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Review Article

Innovative Applications of Mycogenic Metal Oxide Nanoparticles for Eco-friendly and Sustainable Aquaculture Practices: A Review

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ABSTRACT

Nanotechnology has emerged as a transformative field across various industries, with agriculture being a key area of impact. Metal oxide nanoparticles (MONPs) are particularly valuable due to their versatile applications in enhancing agricultural productivity and sustainability. However, the traditional chemical synthesis of MONPs has raised environmental concerns, prompting researchers to seek alternative methods. As a result, green synthesis of MONPs using eco-friendly methods and biological materials has become a popular and sustainable alternative for industrial and agricultural applications. Fungi are highly versatile microorganisms that have the ability to produce

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a diverse range of secondary metabolites that can be utilised as reducing agents for the production of MONPs. Despite this potential, there is a lack of comprehensive review articles that specifically examine the role of fungi from different genera as nanofactories for MONPs, along with their biological capabilities in promoting sustainable aquaculture practices. Therefore, this review seeks to bridge this gap by providing a comprehensive overview of fungal species capable of synthesising MONPs. In addition, this review highlights the recent advancements in mycogenic MONPs, with a focus on their potential applications, particularly in aquaculture.

Keywords: Aquatic health management, aquaculture innovation, eco-aquaculture solutions, environmental sustainability, nanotechnology in aquaculture

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INTRODUCTION

Agriculture is vital to the economy of both developed and developing nations, and rising global populations will significantly increase food demand in the coming decade. However, climate change and global warming are already impacting the efficiency and quality of food production worldwide (Food & Agriculture Organisation, 2019). Moreover, global warming drives shifts in disease epidemiology by altering ecosystems, increasing vulnerability, and enhancing pathogen exposure (Ghazali et al., 2018). A sustainable food system must reduce climate impact, protect ecosystems, restore biodiversity, and ensure a safe, nutritious food production.

Nanotechnology offers innovative solutions in agriculture and environmental management through nanovaccines and nanosupplements for enhanced disease control. It involves manipulating materials at the nanoscale (1–100 nm), where increased surface area enhances applications in biosensors, nanomedicine, and bionanotechnology. Metal oxide nanoparticles (MONPs) offer high stability, easy synthesis, tunable properties, and resistance to swelling. Their charge allows integration into diverse systems and functionalisation for various applications (Nikolovo & Chavali, 2020).

MONPs can be synthesised through green, chemical, and physical methods, with the agricultural sector favouring the eco-friendly approach. The capacity of different biological systems to produce metallic nanoparticles varies significantly. This variation arises from differences in enzymatic activity and intrinsic metabolic processes, leading to the fact that not all living organisms are capable of synthesising nanoparticles (Ojha, 2022). Nonetheless, fungi have emerged as the preferred organisms for nanoparticle production.

Compared to bacteria, fungi are more advantageous in nanoparticle biosynthesis due to their rich repertoire of bioactive metabolites, superior aggregation abilities, and higher overall efficiency (Ghosh et al., 2021). Numerous studies have explored mycogenic MONP synthesis, but their application in aquaculture remains limited despite showing significant potential for system improvement. They are particularly effective in controlling bacterial diseases, mitigating environmental pollutants, and functioning as vaccine carriers or immunostimulants (Danaraj et al., 2022).

By integrating MONPs into aquaculture practices, reliance on antibiotics can be minimised, thereby contributing to sustainable aquaculture and addressing the critical issue of antimicrobial resistance. This review highlights the diversity of fungi in MONP production, their current application in aquaculture, and discusses challenges and future directions, including scalability and commercialisation, environmental and safety considerations, and future research areas.

BIOGENIC METALLIC OXIDE NANOPARTICLES (MONPS)

MONPs are usually produced by hydrolysing metal salts at room temperature or below 100°C. Their remarkable physical and chemical properties stem from their small size and the high density of corner and edge surface sites. In contrast, biosynthesis involves the generation of compounds from simple precursors by living organisms, their metabolites, or biomolecules. This method is recognised as the most efficient, biocompatible, and safe approach for nanoparticle synthesis, making it the preferred choice for many applications (Jadoun et al., 2021).

Both chemical and biological processes use reducing agents for synthesis, but green chemistry approaches are gaining popularity to minimise harmful by-products. Biogenic MONP synthesis employs microbes, plants, or animal-derived substances to generate nanoscale materials via biomolecule production in a bottom-up approach (Figure 1). This widely accepted method enables atoms to self-assemble into nuclei, forming nano-sized particles. Conversely, the microbial synthesis of MONPs is regarded as an eco-friendly

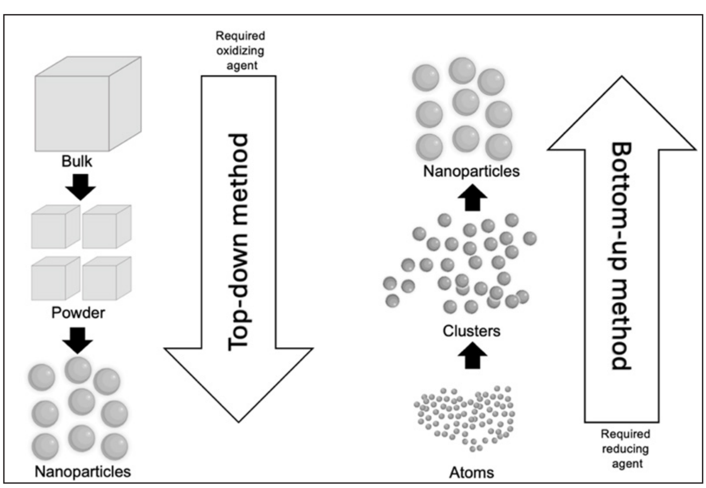

Figure 1. The synthesis of metallic oxide nanoparticles involves both top-down and bottom-up approaches

approach, as it typically takes place in aqueous media, under ambient temperature and pressure conditions, and near-neutral pH levels.

