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Evaluation of antifungal activity of Magnesium oxide (MgO) and Iron oxide (FeO) nanoparticles on rot causing fungi

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ABSTRACT

The antifungal activity of Magnesium oxide (MgO) and iron oxide (FeO) nanoparticles prepared by bio safe method was evaluated for *Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicillium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani*. It was observed from the study that all the concentrations of nanoparticles brought about significant inhibition in the spore germination and mycelial growth of all the rot causing fungi. However, the highest inhibition in the germination of all the test fungi was observed at higher concentrations followed by lower concentrations of nanoparticles. It was observed from the present study that MgO and FeO nanoparticles showed significant antimycotic activity against all the tested fungal pathogens. However, highest reduction in spore germination was observed against *Mucor plumbeus* whereas least reduction of spore germination was observed against *Aspergillus niger* at different concentration of nanoparticles of MgO respectively. Likewise, the maximum inhibition in the fungal growth was observed against *Alternaria alternata* and least inhibition in zone of fungal growth due to MgO nanoparticle was found against *Mucor plumbeus* respectively. Similarly, highest inhibition in spore germination was found against *Penicillium expansum* and least inhibition in spore germination was found against *Aspergillus niger* at different concentrations of nano FeO. Likewise, the maximum inhibition in the fungal growth was found against *Rhizoctonia solani* and *Trichothecium roseum* and least inhibition in zone of fungal growth was found against *Penicillium expansum* and *Mucor plumbeus* at 0.1mg/ml, 0.25mg/ml and 0.5 mg/ml concentrations of FeO nanoparticles respectively. Activity index was recorded highest against *P. chrysogenum* (0.97) in case of MgO and against *T. roseum* (0.97) in case of FeO nanoparticles respectively.

Keywords: Spore germination, mycelial growth, rot causing fungi, tomato, brinjal, MgO and FeO nanoparticles

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1. INTRODUCTION

Nanotechnology is the application of science and technology to control matter at the molecular level. At the nanoscale level, the properties of matter are significantly different from their macroscopic bulk properties. Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular scale. The metal microbe interactions have an important role in several biotechnological applications including the fields of bioremediation, biomineralization, bioleaching, and microbial corrosion[1]. Nanotechnology is also referred to the ability for designing, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale[2]. Nanoparticles are viewed as the fundamental building blocks of nanotechnology[2, 3]. They are the starting points for preparing many nanostructured materials and devices. Their synthesis is an important component of the rapidly growing research efforts in nanoscience and nano engineering. The nanoparticles of a wide range of materials can be prepared by a number of methods. In synthesis and assembly strategies of nanoparticles or nanomaterials, precursors from liquids, solid or gas phase are used[2]. Nanoparticles have great potential in management of different diseases; they have great antimicrobial properties and are stable under harsh process conditions[4]. 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[5].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. Antifungal assay

2.1.1. Test organisms

The test fungal organisms used in this study (*Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor*

plumbeus, *Penicillium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani*) were obtained from Section of Mycology and Plant Pathology, Department of Botany, University of Kashmir, Srinagar.

2.1.2. Spore germination assay

To evaluate the efficacy of Magnesium oxide and Iron oxide nanoparticles on spore germination of some tested fungi, different concentrations, viz. 0.1mg/ml, 0.2mg/ml and 0.5mg/ml of Magnesium oxide and Iron oxide nanoparticles were 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 nanomaterial 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 0.1ml) 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 24h by hand tally counts at different microscopic fields. Percent spore germination of each treatment was calculated by the formula given by [6].

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

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

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

2.1.3. Agar well diffusion method assay

The antifungal activity of the Magnesium oxide and Iron oxide nanoparticles were determined by agar well diffusion method as adopted by[7]. 7–8 days old fungal cultures grown on potato dextrose medium (PDA) were used to check the antifungal activity of synthesized nanoparticles. An aliquot of 0.02ml of inoculum from each fungal pathogen was inoculated in 20ml of molten Sabouraud dextrose agar (SDA) medium in culture tubes. The culture tubes were then homogenized between the hands and poured into 90mm Petri plates. The culture plates were then allowed to solidify in laminar airflow chamber and then wells were made on the agar plate using 5mm standard cork borer. Different concentrations (0.10mg/ml, 0.25mg/ml and 0.50mg/ml) of the nanomaterial were prepared and added to respective wells. Hexahit 0.1mg/ml (20µl/disc) was used as standard (Positive control). The effect of Magnesium oxide and 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 [8].

