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Research Note

Enhanced Disease Resistance in Striped Catfish (*Pangasianodon hypophthalmus*) Fed Jamun (*Syzygium cumini*) Leaf Meal Following *Aeromonas hydrophila* Challenge

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This study evaluated the effects of dietary supplementation with *Syzygium cumini* leaf meal on the disease resistance of *Pangasianodon hypophthalmus* fingerlings against *Aeromonas hydrophila* infection. Fish were fed five experimental diets containing graded levels of *S. cumini* leaf meal (Control: 0%, T1: 0.5%, T2: 1.0%, T3: 1.5%, and T4: 2.0%) for 60 days, followed by an intraperitoneal challenge with *A. hydrophila* and were monitored for 14 days. The T_2 group exhibited the lowest cumulative mortality (13.33%), resulting in the highest post-challenge survival. These findings indicate that dietary supplementation with 1% *S. cumini* leaf meal effectively enhances disease resistance in *P. hypophthalmus*, highlighting its potential as a natural phytobiotic for sustainable aquaculture.

Keywords: Phytobiotic, *Syzygium cumini*, *Pangasianodon hypophthalmus, Aeromonas hydrophila,* Cumulative mortality

Diseases are among the major constraints in freshwater aquaculture and are a limiting factor for economic development in many parts of the world, including India. Bacterial diseases are very common and constitute one of the most challenging health problems in freshwater fish culture. *Aeromonas* species are natural inhabitants of aquatic environments and cause diseases in aquatic animals. Antibiotics are most commonly used in aquaculture to control diseases as therapeutic, prophylactic, or metaphylactic agents. Despite their widespread use, antibiotics pose several challenges, including short-term protection, high costs, and the emergence of antibiotic-resistant bacterial strains (Reverter, Bontemps, Lecchini, Banaigs, & Sasal, 2014). Beneficial products like probiotics, prebiotics, feed enzymes, organic acids, phytobiotics, and plant-based immunostimulants have been explored as alternatives to antibiotics (Bondad Reantaso, Arthur, & Subasinghe, 2012). Among these, phytobiotics are promising alternatives for sustainable aquaculture as they are biodegradable, environmentally friendly, often readily available, and generally have minimal or no side effects on humans (Pudota et al., 2025). *S. cumini* is known to possess various pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antibacterial, anti-HIV, antileishmanial, antifungal, nitric oxide-scavenging, free radical-scavenging, anti-diarrheal, antifertility, anorexigenic, gastroprotective, anti-ulcerogenic, and radioprotective activities (Sagrawat, Mann, & Kharya, 2006). Its leaves are known for potential health benefits, particularly due to their rich content of bioactive compounds such as alkaloids, anthocyanins, acylated flavanol glycosides, quercetin, myricetin, myricitrin, triterpenoids, tannins, galloyl carboxylase, ellagic acid, and kaempferol (Ayyanar & Subash-Babu, 2012). Among the active compounds, alkaloids, glycosides, and polyphenolic substances such as gallic acid and ellagic acid are known for their potent antibacterial properties (Pandey & Singh, 2011). Several previous studies investigated the effects of *S. cumini* administration

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on the growth performance and immunomodulatory responses in fish (Prabu et al., 2018; Prabu, Ebeneezar, Chandrasekar, Kavitha, & Vijayagopal, 2021; Giri et al., 2023). Previous studies have evaluated the effect of phytobiotics such as *Cistus laurifolius* leaf (Bilen, Ali, Amhamed, & Almabrok, 2021) and *Andrographis paniculata* leaf extract (Maiti et al., 2023), demonstrating improved protection against *A. hydrophila* challenge in different fish species. However, no study has yet reported the protective effect of *S. cumini* leaf meal in *P. hypophthalmus* fingerlings challenged with *A. hydrophila*. Therefore, the present study evaluated the disease resistance of *P. hypophthalmus* following a 60-day feeding regimen with jamun (*S. cumini*) leaf meal and subsequent challenge with *A. hydrophila*.

