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Synthesis of Iron Oxide Nanoparticles and Evaluation of their Antimicrobial Efficiency against *Rhizoctonia Solani.*

Rohit Rawat[1], Nidhi Tripathi[2], Harsh pardeshi[3], Aadi Jain[4], Khushi Bhadange[5], Shiksha Gour [6], Rajeshwari Verma[7]

[1]HARI Lifesciences, Bhopal

[2]Department of Biotechnology, Career College, Bhopal

[3, 4, 5, 6, 7]Research Student, Department of Biotechnology, Career College, Bhopal

ABSTRACT:

Iron oxide nanoparticles with particle size 10–30 nm was prepared by a green approach using $FeCl_3$ capping agent. The antifungal activity of iron oxide (FeO) nanoparticles prepared by bio safe method was evaluated for *Rhizoctonia solani*. It was observed from the study that all the concentrations of nanoparticles brought about significant inhibition in the mycelial growth of all the rot causing fungi. However, the highest inhibition in the growth of the test fungi was observed at higher concentrations followed by lower concentrations of nanoparticles. It was observed from the present study that FeO nanoparticles showed significant antimycotic activity against all the tested fungal pathogens. The maximum inhibition in the fungal growth was found against *Rhizoctonia solani* at 10^{-3} concentration of serial dilution of FeO nanoparticles.

Keywords- Iron oxide, nanoparticle, *Rhizoctonia solani*, antifungal activity

Introduction:

NPs with a size of less than 10–20 nm and made of ferromagnetic materials show super paramagnetism, a unique type of magnetism. Elements, alloys, oxides, and other chemical compounds that are magnetised by an external magnetic field are examples of ferromagnetic materials. This is a significant phenomenon that is typically limited to NP systems (Huber D L, 2017). Magnetic iron oxide (Fe3O4 and γ-Fe2O3) nanoparticles (NPs) have garnered significant attention due to their superparamagnetic properties, including surface area and volume ratio, low toxicity, and straightforward separation methodology. These applications include protein immobilisation in diagnostic magnetic resonance imaging (MRI), thermal therapy, and drug delivery (Hasany et al., 2012).

Although iron's reactivity is a big problem at the nanoscale, it has important macroscopic uses (rusting, in particular) (Wang et al., 2005). Although all living forms need iron (Fe), several different crops frequently lack this component (Mimmo et al., 2014). Iron is essential to plants for a number of processes, such as respiration, the production of chlorophyll and the control of redox reactions (Ye et al., 2015, Kobayashi et al., 2012). Fertile soil produces crops that are also weak in iron, which lowers crop quality and production (Liu et al., 2023). However, from the standpoint of the food chain, a deficit in iron can cause anaemia in living things (Laurie et al., 1991). Currently, organic iron, chelated iron fertiliser, and inorganic iron are employed to address this issue (Cesco et al.,2000). High price and possibility for absorption are disadvantages of the current system. Therefore, formulation of using Fe fertilizer need to be improved. Certain plant fungal infections have been successfully controlled by using biogenic nanoparticles, which are nanomaterial-based fungicides (Win et al., 2020). As biocontrol agents, they could find widespread application in agriculture to advance sustainable farming practices (Oluwaseun et al., 2017). In rice, maize, mustard, green grammes, and watermelons, the synthesised Fe3O4-NPs significantly improved the germination and vigour index. Additionally, Fe3O4-NPs shown antifungal action *against Phythium sp., Rhizoctonia solani, Fusarium oxysporum, Fusarium tricinctum, and Fusarium maniliforme*. It is appealing to comprehend the role of NPs in seed germination and growth given the recent and startling advancements in nanotechnology and its use in agriculture (Siddiqui et al., 2013). Rhizoctonia solani attacks maize, rice, wheat, barley, oat, soybean, peanut, dry bean, alfalfa, chickpea, lentil, field pea, tobacco, potato, sugar beet, canola, coffee, cotton, lettuce, pothos, ficus, flax. Seed rot, root rot, hypocotyl rot, crown rot, stem rot, limb rot, pod rot, stem canker, black scurf, seedling blight, and pre- and post-emergence damping off are symptoms on a variety of hosts. Depending on whether anastomosis group is present at the time of infection, seedling disease symptoms on soybean range from seed rot and pre-emergence damping off to root or hypocotyl rot.