2.1.4. 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 [9].

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

2.1.5. Statistical analysis

The data collected during these investigations were subjected to appropriate statistical analysis using SPSS statistical software (version 16.0). The data was statistically analyzed by one way analysis of variance (ANOVA) and comparison of the means was done by Duncan multiple comparison tests at $P \leq 0.05$.Standard deviation was calculated as $\delta = \sqrt{\frac{\sum x^2}{N-1}}$.

3. RESULTS

3.1. Effect of MgO nanoparticles on the spore germination and mycelial growth of rot causing fungi

It was revealed from the results (Table 1, Fig 1) that different concentrations of MgO nanoparticles caused considerable inhibition in the spore germination of tested fungal pathogens, viz. *Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicilllium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani.* The inhibition in the spore germination increased with the increase in the concentration of all nanoparticles. However, the maximum reduction in the spore germination was found at highest concentration 0.5ml. It was followed by 0.2 ml and 0.1 ml concentrations of nanoparticles. The reduction in the spore germination against *Mucor plumbeus* varies from 39.56% to 5.46% in different concentrations of nano MgO, whereas least reduction of spore germination ranges from 64.25 % to 11.86 % in different concentration of nanoparticles against *Aspergillus niger* respectively, as compared to untreated control. Likewise the inhibition in the spore germination of other fungi also varies significantly in different concentrations of MgO nanoparticles and highest concentration brought about highest reduction in the spore germination followed by lower concentrations of MgO nanoparticles compared to control.

It was observed from the results (Table 2, Fig 2) that MgO nanoparticles caused inhibition in the mycelial growth of all the tested fungi. However, the maximum inhibition in the fungal growth was observed in *Alternaria alternata* with the zone of inhibition as 13.00 mm, 15.00 mm and 16.33mm at different concentrations of MgO nanoparticles respectively. The inhibition in zone of fungal growth due to MgO nanoparticle against *Trichothecium roseum* and *Aspergillus niger* was 12.00 mm, 14.00 mm, 15.33 mm and 11.00 mm, 12.66 mm, 14.66 mm respectively. While as moderate inhibitory activity of MgO nanoparticles was shown against *Rhizoctonia solani* with the zone of inhibition of 11.00 mm, 12.66 mm, 14.33 mm; against *Penicillium chrysogenum* with zone of inhibition as10.33 mm, 12.66 mm, 14.33 mm and against *Penicillium expansum* with zone of inhibition of 10.00 mm, 11.00 mm, 12.00 mm at 0.1mg/ml, 0.25mg/ml and 0.5 mg/ml concentration respectively. The inhibition in zone of fungal growth due to MgO nanoparticle against *Mucor plumbeus* was 09.00 mm, 11.00 mm, 13.33mm at 0.1mg/ml, 0.25mg/ml and 0.5 mg/ml concentration respectively. The results were compared with hexaconazole as a positive control.

Table 1: Effect of MgO nanoparticles on the spore germination of rot causing fungi

Concentration / Fungal Pathogens	Spore germination (%)			
	0.1mg/ml	0.25mg/ml	0.5 mg/ml	Control
Penicillium expansum	41.74 ± 0.02^{b}	24.26 ±0.02^{c}	10.27±0.02^{d}	86.74 ± 0.04^{a}
Aspergillus niger	64.25 ± 0.02^{b}	45.37 ±0.01^{c}	11.86±0.01^{d}	91.45 ± 0.01^{a}
Alternaria alternata	43.88 ± 0.01^{b}	32.74±0.015^{c}	9.86± 0.01^{d}	89.48 ± 0.01^{a}
Mucor plumbeus	39.56 ± 0.015^{b}	22.24 ±0.01^{c}	5.46 ± 0.01^{d}	85.57 ± 0.01^{a}
Penicillium chrysogenum	43.64 ± 0.02^{b}	26.36±0.015^{c}	12.27±0.01^{d}	88.75 ± 0.01^{a}
Trichothecium roseum	44.74±0.015^{b}	27.65 ±0.01^{c}	14.46±0.01^{d}	89.66 ± 0.01^{a}
Rhizoctonia solani	45.64 ± 0.02^{b}	29.47±0.015^{c}	16.34±0.02^{d}	91.74 ± 0.01^{a}