Various parameters, such as pH, pressure, temperature, and solvents, influence MONP synthesis using biogenic precursors. For instance, Wijesinghe et al. (2021) used *Tephrosia purpure*a extract for ZnO nanoparticle synthesis, highlighting pH's crucial role in optimization. A key advantage of biologically synthesised MONPs is the natural protein layer on their surfaces, which enhances suspension stability and prevents agglomeration (Durán & Seabra, 2012).

FUNGI AS NANOFACTORIES FOR MONPS

Traditional MONP synthesis relies on hazardous, costly methods with high energy demands and environmental risks. In contrast, green synthesis is sustainable, reducing waste, pollution, and toxicity while using renewable materials. Microbial-mediated green synthesis offers a sustainable alternative, utilising algae, bacteria, and fungi to produce MONPs via extracellular and intracellular mechanisms (Figure 2).

Fungi are promising sources of bioactive compounds, with ascomycetes and other species producing about 6,400 such molecules (Guilger-Casagrande & Lima, 2019). Their diverse intracellular enzymes, surface proteins, and reducing agents make them efficient biological nanoparticle synthesisers (Narayanan & Sakthivel, 2011). Furthermore, fungi uniquely tolerate and accumulate heavy metals, potentially enhancing nanoparticle production from bulk materials (Guilger-Casagrande & Lima, 2019).

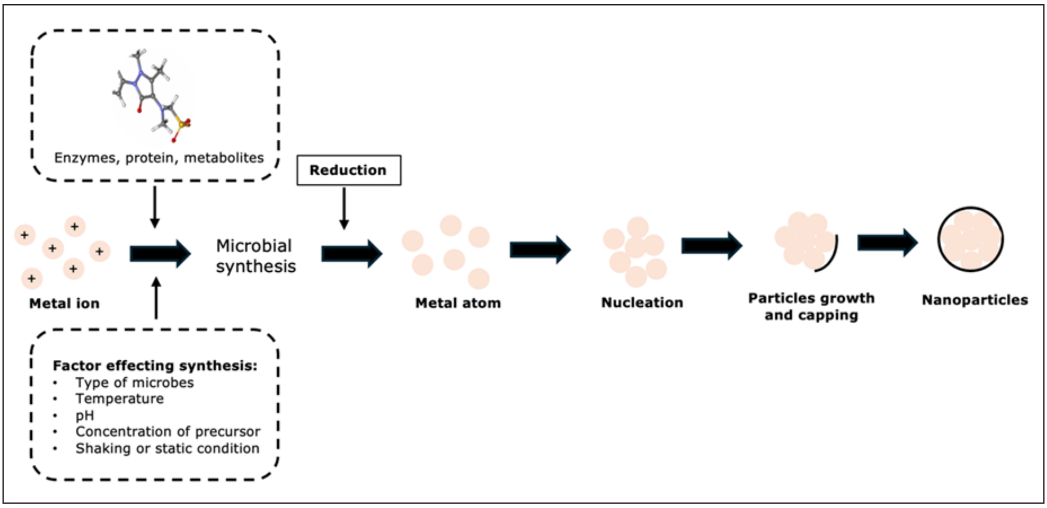

Figure 2. Microbial-mediated synthesis of nanoparticles involves the capture of metals, their enzymatic reduction, and capping, all facilitated by biomolecules such as proteins, enzymes, and metabolites from microbial extracts, which play a crucial role in maintaining nanoparticle stability
Adapted with Modification from Ghosh et al. (2021)

Selecting fungi for nanoparticle biosynthesis offers several notable advantages. As previously stated, fungi demonstrate exceptional tolerance to high concentrations of metal ions and exhibit highly efficient bioaccumulation capabilities, making them ideal candidates for this process. Fungi produce significantly more nanoparticles than bacteria due to their enhanced secretion of extracellular proteins, which aid nanoparticle formation. Additionally, fungal-derived nanoparticles typically exhibit low toxicity, making them ideal for various applications (Karunakaran et al., 2023).

Various genera of fungi, including certain metropolitan and rare fungal species, have been reported to possess the ability to synthesise MONPs. Metropolitan fungi such as *Trichoderma*, *Penicillium*, *Fusarium* and *Aspergillus* have been extensively explored as nanofactories for biosynthesis of MONPs. Beyond these well-known groups, other genera with similar potential are highlighted in the section below:

Trichoderma *Spp*

Trichoderma is a genus within the Fungi Kingdom, specifically classified under the Ascomycota Division, Pezizomycotina Subdivision, Sordariomycetes Class, Hypocreales Order, and Hypocreaceae Family. This genus comprises various species that are economically significant due to their capacity to produce industrial enzymes, antibiotics, and bioactive compounds (Consolo et al., 2020). To date, a total of 375 species of *Trichoderma* have been identified and confirmed worldwide (Cai & Druzhinina, 2021). These organisms are gaining prominence in nanotechnology, especially in the synthesis of MONPs.

Recent studies indicate that they can resist various nanocompounds; however, we still do not fully understand how this tolerance impacts their ability to synthesise MONPs (Ramírez-Valdespino & Orrantia-Borunda, 2021). The synthesis of nanoparticles driven by *Trichoderma* may involve enzymes such as reductases, which act as bioreductive agents in nanoparticle biofabrication. Previous research has shown that *Trichoderma* can accumulate metals through a mechanism involving reductase enzymes like NADPH-dependent nitrate reductase (Simões et al., 2020). This sequential process facilitates the reduction of metal salts, ultimately leading to the formation of nanoparticles.

