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Comparative analysis of the antimicrobial activity of iron and iron oxide nanoparticles against *Trichothecium roseum*

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Abstract

The emergence of antimicrobial resistance poses a significant challenge to global health, necessitating the exploration of alternative antimicrobial agents. Iron and iron oxide nanoparticles have garnered attention due to their potential antimicrobial properties. This study aims to comparatively analyse the efficacy of iron and iron oxide nanoparticles against *Trichothecium roseum*, a common fungal pathogen known for its detrimental effects on various crops and stored grains. The findings showed that the spore germination of the investigated fungal pathogens was significantly inhibited by the various quantities of iron and iron oxide nanoparticles at various serial dilution doses (10^{-3}, 10^{-6}, and 10^{-9}). However, when the concentration of the nanoparticles rises, so does the suppression of mycelial growth. The highest concentration of iron and iron oxide nanoparticles (10^{-3}) against *Trichothecium roseum* was found to inhibit mycelial growth the most, with optical densities of 0.911 and 0.544, respectively. The control group, which did not have any iron or oxide nanoparticles, displayed the highest mycelial growth, with an optical density of 1.011. Iron and iron oxide nanoparticle optical densities at 10^{-6} concentration were found to be 0.929 and 0.880, respectively. Conversely, concentrations of iron and iron oxide nanoparticles at 10^{-9} serial dilution resulted in optical densities of 0.984 and 0.954, respectively. It is discovered that iron oxide nanoparticles have a greater antifungal efficacy than iron nanoparticles against *Trichothecium roseum.* This comparative analysis sheds light on the promising antimicrobial properties of iron and iron oxide nanoparticles and underscores their potential applications in agriculture and food preservation industries for controlling fungal pathogens like *Trichothecium roseum.* Further research is warranted to optimize nanoparticle synthesis and understand their interactions with microbial cells for effective antimicrobial strategies.

Keywords: *Trichothecium roseum;* Nanoparticles; Antifungal Efficacy; Fungal Pathogens; Antimicrobial Properties

1. Introduction

According to Abad et al. (2019), nanotechnology is expected to be at the forefront of the expansion of nanomaterials, which are primarily used in numerous domains of science and technology. The creation of nanoparticles with applications in biology, especially medication administration, is the focus of nanotechnology (Zahin et al., 2020). Nanoparticles are the fundamental units of nanotechnology (NPs). The size range of these nanoscale entities is 1–100 nm (Asha A et. al., 2016, Khan I. et. al., 2019). NPs have distinct physiochemical, electrical, magnetic, and thermal properties compared to their bulk counterparts (Nadeem M. et. al. 2018). Because of their smaller size, superparamagnetic characteristics, and decreased biocompatibility, iron nanoparticles (IONPs) have been utilized in biomedical applications more than other metallic nanoparticles. Additionally, targeted distribution, imaging, tissue engineering, bioprocessing, and disease management (Patil R.M. et. al. 2017, Sangaiya P et. al., 2018, Kaul R. et. al., 2012). In particular, the antimicrobial, anti-larvicidal, and antioxidant therapies are the most notable ones.

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Green synthesis of nanoparticles, or NPs, started with the discovery of magnetic materials in living systems. The study of magnetite (Fe (III)) in intracellular spaces led to the study of Fe (II), which produced a significant discovery: living systems have a metabolic cycle that reduces ferric oxide. This was the first proof that living systems could convert metal to zero valent form. Additionally, he provided the first evidence of magnetite particles smaller than nanometers being present in the cellular structure.

