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Research Article

PROPHYLACTIC EFFECT OF LACTOBACILLUS SPECIES AGAINST RED DISEASE (AFLATOXICOSIS) CAUSED BY *ASPERGILLUS* IN *PENAEUS VANNAMEI* DURING LARVAL STAGES

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ABSTRACT

India is one of the world's leading shrimp-producing country in the world. The shrimp hatchery industry also developed in parallel to this. Seed production is not constant due to various disease threats. Shrimp hatchery seed (*Penaeus vannamei)* production is depressed by various diseases, particularly caused by Aflatoxicosis (red disease), filamentous fungi, and some viruses. Usage of probiotics provides a solution to these problems. The microbial species composition in hatchery tanks by adding selected fungal species to displace deleterious normal fungi. The virulence of filamentous fungi (*Aspergillus)* can be controlled in this manner. The abundance of filamentous fungi strains decreased in hatchery tanks where specially selected, probiotic strains of lactobacillus species were added. The filamentous fungal disease was controlled by using probiotics (Lactobacillus species). In this study also it reveals that the probiotic used tank's water quality and animal health were good.

Keywords: *Penaeus vannamei*, Filamentous fungi, Aspergillus, Probiotics, Lactobacillus species.

INTRODUCTION

Penaeus vannamei live in tropical marine habitats. While postlarvae travel inshore to spend their juvenile, adolescent, and sub-adult phases in coastal estuaries, lagoons, or mangrove habitats, adults live and spawn in the open ocean. By the time they are 6-7 months old, males reach maturity at 20 g, and females at 28 g. A 30-45 g *P. vannamei* will produce 100 000-250 000 eggs with a diameter of about 0.22 mm. About sixteen hours after spawning and fertilization, hatching takes place. The initial stage larvae, known as nauplii, are positively phototactic and swim sporadically. Nauplii rely on their yolk stores for sustenance rather than food. The next larval stages protozoea, mysis, and early post larvae, respectively eat phytoplankton and zooplankton, stay planktonic for a while, and are carried by tidal currents to the coast. About five days after moulting into PL, the post larvae (PL) shift from their planktonic lifestyle to an inshore one, when they start consuming benthic debris, worms, bivalves, and crustaceans. (FAO, 2009). The most prevalent and productive of the cultivated crustaceans is *Penaeus vannamei*, which produced 5.815 million tonnes in 2020, or 52% of the total production of crab aquaculture (FAO, 2023). Cannibalism is the main factor limiting productivity and income potential in commercial prawn production (Romano and Zeng, 2016). The market for seafood has grown over time, and it is regarded globally as a premium, nutrient-dense meal that promotes a balanced diet (Maia M L *et al*., 2022). The world's seafood business is dominated by shellfish, which include prawns, crayfish, oysters, mussels, crabs and more. They offer users a number of health advantages in addition to being a powerful source of nourishment (Venugopal V *et al*., 2017). Because of their distinct flavour and texture, they have a high market value and are seen by consumers as a healthy choice (Das J and Mishra, H N. 2021). Omega-3 fatty acids improve vision, insulin response, and bone mass retention while preventing inflammation, tumours, and cardiac issues (Das P *et al*., 2021). After bacterial infections, fungal infections are the second most common cause of aquaculture's financial losses (Ramaiah N A, 2006). Because they can readily break down cell membranes made primarily of proteins and lipids, fungi that generate the enzymes proteinase and phospholipase-like aid in adhesion and invasion in host

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tissue (Cruz da Silva LR, 2011). The most vital organ in prawns that is in charge of breathing is the gill. Shrimps may die from a fungal infection in their gills because the fungi can obstruct their breathing, raise their chronic mortality rate, and make them more susceptible to other illnesses. The initial clinical sign of a fungal infection is a change in the gill's colour. The gills initially appear opaque white, then become yellow or brown, and finally turn black (Rhoobunjongde W, 2011).

MATERIALS AND METHODS

The study was conducted in a commercial shrimp hatchery in East Coast Road of Chennai. The experiment was conducted to prove the efficacy of probiotic. A commercially available USA based probiotic (BiOWiSH® MultiBio 3PS) used for this experiment. It is a combination of *Lactobacillus plantarum*, *Lactobacillus acidophilus, Lactobacillus rhamnosus,* and *Lactobacillus casei*. The seawater used in the tanks was filtered through a Bio-filter and mesh bag filters (1.0 µm) before being pumped into a reservoir tank for primary disinfection by chlorination (20 ppm sodium hypochlorite for 24 hours). After chlorination and particulate settling the water was filtered through a pressure filter and cartridges 1.0 µm, 0.5 µm and 0.25 µm, then disinfected using ultraviolet light (UV), and pumped to the treated seawater reservoir. Before using the seawater, the residual chlorine was checked: If chlorine was still present, sodium thiosulfate was applied, at a ratio of 1:1 of thiosulfate for each ppm of residual chlorine. General water chemistry was analyzed before the experiment (Van Wyk *et al*., 1999).

