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Occurrence of loose shell syndrome disease in culture operation of shrimp *Litopenaeus vannamei* in different regions of Andhra Pradesh

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Abstract
Pacific white shrimp *Litopenaeus vannamei* is one of the most intensively cultivated shrimp in India as well as world. The present study was aimed to investigate the incidence of Loose Shell Syndrome (LSS) in *L. vannamei* during 2018-2019. Sampling was done in East Godavari, West Godavari, Prakasam and Nellore Districts of Andhra Pradesh and subjected for analysis. Four species of *Vibrio* were identified in infected samples i.e. diseased shrimps and were identified as *V. harveyi, V. alginolyticus, V. parahaemolyticus* and *V. mimicus* based on morphological characteristics and biochemical tests. One cycle of crop data was also monitored from Nellore District. The results clearly demonstrate that LSSD significantly reduces the growth rate which in turn the productivity. The manipulation of water and feed management will pave way for successful culture of *L. vannamei* and to overcome LSSD in shrimp culture.

Keywords: *Litopenaeus vannamei*, loose shell syndrome, *Vibrio*

1. Introduction
Aquaculture is considered as one of the fastest growing food sector in the world with the cultured shrimp production increasing at an annual rate of 16.8%. All over the world, the shrimp farming activity contributes to the huge economic returns, and showed a booming expansion and soon a multibillion dollar industry [1]. The issue of malnutrition has become important for the growing population in the world including India. The pacific white shrimp *Litopenaeus vannamei* has become the main crustacean species produced through culture, with production exceeding that of tiger shrimp *Penaeus monodon* for the past one and half decades [2]. Despite of substantial increase in production of cultured shrimp from different parts of the world, there have been staggering, Periotic losses due to certain pathogenic microorganisms including Bacteria & Viruses, and Fungi and also Protozoan parasites [3-7]. Among the diseases identified in shrimp caused by viruses, White Spot Syndrome Disease (WSSD), Monodon Baculovirus Disease (MBV) were considered to be more recurrent and incidences are relatively higher in penaeid shrimp culture operation [3, 8]. Since 2000 onwards, next to WSSD, Loose-shell Syndrome disease is causing lot of mortalities among penaeid shrimp culture operations. The LSSD occurrence was first reported in 1998 in India and infection occurs in both Juveniles and adults and found to be increased the incidence every year [9]. The LSSD was prevalent in Kerala, Tamil Nadu, Andhra Pradesh, Orissa, Gujarat and West Bengal. In recent times LSSD has been reported in several parts of India including Andhra Pradesh. The present study aims to present certain field results and farming activity pertaining to White Shrimp *Litopenaeus vannamei*.

2. Materials and Methods
The Loose Shell Syndrome (LSS) affected live prawn samples were collected from Aquaculture ponds of Andhra Pradesh i.e. East Godavari, West Godavari, Prakasam and Nellore Districts. All the moribund (LSSD affected) were collected and live – transported to the laboratory in Oxygen-packed Polythene bags with temperature maintained at 26 ± 1^0C using the bags.
Haemolymph samples were drawn from the ventral sinus of each shrimp with the help of a sterile syringe and plated on Tryptone Soya Agar (TSA) with 2% Sodium chloride and

Thiosphosphate Citrate Bile Sucrose Agar (TCBS) to estimate Total Vibrio Counts, following standard techniques incubated at 27^0C for 48 hr [3]. The *Vibrio* sps were enumerated with the help of colony counter and expressed in colony forming units (cfu) per milli liter of haemolymph. Identification was done on the basis of their morphological and biochemical tests as per the methods of Buchanan and Gibbons (1974). Biochemical tests such as Gram's staining, Catalase, Oxidase, MR-VP test, Uriease, Oxidative/ Fermentative test, Citrate Utilization tests were carried out in the laboratory.

2.1 Growth Performance studies

The present study was carried out in commercial shrimp pond located near Ramayapatnam (Latitude 15^0 02^1 55^{11}N; Longitude 80^0 02^1 50^{11} E), Prakasam Dist of Andhra Pradesh, South India. Buckingham canal is passing adjacent to all the culture ponds providing continuous supply of water throughout the year. Water was pumped into a storage pond and appropriate treatments were conducted and the water were stored for 10 days and later pumped into culture ponds for further use. The entire culture farm has a covered area of 16 ha and three ponds of equal size i.e. 1.0-1.2 ha were selected in the present study. In all the culture ponds 1 mts depth of water (minimum) i.e. 1.2 mts was maintained throughout the present investigation. During pond preparation all the precautions were taken to remove the obnoxious gases, Oxygenate the bottom soil and remove H_2S odour and to increase fertility. The soil pH was recorded in the ponds with the help of cone type pH meter. For increasing the availability of nutrients, required amount of lime was applied to neutralize the acid soil, condition of the soil based on the average pH level of the pond.