Previous studies have highlighted the presence of phenolics, proteins, amino acids, aldehydes, ketones, and other functional groups on *Trichoderma*-mediated ZnO nanoparticles, which contribute to the reduction, capping, and stabilisation of the nanoparticles (Zaki et al., 2021). This indicates that compounds found in *Trichoderma* Spp can serve as both bioreducers and biostabilisers in the synthesis of MONPs. The resulting ZnO nanoparticles were found to be between 8 and 23 nm in size, exhibiting fungicidal activity against various soil pathogens.

Similarly, Singh et al. (2024) identified hydroxyl, carboxyl, flavonoid, amine, carbonyl sulfide, and carbonyl groups on ZnO nanoparticles mediated by *T. harzianum*. Their study demonstrated that *T. harzianum* can produce ZnO nanoparticles ranging from 617 to 195 nm, with an average size of 314 nm, which also display biological activity. Despite the beneficial compounds associated with *Trichoderma* Spp, the biogenic synthesis of MONPs using these fungi and the exploration of their biological properties remain relatively underexplored.

Penicillium Spp

The genus *Penicillium* is among the most widespread and commonly encountered groups of soil fungi in nature. It is classified into four subgenera: *Aspergilloides*, *Penicillium*, *Furcatum*, and *Biverticillium*. Recently, the first three subgenera have been reclassified under the *Penicillium* genus, while *Biverticillium* has been reassigned to the *Talaromyces* genus (Perrone and Susca, 2017). It encompasses species with significant applications across medicine, pharmaceuticals, agriculture, and biotechnology. Notably, the discovery of penicillin from *Penicillium* fungi revolutionised the treatment of bacterial infections, profoundly impacting human health.

Additionally, this genus includes diverse species that play vital roles in decomposing organic materials and are recognised for their ability to produce extracellular proteins, particularly enzymes (Otero et al., 2020). Beyond their medicinal importance, *Penicillium* species are also crucial in areas such as food spoilage, biotechnology, and plant pathology, with a current total of 483 accepted species (Houbraken et al., 2020).

Penicillium fungi play a crucial role in the green synthesis of MONPs. Zakariya et al. (2022) demonstrated iron oxide (FeO) nanoparticle production using a cell-free aqueous extract of *Penicillium* Spp, highlighting the role of proteins as reducing and capping agents. Fungal enzymes and compounds like phenolic and amine groups further aid in nanoparticle formation and stabilisation. Their study reported FeO nanoparticles (3.31–10.69 nm) with strong antibacterial activity. Similarly, Ali et al. (2021) found that *P. roqueforti* biosynthesises FeO nanoparticles (5–16 nm) with antibacterial and antifungal properties.

El-Batal et al. (2020) found that the culture filtrate of *Penicillium chrysogenum* can generate copper oxide (CuO) nanoparticles with an average diameter of 9.70 nm, exhibiting notable antifungal and antibacterial properties. Their study revealed the presence of N–H bonds, suggesting that this functional group plays a crucial role in the synthesis and stabilisation of CuO nanoparticles. Apart from that, Honary et al. (2012) demonstrated that *P. aurantiogriseum*, *P. citrinum*, and *P. waksmanii* are capable of synthesising CuO nanoparticles. This research highlights the ability of various *Penicillium* species to produce monodispersed particles and emphasises the role of proteins in fungal culture filtrates as bioreducing and biostabilising agents.

Fusarium Spp

Fusarium is a genus of filamentous fungi that includes many species which produce mycotoxins, are notorius plant pathogens in agriculture and making infections in humans from time to time. These fungi are ubiquitous and can exist in various habitat such as water, soil and plant-associated media (Hernández-Ochoa et al., 2020). The genus Fusarium, with approximately 70 species, shows great genetic and biological/ecological diversity that is reflected in the various production of secondary metabolites (Toghueo et al., 2020). Fusarium is one of the most exploited fungal genera for nanoparticle fabrication, utilised by many researchers.

Some species can produce a diverse array of metabolites, showcasing impressive chemical variety and notable bioactivities. For example, *F. oxysporum* is highly valuable in industry and biotechnology due to its wide range of enzymes. These enzymes have various applications, including the production of cutinases, nitrilases, glycoside hydrolases, fructosyl amino acid oxidase, laccases, lipoxygenase, nitric oxide reductase, decarboxylases, keratinase, phospholipase B, and triosephosphate isomerase (Ibrahim et al., 2021), which may potentially be employed to produce MONPs.

Previously, it was reported that a cell-free filtrate of *F. oxysporum* successfully synthesised vanadium oxide (V_2O_5) nanoparticles, with sizes ranging from 10 to 20 nm (Gholami-Shabani et al., 2021). The study revealed the presence of carbonyl groups from proteins and amino acids in the fungal cell-free filtrate, which acted as bioreducing and capping agents for the mycosynthesis of V_2O_5 nanoparticles. Interestingly, the V_2O_5 nanoparticles synthesised by *F. oxysporum* demonstrated significant biological activity, particularly antimicrobial activity.

Similarly, a study by Gupta and Chundawat (2020) demonstrated that *F. oxysporum* can produce ZnO nanoparticles ranging in size from 18 to 25 nm. This study also identified extracellular proteins in the ZnO nanoparticles, which likely facilitate the reduction of zinc ions. Additionally, phenols, including polyphenols such as terpenoids and flavonoids, were found to act as bioreducing agents in the synthesis process. These nanoparticles were also found to possess biocatalytic activity to produce bioethanol from rice straw.

Apart from that, *Fusarium* Spp has been shown to produce MONPs. Kavitha et al. (2020) synthesised ZrO_2 nanoparticles using *F. solani*, revealing hydroxyl and carbonyl groups on their surface. Similarly, Mohamed et al. (2019) synthesised ZnO nanoparticles using F. *keratoplasticum*, observing two shapes with sizes ranging from 10–42 nm and 8–38 nm. They suggested that proteins or fungal bio-filtrate extracts bind to ZnO nanoparticles via free amino or carboxyl groups, influencing nanoparticle shape.