Filamentous fungal (*Aspergillus*) infected tanks were identified and samples were collected from the neighboring shrimp hatchery. The samples were examined under the microscope in 100X. Affected animals were having filamentous fibrils on the stocked eye balls. The eye balls of disease affected animals were opaque and translucent. The gills of the affected animals were dark in colour and having lot of attachment and the gill was covered like a mat. The animals were brought in to a nearby pathological lab for further confirmation. Collected samples kept it for further experiment. The *Penaeus vannamei* post larvae used in the trial were previously screened for various shrimp diseases including White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV), Infectious Myonecrosis Virus (IMNV), Acute Hepatopancreatic Necrosis Disease (AHPND), and *Enterocytozoon hepatopenaei* (EHP) using polymerase chain reaction (PCR) techniques. The experimental setup was divided in to four groups. There were nine acrylic tanks (0.5 x 0.25 x 0.25 meters = 0.03125 cubic meters each) were first filled with 30 lts of sea water (30 ppt) and stocked with 100 nos of post larvae at the stage of PL_1. All tanks inoculated with filamentous fungi (*Aspergillus*) from the same source. The Group "A" Tanks were maintained as such without any treatment. The Group "B" tanks were added with probiotic End of 1st week (BiOWiSH® Multi Bio 3PS) every day morning and evening each 1.0 ppm till the end of the experiment. The Group "C" tanks were added with probiotic beginning of 2nd week (BiOWiSH® MultiBio 3PS) every day morning and evening each 1.0 ppm till the end of the experiment. The Group "D" tanks were added with probiotic end of 2nd week (BiOWiSH® MultiBio 3PS) every day morning and evening each 1.0 ppm till the end of the experiment.t Water exchange and tank siphoning were identical for all treatments. All the groups were properly fed with artificial pellet feed (INVE Belgium) and freshly hatched live Artemia alternatively. The feeding interval was every four hours in a day. The experiment was continued up to 2 weeks. Water parameters and animal health were analyzed at the end of the experiment.

RESULTS AND DISCUSSION

Information about shrimp hatchery post larvae and water parameters experiment in winter, summer and Rainy seasonal variation is important to understand the quality of hatchery and suitability of water parameters and animal health. All tanks inoculated with filamentous fungi (*Aspergillus*) from the same source. The Experiment of shrimp hatchery in Chennai water and shrimp PL (animal) samples are presented in Group A, B, C and D Respectively. The experiment of Group A and B water parameters in winter season water sample were decreased in the order Hardness>Alkalinity>Water Salinity>Water Tem>Water pH>Total length>Total Ammonia. The experiment of Group A and B water parameters in summer season water sample were increased. A significant increase in Rostral Spines (Group C) and Size Variation (Group D) concentration was noticed in the Summer Season Shrimp PL. when compared with winter and Rainy season. The Result were debited in the Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11 and Table 12. Warm temperatures (typically in summer) accelerate the growth rate and virulence of fungal disease like *Aspergillus* species, leading to increased disease incidence and severity in shrimp populations. These fungi thrive and replicate much faster in warmer condition, making outbreaks more likely during hot months. Cooler temperatures (often in winter) tend to slow fungal growth and reduce the likelihood of outbreaks. Pathogen replication rates are lower, and the overall disease incidence decreases during colder periods. Combined effects with other factors: High temperatures can influence not just fungi but also viral pathogens, and periods of stress (environmental or related to water chemistry) can magnify the effects of temperature changes, creating windows of increased risk. The Tabulated Values in the Tables were represented in (Figure 1- 12).

Table 1. Experiment duration 1st week (Winter Season).