Different approaches are used in the synthesis of nanoparticles, such as chemical, physical, and biological procedures (also known as "green synthesis") (Derakhshani et al., 2023) Both physical and chemical techniques, such as electrodeposition (Zan et al., 2022) and chemical reduction (Liu et al., 2023) yield nanoparticles with regulated size, shape, and composition. But some physical and chemical synthesis techniques frequently use toxic substances, produce dangerous byproducts, and consume a lot of energy, all of which can have a negative impact on biological systems. On the other hand, natural extract-based green synthesis techniques offer environmentally friendly and sustainable approaches to nanoparticle synthesis. (Bibi et al., 2019; Ullah et al., 2023). They cut down on energy use and remove or utilise chemical substances as little as possible (Singh et al., 2019). They also simplify the synthesis process by providing a one-step procedure without the need for extra chemical coatings (Derakhshani et al., 2023). When compared to chemically synthesised nanoparticles, green nanoparticles are more biocompatible and have lower toxicity (Dowlath et al., 2021; Singh et al., 2018).

Materials and methods:

1. Preparation and characterization of iron oxide nanoparticles

Iron oxide nanoparticles were fabricated using green approach. Iron oxide nanoparticle was fabricated using 2M $FeSo_4$ and 1M $FeCl_3$, 1M NH_4OH. In this process, solution of 1M $FeCl_3$ in 50 ml distilled water and 2M $FeSo_4$ in 50 ml distilled water was prepared. Both the solutions were mixed and whole reaction mixture was stirred for 30mins on hot plate magnetic stirrer. 1M NH_4OH in 5O ml was prepared and poured from burette dropwise the Change in colour of the solution indicates the formation of nanoparticles. After completion, the mixture was cooled to room temperature and subjected to centrifugation at 2000 rpm for 10 minutes, resulting in a wet FeO precipitate. The precipitates were further processed b filtration and washed multiple times with distilled water and absolute ethanol. The obtained product was dried at 45°C for overnight, yielding the dry powder of FeO NPs. The overall yield of the prepared FeO NPs was determined to be 65%. UV spectra were used to examine the synthesized nanoparticles, with a wavelength of 390 nm.

2. Antifungal assay

2.1. Test organisms

The test fungal organisms used in this study was *Rhizoctonia solani*.

2.2. Antifungal Activity of FeO against *Rhizoctonia solani*

To evaluate the efficacy of iron oxide nanoparticles on mycelial growth of some tested fungi, serial dilution of different concentrations viz. $10^{-1,}$ 10^{-2},10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9} of iron oxide nanoparticles was prepared from the precipitated sample. 7–8 days old fungal cultures grown on potato dextrose medium (PDA) medium were used to check the antifungal activity of synthesized nanoparticles. Four conical with 50ml nutrient broth in each were prepared. Three selected concentrations were chosen for testing i.e. 10^{-3}, 10^{-6}, ,10^{-9}. In case of control the inoculum was mixed without any nanoparticles and these four conical were incubated for 25 ± 2 °C in a moist chamber to maintain enough humidity. They were examined after 48h by UV-Vis spectroscopy.

Result:

The present study was conducted to characterize the Iron oxide nanoparticles prepared by precipitation method and to investigate their antifungal activity on *Rhizochtonia solani.* It is resistant strain of the family Ceratobasidiaceae. *Rhizoctonia solani* causes devastating diseases in hundreds of plant species. Among these, *R. solani* causes sheath blight, one of the three major diseases in rice.

1. Synthesis and Characterization of FeO NPs

The dark brown coloration that emerged suggested that the nanoparticles were formed. UV–Vis absorption spectrum of iron oxide nanoparticles (FeO-NPs) display a peak at the region 390 nm.