Aspergillus Spp

The genus *Aspergillus* is one of the most prevalent fungal groups, comprising several hundred species (Samson et al., 2014). These saprophytic filamentous fungi are commonly found in soil or organic matter. They are highly adaptive and resilient to changing environmental conditions, capable of growing across a broad temperature range and at relatively low humidity levels. Most *Aspergillus* species produce a wide array of secondary metabolites and fermentative enzymes, establishing a significant connection with human health, daily life, and industrial production (Yang et al., 2022).

Among fungal sources, *Aspergillus* stands out as a particularly promising candidate for MONPs production. This is due to its over 350 species, which exhibit significant biochemical versatility and a high capacity for protein secretion (Rai et al., 2022). Additionally, the compounds produced by *Aspergillus* are classified as generally regarded as safe (GRAS), making them suitable for industrial use.

Previously, a study by Singh et al. (2020) demonstrated that *A. flavus* can biosynthesise ZnO nanoparticles with an average size of 90.05 nm, exhibiting notable antifungal activity against *Alternaria solani*. The research identified various compounds involved in the biosynthesis process, including hydroxide, alkene, alkane, nitric oxide, aliphatic chains, sulphonic acid, alkyl halides, and silicon oxycarbide. This highlights the versatility of *Aspergillus* Spp in nanoparticle biosynthesis, attributed to its rich and diverse metabolite profile.

Furthermore, El-Saadony et al. (2021) reported that *Aspergillus* species, particularly *A. niger*, can synthesise ZnO nanoparticles (~45 nm). Functional groups like alcohols, amines, amides, and phenols contributed to biosynthesis, while proteins in the fungal supernatant stabilised the nanoparticles. These ZnO-NPs also showed strong bactericidal activity against fish pathogens.

Apart from ZnO nanoparticles, *Aspergillus* species have also been reported to synthesise CuO nanoparticles with sizes below 100 nm (Mani et al., 2021). CuO nanoparticles synthesised using *A. terreus* are formed due to the presence of proteins and enzymes in the endophytic fungus, which transform copper nanoparticles into their oxide form, CuO. These biogenic CuO nanoparticles have also been reported to possess several biological activities, including antimicrobial activity.

Other Fungal Genera

Cordyceps Militaris

Medicinal fungi have long been valued in human civilisation, with *Cordyceps* being prominent in traditional Chinese medicine. *Cordyceps militaris*, from the Clavicipitaceae family, is known for its nutritional and medicinal benefits, particularly its bioactive metabolite, cordycepin, which has antibacterial, antitumor, antiviral, and antifungal properties (Choi et al., 2011).

The ability of *C. militaris* to produce MONPs can be seen through research by Dias et al. (2022). This study observes the ability of this fungal species to produce ZnO nanoparticles with an average particle size of 1.83 nm. The FTIR spectral analysis confirmed that various components of the aqueous mushroom extract of *C. militaris* played a role in capping and stabilising the nanoparticles. For example, proteins, alcohols, polyphenols, aromatic acid esters, phenolics, and β-glycosidic bonds of β-glucans were present.

Similarly, a study conducted by Li et al. (2019) synthesised zinc oxide nanoparticles via co-precipitation methods from *C. militaris*. This study revealed the ability of *C. militaris* to produce ZnO nanoparticles with a size of about 10.15 nm. A further characterisation revealed the presence of alcoholic OH groups, CH_2 asymmetric stretching, carbonyl group, carboxylic acid group, CN stretching vibration for phenol and amine group, and alkene group. Despite this, the capacity of *C. militaris* to produce MONPs beyond ZnO is notably limited.

Xylaria Spp

The genus *Xylaria* (Xylariaceae) is a widely distributed fungus in marine and terrestrial environments, known for producing diverse bioactive metabolites with antibacterial, antioxidant, and cytotoxic properties. (Chen et al., 2024). Additionally, this fungal genus has been reported to synthesise ZnO nanoparticles, with particle sizes ranging from 30 to 50 nm (Sumanth et al., 2020). Various compounds, such as phenolic OH groups, hydroxyl groups, polyphenols, keto-enol structures, alkenes, alkanes, and aromatic functional groups, have been implicated in the formation of these nanoparticles, which also demonstrate antimicrobial activity against pathogenic fungi and bacteria.

A study by Nehru et al. (2023) showed that *Xylaria arbuscula* synthesised ZnO nanoparticles (116 nm) with antioxidant, antimicrobial, antidiabetic, and anti-inflammatory properties. Characterisation of the biomolecules associated with the ZnO nanoparticles revealed the presence of compounds such as alcohols, polyphenols, aliphatic hydrocarbons, amides, and aromatic amines, which may serve as capping and reducing agents in the conversion of zinc ions to ZnO nanoparticles. This research highlights the potential of *Xylaria* species for the mycosynthesis of metal oxide nanoparticles (MONPs). However, literature on the synthesis of MONPs other than ZnO by *Xylaria* species remains limited.

Pleurotus Djamor

The genus *Pleurotus* includes several nutritionally and medicinally valuable species, though not all are commonly cultivated (Vega et al., 2022). *Pleurotus djamor* (Rumph.

ex Fr.) Boedijn, found in tropical and subtropical regions, thrives at temperatures up to 30°C and produces fruiting bodies within 1–2 weeks, making it ideal for tropical cultivation (Salmones & Mata, 2015). Studies show its crude extracts possess analgesic, anti-inflammatory, antipyretic, antimicrobial, and antiplatelet properties, along with strong free radical scavenging activity (Susem et al., 2013), highlighting its potential for MONP synthesis.

A study by Manimaran et al. (2021a) demonstrated that P. *djamor* aqueous extract synthesised ZnO nanoparticles (70–80 nm). FTIR analysis confirmed the presence of aromatic rings and carboxylic acid groups, facilitating nanoparticle formation. These mycosynthesised ZnO nanoparticles exhibited strong larvicidal, antibacterial, anticancer, and antioxidant properties, highlighting their potential for biomedical and environmental applications.