Litopenaeus vannamei of 0.79 ± 0.03 g were selected for present investigation and were stocked. The 1st group of shrimp were treated as control group fed with CP Brand commercial Feed. The 2nd group was fed with Probiotic feed (Commercial Feed + 10 g of UB Probizyme + *Lactobacillus*) along with soil, water Probiotics Super PS (10 lit/ha) Super Biotic (10 Kg/ha) added. The 3rd group was fed with commercial feed but incidences of LSS recorded. The culture operation was continued for a period of 130 days.

Growth Performance studies were conducted Continuously with every 15 days interval and observations were recorded. Growth parameters including Average Weights, Average daily growth rates (ADGR) Specific growth Rates (SGR), Feed Conversion Ratio (FCR), Percent Survival rates were calculated and tabulated.

$$\text{Survival Rate (\%):} \quad \frac{\text{Total Number of Live shrimp}}{\text{Total Number of Shrimp Stocked}} \times 100$$

$$\text{Weight gain (g):} \quad \text{Weight of the Shrimp (g) At the end of culture period} - \text{Weight of the Shrimp (g) at the start of the Experiment}$$

$$\text{Feed Conversion Ratio (FCR):} \quad \frac{\text{Total amount of Feed broadcasted (Kgs)}}{\text{Total Biomass (Weight) of Shrimp (Kgs)}}$$

$$\text{Specific Growth Rate (SGR):} \quad \frac{\text{Log weight of the shrimp At the end of the Experiment} - \text{Log weight of the shrimp at the start of the Experiment}}{\text{Total days of culture period of operation.}}$$

All the Growth and Ecological parameters obtained were subjected to Statistical Analysis (SPSS). Data were analysed by Descriptive analysis, Multiple comparisons, Correlation analysis for each set of data obtained.

3. Results and Discussion

A field survey has been made in E. Godavari, W. Godavari, Prakasam and Nellore Districts of Andhra Pradesh and 291 Farm samples were collected. The details of Farms selected Dist wise and LSS incidence occurred in farms of *L. vannamei* culture ponds are presented in Table 1.

Diseased shrimps *L.vannamei* were sluggish, weak with soft muscle, loose shell with condensed and melanised hepatopancreas. The affected shrimps exhibited erratic swimming behaviour, regularly coming to the shore and stopped feeding leading to mortalities. Several authors reported similar kind of observations including flaccid spongy abdomen, caused by muscular dystrophy and a gap was observed between muscle tissue and carapace in Penaeid shrimp *Penaeus monodon* culture operation affected with LSS [9-13]. In the present study, data pertaining to LSS revealed that the rate of infection was more in Prakasam (32%), followed by Nellore (30%) E. Godavari (22%) and W. Godavari (17%). Out of 291 farms selected for sampling 77 shrimp farms were found to be LSS infected i.e. 26% of the shrimp farms. Due to the prevalence of LSS, the mortality rates were found relatively not high, but significantly related growth potentials were leading to heavy economic losses. Previously several authors reported that 23% and 14% of farms in Tamil Nadu were affected by LSS [14], 27% in w. Godavari District, and 5% in Nellore Dist of Andhra Pradesh [15]. In the present study Bad management practices like inadequate nutrition, poor water quality maintenance were corelated with the incidences of loose shell syndrome in *L. vannamei*. In the present investigation every 15 days water quality parameters like Dissolved Oxygen (DO), pH and Total Ammonia Nitrogen (TAN) were found to be affected during LSS infection. The DO content was significantly reduced with a progress in the days of culture operation (DOC) during LSS infected farms (Figure.1). The pH of the culture water was found to be significantly increased during LSS infection, where as pH was found to be almost constant in the control ponds (Figure.2). But one of the key factors of water quality parameters, TAN content are found to be significantly increased during LSS infected shrimp farms (Figure.3).

