Nanotechnology: An insight into silver nanoparticles in aquaculture disease management

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Abstract
In recent decades, the occurrence of diseases has been one of the major constraints in the projected development of the aquaculture industry across the globe. Despite advancements in aquaculture techniques, a massive loss occurs annually because of infectious diseases. New technological approaches have been paved to effectively deal with such challenges to avoid the harmful impact of chemotherapeutics, used for ages to control diseases in the aquaculture system. Nanotechnology is such an emerging technology acting as a novel, and the innovative tool has tremendous potential in the fisheries sector in promoting sustainable aquaculture production through better fish health management. The use of nanoparticles (NPs) due to their broad-spectrum mechanism of action and high efficiency has attracted researchers to control a range of infectious agents of fish and shellfish like bacteria, viruses, fungi and parasites. The nanoparticles target several structural components, metabolites, replication mechanisms, and reproduction; hence, they could be a promising candidate in the potential control and management of diseases in the aquaculture system. This chapter presents a brief idea of traditional methods of disease management. Also, it sheds light on silver nanoparticles and their possible mechanism of action on fish pathogens, particularly emphasizing the role of AgNPs as potent antiparasitic agents. The chapter may contribute to enhancing the management of parasitic diseases, thereby promoting sustainable aquaculture production and strengthening the global economy.

Keywords: Aquaculture; Nanotechnology; silver nanoparticles; antimicrobial action

I. Introduction

With the advancement in culture practices and the unresponsible approach towards the aquatic environment, the aquaculture sector is suffering from severe disease outbreaks, resulting in significant losses globally (Mishra et al., 2017; Sahoo et al., 2020; Behringer et al., 2020). Over the years, an array of biotechnological approaches has shown promising responses to improve the health of cultured organisms and to enhance the production while safeguarding the aquatic environment. Such biotechnological interventions include pathogen-free best management practices by avoiding the possible use of chemical remedial agents and applying biocontrol approaches against pathogens of diverse origins. However, disease outbreaks significantly threaten aquaculture production and trade (Mishra et al., 2020). In the latest review on fish disease management in India by Mishra et al. (2017), it is reported that parasitic infestation is most common in freshwater aquaculture, with an estimate of 45%, followed by bacteria and viral disease, which counts together 30%. Therefore, to mitigate this issue, there is an urgent need for novel technologies to control parasitic diseases potentially.

Nanotechnology is an emerging technology that has gained attention among the scientific community to address the challenges involved in the increase of aquaculture production, mitigation of environmental degradation, disease control, and food safety (Huang et al., 2015; Marquez et al., 2018; Mishra et al., 2019). In aquaculture, a wide variety of metal nanoparticles are being used which are effective against bacteria, viruses, fungi (Paul et al., 2016; Meza et al., 2019; Alkie et al., 2019; Truong et al., 2020) and parasites (Daniel et al., 2016; Saleh et al., 2017). Among the metallic NPs, applied silver

NPs are most commonly used due to their unique physical, chemical, and biological properties (Pinto et al., 2009). However, there is very few documented literatures on the effectiveness of NPs for the treatment of parasitic diseases (Acosta et al., 2019) compared to the extensive research on its application against bacterial and viral infections in aquaculture (Marquez et al., 2018; Shaalan et al., 2020). Therefore, the present article mainly emphasizes the various silver nanoparticles, mode of action, and prospects for controlling infectious pathogens, especially the fish parasites in the aquaculture system.

II. Management of diseases in aquaculture

To manage the infectious diseases of fish and shellfish, a wide range of chemicals, including antibiotics, antiparasitic drugs, antifungal and antivirals; herbal medicines; biological control measures like use of probiotics, cleaner fishes, biosecurity measures at farms and use of vaccines as a disease preventive method in the aquaculture is practiced as a measure of better health management presented in the (fig. 1) (Mishra et al., 2017; Bchmann, 2022; Dinesh et al., 2023). However, the harmful effects on the host and the environment, the development of resistance against many of the antibiotics in bacteria, and the limited stability of phytotherapeutic-based drugs are demanding some technology-based intervention in the effective management of diseases in the aquaculture system to mitigate the significant losses to the aqua farmers globally (Kumar & Kumari, 2022; Jeyavani et al., 2023).