Similarly, Manimaran et al. (2021b) reported that *P. djamor* aqueous extract synthesised TiO_2 nanoparticles (31 nm). FTIR analysis identified functional groups like hydroxyl, amine, and carboxylic acid. These TiO_2 nanoparticles showed potent larvicidal, antibacterial, and anticancer activities, making them promising for pest control, antimicrobial therapies, and cancer treatment.

APPLICATIONS OF MYCOGENIC MONPs IN AQUACULTURE

Aquaculture is shifting from traditional to intensive farming, enabling higher stocking densities in limited water spaces. This shift significantly raises the risk of transmissible and infectious diseases (Santos & Ramos, 2018). The overuse and misuse of artificial feeds in aquaculture, driven by poor management and limited farmer training, often degrade the environment and increase disease prevalence (Hossain et al., 2022). Global aquatic products, encompassing aquatic plants, fish, crustaceans, molluscs, and other species such as bullfrogs and jellyfish, constitute the third largest source of food protein for humans, after cereals and milk, and account for 16.4% of the total animal protein supply (Du et al., 2022). Environmental contamination and disease prevalence pose major challenges in aquaculture, driving recent technological advancements to address these issues.

Mycogenic MONPs, acknowledged as an innovative and transformative method, offer a wide range of applications and significant potential in the field of aquaculture (Figure 3). It enables new methods for drug management and vaccine development, protecting fishery resources from pathogens. The rapid rise of nanotechnology presents promising solutions for advancing aquaculture. MONPs have gained interest in agriculture for their high surface area, stability, and tunable properties. However, despite their eco-friendly and biocompatible nature, mycogenic MONPs remain underexplored compared to other synthesis methods.

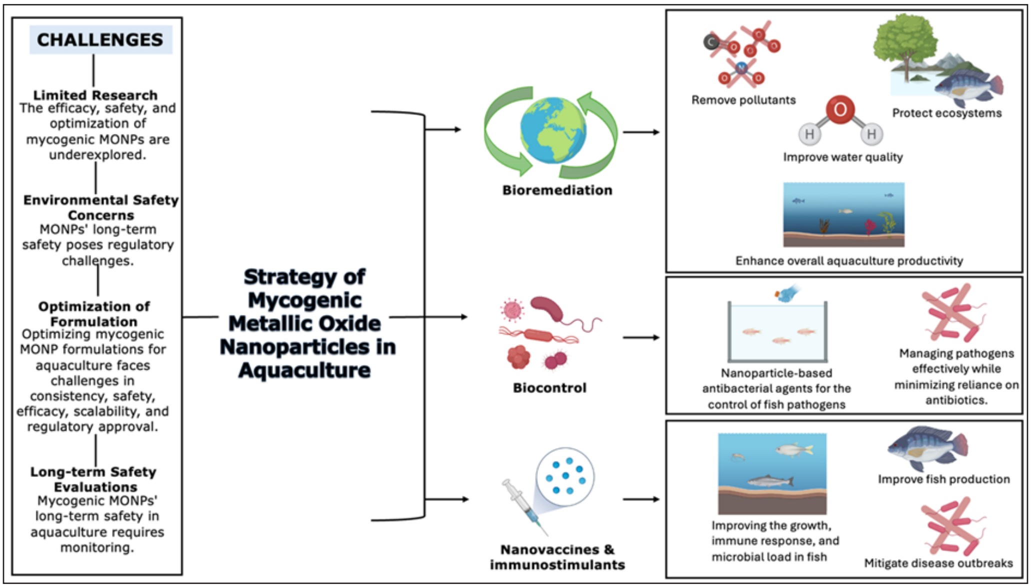

Figure 3. Strategy of mycogenic metallic oxide nanoparticles (MONPs) in aquaculture with applications in bioremediation, biocontrol, nanovaccines, immunostimulants, and associated challenges

Nanovaccines and Immunostimulants

The rise of infectious diseases in aquaculture demands advanced therapeutic strategies. MONPs offer superior biocompatibility, targeted delivery, and immune enhancement (Singh et al., 2021), making them ideal for nanovaccines and immunostimulants. Mycogenic MONPs stand out for their eco-friendly production, scalability, and unique biological functionalities. Their synthesis relies on enzymatic and metabolic secretions that control particle size, morphology, and function, ensuring suitability for aquaculture applications.

In this context, El-Saadony et al. (2021) found that mycogenic ZnO nanoparticles from *Aspergillus niger* improved *Oreochromis niloticus* performance, feed efficiency, and behaviour. Treated fish showed enhanced liver function, higher LYZ and NBT activity, lower cortisol, and increased testosterone and growth hormone levels, indicating better stress management and growth regulation.

Additionally, mycogenic ZnO nanoparticles exhibited strong antibacterial properties, achieving the lowest bacterial loads in water and fish tissues while effectively targeting key pathogens, including *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Aeromonas hydrophila*. These findings highlight their potential as a powerful therapeutic in aquaculture.

The integration of nanotechnology, particularly MONPs, is revolutionising therapeutic approaches in aquaculture. As nanovaccines and immunostimulants, MONPs enhance immune activation and provide sustained protection against infections. However, research on mycogenic MONPs in these roles remains scarce, highlighting the need for further studies. Future research should compare the efficacy of different mycogenic MONPs, optimise formulations, assess long-term safety, and expand applications across aquaculture systems to maximise their potential as sustainable disease control tools.

Biocontrol Potential

Aquaculture plays a vital role in global food security and economic sustainability. However, the industry is continually challenged by the prevalence of infectious diseases, which cause significant losses in fish populations. In recent years, MONPs have emerged as a promising tool in combating fish diseases due to their unique physicochemical properties, including antimicrobial activity, biocompatibility, and stability, offering innovative solutions to safeguard aquatic health.

