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ORIGINAL ARTICLE

CULTURE OF *L.VANNAMEI* (PREPARATION, WATER QUALITY PARAMETERS) COMPARATIVE STUDY WITH DIFFERENT STOCKING DENSITIES WITH SLANDERED OPERATIONAL PROCEDURES.

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ABSTRACT

The prevalence of Arbuscular mycorrhizal fungal association has been well reported from several natural ecosystems, information on AM fungal association and their abundance are unknown for Shola ecosystems. In the present study, 71 plant species (in 35 families) examined all the families were colonized by AM fungi except two species in a genus *Psychotria*. AM association was observed in members of supposedly non-mycorrhizal families Commelinaceae, Cleomaceae and Convolvulaceae. Thirty four of the plant species had *Arum*-type morphology, 25 had Intermediate- type and 12 had typical *Paris*-type morphology. There were large differences in the extent of AM colonization and root lengths with AM fungal structure between plant species.

Keywords: : AM fungi, Arum-type, Shola forest

1.INTRODUCTION

In India, the shrimp culture today has industrialized into an improved farming system and is evolved day by day into a well talented for the management. The last half a decade has proved adequately that the possible for its shrimp production is quite good in India with its warm tropical climate, suitable soil, along the main estuaries and lagoons, suitable water availability and potential force of highly industrious farming community (Lightner, 1996; Flegel, 1997). Due to last outbreak of White Spot Syndrome (WSSV) in *P.monodon*culture indications to devastating of shrimp culture in India. In the meantime, the Coastal Aquaculture Authority of India (CAA) has presented a new species *(L.vannamei*) in India and the Coastal Aquaculture Authority of India is actual intense in the Biosecurity and endorsement for the cultures of *L.vannamei.*

There is very limited research work was done on the culture and growth performance of *L.vannamei* with different high stocking densities in Tamil Nadu. So, hence the present study was investigated to assess the water quality, survival, growth and FCR of *P. Vannamei* cultured in different stocking densities.

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2.MATERIALS AND METHODS

The current work was commenced at a shrimp farm culture in Tranqubar, Nagai district, Tamil Nadu, India. The study was carried out in four shrimp rearing culture ponds. Three ponds (A1, A 2, and A3) were 0.5ha, pond A4 and A5 was 1.0ha in area. Besides that there was a reservoir present in the A4 pond with a size of 0.5ha, a sedimentation pond and a chlorination pond are in the size of 0.5 ha. Water re-circulation method was trailed to prevent cross infection during the culture period. All the investigational cultural ponds were measured to about 1.2 – 1.5m depth. The type of the soil should be maintained as sandy clayey soil. Ponds were primarily organized by drying, tilting (to remove the pests and predators and oxidize bottom soil) and liming to adjust the pH of the soil (Plates 2-5).

Many inorganic fertilizers like urea and triple superphosphate were applied to enrich the natural food organisms present in the water. Bird netting and crab fencing were performed before pumping water to prevent the auto entrants (Plates 6 & 7). The filter bags (Plate 8) were periodically checked, and it was fitted in the inlet and outlet pipe followed by the pumping the water to the whole ponds. After satisfying, the water was allowed to stand for 1 day deprived of any trouble

and allowed for sedimentation. Then the water was chlorinated (60 ppm/ha) and excess of chlorine was neutralized by dechlorination process takes nearly 72 hours. After that, the water was supplemented with probiotic to provide the good beneficial bacterial environment. After 7 days, the algal bloom was noticed slowly on the external of the waterin the ponds. The Post larval *L.vannamei*seeds (stage 14)were procured, it was acclimatised maintained to a salinity of 17 ppt and it's an evidence for the negative symptoms of the WSSV and Taura

Plate 3. Scraping the bottom of pond (using man power)

Plate 4.Ploughing or tilting the bottom of pond

Plate .5 Lime applications in the bottom of pond

Syndrome Virus (TSV) confirmed by using molecular tools like(PCR – Polymerase Chain Reaction) were obtained from Sigma, India and the seeds were procured from Rank marine hatchery, Marakaanam, Pondicherry. The seeds were transported carefully in double-layered oxygenated polyethylene bags with wrinkled ice packs placed in between the covers of the bag to maintain an optimal temperature for the less stress shrimps and the whole set up was packed in a container.

Plate 6. Bird fencing

Plate 7. Crab fencing

Plate 10. Feed Distribution in the entire culture pond

The seeds were immediately transported to the farm site and the seed bags were kept in the pond water for one hour to acclimatize the seeds. Then, the pond water was further added gradually into the seed bag to adjust the physical factors like salinity and pH. Next, the shrimp seeds were released gradually and deliberately in to the ponds. The density of the stocking must be sustained with 80/ m², 120/m² and 160/m² for ponds A1,A2 and A3 respectively. RNK feed pellets (Plates 9 & 10) were fed to the stocked post larvae for four times daily at 7am,10am, 1pm and 4pm respectively.