S. No	Parameters	A (Control)	B (Probiotic End of the 1st week)
1	Water Salinity	30±1.0 ppt	30±1.0 ppt
2	Water Tem	31±1° C	31±1° C
3	Water pH	7.8±1	7.8±1
4	Alkalinity	138.0mg/L	138.0mg/L
5	Hardness	4850 mg/L	4850 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Total Length	6.0±1 mm	6.5±1 mm

Fig 1. Experiment duration 1st week (Winter Season)

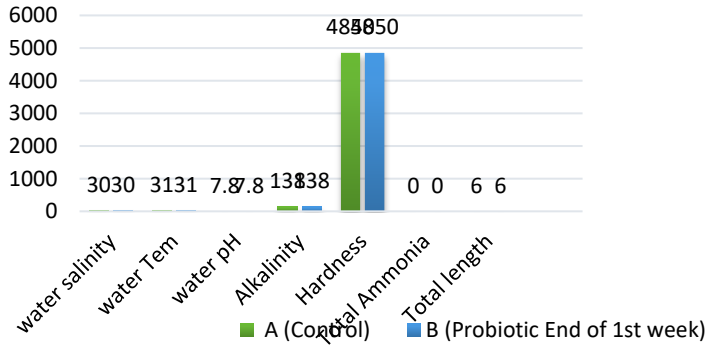

Table 2. Experiment duration 1st week (Winter Season).

S.No	Parameters	A (Control)	B (Probiotic)
1	Water colour	Light Greenish	Light Greenish
2	Animal colour	Transparent	Transparent
3	Appendages	No attachment	No attachment

S.No	Parameters	C (Beginning of 2nd week)	D (Probiotic End of the 2nd week)
1	Rostral spines	2.5	2.8
2	Size variation	2.0%	2.2%

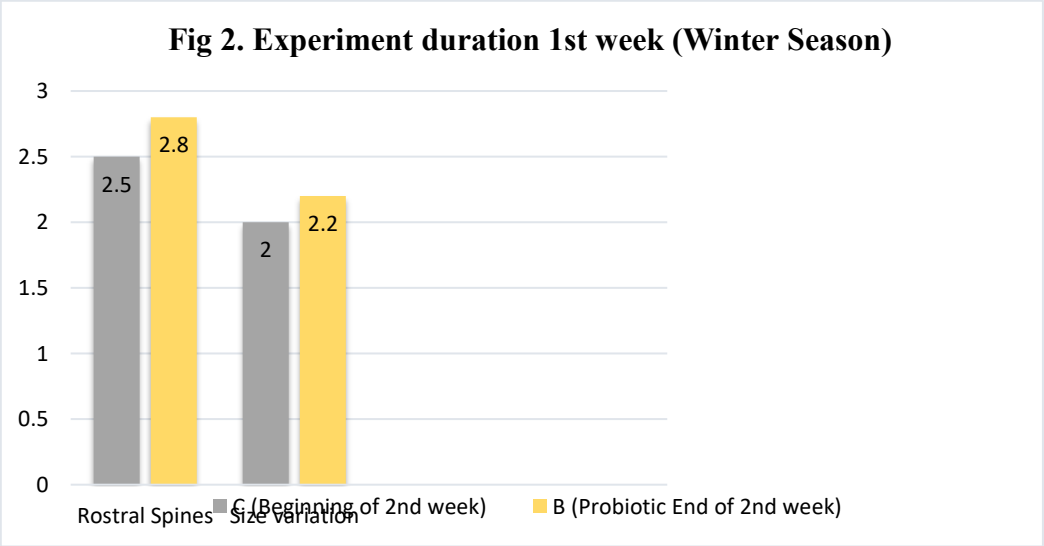

Table 3. Experiment duration 1st week (Summer Season).

S. No	Parameters	A (Control)	B (Probiotic End of the 1st week)
1	Water Salinity	35±1.0 ppt	35±1.0 ppt
2	Water Tem	33±1° C	33±1° C
3	Water pH	7.9±1	7.9±1
4	Alkalinity	142.0mg/L	142.0mg/L
5	Hardness	4853 mg/L	4853 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Total Length	6.4±1 mm	6.7±1 mm