A 60-day experimental feeding trial was conducted to evaluate the effect of dietary supplementation with *S. cumini* leaf meal powder on the disease resistance of *P. hypophthalmus* against *Aeromonas* infection. The experimental design was a completely randomised design with five treatments, with three replicates each. Six hundred healthy fingerlings (8.4 ± 0.45 g) were procured and acclimatised for two weeks under laboratory conditions at the College of Fishery Science, Muthukur, in a HDPE reservoir tank of 5000 L capacity. The experiment was conducted in 15 collapsible tarpaulin tanks (R.S Polymers, New Delhi) measuring 0.8 m x 0.7 m (height x diameter) with a water-holding capacity of 270 L. A total of 10 fishes were stocked in each tank for the challenge study. The water quality parameters were maintained at optimum levels *viz*., temperature (25-28 °C); pH (7.0-7.2); DO (5.7- 6.2 ppm); total alkalinity (112-118 ppm CaCOƒ); total hardness (173-182 ppm CaCOƒ); TAN (0.01-0.12 ppm); NO_2-N: (0.00-0.024 ppm); and NO_3-N (0.01 - 0.26 ppm). Fresh tender leaves of *S. cumini* were collected, washed, and dried in the shade to reduce moisture content to around 10%. The leaves were then ground and sieved (150 μm) to fine powder to make jamun leaf meal. The trial-and-error method was used for formulating the feed. The experimental feeds were prepared by mixing different feed ingredients such as fish meal, groundnut oil cake (GNOC), de-oiled rice bran (DORB), soya bean meal, wheat flour, fish oil, and vitamins-mineral mixture (Agrimin, India) with jamun leaf meal supplemented at 0.5% (T_1), 1.0% (T_2), 1.5% (T_3), and 2.0% (T_4), and control (0%). All the diets were isocaloric (15 MJ/kg) and isonitrogenous (30 % crude protein). The dry feed pellets were stored in airtight containers and labelled according to the treatments. The proximate composition of experimental diets was as follows: crude protein content (30%), crude fibre (6%), moisture (8%), crude fat (2%), total ash (11%), and nitrogen-free extract (43%).

The median lethal dose (LD_{50}) of *A. hydrophila* in *P. hypophthalmus* fingerlings was determined to be 2.3 × 10^7 CFU/mL using the method of Reed and Muench (1938). At the end of the feeding trial, the fish were challenged with the pathogenic strain of *A. hydrophila* (ATCC 35654). The bacterial concentration was adjusted using a spectrophotometer (T60, LABINDA) to an optical density (OD_{600}) of 0.5. Serial dilutions (10^{-2} to 10^{-7}) of the OD-adjusted bacterial culture were prepared, and bacterial enumeration was performed by the spread plate method on TSA plates for all dilutions. The plates were incubated at 30 °C for 24 h, after which colonies were counted to determine the injection dosage as CFU/mL. The fish were anesthetised with MS-222 (150 mg/L) and injected intraperitoneally with 0.1 mL of bacterial suspension containing the LD_{50} dose (H" 10w CFU/mL). Fish injected with 0.1 mL sterile phosphate-buffered saline (PBS) solution served as the control. During the challenge study, the fish were fed the control diet twice daily (8:00 am and 5:00 pm) (Maiti et al., 2023). Fish mortality was assessed daily up to 14 days to evaluate disease resistance. The causative organism was confirmed by re-isolating and identifying it from the liver of diseased fish, thereby fulfilling Koch's postulates. Relative percent survival (RPS) (%) was calculated using the following formula (Yilmaz, Çelik, Ergün, Ahmadifar, & Abdel-Latif, 2023):

RRPS = [1 - (Mortality in the jamun leaf supplemented group / Mortality in the control group)] × 100

Phytochemical profiling of *S. cumini* leaf meal was performed using Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS) (Orbitrap Exploris 120, Thermo Fisher, USA) (Jaiju et al., 2025). The data obtained in the present study were subjected to one-way analysis of variance (ANOVA) using SPSS version 23. Post-hoc analysis was carried out with Duncan's multiple range test, and the means were compared at a significance level of $p < 0.05$.