Plate 9.RNK SUNNY feed

In the meantime, water should be added from the water reservoir at steady intermissions to maintain the water loss due to evaporation or soil seepage. During harvesting time, the water from entire culture ponds were drained completely by allowing them to form sediment in the pond and eventually reached to reservoir pond. At any occasion, the culture pond water should not be driven out side from the farm due to bio secure reasons. From the 40th day of culture the DOC, forwards cast net sampling method was employed at each week for monitoring the status of growth and health of the shrimps (Plates11a-f).

Plate 11 e & f. Calculation of the average body weight

The water level was measured periodically by using a usual scale with centimetre marking. The physiological water quality parameters like salinity by refractometer, pH was measured by using readymade pH pen, temperature by thermometer, dissolved oxygen by DO meter and light transparency were measured respectively. Aeration equipment was fitted and supplied to the entire ponds (Plate 12).

Totally 20horse power (hp) aerator was fitted for the individual culture pond. The aerators are kept can perform dissolve maximum dissolved oxygen level (DO) into the pond water and makes the culture pond eco- friendly. Feed conversion ratio (FCR) and Average daily growth (ADG) were calculated as follow

FCR = Total weight of the harvested shrimps / total feed used

ADG = Total weight gained by the shrimps / Total days of culture

a

b

c

d

e

f

Plate 11 a- d. Checking the animal's health during the sampling

Plate 11 a- d. Checking the animal's health during the sampling

Collection of water samples

Periodically, the water samples were collected from the culture ponds. The samples for dissolved oxygen were collected at the depth of water in order to prevent the direct contact with air. The determination of turbidity, temperature, pH, salinity, calcium, nitrate and ammonia was carried using the similar sample was taken to reduce the duration between the sample collection and analysis of sample. The samples were investigated by using standard methods.

Analysis of water quality parameters

The below mentioned parameters are analysed at each ten days once for all the entire culture ponds.

Satellite picture of the pond

The level of water was measured by standard scale (cms) with marking. Normally temperature measurement is made with good, Celsius thermometers as a minimum the thermometer must have a scale marking at each 1°C with marking edged on the capillary glass, for field operators a thermometer having a metal case to movement breakage (APHA 1998). Turbidity of water is measured by a standard Sachi disc meter scale, with pin (APHA 1998). The water salinity was measured by using a hand refractometer. Hydrogen ion concentrations of the culture pond water were estimated with the help of pH meter manufactured by Hanna Instrumental Company, Japan. The water samples were collected and transferred to the beaker.

There are many methods for determining the pH of the water samples. These may be broadly divided into electrometric method and calorimetric method. Here the hydrogen ion concentration was determined with wide range pH papers in the field and later the values were collected with pH meter in the laboratory. In the electrometric method the glass electrodes were cleaned and allow drying with filter paper and immersed into the sample. And the pH was recorded calibration of the scale was necessary and so buffer solution was prepared. The instrument was standardized with the help of standards. A buffer tablet (commercial) dissolved in100 ml of distilled water will form a buffer solution of pH. The presence of calcium in the pond water was estimated by using EDTA Titration method. Brucine method was used widely to calculate the nitrate level in the pond water followed by ammonia using Nessler method.

Microbiological Analysis

The sediment and pond water samples were collected aseptically stored in sterile polythene bags from various location of the ponds and were mixed to make a one. The similar procedure was repetitive for every culture pond and the final samples were transported to the laboratory immediately and were analysed for their microbial counts. The samples were transferred to a sterile 150 ml conical flask containing 99ml of sterile dist. water and 1 gm of sample thoroughly mixed followed by serial dilution for estimating the total microbial load in different dilutions like $10^{-1}$to 10^{-5} suspension samples. The Zobell marine agar medium was used to enumerate the Total Heterotrophic Bacteria (THB), (Table 5), for *Vibrio* spp TCBS MEDIA was purchased from Hi-media, Mumbai.

Table 5. Composition of Zobell Marine Agar

Composition	Amount (g)
Peptone	5.0
Yeast extract	1.0
K2 HPO4	0.5
Feso4	Trace
Agar	15
50% seawater	1000ml
pH	7.2

Isolation and enumeration

Spread plate methods were employed for enumerating the microbial load. The sterilized medium was decanted in Petri dishes under aseptic conditions, to allow the media to solidify. 0.1µl of the sample was pipetted out and the sample was spread by using sterile L-rod by rotating in clock and anticlockwise direction for 2-3 times. Ensure the sample should be spread the entire area for proper microbial growth. Then the plates were placed in inverted position and incubated at 28±2°C. All the experiments were carried out in triplicates. After 2-3 days, the colonies were counted in all the plates using colony counter and the Colony Forming Units (CFU's) in one gram of sample was calculated by using formula,

Total microbial load

in the given sample (CFU/g)= Total number of colonies

Samples of volume plated (0.1) X Dilution

Statistical Analysis

Two way analysis - ANOVA was employed to distinguish the statistical significance between growth and stocking densities of the shrimp. Data was expressed in mean ± standard error (SPSS- package).