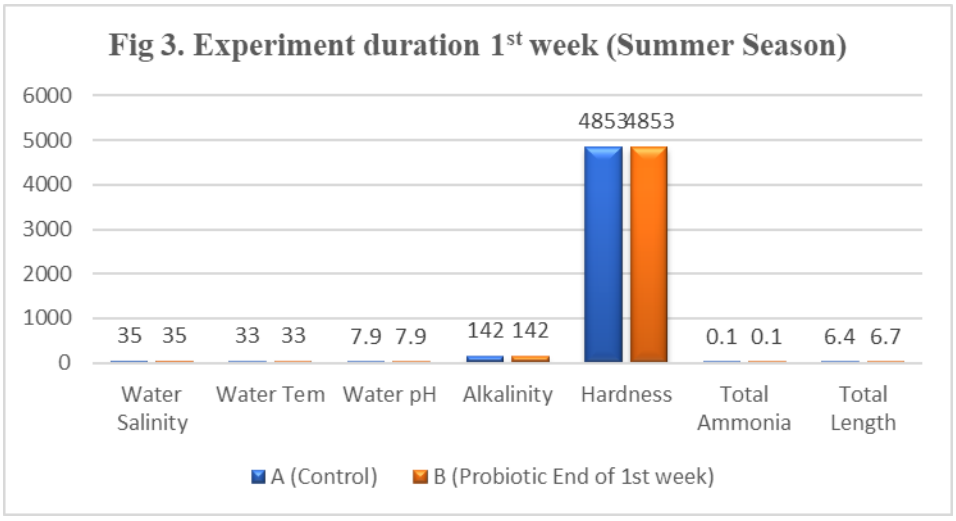

Table 4. Experiment duration on 1st week (Summer Season).

S. No	Parameters	A (Control)	B (Probiotic)
1	Water colour	Light Greenish	Light Greenish
2	Animal colour	Transparent	Transparent
3	Appendages	No attachment	No attachment

S. No	Parameters	C (Beginning of 2nd week)	D (Probiotic End of the 2nd week)
1	Rostral spines	2.7	3.0
2	Size variation	2.4%	2.8%

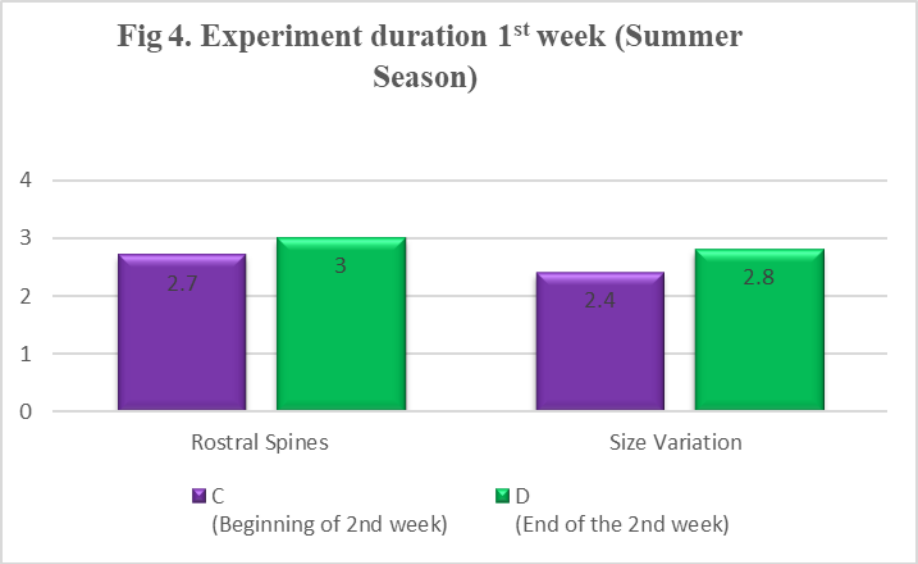

Table 5. Experiment duration on 1st week (Rainy Season).

S. No	Parameters	A (Control)	B (Probiotic End of the 1st week)
1	Water Salinity	32±1.0 ppt	32±1.0 ppt
2	Water Tem	32±1° C	32±1° C
3	Water pH	7.8±1	7.8±1
4	Alkalinity	139.0mg/L	139.0mg/L
5	Hardness	4852 mg/L	4852 mg/L
6	Total Ammonia	≤ 0.1 ppm	≤ 0.1 ppm
7	Water colour	Greenish	Greenish
8	Total Length	6.5±1 mm	6.8±1 mm