3.1 Growth Performance Studies

Growth performance studies of *L. vannamei* were conducted in control pond, Probiotic added pond and LSSD affected ponds and the results are presented in Table 2. Shrimp were harvested after the completion of 130 days of culture operation. One third of water from each pond was drained through outlet Just before catching. While two third of water let out, the prawns were collected and handpicked. Shrimps were immediately transferred to changing tubes provided near the pond. They were rinsed and chilled before packing. Random samples were collected during the weighing process for determination of individual weights. After the quantifying the biomass, mean final yield, Feed conversion Ratio, and Survival rates were calculated by using standard formulae.

Among the experimental groups, the group fed with Probiotics recorded maximum growth rates followed by control group and finally LSS affected group of shrimp (Table 2). The final weights and production rates were recorded to be 32.94 g & 3022 Kgs, 25.98 g & 2313 Kgs and 21.25 g & 1296 Kgs, with Probiotic fed, Control and LSS affected groups, respectively. The results obtained for growth parameters

including Average daily growth rate (ADG), Specific Growth rates (SGR), Feed Conversion ratio (FCR), Production yields and Feed consumed in the present investigation clearly demonstrate that, the experimental feeds selected are fairly relatively better. The FCR values obtained are in and around 2, therefore the feeds selected in the present investigation are considered to be of superior quality feeds. Though the same feed was used, but due to LSS affected for shrimp, there was a drastic reduction in feeding rates followed significant reduction of growth rates produced lower yields and average body weight along with lowest survival rate of 61% was recorded. As for as prawn culture is concerned there are many factors relates the growth and feed activity, which include a functional digestive system to efficiently utilize the nutrients present in the food offered [16] and the physiological conditions and the rearing environment [17]. In the present study, Probiotics including soil, water and feed Probiotics were introduced into the culture environment. The Probiotics including Lactic acid Bacteria, *Bacillus, Lactobacillus* sps were introduced into the culture environment as Biofriendly agents to control the pathogenic organisms [18]. Probiotics in Aquaculture are relatively recent and widely administered to modify and manipulate selected material populations in the pond environment and to reduce or eliminate selected pathogens and leading to better survival of the culture species [19]. The newest attempt to improve water quality in aquaculture is the application of Probiotics to ponds. The application of Probiotics in shrimp benefited the improvement of the intestinal microbial balance, enhancement of the disease resistance by supressing the pathogens and also the enhancement on the immunity or improvement of water quality by the decomposition of organic matter, reduction in nitrogen and Phosphorus concentrations for controlling of ammonia, Nitrite and Hydrogen sulfide level [20]. So with the usage of Probiotics in the present investigation, clearly not only improve water quality and also improve animal health, thereby production rates increased substantially. Moreover there is a significant improvement in FCR, SGR and Average final weights clearly demonstrated in the present study. In addition lower incidence of diseases, greater survival rates also clearly demonstrate that, Probiotics use facilitates a clear increase in the production rates, through manipulation of water and Feed management practices. Several authors also reported the results, which are in favour of the results obtained in the present study [20-22]. Rangipat *et al.* [23] observed that the increased survival rate of shrimp *Penaeus monodon* after feeding with Probiotics *Bacillus* for 90 days. Probiotics have multiple mechanisms of action to inhibit pathogens which include competitive exclusion, production of substances that inhibit growth of opportunistic pathogens, stimulation of the immune response, increase digestive function through production of enzymes, improved nutrition by providing essential nutrients and improved water quality [24, 25].

In the case of one of the culture ponds which was affected by LSS. Through we tried to maintain a good water quality and other parameters, but due to the invading of certain pathogenic microorganisms like *Vibrio* sps, causes the incidence of LSS in the culture pond. The DO content was significantly decreased and there by imposing the hypoxic stress on the shrimp. Similarly another two important water quality parameters including pH and TAN were found to significantly increased there by disturbing the water management leading to the occurrence of LSS in the culture pond. The affected shrimp were lethargic and amorexic, carapace became very thin, a gap was observed in between the muscle tissue and the carapace. The affected also losses their weight significantly during culture operation. In the present study, four major species of *Vibrio* bacteria were associated with LSS affected shrimp *L. vannamei*. All the Morphological and Biochemical characteristics of four *Vibrio* sps were identified are presented in Table 3. Total *Vibrio* sps individually estimated and presented in Table 4. By virtue of Biochemical tests conducted in the laboratory, the isolated species of Bacteria were identified as *Vibrio harveyi, V. alginolyticus, V. Parahaemolyticus* and *V. mimicus*. Previously, several authors reported that LSS affected shrimps displayed clinical symptoms such as loose shell, soft muscle, condensed & melanised hepatopancreas, loss of appetite, erratic movements, reduction in the growth rates and survival rates in penaeid prawn *Penaeus monodon* [6, 11-13]

