



Influence of Chitosan enriched Zinc Nanoparticles for the growth performance, feeding and metabolic process of aquatic animals-A Nanotechnology Approach

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Abstract

Multifunctional nanoparticles (NPs) production from biomaterials is a sustainable process and an eco-friendly method, and this technique has a variety of uses. This study intends to address the synthesis of Mud shrimp mud shrimp (*Austinopecten edulis*) chitosan-zinc oxide nanocomposite (MS-Ch-ZnONPs) and its biological functions. The zinc oxide nanoparticles (ZnONPs) were successfully synthesized using mud shrimp and coated with the chitosan (Ch) polymer through a cost-effective approach. Instruments such as UV-Vis, XRD, FTIR, DLS, Zeta potential, FE-SEM with EDX and high-resolution TEM were applied to characterize the biosynthesized MS-Ch-ZnONPs. Chitosan, a natural polymer derived from chitin found in the exoskeleton of crustaceans, has gained attention for its potential benefits in various applications, including aquaculture. Chitin is a constituent of different exoskeletons of marine arthropods such as crustaceans (crab, shrimp, lobster, krill, crayfish, barnacles), cuttlefish, and squid pen. Chitin is made up of a linear chain of acetylglucosamine groups. Chitosan extraction from mud shrimp (*Austinopecten edulis*) involves three consecutive stages: demineralization, deproteinization, and deacetylation. In the present study, we performed Growth Performance of Ornamental fish, *Poecilia sphenops* fed Indigenous feed ingredients formulated with various Levels of mud shrimp Chitosan nanoparticles for 60-Days. Feeding trials have been conducted to estimate food intake, growth studies and food utilization measures. Studies have undertaken to estimate digestive enzymes such as protease, amylase and lipase activities in the midgut regions *Poecilia sphenops* after fed with the mud shrimp chitosan zinc nanoparticles. We have also evaluated stress enzymes (SOD, CAT and LPO) levels in the experimental fish that were adopted with different food regimes (with and without chitosan). In general, a chitosan-enriched diet could potentially promote the growth and food intake of ornamental fish: Moreover, Chitosan is rich in nitrogen and can potentially serve as a source of dietary protein for fish. Its digestibility and nutrient profile can contribute to improved growth rates in ornamental fish species. The mud shrimp chitosan Zinc Nanoparticles enriched diet fed insects performed better growth, than the without chitosan enriched diet. The body weight gain (WG), initial weight gain, final weight gain, specific growth rate (SGR) were greater. The feed conversion ratio (FCR) among fish fed with different levels of dietary chitosan was also enhanced than the control. The activities of digestive enzyme profiles showed to have higher concentrations in the chitosan nanoparticles treatment than the control. This may be due to the fact that Chitosan has been shown to enhance the absorption of nutrients such as amino acids and minerals in the digestive tract of fish. This could lead to better utilization of the feed and ultimately promote growth. By potentially modulating oxidative stress, immune responses, and overall stress resilience, chitosan may contribute to improving the welfare and health of ornamental fish in aquaculture settings. The changing profiles of SOD, CAT and

LPO have been evaluated with mud shrimp enriched chitosan zinc nanoparticles. In the present study the levels of SOD decreased when fed on without a chitosan diet. Chitosan is known to possess antioxidant activity, which can scavenge free radicals and reduce oxidative stress in cells. This may lead to a decrease in the activity levels of oxidative stress markers such as catalase and SOD, as the antioxidant capacity of the fish is enhanced. The decrease in the activities of CAT in the body of *Poecilia sphenops*, may be due to the Ornamental fish requiring a balanced diet to maintain health and antioxidant defenses. We have also proved chitosan enriched diets have an influential role in boosting the predatory behavior of *Poecilia sphenops* when fed on nanoparticle contaminated environments. The chitosan enriched diet reared *Poecilia sphenops* consumed a higher number of *Aedes aegypti* mosquito larvae at the aquarium tank. Total predation of mosquito fish against first, second, third and fourth instars *Aedes aegypti* without nanoparticle treatment was 150.6, 131.0, 102.6 and 80.1, respectively. At the nanoparticle exposed situation, the predatory efficiency was increased and total predation of mosquito fish was 183.2, 150.6, 126.2 and 126.2, respectively. Chitosan is a biopolymer derived from chitin, which is found in the exoskeletons of crustaceans like shrimp and crabs. It has various applications due to its biocompatibility, biodegradability, and non-toxic nature. It is also suggested that chitosan can enhance the olfactory responses of mosquito fish to cues from mosquito larvae. This increased attraction to larvae could lead to more effective hunting and feeding by the fish. The Mud shrimp mud shrimp (*Austinopecten edulis*) chitosan-zinc oxide nanocomposite (MS-Ch-ZnONPs) tested against the dengue mosquito as larvicidal properties and also for the water purification properties. After characterization of nanoparticles and the antibacterial efficacy of these nanoparticles against both Gram-positive and Gram-negative human pathogenic pathogens was evaluated. Finally, cytotoxicity effects of MS-Ch-ZnONPs were done and after 24 h incubation, the concentration dependent inhibition of MS-Ch-ZnONPs against A549 lung cancer cells and MCF-7 breast cancer cells were observed in cytotoxicity assay. It revealed that mud shrimp mediated MS-Ch-ZnONPs was an excellent anti-cancer agent against A549 and MCF-7 cancer cells. Mosquitoes propagate quite successfully in a variety of aquatic habitats such as drinking water systems, manmade water bodies, and sewage contaminated aquatic systems. Since water is essential for all life, without it, life won't progress and at the same time, the quality of the water is also important. With the unique structural features present in chitosan, bio-polymer helps to bind effectively with fine suspended particles, pollutants, bacteria, heavy metals etc. The biocompatible and biodegradable nature of chitosan makes it a potential candidate for mosquito breeding water purification purposes. In this regard, chitosan has been extracted from freshwater crab shells mud shrimp. It has been utilized as a potent tool in control of young instars of *Aedes aegypti* and its breeding water purification. After nanoparticle administration, the cell migration and wound healing, the "in vitro scratch assay" method was performed. HMEC-1 cells were seeded in a 24-well plate with 10⁵ cells per well and incubated for 24 h. Endothelial cell migrations were assessed at 6, 12 and 24 hours after in vitro wound induction. The results of the scratch test proved that the nanoparticles could cause the cell migration and the percentage of wound closure increased with respect to time and the maximum wound closure observed was 85% after 24 hours. Antibacterial bioassay was also conducted and this evaluation typically involves conducting antibacterial assays or tests to determine the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) against various bacterial strains. Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) are commonly used as model pathogens in such studies due to their relevance to human health.

Keywords: *Austinopecten edulis*; chitosan; nanotechnology; digestive enzymes; stress physiology; predation; *Poecilia sphenops*; larvicidal; anti-bacterial; wound healing; anti-cancer.