After challenge with *A. hydrophila*, the uninfected control fish exhibited no signs of disease, whereas the infected fish showed characteristic clinical signs of infection. The clinical signs observed in the challenged fish are presented in Fig. 1 (a-f).

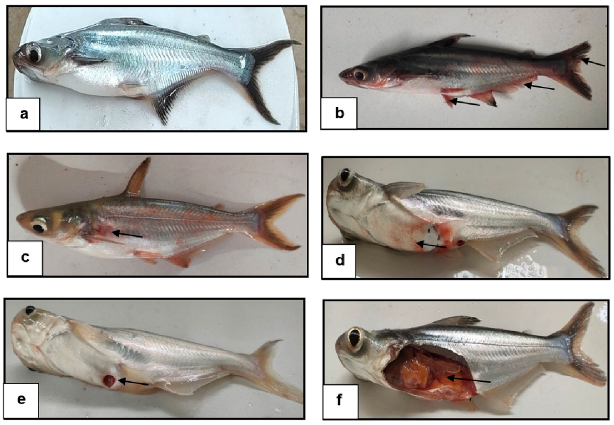

Fig. 1. External signs observed in *P. hypophthalmus* challenged with *A. hydrophila***.** a) Healthy fish from the unchallenged control group; b) Haemorrhages in the fins and tail rot in the tail fin; c) Paint brush haemorrhages on the body; d) Exophthalmia in the eye and haemorrhages on the abdomen; e) Ulcer formation on the abdomen; f) Fluid accumulation in the visceral cavity.

The cumulative mortality in *P. hypophthalmus* over the 14-day post-challenge period differed significantly ($p < 0.05$) among the five challenged treatment groups. No mortality was observed in the unchallenged control group, confirming that injection stress alone did not cause any mortality. In the challenged control group, mortality began on day 1 with 13.30% and increased significantly to 66.67% by day 7, remaining stable thereafter. In the T_1 group, mortality began on day 1 (6.67%) and peaked by day 7 (43.33 %), with no further mortality thereafter. The T_2 group (1% *S. cumini* leaf meal) exhibited the lowest mortality, which began on day 3 (6.67%) and reached 13.33% by day 4, after which no additional mortality was observed. In the T_3 group, mortality started on day 1 (3.33%), increased to 20.00% by day 5, and remained stable until day 14. Similarly, the T_4 group exhibited an initial mortality of 3.33% on day 1, which increased to 26.67% by day 5, with no further mortality thereafter. Overall, most mortalities occurred between days 4 and 7 post-challenge with *A. hydrophila.* Fish fed jamun leaf meal-supplemented diets showed significantly reduced mortality compared to the challenged control group. Among the treatments, the T_2 group exhibited the lowest cumulative mortality, indicating the highest level of protection against *A. hydrophila* infection (Fig. 2).

The highest RPS was recorded in the 1% *S. cumini* leaf meal-fed group (86%), followed by 1.5% fed group (80%), 2% fed group (73%), and 0.5% fed group (56%), whereas the challenged control diet fed group recorded the lowest RPS (33%). LC-HRMS analysis of *S. cumini* leaf meal identified major bioactive compounds including flavonoids, phenolic acids, terpenoids, phenolic compounds, triterpenoids, phenolic glucosides, and flavonoid glycosides.

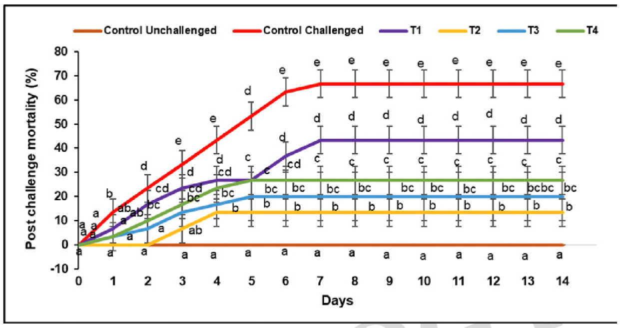

Fig. 2. Day-wise post-challenge mortality recorded in *P. hypophthalmus* challenged with *A. hydrophila* fed on *S. cumini* leaf meal supplemented diets