From the results obtained in the present investigation it may be concluded that East Godavari, West Godavari, Prakasam, and Nellore Districts were selected for sampling and 291 Aquafarms were identified for Sampling. Out of 291 Aquafarms selected 77 (26%) of aquafarms were identified with LSS occurrence. The occurrence of LSS were found to be 32% aqua farms in Prakasam, followed by 30% Nellore, 22% with East Godavari and minimum of 17% with West Godavari districts. Among the aqua ponds selected, the LSS were found to be occurred only after 50 DOC. The Growth Parameters were found to be significantly increased consequent upon the addition of Probiotics i.e. Soil, Water and Feed Probiotics into the culture environment. Final weights, Average daily weights, Survival rates productivity rates were found to be significantly increased in Probiotic added shrimp, suggests that Probiotics are influencing not only Growth parameters and also immune parameters for successful elimination of pathogenic microorganisms, there by promoting highest and best growth rates. In the LSS affected ponds, the survival, growth and productivity rates were significantly reduced, due to bad water management during culture operation. The Water quality parameters were severely affected due to LSS in the culture operation, significantly reduced the growth rates subsequently production rates. WSSV infection rates were also identified in certain aquafarms selected in the present study. Among the 10 Aquafarms selected, 5 were from Prakasam, 2 from Nellore, 2 from E. Godavari and 1 from W. Godavari. Multiple infection i.e. WSSV, Vibrio and Protozoans were also identified in 6 Aqua farm, 3 from Prakasam, 1 each from other places including Nellore, W. Godavari and E. Godavari. From the LSS affected Aquafarms four major species of *Vibrio* sps were identified by following Morphological and Biochemical characteristics. The four *Vibrio* sps are *V. harveyi, V. Parahaemolyticus, V. mimicus* and *V. alginolyticus.* Among the four Vibrio sps, *V. harveyi*, found to more dominant among the aquafarms of Nellore, *V. Parahaemolytics and V. alginolyticus* in Prakasam, *V. harveyi and V. mimicus* from E. Godavari, where as *V. Parahaemolyticus* is dominant in W. Godavari District. Out of Total Vibrio Count (TVC), the four sps of vibrio were found to be more predominant sps *V. harveyi*, followed by *V. alginolyticus, V. Parahaemolyticus*, and *V.mimicus*. The abundance of TVC and *Vibrio* sps occurrence was more in the case of LSS affected farms compared to control or Probiotic added farms. A minimum were observed in Probiotic added farms. From the observations recorded in the present study will pave way for the successful culture of *L. vannamei*.

Table 1: Field results of Loose Shell Syndrome incidences in Andhra Pradesh

District	Number of Farms sampled	Number of LSS incidences
East Godavari	55	12 (22%)
West Godavari	48	8 (17%)
Prakasam	73	23 (32%)
Nellore	115	34 (30%)
Total	291	77 (26%)

Table 2: Growth Performance parameters in *L. vannamei*

Parameter	Control	Probiotic Added	LSSD
Stocking density (nos/ha)	1,00,000	1,00,000	1,00,000
Culture period (days)	130	130	130
Survival rate (%)	89	94	61
Initial weight (g)	0.79 ± 0.03	0.79 ± 0.03	0.79 ± 0.03
Final weight (g)	26.77^{a} PDC	32.94^{b} (+23)	$22.04^{b,c}$ (-18)
Weight gain (g)	25.98^{a}	32.15^{b}	$21.25^{b,c}$
Average Daily Growth Rate (g)	0.20^{a}	0.25^{b} (+25)	$0.16^{b,c}$ (+20)
Productivity (kgs)	2313^{a}	3022^{b} (+31)	$1296^{b,c}$ (+20)
WSSV Infection Rate (%)	1	Nil	10
Multiple Infection Rate (%) (WSSV, Vibrio, Protozoas)	Nil	Nil	6

All Values are Mean ± SD of Six individual observations.
Values presented in Parenthesis are Percent Change over their respective Control.
Values with different Superscripts are significantly different from each other @ P<0.05.