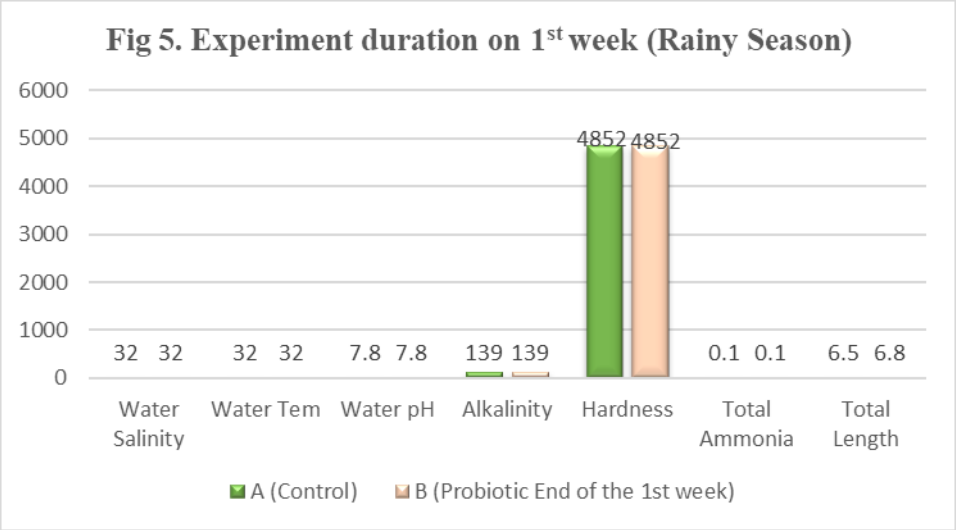

Table 6. Experiment duration on 1st week (Rainy Season).

S.No	Parameters	A (Control)	B (Probiotic)
1	Water colour	Light Greenish	Light Greenish
2	Animal colour	Transparent	Transparent
3	Appendages	No attachment	No attachment

S.No	Parameters	C (Beginning of 2nd week)	D (Probiotic End of the 2nd week)
1	Rostral spines	2.4	2.9
2	Size variation	2.2%	2.4%

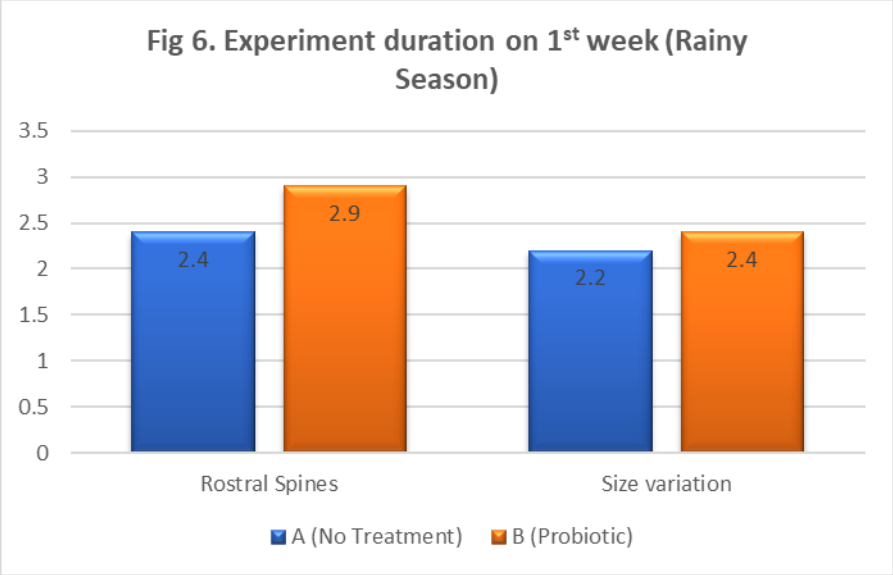

Table 7. Experiment duration on 2nd week (Winter Season).

S.No	Parameters	A (Control)	B (Probiotic End of the 1st week)
1	Water Salinity	30±1.0 ppt	30±1.0 ppt
2	Water Tem	29±1° C	29±1° C
3	Water pH	7.9±1	8.1±1
4	Alkalinity	138.0mg/L	138.0mg/L
5	Hardness	4850 mg/L	4850 mg/L
6	Total Ammonia	0.20 ppm	0.06 ppm
7	Total Length	6.5±1 mm	7.3±1 mm

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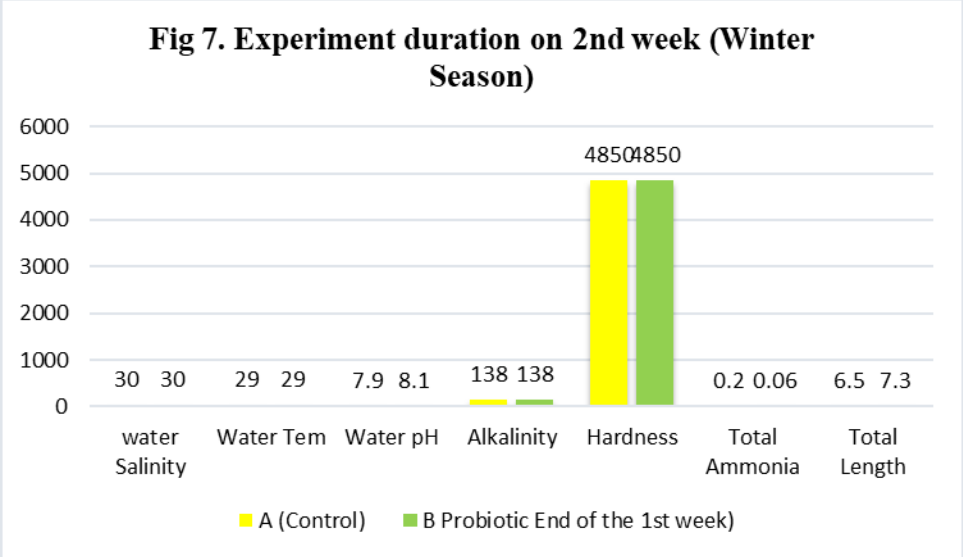


Table 8. Experiment duration on 2nd week (Winter Season).