Al-Shammari et al. (2022) demonstrated the efficacy of mycogenic ZnO nanoparticles synthesised using *Fusarium* sp. against *Saprolegnia parasitica*. Species from the Saprolegniaceae family, including *Achlya* and *Saprolegnia*, infect fish eggs in both aquaculture and natural environments, posing challenges during incubation. At 100 ppm, mycogenic ZnO nanoparticles achieved 79% inhibition of *Saprolegnia* Spp, comparable to malachite green. This highlights their potential for controlling saprolegniasis in fish hatcheries.

Moreover, the potential of mycogenic MONPs as antibacterial agents against aquatic pathogens is evident in a study by Hassan et al. (2021). Mycogenic magnesium oxide (MgO) nanoparticles synthesised using *Rhizopus oryzae* exhibited antibacterial properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which cause significant health issues in fish and fisheries products (Sivaraman et al., 2022; Shahrokhi et al., 2022). Their presence in fish products also poses a risk of foodborne illnesses, emphasising the need for nanoparticle-based pathogen control.

Similarly, a study by Flores-Rábago et al. (2023) demonstrated the efficacy of mycogenic CuO nanoparticles synthesised using *Ganoderma sessile* extract as antibacterial agents against *S. aureus* and *P. aeruginosa*. These mycogenic nanoparticles effectively inhibited both bacteria at low concentrations, with *P. aeruginosa* being the most susceptible. Despite the potential of mycogenic MONPs in aquaculture, research in this area, particularly for combating aquatic diseases, remains limited. Further studies are needed to explore their application in aquaculture systems, ensuring environmental safety, and evaluating their impact on aquatic organisms and overall productivity.

Bioremediation in Aquaculture

Water pollution occurs when unnatural substances enter water bodies, disrupting the natural cycle. Aquatic systems process waste, but excessive pollutants and overuse from agriculture, industry, and development reduce their capacity to assimilate waste (Malik et al., 2020). Fish species are crucial to human-related aquatic communities. Furthermore, Environmental pollution is rapidly increasing due to urbanisation, industrialisation, and population growth. The textile and dyeing industries are major contributors, releasing synthetic dyes and heavy metals that harm aquatic life, degrade water quality, and threaten human health.

Previously, MONPs have been reported to show a promising use in wastewater purification, removal of heavy metals and dyes, and microbial contaminants (Nizamuddin et al., 2019). Due to the growing demand for oxide nanoparticles and nanomaterials, research into their synthesis using fungi has been rapidly advancing in recent years (Loshchinina et al., 2023). A study by Kumar et al. (2024) reported the application of mycogenic FeO nanoparticles synthesised by *Myceliophthora thermophila* as a catalytic reducer of *p*-nitrophenol (PNP) into *p*-aminophenol, a toxic organic compound commonly found in industrial wastewater. Additionally, these nanoparticles demonstrated the ability to decolourise bromophenol blue and malachite green.

PNP is highly soluble in water, resistant to biodegradation, and threatens aquatic ecosystems by disrupting enzymatic processes, bioaccumulating in the food chain, and harming biodiversity. Malachite green, widely used in aquaculture, is highly toxic and poses long-term ecological and human health risks (He et al., 2023). Similarly, improper disposal of bromophenol blue degrades water quality and disrupts aquatic ecosystems (Saad et al., 2024). These findings highlight the need for effective waste management to reduce environmental impact.

A study by Darwesh et al. (2023) reported the ability of CuO nanoparticles synthesised by *Fusarium oxysporum* OSF18 as a microbial, heavy metals, and textile dye remover from industrial wastewater. The mycosynthesised CuO nanoparticles embedded in alginate beads demonstrated exceptional performance, achieving microbial disinfection efficiency of 99.995%, removal of heavy metals (93% for Pb, 55% for Cr, and 30% for Ni), and dye decolourisation efficiency of 90%. These findings mark a significant advancement toward developing an eco-friendly, cost-effective, and user-friendly solution for the bioremediation of wastewater from the textile industry, ultimately contributing to the preservation of aquatic ecosystems.

Next, a study by Mahanty et al. (2023) reported the ability of FeO nanoparticles synthesised by *Corynespora cassiicola* as a bioremediation to adsorb heavy metals, viz. Pb, Ni, Cu, Zn, Cd, and Cr from water. The comparative evaluation of mycogenic FeO nanoparticles against other green adsorbents clearly demonstrated their superior effectiveness as heavy metal adsorbents. Among these heavy metals, chromium (Cr) stands out as one of the most hazardous, posing severe risks to both health and the environment. This study highlights the potential of mycogenic FeO nanoparticles for efficient heavy metal removal in treating industrial effluents and aquaculture water.

Although previous studies have highlighted the potential of mycogenic MONPs as a photocatalytic and bioremediation agent, it is important to note that future efforts should focus on advancing their application, understanding their mechanisms, and ensuring environmental and economic sustainability. For example, conducting field trials and pilot studies by implementing MONP-based water remediation in small-scale aquaculture farms can help validate lab-scale results in real-world conditions.

CHALLENGES AND FUTURE DIRECTIONS

The successful use of mycogenic MONPs in aquaculture requires overcoming key challenges and exploring future advancements. This includes scaling up production for commercial viability, ensuring environmental safety, and enhancing efficacy. Key aspects discussed include scalability, commercialisation, regulatory compliance, and future research to drive innovation. Tackling these challenges will help transition mycogenic MONPs from lab innovations to sustainable aquaculture solutions.

Scalability and Commercialisation

Scaling up mycogenic MONP synthesis is crucial for commercial aquaculture applications. While lab-scale production shows promise, industrial-scale synthesis requires optimising fungal cultivation, standardising protocols, and improving yield efficiency. This section explores key aspects of scalability, including optimising fungal cultivation processes, standardising synthesis protocols, and improving yield efficiency to ensure consistent nanoparticle quality. Additionally, it highlights the need to address economic feasibility, regulatory compliance, and environmental sustainability. Overcoming these challenges can make mycogenic MONPs a viable and eco-friendly solution for improving aquaculture.