3.RESULTS

Present work was carried out in the shrimp *Penaeus vannamei* culture in Sattanathar aqua farm in the east coast of Nagai District, Tamil Nadu, India from April 2015 to August2015 (Table. 1 & 2).Water quality parameters for the entire culture ponds are summarized in tables 1. Pond water pH, temperature and DO readings is measured in early mornings and in the late evenings. For the three culture ponds, the average pH value was found between 7.9 and 8.6 in the early morning, while fluctuation of pH value was between 8.2 and 8.6 in the evening. DO values varied between 4 mg/l to 8 mg/l. In overall, early morning readings recorded lesser and the cycle proceeded and the standing crop was enlarged. Average pond temperatures were 26 to 31° C, respectively (Table 1). Generally, the temperature tendency in the production cycle started around 28 °C and got reduced to 26 °C due to cold conditions were found during the 21st and 28th day, and it was found to be increased at 29–31°C. During the culture period, the highest salinity was recorded as 41ppt and reduced salinity was found as 25 ppt in all the culture ponds.

Table : 1 Mean water quality parameters of the culture ponds

Parameters	A1	A2	A3
Salinity (ppt)	25 – 40	25 – 38	25 – 41
Temperature °C	26 – 31	26 – 31	26 – 31
pH - AM	7.9 - 8.5	7.9 - 8.6	7.9 - 8.6
pH– PM	8.2 – 8.5	8.2 – 8.6	8.2 – 8.6
Transparency	60 – 30	65 - 30	65 – 25
Dissolved oxygen	5 – 8	4 - 8	4 – 8
Ammonia	.1 - .3	.1 - .4	.1 - .5
Calcium	500 – 650	400 - 650	400 – 700

	Days of culture (DOC)											
PONDS	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**
A1	90	100	110	130	160	200	320	270	250	220	300	210
A2	90	90	100	100	90	210	130	110	100	190	180	160
A3	120	150	214	200	320	312	250	520	352	450	380	415

	Days of culture (DOC)											
PONDS	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**
A1	60	10	30	100	80	40	32	100	90	120	100	80
A2	40	50	40	40	60	50	60	40	50	40	50	40
A3	50	60	90	90	130	160	200	180	200	200	180	150

The population of bacterial communities were changed during every sampling. The bacterial population was found to be yellow colony (beneficial bacteria count) in pond A1 maximum320 and minimum 90 was recorded, in pond A2, A3 the maximum yellow colony were recorded 190,520 and minimum 120,90 were recorded. The bacterial population green colony count (harmful bacteria) was recorded in maximum 120,60and 200 at pond A1,A2 and A3, respectively. The minimum 10 was recorded in pond A1.

Weekly growth of the shrimp is presented in table 4. After 130thdays of culture, the average growth were recorded as 28.5g, 26.8g and 25.3g for ponds A1, A2& A3 respectively (Table 4). Survivals were 80, 72 and 66% for ponds A1, A2 & A3 respectively; FCR was 1.4, 1.5 and 1.7 for ponds A1, A2 & A3, respectively. The average production was 18250, 23478 and26640 kg/ha for ponds A1, A2 & A3, respectively (Table 5).

Table : 4 Average body weight of the "A1,A2 & A3" culture ponds.

DOC	A1	A2	A3
40	6.3	6.1	5.4
50	8.5	8.4	7
60	10.8	10	9.2
70	13.1	12.8	11.4
80	15.8	15.2	13.6
90	18	17.1	15.8
100	20.6	19.5	18
110	23.1	22	20.4
120	25.8	24.5	21.9
130	28.5	25.5	23.7
135	0	26.8	0
140	0	0	25.3

Details	A1	A2	A3
Area (Ha)	0.5	0.5	0.5
Stocking Pcs	400000	600000	800000
Density Pcs/m²	80	120	160
Stocking Date	02-Apr-15	02-Apr-15	02-Apr-15
Harvest Date	10-Aug-15	15-Aug-15	20-Aug-15
Culture Period	130	135	140
Harvest Size (GM)	28.5	26.8	25.3
Count(Pcs/Kg)	35	37	39.5
Shrimp Harvest (Kgs)	9125	11739	13320
Survival %	80	72	66
Total Feed Used (Kgs)	12775	17608	22644
FCR	1.4	1.5	1.7
Production (Kg/ha)	18250	23478	26640
ADG	0.22	0.2	0.18