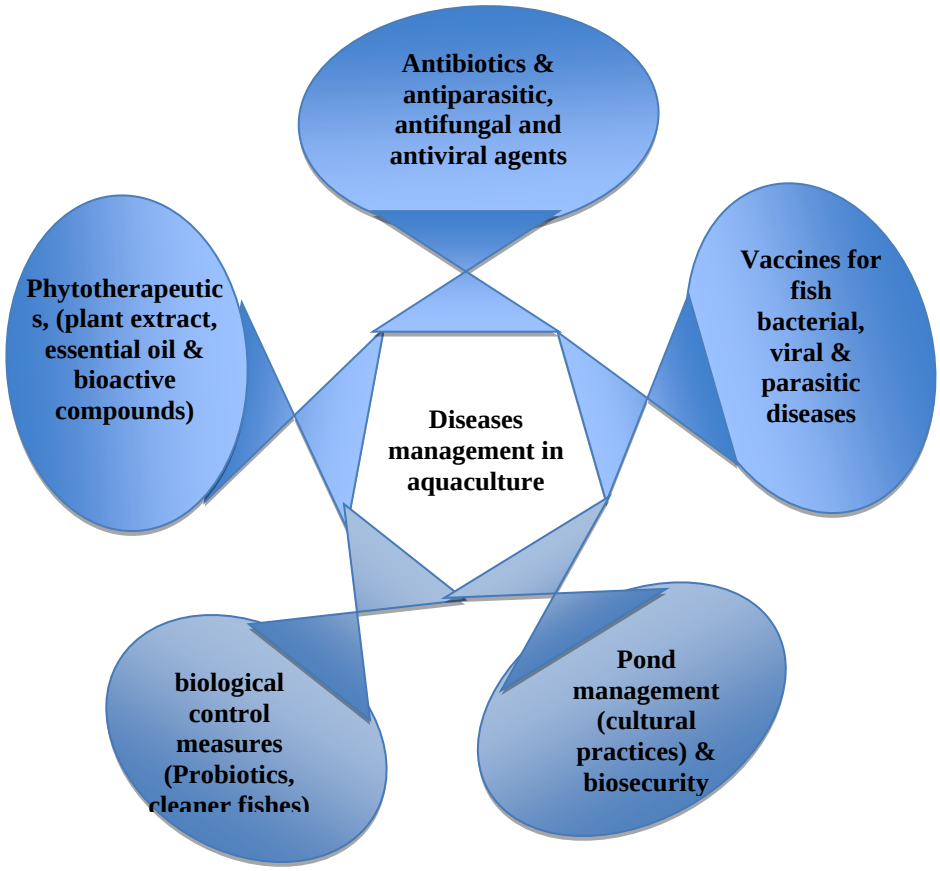

Figure. 1. Showing various methods of disease management in aquaculture system

III. Nanotechnology and nanoparticles

For sustainable development, the aquaculture industry must adopt innovative technologies to overcome the challenges of disease outbreaks. Nanotechnology is a novel approach that has attracted widespread interest among the scientific community in the aquaculture system (Marquez et al., 2018). In nanotechnology, the word nano indicates one billionth (10-9) of a meter, and nanoparticles (NPs) are a group of molecules in the range of 1-100 nm dimension (Masciangioli and Zang, 2003). Nanoparticles (NPs) exhibit large surface area to volume ratio and characteristic morphology, which imparts them significantly new or improved properties compared to their bulk form (Willems, 2005). This technology offers the ability to engineer the properties of materials by controlling their sizes, which has driven

research toward many potential uses for nanomaterials (Verma & Florence, 2012). Nanoparticle (NPs) synthesis through physical and chemical methods is associated with many drawbacks like high energy consumption, altered surface chemistry, and generation of toxic by-products (Raliya & Tarafdar, 2014). On the other hand, the biological systems (eco-friendly "nano-factories") offer an environmentally safe green synthesis of NPs (Rai et al., 2016).

The most frequently used biological agents for NPs synthesis are plants, algae, fungi, yeast, bacteria, actinomycetes, and viruses (Saratale et al., 2018). The synthesis of metal and metal oxide NPs by plants and phytochemicals is advantageous due to its easy availability, economic feasibility, high scalability, production of stable materials, non-toxic, environmental friendliness, and higher efficiency (Benelli, 2016). Among several metal NPs types, AgNPs have exhibited potent and broad-spectrum antimicrobial properties established by previous study trials (Marquez et al., 2019). However, exploring biosynthesized metal NPs for treating fish diseases in aquaculture farms needs to be more extensive and creates new research opportunities for researchers.