Each value is mean of 3 replicates ± SD

Mean values followed by different superscript in a column are significantly different (p≤ 0.05)

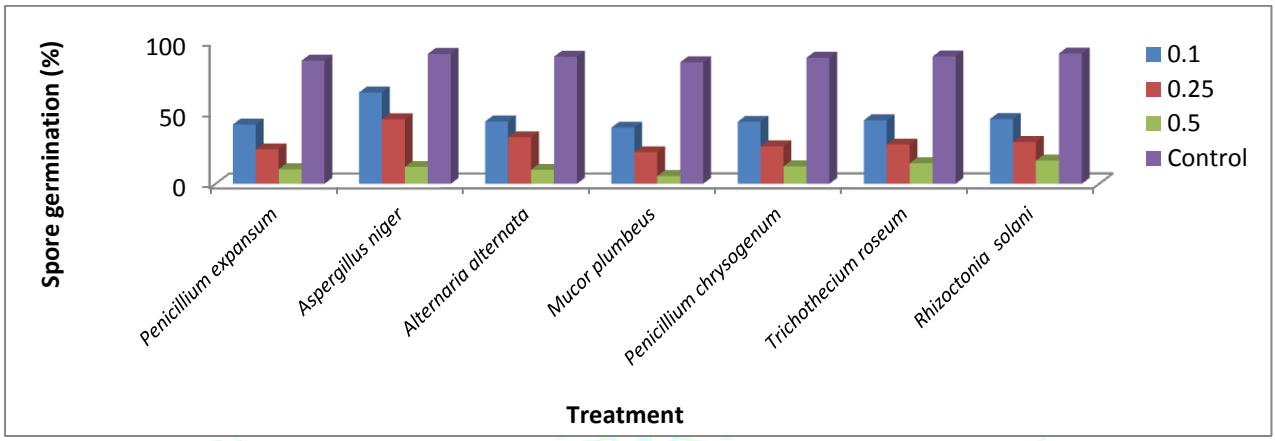

Figure 1: Effect of MgO nanoparticles on the spore germination of rot causing fungi.

Table 2: Effect of MgO, nanoparticles on the mycelial growth of rot causing fungi

Concentration / Fungal Pathogens	Zone of mycelial Inhibition (mm)			
	0.1mg/ml	0.25mg/ml	0.5 mg/ml	Control
Penicillium expansum	10.00 ± 1.00^{c}	11.00 ±1.00^{bc}	12.00 ± 1.00^{b}	17.33 ± 0.57 ^{a}
Aspergillus niger	11.00 ± 1.00^{d}	12.66 ± 1.00^{c}	14.66 ± 0.57^{b}	19.00 ± 1.00^{a}
Alternaria alternata	13.00 ± 1.00^{c}	15.00 ± 1.00^{b}	16.33 ± 1.15 ^{b}	19.33 ± 0.57 ^{a}
Mucor plumbeus	09.00 ± 1.00^{d}	11.00 ± 1.00^{c}	13.33 ± 0.57^{b}	16.00 ± 1.00^{a}
Penicillium chrysogenum	10.33 ± 0.57^{c}	12.66 ± 0.57^{b}	14.33 ± 0.57^{a}	14.00 ± 1.00^{a}
Trichothecium roseum	12.00 ± 1.00^{c}	14.00 ± 1.00^{b}	15.33 ±1.15^{ab}	17.00 ± 1.00^{a}
Rhizoctonia solani	11.00 ± 1.00^{d}	12.66 ± 0.57^{c}	14.33 ± 0.57^{b}	17.33 ± 0.57^{a}

Each value is mean of 3 replicates ± SD

Mean values followed by different superscript in a column are significantly different (p≤ 0.05).