4.DISCUSSION

The present investigation is on the culture of *P.vannamei* in the estuarine or brackish water shrimp farms in Tranquebar, Nagai district, Tamil Nadu, India. This work shows that high stocking density with proper water quality and feed management can give good growth of *P. vannamei.* Many researchers reported that the growth and survival of *L. Vannamei* is depends on various salinities and densities (Wyban *et al.*, 1988; Samocha *et al.*, 1993, 1999; Emberson *et al.*, 1999, Gunalan *et al*., 2011). The water quality should be upheld properly and it's very much important for their optimum survival and growth. The quality of water is very much considered for many factors like DO, temperature, salinity and pH. The surplus of feed, their waste matter and other metabolites will employ incredible effect on the water in shrimp culture pond (Soundarapandian and Gunalan, 2008).

The salinity was maintained with an average of 25 – 40ppt in all the culture ponds. *L. vannamei*, is extensively cultured in Central and South America (Wen-Young Tseng, 1988) and it tolerates the salinity of 2-45 ppt (Parker *et al.*, 1974; Samocha *et al.*, 1998). Numerous authors have stated survival and good growth of *L.vannamei* in brackish water ranges from 1.7-2.3 ppt (Bray *et al.*, 1994; Samocha*et al.*, 1999; Embersone*t al.*, 1999; Moya*et al.*, 1999). Karthikeyan (1994) and Gunalan et al. (2010) recommended a salinity range of 10 –35 ppt is perfect for shrimp culture. Samocha et al.(2004) described that development of shrimps is high in lower salinity at 2 ppt than in sea water.

The pH value is ranged from 7.9 – 8.2 in the early sunshine morning and 8.2- 8.6 in the sunset. The pond water pH is prejudiced by many factors, which includes pH, water source, acidity of bottom soil, inputs of shrimp culture and various biological activities. Wang et al.(2004) suggested the optimum pH ranges from 7.5 -8.7in *L. vannamei*. The levels of dissolved oxygen in the entire ponds were ranged from 5.0-8.0 mg/l during the whole culture period. The water quality parameters values revealed that all are in the appreciable range for survival and growth of *L.vannamei* (Van Wyk and dan John Scarpa,1999). In the present work, RNK sunny feed pellets were used for the entire ponds and the same quantity was followed as per standard feed chat. The extreme feeds were used in pond A3 followed by A2 and A3. From this present investigation, the average FCR was 1.4 to 1.7 for the whole ponds. Related reports were previously recorded by Paul Raj (1999), Ramakrishna (2000) and Soundarapandian and Gunalan (2008).

In the present work, the bacterial population yellow colony (beneficial bacteria count) in pond A1 maximum 320 and minimum 90 were found in pond A2, A3 the maximum yellow colony were recorded 190,520 and minimum 120, 90 were recorded. The bacterial population green colony counts (harmful bacteria) were found maximum in 120,60,200 at pond A1,A2 and A3, respectively. The minimum 10 was found in pond A1.

Ruangpan and Kitao (1991) described that the high occurrence of luminescent *Vibrio* is reliable with incidence of disease and poor or zero harvest results. Thebacterial pathogen like *Vibrioharveyi*will affects the *P. monodon* and it leads to drastic loss (Baticados*et al.,* 1990). The probiotic bacteria application was used either in higher or a very low profusion or a comprehensive absence of luminous *Vibrio* in pond water results in good harvest. The steady and higher productivity will occur, even in the percentage of luminescent *Vibrio* in the water was found to be higher in the sea water source, and the richness of total green colony in the pond water was higher than in source of water. Moreover, luminescent *Vibrio* was totally absent in all the stages of growth and the presence of the super biotic *Bacillius* species was recorded.

In the present study, first sampling was carried out in all ponds at the 40th DOC of the culture. During harvest in A1 pond the shrimps are ranged at a size of 28.5 g, in A2 pond 26.8 g and in A3 pond 25.3 g. Higher survival (80%) was noted in pond A1 and lower survival (66%) was noted in pond A3. Bray et al. (1994) was observed similar finding in their research. The survival of shrimp was quite well were considering the area and the size of pond and the hygienic risks of outdoor-reared shrimp (Green *et al.,* 1997; Martinez-Cordova *et al.,* 1998). The considerable FCR revealed better rearing practices shared with a appropriate environment and a good shrimp biological responsiveness. In the present work, higher growth was reported in pond A1 followed by pond A2. This accepts the basic concept of aquaculture that better water quality management, proper aeration and good seed feed management leads to better survival and growth. From the present study it was determined that *L.vannamei* culture is fruitful in brackish water environment and the growth is directly proportional to proper water quality, aeration and feed management.

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