IV. Role of nanotechnology in aquaculture

Nanotechnology has extensive applications in the aquaculture industry in the field of water treatment, pond sterilization, nano-food particles for fishes, enhancement of fish growth, targeted drug delivery, to develop devices for aquatic environment management, clinical disease diagnosis and therapeutics development (Marquez et al., 2018; Udo et al., 2018). Several classes of nanoparticles, such as metal and polymeric NPs have shown effective antibacterial activity against various fish pathogens (Meza et al., 2019). Many studies have revealed the potential antimicrobial property of metal-based nanoparticles against a broad range of fish and shellfish pathogens like bacteria (Paul et al., 2016; Vijayakumar et al., 2017), viruses (Alkie et al., 2019) and parasites (Saleh et al., 2017). Further, prophylactic agents' development as vaccines and immunostimulant diets to prevent fish diseases are also reported (Tawwab et al., 2019). Nanoparticle-mediated drug delivery aims for enhanced delivery or uptake by target cells, reducing the toxic effects of free drugs to non-target organs (Benelli, 2018). The nano-encapsulated vaccines against *Listonella anguillarum* in Asian carp (Rajeshkumar et al., 2009), white spot syndrome virus (WSSV), and infectious myonecrosis virus (IMNV) (Chalamcheria, 2015) have been developed very successfully. Nanotechnology also presents an excellent opportunity to develop fast, accurate, and cost-effective diagnostic techniques to detect particular molecular targets in biodiagnostic applications and detect pathogenic infectious agents in aquaculture (Kaittanis et al., 2010; Mishra et al., 2020). In the last two decades, nanoscience and nanotechnology have seen a plethora of new developments in almost every field of science and technology, especially in biology and medicine (Mishra et al., 2020) due to their characteristic properties. Further, NPs bypass the development of multi-resistance in the microbes due to their multiple modes of actions, which is an added advantage (Prakash et al., 2013). Among an array of metal-based NPs, silver NPs are being studied extensively due to their broad & excellent mechanism of action as an antimicrobial agent. The application of nanotechnology in aquaculture is still in its infancy stage. It needs to broaden the current research to explore the prospects of this technology in fish health management.

V. Antimicrobial mechanism of action of silver nanoparticles

A. Antibacterial activity

Among the various engineered nanoparticles used in antibacterial treatments, AgNP is the most widely explored due to its robust antimicrobial properties (Das et al., 2020). Silver NPs have shown very effective results against numerous fish pathogenic bacteria like *Aeromona hydrophila* and *Pseudomonas aeruginosa* (Adawy et al., 2021), *A. salmonicida* (Shaalan et al., 2018), *Aeromonas sp.* and *Vibrio sp.* (Van et al., 2019), *Pseudomonas flourescens, E. tarda*, *Flavobacterium* spp., and multiple drug-resistant isolates of *Staphylococcus aureus*, *Y. ruckeri*, etc (Shaalan et al., 2018). Silver NPs have also been explored to control many of the potential bacteria of penaeid shrimps (*Litopenaeus vannamei*) including *Vibrio parahaemolyticus, V. harveyi, V. cholarae,* (Sivaramasamy et al., 2016; Cirerol et al., 2019) and *Rickettsia* like bacteria (Acedo-Valdez et al., 2017) very effectively. The antimicrobial properties of silver nanoparticles are derived from the chemical nature of Ag+ ion as follows: (1) Ag+ ions strongly binds with peptidoglycan of the bacterial cell walls and inhibit the transport of oxygen into the cell to paralyze the bacteria, (2) Silver is known to react with phosphorous and sulfur the main components of the cell membrane, proteins, and DNA and thus can accumulate on the cell wall and membrane causing the shrinkage of the cytoplasm, membrane detachment, pore formation, and finally disrupted membrane and bacterial cell death (3) Intracellularly, Ag+ binds to nucleic acids and

cytochrome, and disable the power of phosphate thus preventing the DNA replication process and cell division, (4) AgNPs with size 1–100 nm and high surface energy are capable of slowly releasing Ag+ ions into the solution; therefore, silver nanoparticles have prolonged antimicrobial efficacy (Huang et al., 2011; Shaalan et al., 2018; Tang and Zheng, 2018). One advantage of silver nanoparticles over conventional antibiotics is that their antimicrobial action arises through interference with multiple cellular processes of the bacteria, so the emergence of resistance is less likely. The mechanism of action on various bacterial pathogens of fish is presented in Table (1. a.).

