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Preparation, characterization and antifungal activity of iron oxide nanoparticles

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ABSTRACT

Iron oxide nanoparticles with particle size 10–30 nm were prepared by a green approach using tannic acid as reducing and capping agent. These nanoparticles were characterized by X-ray diffractometry (XRD), high resolution field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). The prepared iron oxide nanoparticles were also evaluated for their antifungal activity against *Trichothecium roseum, Cladosporium herbarum, Penicillium chrysogenum, Alternaria alternata* and *Aspergillus niger*. The antifungal activity was observed as inhibition in spore germination and by determining the zone of inhibition of fungal pathogens caused by different concentrations of iron oxide nanoparticles on culture media. It was observed from the present study that Fe_2O_3 nanoparticles showed significant antimycotic activity against all the tested fungal pathogens. Highest inhibition in spore germination was caused against *T. roseum* (87.74%) followed by *C. herbarum* (84.89%). The highest zone of inhibition by iron oxide nanoparticles was reported against *P. chrysogenum* (28.67 mm) followed by *A. niger* (26.33 mm), *T. roseum* (22.67 mm), *A. alternata* (21.33 mm) and least against *C. herbarum* (18.00 mm). Activity index was recorded highest against *P. chrysogenum* (0.81). The MIC value of Fe_2O_3 NP varies between 0.063 and 0.016 mg/ml for different fungal pathogens that is comparable with the MIC value shown by the standard, revealing the efficacy of iron oxide NP's against different fungal pathogens.

1. Introduction

Nanotechnology is the synthesis, manipulation and use of materials that are of nanoscale size. In last few decades, nanoparticles have attracted much attention due to their unique size dependent properties and applications [1]. Nanoparticles have great potential in management of different diseases, they have great antimicrobial properties and are stable under harsh process conditions [2]. The use of nanoparticles as antifungal and antibacterial agents, have been considered as alternate, cost effective and ecofriendly management strategy for the control of pathogenic microbes [3]; [4]. Metal oxide nanoparticles are stable and regarded safe to human beings [5]; [6]. Metal oxides constitute an important class of materials that are involved in electrochemistry, magnetism, biology, environmental science [7]. Iron oxide nanoparticles has been widely used as a catalyst, solar energy conversion, environmental protection, sensors, biomedical applications, tissue repair, magnetic storage devices, drug delivery [8,9]. Iron oxide nanoparticles has excellent property to remove various water pollutants and are used as adsorbent in waste water treatment [10].

Nanoparticle synthesis and their characterization is the area of immense interest due to their broader applicability in every field [11]. Various methods have been developed for the fabrication of nanoparticles that include physical, chemical and biosynthetic methods. The problem with the physical and chemical methods includes short term stability and safety issues [12]. So keeping in view the present scenario of environment and safety issues, there is currently widespread interest in developing simple, easy, benign and environmental friendly green process to synthesize nanoparticles [13]. Because of excellent properties and less environmental pollution, iron oxide nanoparticles were prepared by green approach and characterized by XRD, FESEM and TEM. The synthesized iron oxide nanoparticles were also evaluated for their antifungal activity both qualitatively and quantitatively against *Cladosporium herbarum*, *Trichothecium roseum, Penicillium chrysogenum, Alternaria alternata* and *Aspergillus niger,* using spore germination assay, agar well diffusion assay and by determination of minimum inhibitory concentration. The fungal species that were used during the present

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study are the common fungal pathogens that cause majority of the fruit and vegetable rot diseases under storage conditions resulting in severe losses to the agricultural and horticultural crops every year [14–16]. Although a number of management methods have been used, but these methods have some limitations [17,18]. Therefore, in this study an attempt was made to use an alternate ecofriendly management strategy in terms of use of synthesized nanoparticles.

2. Materials and methods

2.1. Preparation of iron oxide nanoparticles

Iron oxide nanoparticles were fabricated using green approach. 1% of iron oxide nanoparticle was fabricated using tannic acid in alkaline medium. In this process, 0.5 g of iron was dissolved in 50 ml of double distilled water to it 5 ml of 0.004 M tannic acid was added followed by 2 ml of NaOH (0.5 M). The whole of reaction mixture was stirred for 30mins. Change in colour of the solution indicates the formation of nanoparticles.

2.2. Characterization of iron oxide nanoparticles

2.2.1. X-ray diffractometry

XRD is the widely used technique to analyze the crystalline/amorphous nature of nanoparticles along with its phase and purity of sample. The XRD pattern of finely ground NP's was observed under wide range of bragg's angle (θ). Philips X'pert PRO analytical instrument operated at 40 kV and 30 mA current with Cu Kα radiation (λ = 0.15419 nm). The average particle size was calculated by Debye Scherrer formula

$$D = \frac{0.9\lambda}{\beta\cos\theta}$$

Where, D is thickness of nanoparticle, λ is wavelength of X-ray, β is half maxima of reflection at bragg's angle 2θ and θ is diffraction angle or bragg's angle.