Optimising Fungal Cultivation and Improving Yield and Efficiency

Large-scale mycogenic MONPs synthesis faces challenges in maintaining consistency and Optimising reaction conditions. It also requires cost-effective fungal cultivation with robust strains for high-yield production (Javed et al., 2024). Apart from that, maximising nanoparticle yield without compromising quality depends on enhancing the reaction efficiency between fungal metabolites and precursor materials. Production efficiency can be further improved by Optimising reaction conditions such as pH, temperature, and reaction time using advanced models like Response Surface Methodology (RSM). Available in software like MINITAB, RSM combines statistical and mathematical methods to optimise processes effectively (Lamidi et al., 2022). It helps determine the optimal factors for the highest nanoparticle yield.

Genetically engineered fungal strains could enhance metabolic capabilities, improving nanoparticle yield and consistency. Genome mining has recently identified genes encoding enzymes for peptide assembly, regulation, resistance, and secondary metabolite synthesis (Leal et al., 2024). These genes form biosynthetic gene clusters (BGCs), allowing coordinated pathway expression. By introducing or modifying specific genes, fungi can be engineered to overproduce metabolites that act as reducing and stabilising agents in nanoparticle synthesis. Several transformation systems have been developed, including chromosomal engineering, Agrobacterium-mediated transformation, and CRISPR/Cas9-based genome editing (Jin et al., 2021). Genetic modifications can also enhance fungal tolerance to temperature and pH fluctuations, improving their robustness in large-scale cultivation.

Standardising Production Processes and Ensuring Economic Feasibility

A major challenge in applying mycogenic MONPs is optimising their synthesis for consistency, scalability, and cost-efficiency. Standardising the process ensures uniform nanoparticle size, shape, and properties, which are crucial for efficacy. Integrating automation and bioreactor technologies can significantly enhance process efficiency and reproducibility, making large-scale production both feasible and reliable (Wainaina & Taherzadeh, 2023). Additionally, it is essential that the production process remains cost-competitive compared to conventional nanoparticle synthesis methods, which can be evaluated through comprehensive cost-benefit analyses (Hassaan et al., 2020). Scaled-up production must also comply with environmental and safety regulations, focusing on effective waste management and reducing the ecological footprint.

Demonstrating Efficacy in Real-world Conditions

A major challenge in promoting mycogenic MONPs in aquaculture is proving their effectiveness and addressing adoption barriers. Field trials and pilot studies on farms are crucial to demonstrating their benefits in water quality, pathogen control, and fish health, as mentioned earlier. These studies will also offer insights into environmental impact, economic feasibility, and long-term sustainability.

Additionally, collaboration with aquaculture practitioners and industries is crucial for integrating this innovation into existing systems. Engaging stakeholders through workshops, knowledge exchange, and partnerships will enhance adoption. Overcoming challenges through collaboration will help mycosynthesised MONPs evolve from lab innovation to a scalable, sustainable solution for aquaculture.

Environmental and Safety Considerations

As the use of mycogenic MONPs in aquaculture grows, it is essential to address their potential environmental and safety impacts (Liu et al., 2024). This includes evaluating their effects on aquatic ecosystems, ensuring sustainable practices, and minimising risks to both target and non-target organisms.

Comprehensive Risk Assessment

Although research on the toxicity, bioaccumulation, and environmental effects of mycogenic MONPs in aquaculture is limited, studies on other nanoparticles reviewed by Nam et al. (2014) provide valuable insights. This underscores the need to assess their interactions with environmental substances. Toxicological studies should examine their effects on target and non-target aquatic species, including plants, invertebrates, and microbial communities. Bioaccumulation studies are also essential to determine their accumulation in aquatic organisms and potential transfer through the food chain. Additionally, environmental impact assessments should focus on their degradation, persistence, and behaviour in aquatic ecosystems.

Develop Safer Nanoparticle Formulations

Nanoparticles function differently from their bulk counterparts due to their small size and large surface area (Tirumala et al., 2021). This enhances interactions with biological systems, making surface modifications essential to reduce the reactivity and toxicity of MONPs. Mycogenic MONPs may naturally acquire protein or biomolecule coatings from fungal byproducts, improving stability, reducing toxicity, and enhancing biocompatibility (Campaña et al., 2023). Thorough testing of these eco-friendly MONPs is crucial to assess toxicity and ensure compliance with environmental and aquatic health regulations.

Establish Regulatory Frameworks

Currently, no standardised regulatory frameworks govern nanotechnology in food and agriculture (Fajardo et al., 2022). Therefore, developing robust policies is crucial for the safe use of nanoparticles in these industries. Collaboration with regulatory agencies ensures mycogenic MONPs comply with environmental and aquatic health regulations, promoting responsible innovation while minimising risks. Clear guidelines also support the adoption of mycogenic MONPs in aquaculture, fostering sustainable advancements while maintaining public and environmental safety.

Regular Monitoring and Surveillance

A key challenge in applying mycogenic MONPs in aquaculture is understanding their long-term effects on aquatic ecosystems, especially their influence on antimicrobial resistance. Biomonitoring programmes are crucial for assessing aquatic health and tracking resistance patterns where mycogenic MONPs are used. These programmes provide insights into their impact on microbial populations (Parada et al., 2019) and their role in resistance development. Regular monitoring helps detect adverse effects early, allowing timely interventions to reduce risks and support the sustainable use of mycogenic MONPs in aquaculture.

Future Research Areas

The green synthesis of MONPs is rapidly growing, yet fungal-based MONP synthesis and its aquaculture applications remain underexplored. Hence, this section highlights future research directions in this area.