B. Antiviral activity

Very little work is explicitly published on antiviral effects of silver nanoparticles in fish and shrimps (Table 1. b). Only a handful of information is available using AgNPs to treat white spot disease caused by WSSV in penaeid shrimps (*Littopineus vannamei*), which enhances the survival of the treated group compared to the control shrimp. AgNPs interact with viral glycoproteins, hamper viral attachment and penetration to host cells. Inhibition of viral proliferation could be mediated by the blocking of nucleic acid synthesis (DNA and RNA) through the binding of AgNPs to the genetic material of the virus (Sun et al., 2008; Speshock et al., 2010; Aravena et al., 2015; Meza et al., 2019; Romo-Quionez et al., 2020).

C. Antifungal activity

Silver nanoparticles as an antifungal agent exhibited high inhibitory effects similar to Amphotericin B (commercial antifungal) against *candida sp., Dermatophytes* and *Aphanomyces invadans* reported by (Mallmann et al. 2015; Shaalan et al., 2018). Further, the colloidal AgNPs hold excellent fungicidal activity against *Saprolegnia* sp., mainly infecting fish eggs and larvae. Silver NPs induce mitochondrial dysfunctional apoptosis through increased oxidative stress via ROS generation, reduce membrane fluidity, and damage cell walls and membranes **(**Johari et al., 2015; Radhakrishnan et al., 2018) presented in (Table 1. c).

D. Antiparasitic activity of AgNPs against fish parasites

Only a handful of studies has been conducted on application of NPs especially silver nanoparticles to combat parasitism in aquaculture. Nanoparticles have been reported to be highly effective with probable antiparasitic mechanism of action against Ich (protozoan parasites), monogeneans, copepoda parasite, *Lernaea* and tetrahymena parasites of infecting fish species by Acosta et al. (2019), Saleh et al. (2017), Elala et al., 2018 (presented in Table. 1. d). It has been found that the Silver NPs get adsorbed on the exoskeleton of aquatic invertebrates, and since the crustacean cuticle is a constituent of crystalline chitin, sugars, and silk-like proteins, which are attached through specific H-bonds and globular proteins, which confer a net negative surface charge (Vincent, 2002). This negatively charged cuticle develops an affinity for the cationic NPs, which anchor onto the microbial cell wall, penetrate it, form pits and affect osmotic stability, resulting in the subsequent leaking of cellular constituents (Prabhu and Poulose, 2012). It has been observed that silver ions (Ag+) directly bind with the thiol, sulfur of protein and phosphorous of nucleic acid, decreasing the membrane permeability and resulting in enzyme denaturation and cell death (Duran et al., 2017; Benelli, 2018). Further, AgNPs stimulate the ecdysone receptor gene and reproductive hormones, resulting in reproductive failure and impaired developmental stages (Nair and Choi, 2011). Silver NPs were also found to reduce copper-dependent enzymes and acetylcholinesterase of the crustacean parasites, resulting in cuticular demelanization and nervous imbalances (Fouad et al., 2018). Although the AgNPs exhibit a broad-spectrum mechanism of action against parasites and insects (Benelli, 2018) and *Argulus* is a crustacean parasite that has similar biology and physiology as that of insects and various treatment measures have been applied but limited success (Kumari et al., 2020). Phytotherapeutics is very effective against *Argulus* parasite (Kumari et al., 2019); however, high dose, lack of purity and standard protocols limits its applications. The consequences of global warming have directly affected the enhanced incidences of infection with arguluosis, a serious matter of concern (Brahmchari et al., 2023). This broad mechanism of action of AgNPs enables the current researchers to utilize it to combat the detrimental parasites in the aquaculture system. It widens the scope of using AgNPs through feed or dip/bath treatment, which would be a potential therapeutic agent, especially against fish parasites in the aquaculture system, in the forthcoming years.