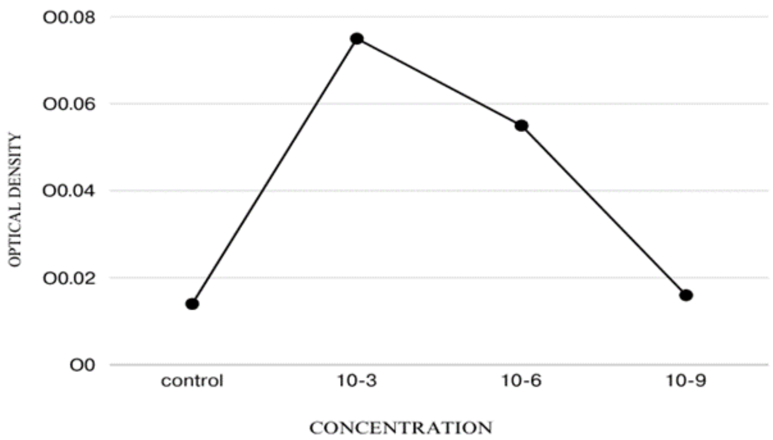
2. Antifungal Activity of FeO

2.1. In Vitro Effect of FeO on the Growth of *Rhizoctonia solani*

S.NO.	CONCENTRATION	OPTICAL DENSITY
1.	Control	0.014
2.	10^{-3}	0.075
3.	10^{-6}	0.055
4.	10^{-9}	0.016

Table 1- Results showing optical density

It was revealed from the results that the different concentrations of iron oxide nanoparticles at different concentrations of serial dilution ($10^{-3,}$ $10^{-6,}$ 10^{-9}) brought about significant inhibition of spore germination of tested fungal pathogens. However, inhibition in mycelial growth increases with the increase in concentration of the nanoparticles. The maximum inhibition in mycelial growth was found by highest concentration of iron oxide nanoparticles (10^{-3}) against *Rhizoctonia solani* followed by control which lacked the nanoparticles of iron oxide showed maximum growth of mycelium.



Discussion:

The iron oxide nanoparticles used in this investigation were made using a green method. This approach of nanoparticle preparation is easy, quick, affordable, and safe for the environment. An environmentally safe, non-toxic, and clean way to create NPs with a variety of size, shape, composition, and physiochemical properties is through the use of environmentally green chemistry (Shah et al., 2015). The findings clearly show that the various concentrations of iron oxide nanoparticles utilized in this investigation significantly inhibited the mycelial growth of fungal pathogen studied. It was discovered that the highest concentration performed best, with smaller amounts following in next (Wani et al., 2012), Because of their small size and high area to volume ratio, nanoparticles exhibit important changes in characteristics that are not present in the same material in its bulk forms(Issa et al., 2017). These NPs are utilized in pharmaceutical products, medical diagnostic imaging, and therapy protocols because of their distinct physiochemical and biological characteristics (Khan et al., 2017). The concentration of fungal spores and nanoparticles determines the extent to which inhibition occurs. According to a related study, iron oxide nanoparticles (NP) exhibit encouraging antimicrobial activity against a variety of human diseases (Abdeen et al., 2013). Thus, it can be stated that iron oxide nanoparticles have the potential to be employed as an antifungal agent to fight a variety of plant pathogenic fungi. The smaller size and higher surface area to volume ratio of nanoparticles, which effectively cover the pathogen and decrease the supply of oxygen for respiration, are the mechanisms underlying their antibacterial activity (Abdeen et al., 2013). Certain metal oxide nanoparticles (NPs) produce metal ions that enter cells through the membrane and interact with proteins and nucleic acids to damage enzyme activity (Zakharova et al., 2015, Abdeen et al, 2013)

Conclusion:

Significant advancements have been achieved in the manufacturing of monodisperse iron oxide nanoparticles for use in nanobiotechnology. Numerous simple techniques are developing rapidly providing a range of monodispersed spherical nanocrystals with tunable particle sizes, compositions, shapes, and magnetic characteristics. The primary factor to be taken into account when choosing synthesis methods is iron oxide's solubility in aqueous solution and in colloidal form due to the biological environment. Therefore, this requirement is satisfied by wet-chemical techniques including coprecipitation and thermal breakdown of organometallic precursors. The last several years have seen a significant effort to change the surface chemistry of iron oxide nanoparticles to make them hydrophilic and biocompatible. The creation of magnetic nanoparticles with efficient surface coatings that offer the best results in in vitro and in vivo biological applications is a significant problem for all of the approaches. This study concluded that Iron oxide NPs synthesized using $FeSo_4$, $FeCl_{3,}$ NH_4 OH were $FeCl_3$ is the capping agent The method is simple, fast, economical and environmentally safe. Iron oxide NPs fabricated using this method were stable, crystalline and possess promising antifungal activities against many fungal pathogens., hence can be used for the control of various fungal diseases. The formation of FeO was confirmed by UV spectroscopy and the biosynthesized FeO showed an inhibitory effect on *R. solani* growth. The synthesized nanoparticles possess a great capacity of suppressing *R. solani* infections.

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