This sparked curiosity about the potential of microbes for bio-reduction, which is why metabolites, plant extracts, cells, tissues, seeds, or other vegetative reproduction structures, as well as organs and entire organisms, are used in the biosynthesis of NPs. This process is referred to as "green" because, in contrast to physical and chemical methods, it produces no harmful byproducts and, among other benefits, is less expensive because it uses less reagents, infrastructure, and energy (A. Morales-Díaz, et al. 2017, A. Singh, et al. I 2020, D. Sravanthi et al., 2019). Due to its extensive application in both biological and geological processes, iron is one of the most topical infrastructures (Abdollahi et al., 2019). Because of its significant utility in daily life and lower toxicity, iron oxide nanoparticles are one of the most widely employed particles (Abbaszadeh and Hejazi, 2019). Hematite and magnetite, the two forms of iron oxides found in nature, are the only ones that are used in scientific research (Markeb et al., 2019). In nanotechnology, iron oxide nanoparticles with sizes and widths between 10 and 100 nm were crucial. Various techniques, including physical, chemical, and biological procedures, can be employed to create iron oxide nanoparticles (NPs). The use of nano-formulations of agrochemicals as fertilizers and pesticides to improve crop yields, nano-biosensors for crop protection to identify pesticide residues and diseases, nano-devices for plant genetic engineering, and other applications of nanotechnology can increase agricultural productivity. Nanobiotechnology is utilized in agriculture to increase food production, resulting in food that is more nutritious, safe, and of higher quality (Rawat R. et. al., 2024).

A precursor that supports a particle size in the nanometer range is provided by the physical approach of creating nanoparticles (Alphandéry, 2019). The very basic chemical preparation process controls the nanoparticles' shape, size, and a few other characteristics (Amanzadeh et al., 2019). The biological technique of nanoparticle synthesis is more economical and effective for large-scale production than other approaches. In contrast, plants offer a faster, more environmentally friendly, and less expensive method of synthesizing nanoparticles (Abou El-Nour et al., 2010). For biological applications, plant-based synthetic nanoparticles work better (Ahmad et al., 2022). A fungus belonging to the Ascomycota division called *Trichothecium roseum* was originally identified in 1809 (Batt et al., 2014). Koch (1934) documented the antagonistic behaviors of T. roseum towards several plant pathogenic fungi. Black knot disease in cherry, plum, and apricot trees is caused by *Dibotryon morbosum*, which *T. roseum* was found to actively parasitize (Freeman, G.G et al., 1949).

2. Materials and Methods

2.1. Synthesis of nanoparticles

2.1.1. Synthesis of iron nanoparticles:

4 gm tea powder was weighed and soaked in 20 ml hot distilled water for 15 minutes. After 15 minutes it was filtered using filter paper. 0.1 M $FeCl_3$ solution was prepared in20 ml distilled water. It was mixed for 2 minutes at magnetic stirrer. Tea filtrate was filled in burette and poured drop by drop in $FeCl_3$ solution. This step is also performed on magnetic stirrer. After sometime the light brown coloured mixture will convert to dark black brown coloured ppt. This solution was centrifuged at 3000 rpm for 15 minutes. Supernatant was discarded and pallets were collected followed by ethanol wash several times. These p were left overnight for drying.

2.1.2. Synthesis of iron oxide nanoparticles:

Iron oxide nanoparticles were fabricated using green approach. Iron oxide nanoparticles were synthesized using 2M $FeSO_4$ and 1M $FeCl_3$, 1.5 ml ammonia. In this process, solution of 1M $FeCl_3$ in 20 ml distilled water and 2M $FeSO_4$ in 20 ml distilled water was prepared. Both the solutions were mixed and whole reaction mixture was stirred for 15mins on hot plate magnetic stirrer. 1.5 ml ammonia was poured with the help of pipette in the above solution dropwise. The Change in colour of the solution from light brown to dark black brown indicates the formation of iron oxide nanoparticles. After completion, the mixture was cooled to room temperature and subjected to centrifugation at 3000 rpm for 10 minutes, resulting in a wet iron oxide precipitate. The precipitates were further processed by filtration and washed multiple times with absolute ethanol. The obtained product was dried at room temperature, yielding the dry powder of iron oxide NPs.

2.2. Antifungal Assay

2.2.1. Test organisms

The test fungal organisms used in this study was *Trichothecium roseum.* The strain was inoculated on PDA slants and incubated for 5-7 days at 21 ℃ and humidity provided was 60- 70.