VI. Challenges associated with Nanoparticles application in Aquaculture

Though various advancements in the nanotechnology applications for sustainable aquaculture and fisheries development have been moving toward (Shah and Mraz, 2020), it is only partially free of challenges. Several studies have investigated the toxicity of NPs on fish and other aquatic organisms (Rivas-Aravena et al., 2015; Khosravi-Katuli et al., 2017). Paul et al. (2016) proposed that smaller the size of AgNPs better the antibacterial activity with an improved survival rate of *Labeo rohita*. The toxicity of AgNPs in the fish increases with size as the LD50 for larger Ag-NPs (103 nm) was 40.164 mg/kg and 53.630 mg/kg, and the LD50 for small Ag-NPs (27nm) was 68.631 mg/kg and 80.439 mg/kg respectively via injection and feed (Paul et al., 2016). The earlier studies also showed that AgNPs are toxic to fish at higher concentrations (1000 mg kg-1), and no mortality was observed in the lowest levels, such as 25 and 50 mg kg-1 with LC50 of 100 mg kg^{-1} (Rajkumar et al., 2016). Further, Fuentes-Valencia et al. (2020) found that when Pike silverside was exposed to UTSA AgNPs at 31.8 and 95.4 ng/L for 96 h, it showed no histopathological damage or gut microbial changes. On a note, there is some evidence that very high concentrations of nanomaterials are acutely toxic to fish embryos in the laboratory, e.g., Zebrafish embryos (Zhu et al., 2009). Moreno et al. (2017) revealed an enhanced survival rate (80%) of WSSV-infected shrimps (*L. vannamei*) after Ag-NP administration with minimal stress in comparison to untreated organisms with only 10% survival in 96 h after infection. These studies demonstrate that the toxicity of nanoparticles depends on the exposure duration, concentration, size, age, and fish species.

VII. Conclusion and future directions

Nanotechnology undoubtedly presents a significant opportunity for aquaculture to achieve best fish health management against diseases. However, limited studies are available on the nanoparticle dose, application route and mechanism of action against infectious pathogens, including bacteria, viruses & fungus. Further, detailed information on nanoparticles potentiality in treating fish parasitic diseases is explained. Additionally, the article sheds light on the broader mechanism of action and nobleness of silver nanoparticles in treating the most hazardous crustacean parasite *Argulus* causing disease argulosis in the aquaculture system. Hence, the present review will provide an extensive outlook on the multidimensional application of nanoparticles in disease management; however, future studies could be warranted in identifying the significant impact of nanoparticles on the host and environmental health.

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Table 1. Nanoparticles (NPs) efficacy against fish and shrimp pathogens with its possible mechanism of action

Bacterial species	NPs, size, Concentration, contact time & route (s) of administration	Mechanism of action	Outcomes	References
a. NPs as antibacterial				
Aeromonas hydrophila & *E. ictaluri*	AgNPs/chitosan/diatomite suspension, 6.5 nm, 2.6 mgL^{-1}, at 24 h exposure	Increases cell permeability and disturbing respiration, also causes DNA damage and cell membrane breakdown	Potentially inhibited the growth of bacteria to 100% and enhanced the survivability of *Pangasianodon hypophthalmus*	Truong et al., 2020
A. hydrophila *A. salmonicida*	AgNPs, 44.5 nm, 100 µg/mL immersion (3h), IP injection 17 µg/mL	Bactericidal agent through disruption of the bacterial cell membrane integrity and functionality	Potent antibacterial without any residual effect in fish *Oncorhynchus mykiss*	Shaalan et al., 2018
Pseudomonas aeruginosa	AgNPs, 31-35 nm, 10 mg/l for 14 days, immersion	Ag+ ion bind with –ve charge of bacterial cell wall, rupture cell membrane causes bacterial death	Restrict the bacterial proliferation in treated fish *Labeo rohita*	Kanwal et al., 2019
Vibrio fluvialis	AgNPs, 60 to 170.8 nm,	Bacteriostatic effects	88% survival of infected fish angelfish was obtained	Marquez et al., 2019
Vibrio parahaemolyticus (AHPND)	*Ulva clathrata* synthesized AgNPs, 9.5 nm, 10000 ppm, for 7 days through feeds	Ag^{+} penetrate bacterial cells by binding with metal-binding proteins present in the hepatopancreas of shrimps	Potential management of Vp AHPND+ without exerting toxic effect on the host *Litopenaeus vannamei*	Muniz et al., 2020
V.salmonicida, E. tarda	AgNPs embedded chitosan, 25 - 45 µm, 25 to 150 µg/mL	Ag^{+} directly by interfering with cell permeability, triggers inactivation of respiratory enzymes, interrupt DNA replication, and cause bacteriolysis.	MIC: 75 µg/mL for both *V. salmonicida*. MBC: 75, 125 and 75 µg/mL for *V. salmonicida, V. tapetis and E. tarda*	Dananjaya et al., 2017
Aeromonas hydrophila	polysaccharide coated AuNPs, 10–100 nm,100 µg mL^{-1}	Disturbs ATP synthesis, destroy ribosome subunit for tRNA binding, collapse biological action, hinder exopolysaccharide production.	Reduced mortality of fish from 90 % to 30% of treated fish *O. mossambicus*	Vijayakumar et al., 2017
Aliviibrio salmonicida	AgNPs with chitosan, 281 nm, MIC, 50 µg mL^{-1} and MCB, 100 µg mL-1	Enhances bacterial cell membrane permeability, generate oxidative stress, extensive DNA degradation, proteins, cause bacterial cell death	CAgNPs is potential antibacterial agent to control fish pathogenic bacteria.	Dananjaya et al., 2017
A. hydrophila	AgNPs with *Azadirachta indica*, 35.4 nm, (50 µL/300L) immersion for 20 days	Penetration of Ag^{+} through the cell wall affects DNA and protein synthesis in bacteria	Ag-NPs treated *Cirrhinus mrigala* showed immunomodulatory and antibacterial effects with relative percentage survival of	Rather et al., 2017