S.No	Parameters	C (Beginning of 2nd week)	D (End of the 2nd week)
1	Rostral spines	2.5 Nos	3.7 Nos
2	Size variation	2.2± 0.5%	2.5%

S.No	Parameters	A (Control)	B (Probiotic)
1	Water colour	Transparent	Grayish
2	Animal colour	Slightly Pigmented	Pigmented
3	Appendages	Huge attachment	Very mild attachment

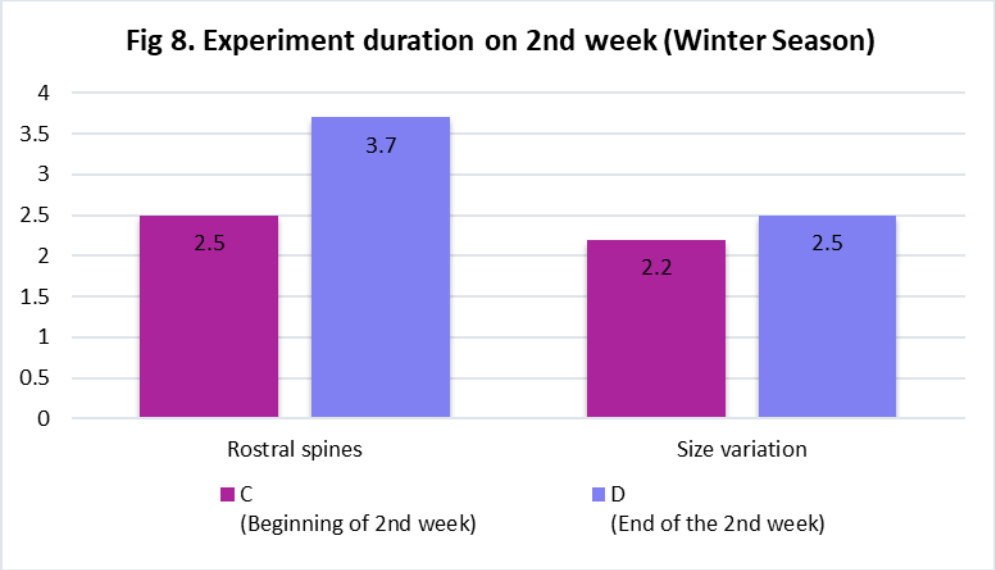


Table 9. Experiment duration on 2nd week (Summer Season).

S. No	Parameters	A (Control)	B (Probiotic End of the 1st week)
1	Water Salinity	35±1.0 ppt	35±1.0 ppt
2	Water Tem	32±1° C	32±1° C
3	Water pH	8.2±1	8.4±1
4	Alkalinity	140.0mg/L	140.0mg/L
5	Hardness	4855 mg/L	4855 mg/L
6	Total Ammonia	0.25 ppm	0.09 ppm
7	Water colour	Transparent	Grayish
8	Total Length	6.8±1 mm	7.6±1 mm

