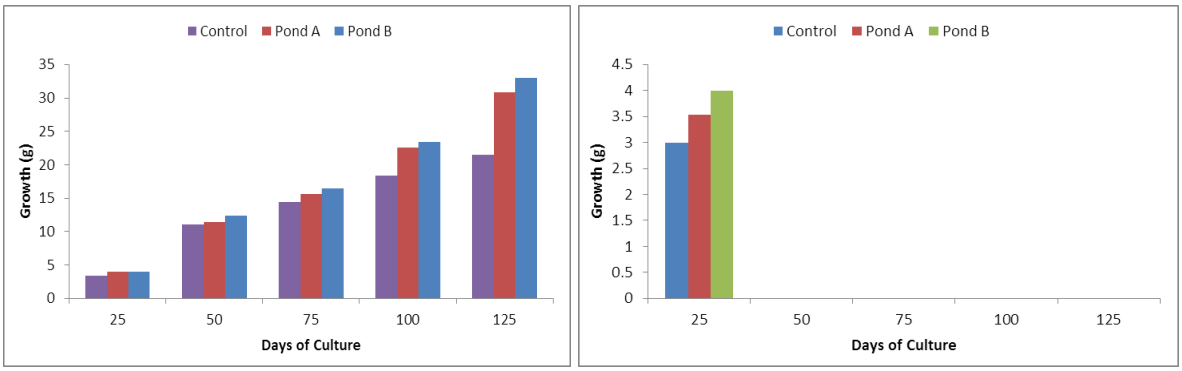
**Table 14: Growth of *Litopenaeus vannamei* (grams) at Vadacheepurupalli during the year 2013**

**Summer - 2013**

| Days of culture | Control | Pond A | Pond B |
|---|---|---|---|
| 25 | 3.42 ± 0.58 | 4.00 ± 0.42 | 3.97 ± 0.56 |
| 50 | 11.00 ± 0.77 | 11.38 ± 0.58 | 12.37 ± 0.81 |
| 75 | 14.38 ± 0.70 | 15.57 ± 1.07 | 16.43 ± 1.02 |
| 100 | 18.42 ± 0.79 | 22.52 ± 0.43 | 23.40 ± 0.75 |
| 125 | 21.52 ± 0.56 | 30.89 ± 0.78 | 33.00 ± 0.62 |

**Winter - 2013**

| Days of culture | Control | Pond A | Pond B |
|---|---|---|---|
| 25 | 3.00 ± 0.45 | 3.53 ± 0.53 | 4.00 ± 0.37 |
| 50 | - | - | - |
| 75 | - | - | - |
| 100 | - | - | - |
| 125 | - | - | - |




**Summer Crop** **Winter Crop**

**Fig 7: Growth of *Litopenaeus vannamei* at Vadacheepurupalli during the year 2013**

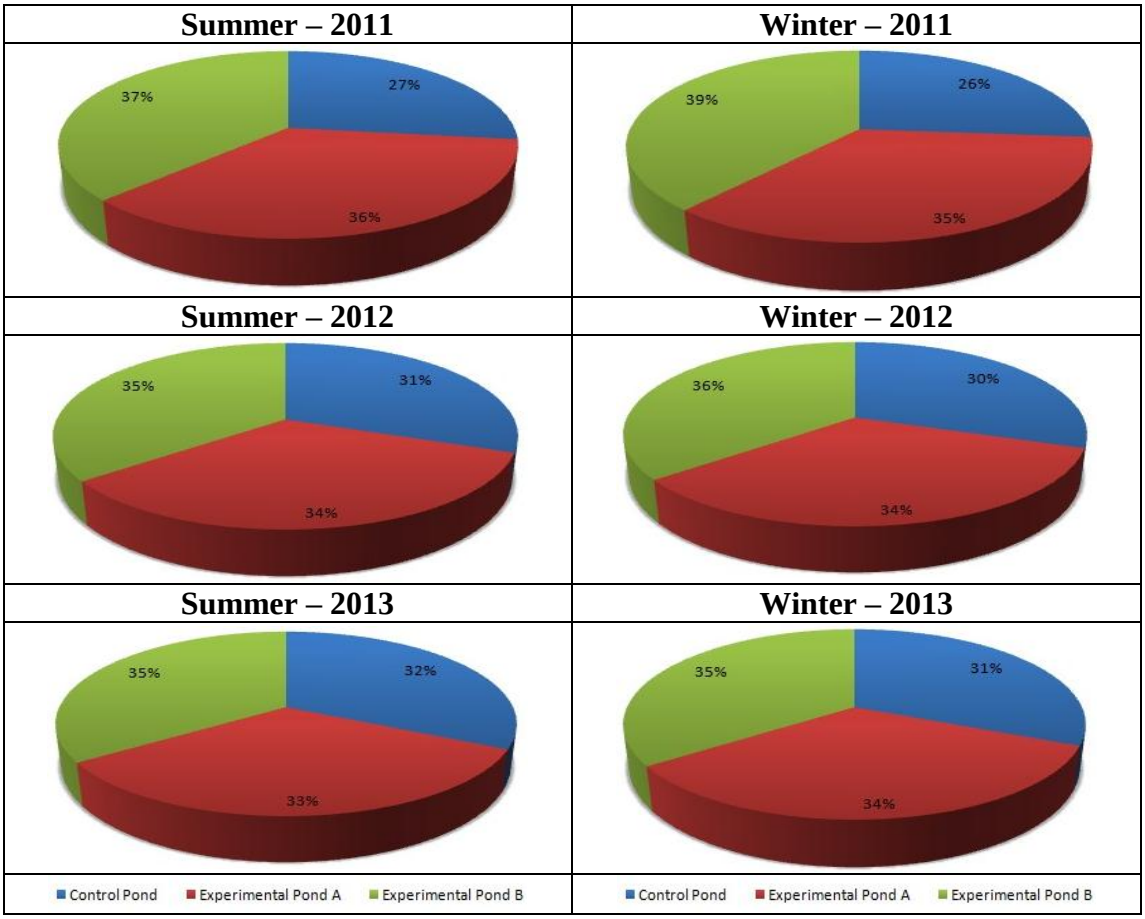



**Fig 15: Survival percentage of *Litopenaeus vannamei* in the culture ponds of Vadachepurupalli during the years 2011 to 2013**

**Table 21: Percentage Survival of Litopenaeus vannamei in the culture ponds during the years 2011 to 2013**

| S. No | Station | 2011 | | | | | | 2012 | | | | | | 2013 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Summer Crop | | | Winter Crop | | | Summer Crop | | | Winter Crop | | | Summer Crop | | | Winter Crop | | |
| | | Control | Pond A | Pond B | Control | Pond A | Pond B | Control | Pond A | Pond B | Control | Pond A | Pond B | Control | Pond A | Pond B | Control | Pond A | Pond B |
| 1 | Vaada-cheepurupalli | 65 | 86 | 90 | 60 | 80 | 88 | 76 | 84 | 88 | 72 | 81 | 85 | 85 | 88 | 91 | 65 | 70 | 72 |

## CONCLUSION

## ACKNOWLEDGEMENT

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