Table 3: Morphological and Biochemical characteristics of Vibrio species isolated from Loose Shell Syndrome Disease (LSSD) affected *L. vannamei*.

Test	V.h	V.p	V.m	V.a
Gram's test	-	-	-	-
Shape	Rod	Rod	Rod	Rod
Motility	+	+	+	+
Oxidase Reduction	+	+	+	+
Catalase Reduction	+	+	+	+
O/F test	F	F	F	F
Decarboxylation of Amino acids				
Arginine dihydrolase	-	-	-	-
Ornithine dihydrolase	+	+	+	+
Lysine decarboxylase	+	-	+	+
Methyl red test	+	+	+	+
Voges Proskauer test	-	-	-	+
Indole Production test	+	+	+	+
Starch hydrolysis	+	-	+	+
Urea hydrolysis	+	-	+	+
Gelatin liquefaction	+	+	-	+
H_2S production	+	+	+	+
Nitrate Reduction	+	-	+	+
Citrate utilization	+	-	+	+
Growth with NaCl				
4%	+	+	+	+
8%	+	+	+	+
Utilization of Carbohydrates				
Sucrose	+	+	+	+
Arabinose	+	-	+	-
Fructose	+	+	+	+
Glucose	+	+	+	+
Galactose	+	+	+	-
Dextrose	+	+	+	+
Mannose	+	-	+	-
Trehalose	+	+	+	+
Xylose	-	-	-	-
Lactose	-	-	-	-
Cellobiose	-	-	-	-
Urease	-	+	+	+

V.h: *Vibrio harveyi*
V.p: *Vibrio parahaemolyticus*
V.m: *Vibrio mimicus*
V.a: *Vibrio alginolyticus*

Table 4: Total *Vibrio* count (TVC) and *Vibrio* sps distribution in different culture ponds.

Vibrio Species	Days of Culture Operation (DOC)			
	30	60	90	120
Control Pond				
Total Vibrio count (x 10^3 cfu/ml)	10.12	8.42	6.48	6.74
V.harveyi	3.14 (31%)	2.78 (33%)	2.27 (35%)	2.43 (36%)
V.alginolyticus	2.93 (29%)	2.53 (30%)	2.33 (36%)	2.43 (36%)
V.parahaemolyticus	1.62 (16%)	1.52 (18%)	0.91 (14%)	1.01 (15%)
V.mimicus	1.52 (15%)	1.09 (13%)	0.82 (12%)	0.67 (10%)
Unidentified	0.92 (9%)	0.51 (6%)	0.21 (3%)	0.14 (2%)
Probiotic Added Pond				
Total Vibrio count (x 10^3 cfu/ml)	8.41	7.03	5.03	5.10
V.harveyi	2.94 (35%)	2.67 (38%)	1.81 (36%)	1.94 (38%)
V.alginolyticus	2.35 (28%)	2.25 (32%)	1.71 (34%)	1.84 (36%)
V.parahaemolyticus	1.68 (20%)	1.48(21%)	1.01 (20%)	1.02 (20%)
V.mimicus	1.01 (12%)	0.49 (7%)	0.40 (8%)	0.20 (4%)
Unidentified	0.42 (9%)	0.14 (2%)	0.10 (2%)	0.10 (2%)
LSSD Affected Pond				
Total Vibrio count (x 10^3 cfu/ml)	10.34	15.45	16.74	17.42
V.harveyi	3.93 (38%)	5.41 (35%)	6.36 (38%)	6.97 (40%)
V.alginolyticus	3.83 (37%)	5.10 (33%)	5.36 (32%)	6.45 (37%)
V.parahaemolyticus	1.45 (14%)	3.40 (22%)	3.35 (20%)	3.14 (18%)
V.mimicus	0.62 (6%)	0.77 (5%)	1.17 (7%)	0.52 (3%)
Unidentified	0.52 (5%)	0.77 (5%)	0.50 (5%)	0.35 (2%)