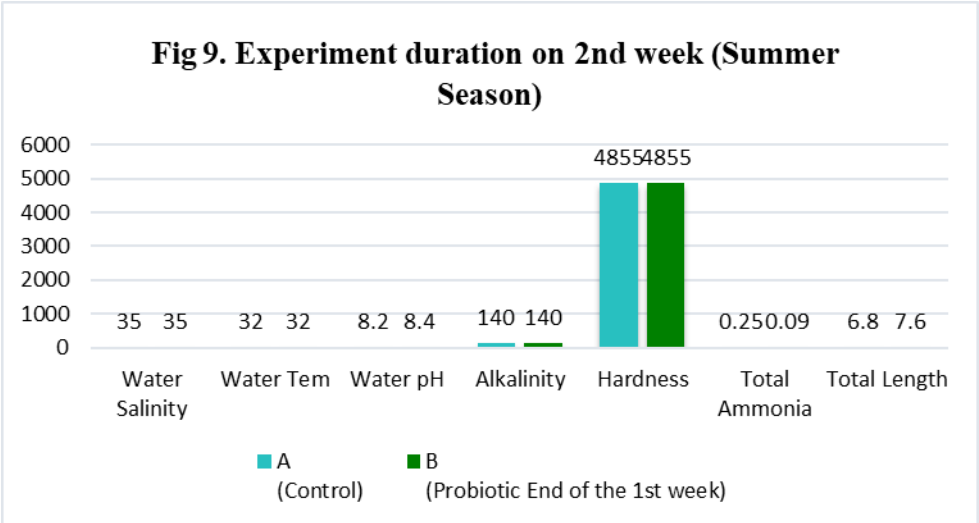


Table 10. Experiment duration on 2nd week (Summer Season).

S. No	Parameters	C (Beginning of 2nd week)	D (Probiotic End of the 2nd week)
1	Rostral spines	2.9 Nos	3.9 Nos
2	Size variation	2.6± 0.5%	≤ 2.8%

S.No	Parameters	A (Control)	B (Probiotic)
1	Water colour	Transparent	Grayish
2	Animal colour	Slightly Pigmented	Pigmented
3	Appendages	Huge attachment	Very mild attachment

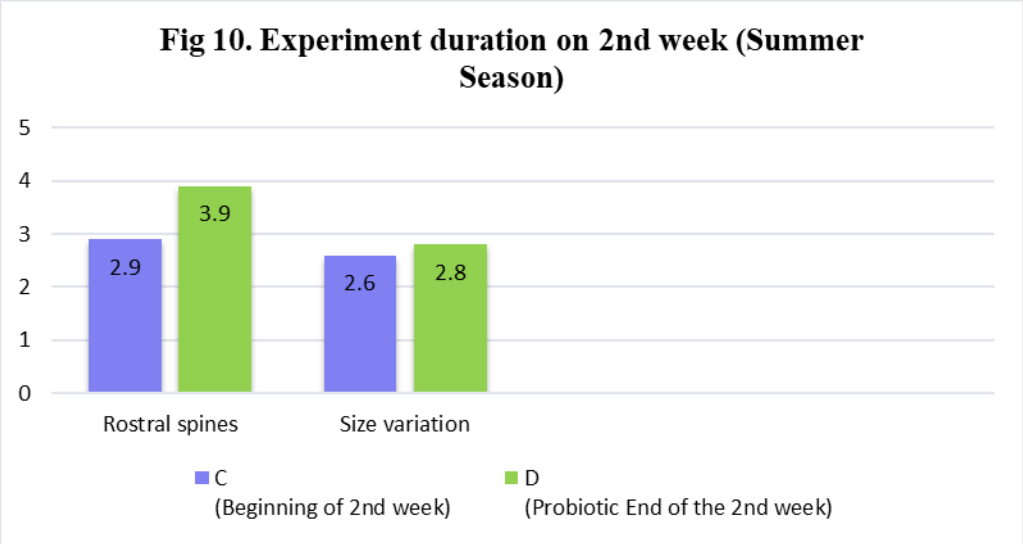


Table 11. Experiment duration on 2nd week (Winter Season).

S.No	Parameters	A (Control)	B (Probiotic End of the 1st week)
1	Water Salinity	33±1.0 ppt	33±1.0 ppt
2	Water Tem	31±1° C	31±1° C
3	Water pH	8.1±1	8.3±1
4	Alkalinity	139.0mg/L	139.0mg/L
5	Hardness	4853 mg/L	4853 mg/L
6	Total Ammonia	0.24 ppm	0.07 ppm
7	Water colour	Transparent	Grayish
8	Total Length	6.7±1 mm	7.5±1 mm