2.2.2. Antifungal Activity of iron and iron oxide nanoparticles on Trichothecium roseum

To evaluate the efficacy of iron and 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 and iron oxide nanoparticles was prepared. The fungal cultures grown on potato dextrose medium (PDA) medium were used to check the antifungal activity of synthesized nanoparticles. 7 conical with 50ml nutrient broth in each were prepared separately for both nanoparticles. Three the dilution concentrations selected for testing were 10-3, 10-6, 10-9 for each nanoparticle. 1 conical was kept as control. 1ml of the desired concentration was added in each conical. 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.

3. Result and Discussion

The present study was conducted to synthesize iron and iron oxide nanoparticles and evaluate the antifungal activity of Iron and iron oxide nanoparticles against *Trichothecium roseum* prepared by precipitation method. *Trichothecium roseum* causes devastating diseases in hundreds of plant species. It was revealed from the results that the different concentrations of iron and 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 and iron oxide nanoparticles (10^{-3}) against *Trichothecium roseum* with optical density of 0.911 and 0.544 respectively followed by control which lacked the nanoparticles of iron and iron oxide showed maximum growth of mycelium with optical density of 1.011. The optical density of 10^{-6} concentration of iron and iron oxide nanoparticle came out to be 0.929 and 0.880 respectively. Whereas concentration with 10^{-9} serial dilution of iron and iron oxide nanoparticle comprised the optical density of 0.984 and 0.954 respectively. The effectiveness of antifungal activity of iron oxide nanoparticles against *Trichothecium roseum* is found out to be more than iron nanoparticles.

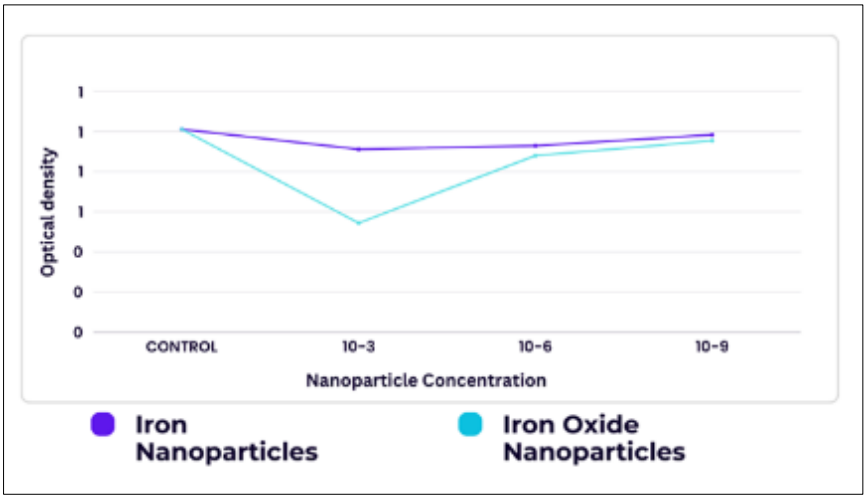

Figure 1 Comparative optical density of different concentrations of nanoparticles

4. Conclusion

In conclusion, the comparative analysis of the antimicrobial activity of iron and iron oxide nanoparticles against *Trichothecium roseum* provides valuable insights into the potential effectiveness of these nanoparticles as antifungal agents. Through a series of experiments, it was observed that both iron and iron oxide nanoparticles exhibit antimicrobial properties against *Trichothecium roseum,* with iron oxide nanoparticles demonstrating greater efficacy in inhibiting fungal growth compared to iron nanoparticles, particularly at higher concentrations. The results suggest that

the choice of nanoparticle composition significantly influences their antimicrobial activity, likely due to variations in physicochemical properties and interactions with the fungal pathogen. Additionally, the concentration of nanoparticles plays a crucial role in determining their effectiveness, with higher concentrations generally resulting in greater inhibition of fungal growth. These findings contribute to our understanding of the potential applications of iron and iron oxide nanoparticles in combating fungal infections, both in biomedical and environmental contexts. However, further research is warranted to elucidate the underlying mechanisms of nanoparticle-fungus interactions and optimize nanoparticle formulations for enhanced antimicrobial efficacy and biocompatibility.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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