In the present study, the challenged control fish exhibited more severe gross pathological signs, including haemorrhages on the body, dorsal fin erosion, tail rot, exophthalmia, ulcer formation, and fluid accumulation in the visceral cavity during the first 96 h post-challenge with *A. hydrophila*, compared to the treatment groups. The gross pathological changes observed in this study are consistent with those reported by Lavanya, Neeraja, Ramana, Balasubramanian, and Lakkoju (2025). Bacterial challenge studies are crucial for evaluating the effectiveness of phytobiotics in conferring protection against pathogen attack (Pudota et al., 2025). Following the 60-day feeding trial, *P. hypophthalmus* fingerlings fed *S. cumini* leaf meal-supplemented diets exhibited significantly lower ($p < 0.05$) post-challenge mortality than the challenged control group 96 h after intraperitoneal injection with *A. hydrophila.* Among the challenged groups, the post-challenge mortality was significantly ($p < 0.05$) lower in all *S. cumini* leaf meal-supplemented treatments, with T_2 (13.33%) showing the lowest mortality, followed by T_3 (20%), T_4 (26.67%) and T_1 (43.33%), whereas the control group recorded the highest mortality (66.67%).

The reduced mortality in the treatment groups, especially in T_2, can be attributed to the antibacterial properties of bioactive compounds (flavonoids, phenolic compounds, terpenoids, and phenolic acids) present in *S. cumini* leaves. The bioactive substances in *S. cumini* are known to enhance antibacterial responses and promote general health in fish (Ahmadifar et al., 2021). Flavonoids and terpenoids have been reported to act as effective antibacterial agents against various bacterial infections (Huang et al., 2022). Additionally, phenolic compounds such as gallic acid and ellagic acid act as potent antibacterial agents by disrupting bacterial metabolic processes, inducing coagulation of cytoplasmic components, and causing bacterial cell leakage (Pandey & Singh, 2011). The beneficial effects of dietary supplementation with *S. cumini* leaf meal on disease resistance observed in the present study are consistent with earlier reports (Prabu et al., 2021; Giri et al., 2023). Although dietary supplementation with S. cumini leaf meal improved survival, higher concentrations of leaf meal in diets did not confer greater survival than the 1% inclusion level. Higher levels of jamun leaf powder can increase metabolic demands, resulting in unfavourable effects in fish (Giri et al., 2023). Furthermore, the presence of anti-nutritional factors, such as tannins and polyphenols, in jamun leaves can adversely affect fish health when incorporated at higher dietary levels (Kannan, Felix, Panigrahi, & Ahilan, 2022). Comparable findings have been reported by Prabu et al. (2021) and Giri et al. (2023), who also observed that higher levels of phytobiotic incorporation are not effective in increasing survival in fish following infection. Overall, the findings indicate that mortality decreased from T_1 (0.5%) to T_2 (1%), but increased again in T_3 (1.5%) and T_4 (2.0%), which suggests a non-linear dose-response, as reported in previous studies. In this study, major bioactive substances with specific roles in fish health were found in *S. cumini* leaf meal. The presence of flavonoids, phenolic compounds, and terpenoids in *S. cumini* prevented bacterial growth by interfering with protein synthesis, interrupting vital enzyme functions, and rupturing cell walls. It has been observed that phenolic compounds such as gallic acid and ellagic acid have bactericidal effects that can cause membrane leakage, coagulate intracellular contents, and interfere with bacterial metabolic activities (Pandey & Singh, 2011). These benefits may be attributed to the synergistic effects of various bioactive compounds, amino acids, minerals, and vitamins, which are known for their antimicrobial properties (Pudota et al., 2025).

The present study demonstrated that incorporating 1% *S. cumini* leaf meal into the diet of *P. hypophthalmus* significantly lowered cumulative mortality following *A. hydrophila* challenge in an *in vivo* model under controlled laboratory conditions. However, further *in vitro* assays and on-farm trials are warranted to validate its antibacterial potential and assess its practical applicability and efficacy under field conditions.

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