			74%.	
Vibrio harveyi	*Camellia sinensis* synthesized AgNPs, 10 µg through feed	Ag-NPs bind with sulfur-rich proteins of membrane surfaces of bacteria, also binds with DNA and inhibit cell cycle	Inhibited bacterial growth by 70% compared to control group of *Feneropenaeus indicus*.	Vaseeharan et al., 2010
E. tarda	Ag-NPs, 27 nm, @ 0.2 mg/L, injection for 21 days	Smaller size NPs interact with bacteria more efficiently and produce electronic effects which enhances the bacteriostatic and bactericidal reactivity	Complete inhibition of bacterial growth & high host survival percentage	Paul et al., 2016
A. hydrophila, E. tarda, Vibrio spp, S. aureus,	NPs of CuO (93 nm), ZnO (122.4 nm), Ag-NPs (58.7 nm) @ 0-5 mg mL^{-1}	Inhibits synthesis of functional biomolecules or alter cellular activities to kill pathogens	Showed excellent antimicrobial activity in tested strains, acts as substitute of antibiotics	Swain et al., 2014
Vibrio angularium	Squilla chitosan Ag-NPs, 22.73–39.77 nm, 0.5 mg ml^{-1} via encapsulation	Ag^{+} binding with the sulfur-rich proteins of membrane surfaces, and inhibit metabolic pathways	Ceased bacterial growth with enhanced survival (72.5-75 %) of treated fish group	Barakat et al., 2016
A. Hydrophila,	*Carica papaya* synthesized Ag-NPs, 25–40 nm @153.6 µg mL^{-1} (well diffusion method)	Ag+ reduces phosphate uptake in and drives the efflux of accumulated phosphate and crumple the proton motive force thereby triggering microbial cell death.	*Carica papaya* Ag-NPs showed significantly high antimicrobial activity	Mahanty et. al., 2013
Aeromonas veronii	Ag-NPs (*Oreochromis niloticus*) At 750 µg/L using bath treatment	Inactivation of cellular proteins, formation ROS which inhibit respiratory enzymes and proteins leading to physiological malfunctioning responsible for morality of *A.veronii*.	Ag-NPs shows the significant antibacterial activity against fish pathogen.	Elgendy et al., 2022
b. NPs as Antiviral				
Lymphocystis disease virus	PLGA NPs + DNA vaccine, <500 nm, 0.5 ml with 30µg DNA vaccine, oral administration	Maintain the integrity and efficacy of vaccine and allows its slow and effective release at the site	Effective control of LVDV in Japanese flounder	Tian and Yu, 2011
MrNV- XSV	chitosan NPs, 297 nm, oral (100 ng μL^{-1} of each plasmid DNA was mixed	NPs stabilizes DNA by protecting it from enzyme degradation, facilitates sustained release & better	The prawns treated with chitosan-conjugated XSVAS gave better	Ramya et al., 2014