Vaccine Development and Delivery System

The potential of mycogenic MONPs as a platform for vaccine development and delivery represents an exciting and promising area for future research. These nanoparticles offer distinct advantages, including biocompatibility, stability, and the ability for surface functionalization, which are crucial for effective vaccine delivery. Additionally, the mycosynthesis of MONPs enhances their safety and eco-friendliness, further establishing their suitability for this purpose.

One of the key areas of focus is the ability of MONPs to serve as carriers for antigen delivery through surface modification. These nanoparticles can be functionalized to carry specific antigens, enabling targeted immune responses (Wang et al., 2024). Future research can explore ways to optimise the functionalisation process, ensuring that antigen integrity is preserved and immunogenicity is enhanced.

Another key aspect is the adjuvant potential of MONPs, which enhance vaccine effectiveness through immunostimulatory properties (Wang et al., 2024). They prolong antigen exposure, target immune cells, and boost responses in aquaculture. Research can explore their synergy with traditional adjuvants and their role in both innate and adaptive immunity. Integrating mycogenic MONPs into aquatic vaccines offers a novel strategy against bacterial infections. Studies may assess their ability to elicit strong immune responses while maintaining fish health and growth. Additionally, research could examine their role in protecting antigens from degradation for safe delivery to target tissues.

The biodegradability and safety of mycogenic MONPs are also critical considerations for their application in vaccine delivery. Future investigations should include long-term safety assessments, examining cytotoxicity, immunogenicity, and the potential for accumulation in biological systems. The fungal-based synthesis pathway provides a unique advantage in ensuring eco-friendly production, but thorough studies are needed to confirm these benefits.

Advancing research in these areas could pave the way for mycogenic MONPs to revolutionise vaccine delivery systems. Their scalable, cost-effective, and efficient properties hold significant promise for enhancing aquatic animal health and addressing the challenges of infectious diseases in aquaculture.

Biosensor Application

Aquatic ecosystems face growing threats from pollutants, including heavy metals, pesticides, and harmful microorganisms, which highlight the urgent need for efficient monitoring tools. Mycogenic MONPs offer significant promise for developing sensitive and eco-friendly biosensors tailored for aquatic environments, owing to their distinctive physicochemical properties. One critical area of focus is the detection of heavy metals. Mycogenic MONPs, such as ZnO nanoparticles synthesised through fungal methods, exhibit remarkable conductivity and photocatalytic properties, making them ideal for detecting trace levels of metals like mercury (Hg), lead (Pb), and cadmium (Cd). Despite the broad synthesis of metal oxide nanoparticles from transition metals, the application of mycogenic MONPs in heavy metal detection remains relatively limited.

Another key application is monitoring aquatic pathogens. Biosensors with MONPs detect harmful microorganisms like *Vibrio* Spp, *Aeromonas* Spp, and *Streptococcus* Spp. Electrochemical biosensors use biologically relevant materials such as proteins, antibodies, or DNA to interact with target analytes, generating measurable electrical signals (Channabasavana Hundi Puttaningaiah, 2024). Functionalised MONPs, when combined with DNA or antibodies, enable rapid and specific detection of pathogens (Zimina et al., 2022). Additionally, MONPs are instrumental in assessing pesticides and organic pollutants in wastewater. Their photocatalytic properties, particularly in FeO, ZnO, or TiO_2 nanoparticles, can be harnessed for sensing and degrading organic pollutants in diverse environments (Ashour et al., 2023). Fungal-derived MONPs offer enhanced specificity and a reduced environmental footprint compared to chemically synthesised alternatives. By capitalising on the unique capabilities of mycogenic MONPs, researchers can drive innovations in aquatic biosensing technologies, fostering environmental sustainability and advancing aquaculture management practices.

Aquatic Feed Supplement

The use of mycogenic MONPs in aquatic feed supplementation offers great potential for improving aquaculture productivity. Synthesised through eco-friendly fungal-based methods, these nanoparticles have high surface area, bioavailability, and biocompatibility, making them ideal for feed enhancement. ZnO nanoparticles, in particular, serve as effective mineral supplements, providing essential micronutrients for growth, skeletal development, and metabolism (Yusof et al., 2023). Their nanoscale size ensures efficient absorption, addressing nutritional deficiencies in aquaculture diets.

Mycogenic MONPs enhance immunity, improving disease resistance and reducing antibiotic reliance in aquaculture. They also interact with gut microbiota (Xia et al., 2017), promoting gut health and feed efficiency, leading to better growth and overall fish health. Additionally, their antimicrobial properties suppress harmful pathogens, lowering disease incidence and improving survival rates. By leveraging these multifunctional benefits, integrating MONPs into aquaculture feed can drive sustainability, productivity, and environmental stewardship.

CONCLUSION

Numerous studies have highlighted the potential of various fungal species to synthesise MONPs and their diverse biological activities. Fungal genera such as *Trichoderma*, *Penicillium*, *Fusarium*, *Aspergillus*, and others have demonstrated considerable promise as natural nanofactories for MONP production due to the presence of various secondary metabolites and proteins. In addition, nanoparticles synthesised by these fungi have demonstrated a range of biological activities. Harnessing these fungi for MONP synthesis could significantly contribute to sustainable aquaculture practices. Nevertheless, additional research is necessary to thoroughly investigate their maximum potential. This review also discusses the challenges while highlighting future directions focused on mycogenic MONPs and assessing the long-term environmental impact of using MONPs in aquaculture. In conclusion, fungal-synthesised MONPs offer a promising alternative for sustainable aquaculture, though further research is needed to overcome challenges and enhance application.

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AI USAGE STATEMENT

The authors confirm that no generative artificial intelligence (AI) tools were used in the writing or preparation of this manuscript. The manuscript was prepared entirely by the authors. We believe the high AI similarity score may represent a false positive result due to conventional scientific writing structure and terminology.

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