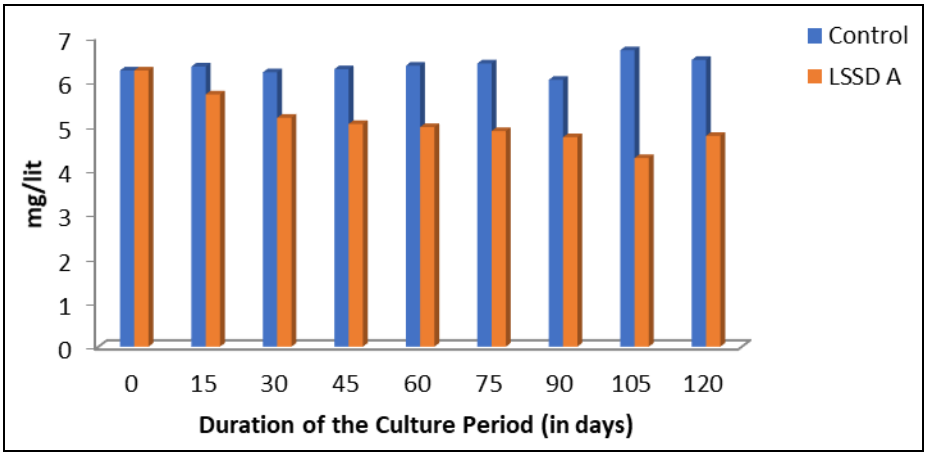

Fig 1: Changes in Dossolved Oxygen (DO) content in Control and LSS affected ponds of different periods of DOC

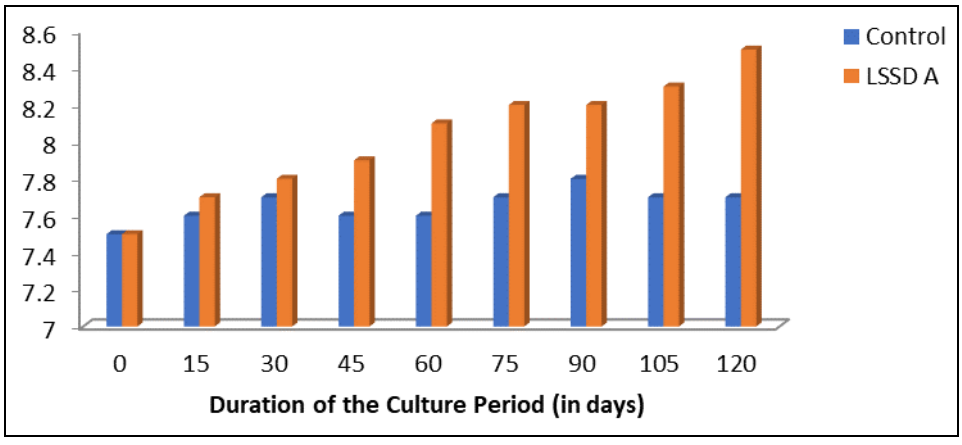

Fig 2: Changes in pH in Control and LSS affected ponds of different periods of DOC

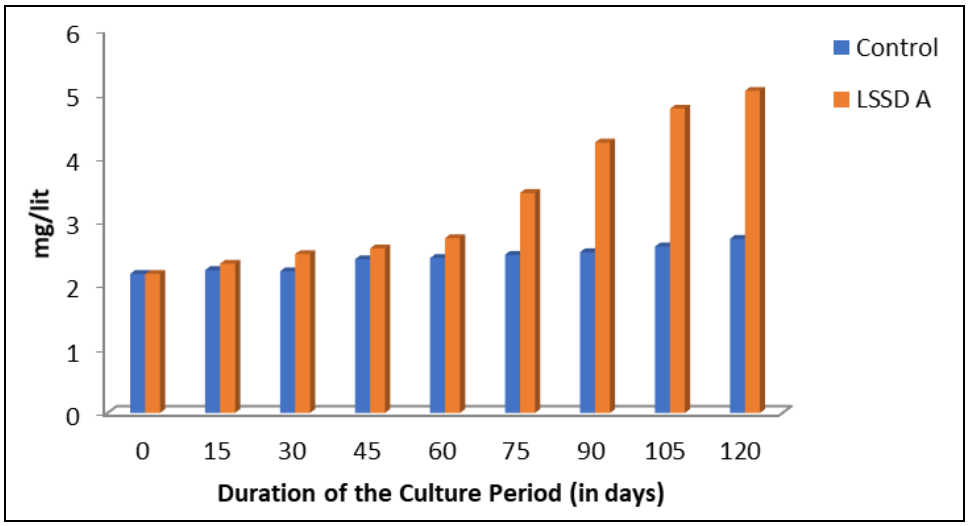

Fig 3: Changes in Total Ammonia Nitrogen (TAN) in Control and LSS affected ponds of different periods of DOC

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