	with feed at 100 µg per gram	expression	protection than control group.	
White spot syndrome virus (WSSV)	Ag-NPs (Argovit®), 12 ng/mL through injection	Ag-NPs induce 2-fold increase of LGBP expression (key gene of immunological response).	Improved survival of 80% by inducing shrimp *(Penaeus vannamei*) immune system without any toxic effects on	Meza et al., 2019
Infectious salmon anaemia virus (ISAV)	Chitosan NPs (40 nm), 7 mg of DNA & $1x10^5$ $TCID_{50}$ of virine/fish, orally for 7 days	NPs stimulated the expression of immune molecules,	Protection up to 77% of fish against ISAV in vaccinated group of fish with NP-V & NP-Ad together	Aravena et al., 2015
Viral hemorrhagic septicemia virus	Poly (I:C) + cationic phytoglycogen NPs, 70 nm, 31.25–1000 ng/mL	More effective inducer of IFN-related antiviral immune responses compared to HMW poly (I:C) alone induced significantly higher Mx1 expression	Nano-HMW effectively reduced virus replication in RTgutGC cells to a great extent than to poly(I:C) alone	Alkie et al., 2019
Infectious haematopoietic necrosis virus	DNA vaccine with PLGA coumarin-6 NPs, 300 to 1000 nm, 24 mg oral administration	PLGA NPs stimulate expression of DNA vaccine be by cells and also induce immune gene expressions at mild rate.	Results in slight increase in survivability of vaccinated fish (*Oncorhynchus mykiss)* with NPs	Adomako et al., 2012
c. NPs as Antifungal				
Penicillium and Mucor species.	ZnO-NPs (122.4 nm), 5.0 mg/ml under 7 days incubation	Suppression of extracellular enzymes and metabolites	Effective as an antifungal agent	Swain et al., 2014
Fusarium oxysporum	Ag-NPs embedded chitosan, 25 - 45 µm, 25 to 100 µg/mL) 10 days inoculation	damages its mycelial structure and its growth	Inhibited the growth of *F. oxysporum* compared to the control	Dananjaya et al., 2017
Aphanomyces invadans	Ag-NPs with chitosan, 10ng/ 20ml water, for 20 seconds bath for 3 days	Disruption of fungal cellular structure and impair with its functions leads killing of fungi.	Complete cure from red spot disease & disappeared symptoms of infection with long term protection from reinfection.	Daniel et al., 2016

d. NPs as antiparasitic agent				
Parasitic sp.	**Fish species**	**NPs Concentration, size, route of administration**	**Mechanism of action**	**References**
Schistosoma japonicum	*Oncomelania hupensis*	Ag-NPs, 125 μg mL^{-1} (30 nm, Bath)	Tail-shedding of cercaria or cercariocidal	Cheng et al., 2013.
Microsporidian	*Heterosporis saurida*	Au-NPs, 1.0 μg mL^{-1}, 11-14 nm (7 days) Bath	Antimicrosporidial	Saleh et al., 2016
Ichthyophthirius multifiliis (Ich)	*C. auratus*	Starch Ag-NPs, 10 ng/g of body weight, 10-20 nm, Dip for 3 days	Tissue damage	Daniel et al.,2016
I. multifiliis	*Oncoryhnchus mykiss*	Ag-NPs,10 ng mL^{-1}, 21 nm (2 h)	Disruption of *I. multifiliis* membranes	Saleh et al., 2017
Lernaea cyprinacea (Anchor worm)	*Carassius auratus*	Chitosan based Ag-NPs, 5.5 mg L^{-1}, 217 nm, Bath (1 h)	Anchor degeneration, distorted mouth, shrunken carapace	Elala et al., 2018
Monogenean (*Cichlidogyrus* sp.)	*Oreochromis mossambicus*	Ag-NPs, 36 μg L^{-1}, 1-3 nm, Bath (1 h)	Swelling, loss of corrugations, & disrupted parasite's tegument.	Acosta et al., 2019
Tetrahymena (Ciliates)	(*Chirostoma estor*) Pike	Ag-NPs, 3300 ng L^{-1},15-35 nm, Immersion (15 min)	Perforated cell surfaces, ruptured cell membrane & cilia	Valencia et al., 2020