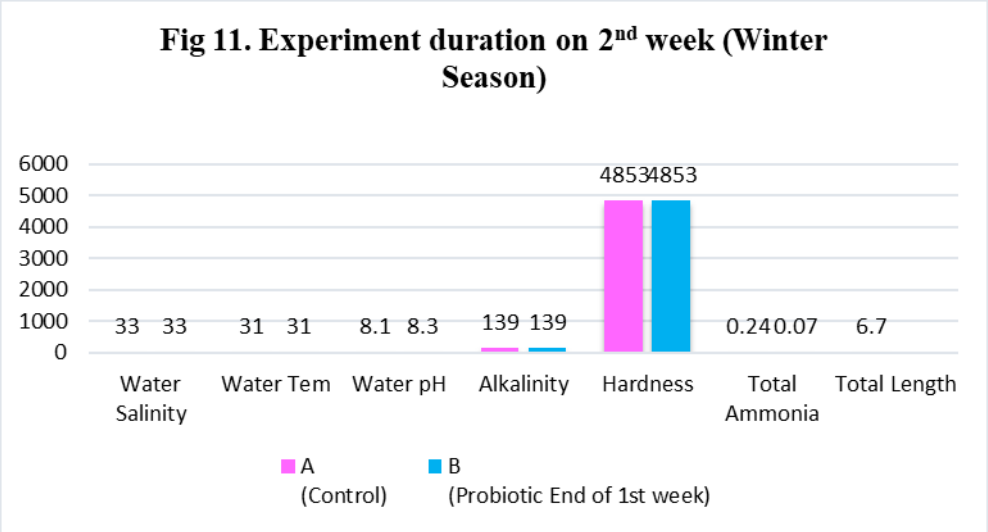
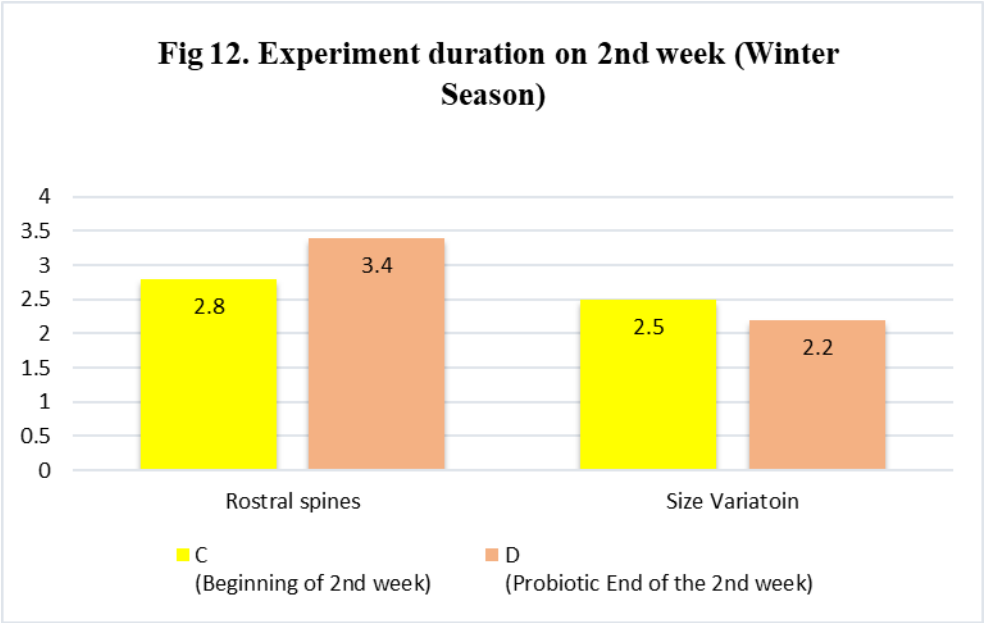


Table 12. Experiment duration on 2nd week (Winter Season).

S.No	Parameters	C (Beginning of 2nd week)	D (Probiotic End of the 2nd week)
1	Rostral spines	2.8 Nos	3.4 Nos
2	Size variation	2.5± 0.5%	2.7%

S. No	Parameters	A (Control)	B (Probiotic)
1	Water colour	Transparent	Grayish
2	Animal colour	Slightly Pigmented	Pigmented
3	Appendages	Huge attachment	Very mild attachment



CONCLUSION

Usually making up a larger portion of biomass than bacteria, fungi are crucial parts of the ecosystem and are thought to be the largest biotic community after insects (Sarbhoy *et al*., 1996). Fungi are essential to biogeochemical cycles, industry, agriculture, and medicine (Cowan, 2001; Gates *et al.*, 2005). According to Molina *et al*., (1993), Keizer (1998), Pilz and Molina (2001), Hunt (1999), Manoharachary *et al*., (2005), and others, fungi are also utilised in the textile business, bioremediation, biotechnology, fish industry, and bio-fertilizers. With increasing demands for eco-friendly aquaculture practices, the application of probiotics to prevent diseases in prawn farming is gaining popularity, although many extensive studies are still required (Dawicki S, 2012). Probiotics enhance the health of shrimp by competing against pathogens for colonization, producing metabolites that inhibit pathogen growth and consequently boosting the shrimp's resistance to diseases (David JW, 1999). The optimal definition of probiotics is "a live microbial feed additive that positively influences the host animal by enhancing its intestinal microbial balance," as stated by (Fuller R, 1987). As noted by (Wang et al., 2005), these effective probiotic bacteria should be utilized in aquaculture to safely handle prawn diseases.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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