2.2.2. Field emission scanning electron microscopy (FESEM)

The size and morphology of the iron oxide nanoparticles was determined by high resolution field emission scanning electron microscope (FEI NOVA NANOSEM-600). Thin film of the Iron oxide NP sample was prepared on carbon coated copper SEM grids by just dropping the suspension of nanoparticles in water on the grid, extra sample solution was removed by blotting paper and then the sample was allowed to dry for 5 min and then sample images were then taken.

2.2.3. Transmission electron microscopy (TEM)

The features and shape of prepared Iron oxide nanoparticles was also imaged by TECNAIG^2 – 30 U TWIN transmission electron microscope operating at 300 kV.

2.3. Antifungal assay

2.3.1. Test organisms

The test fungal organisms used in this study (*Trichothecium roseum, Cladosporium herbarum, Penicillium chrysogenum, Alternaria alternata* and *Aspergillus niger*) were obtained from Section of Mycology and Plant Pathology, Department of Botany, University of Kashmir, Srinagar.

2.3.2. Spore germination assay

To evaluate the efficacy of iron oxide nanoparticles on spore germination of some tested fungi, different concentrations, *viz*. 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml and 0.5 mg/ml of iron oxide nanoparticles was prepared from the precipitated sample. Spore suspension with 1×10^3 conidia/ml was prepared in sterilized distilled water. Equal volume of spore suspension and the nanomaterials solutions were mixed in a test tube and then shaken. The mixture then contained the particular concentration of test nanoparticles. In case of control spore suspension was mixed with equal volume of distilled water. A drop of the mixture (about 0.1 ml) was then placed in the cavity slide and these were incubated for 25 ± 2 °C in a moist chamber to maintain enough humidity. Three replicates were maintained for each treatment including the control. The slides were examined after 24 h by hand tally counts at different microscopic fields. Percent spore germination of each treatment was calculated by the formula given by Ref. [19].

$$\text{Percent spore germination} = \frac{\text{No. of spores germinated}}{\text{Tota no. of spores examined}} \times 100$$

$$\text{Inhibition of spore germination (\%)} = \frac{Gc - Gt}{Gc} \times 100$$

Where Gc and Gt represent the mean number of germinated conidia in control and treated plates, respectively.

2.3.3. Agar well diffusion assay

The antifungal activity of the iron oxide nanoparticles was determined by agar well diffusion method as adopted by Ref. [20]. 7–8 days old fungal cultures grown on potato dextrose medium (PDA) medium were used to check the antifungal activity of synthesized nanoparticles. An aliquot of 0.02 ml of inoculum from each fungal pathogen was inoculated in 20 ml of molten Saboured dextrose agar (SDA) medium in culture tubes. The culture tubes were then homogenized between the hands and poured into 90 mm Petri plates. The culture plates were then allowed to solidify in laminar airflow chamber and then wells were made on the agar plate using 5 mm standard cork borer. Different concentrations (0.10 mg/ml, 0.25 mg/ml and 0.50 mg/ml) of the nanomaterial were prepared and added to respective wells. Hexahit 0.1 mg/ml (20 µl/disc) was used as standard (Positive control). The effect of iron oxide nanoparticles against the fungal pathogens were evaluated and compared with the standard used during the present study. The plates were then sealed and incubated at 25 ± 2 °C for 5 days. The antifungal activity was calculated by measuring the zone of inhibition by using standard scale [21].

2.3.4. Assessment of activity index

The assessment of activity index was calculated by comparing the inhibition zones of nanoparticles with the standard using the formula given by Ref. [22].

$$\text{Activity Index} = \frac{\text{Inhibition zone by the NP sample}}{\text{Inhibition zone by the standard}}$$

2.3.5. Determination of minimum inhibitory concentration (MIC)

Broth dilution method [23] was followed for determination of minimum inhibitory concentration (MIC) values for iron oxide nanoparticle showing antimicrobial activity against test pathogens. MIC is the lowest concentration of the test sample at which the fungi will not show any growth. To measure MIC values, various concentrations of the iron oxide nanoparticles (1.000–0.002 mg/ml) 1.000, 0.500, 0.250, 0.125, 0.063, 0.032, 0.016, 0.008, 0.004 and 0.002 mg/ml were assayed against the test pathogens. Initial concentration of 1 mg/ml was prepared from the precipitated nanosamples and then two fold serially diluted. 1 ml of the each nanoparticle concentration was added to the test tubes containing 1 ml of the SDA broth. The tubes were then inoculated with standard size of fungal spore suspension 3×10^3 CFU/ml, made from 5 days old fungal culture and incubated at 25 °C for 48 h in BOD incubator.

3. Results

3.1. Preparation of iron oxide nanoparticles

This is the simplest method for the preparation of iron oxide