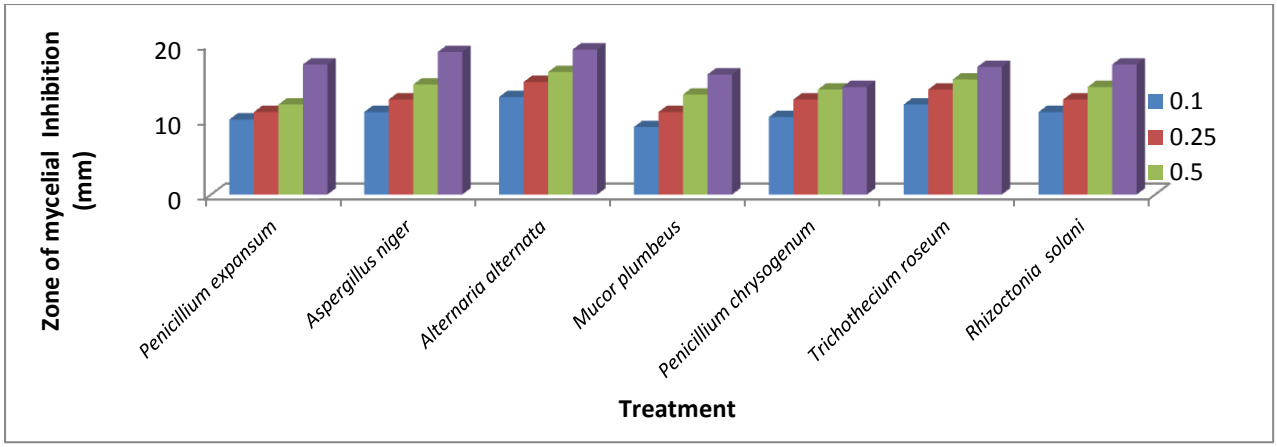

Figure 2: Effect of MgO nanoparticles on the mycelial growth of rot causing fungi.

3.2. Assessment of activity index

The activity index of Magnesium oxide and iron oxide nanoparticles at different concentrations against all the selected fungi is given in Table 3, 6. The activity index depends on the zone of inhibition. The activity index of Magnesium oxide NP was highest against *P. chrysogenum* (0.97) at 0.5mg/ml concentration followed by *T. roseum* (0.90), *A. alternata* (0.84), *M. plumbeus* (0.83), *R. solani* (0.82), *A. niger* (0.77) and least activity was shown against *P. expansum* (0.69) at the same concentration. However, the activity index of iron oxide NP was highest against *T. roseum* (0.97) at 0.5mg/ml concentration followed by *M. plumbeus* (0.94), *A. alternata* (0.93), *P. expansum* (0.92),*A. niger* (0.90), *R. solani* (0.90)and least activity was shown against *P. chrysogenum* (0.85) at the same concentration.

Table 3: Activity index of MgO, nanoparticles at different concentrations against different fungi

Fungal Pathogens	Activity index		
	0.1mg/ml	0.25mg/ml	0.5 mg/ml
Penicillium expansum	0.57	0.63	0.69
Aspergillus niger	0.57	0.66	0.77
Alternaria alternata	0.67	0.77	0.84
Mucor plumbeus	0.56	0.68	0.83
Penicillium chrysogenum	0.72	0.88	0.97
Trichothecium roseum	0.70	0.82	0.90
Rhizoctonia solani	0.63	0.73	0.82

3.3. Effect of FeO nanoparticles on the spore germination and mycelial growth of rot causing fungi

It was observed from the results (Table 4, Fig 3) that different concentrations of FeO nanoparticles caused inhibition in the spore germination of all tested fungi such as *Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicillium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani*. However, the maximum reduction in the spore germination was found at highest concentration (0.5ml). It was followed by 0.2 ml and 0.1 ml concentrations of nanoparticles. The nano FeO at highest concentration was found most effective in reducing the spore germination. Highest inhibition in spore germination against *Penicillium expansum* varies from 54.34% to 21.37% in different concentrations of nano FeO. However, least inhibition in spore germination was found in *Aspergillus niger* which varies from 76.33% to 40.76% in different concentration of nanoparticles of FeO as compared to control. Likewise the inhibition in the spore germination of all other fungi was also observed in different concentrations of FeO nanoparticles and highest inhibition was found in highest concentration of FeO nanoparticles followed by lower concentrations of FeO nanoparticles.

Further it was revealed from the results (Table 5, Fig 4) that FeO nanoparticles caused considerable inhibition in the mycelial growth of all the tested fungi at different concentrations. However, the maximum inhibition in the fungal growth was found in *Rhizoctonia solani* and *Trichothecium roseum* with zone of inhibition as 12.00 mm, 14.33 mm and 16.33 mm and 12.00 mm, 13.33 mm and 16.00 mm at 0.1mg/ml, 0.25mg/ml and 0.5 mg/ml concentrations of FeO nanoparticles respectively. The inhibition in zone of fungal growth due to FeO nanoparticle against *Alternaria alternata* was 12.33 mm, 13.00 mm, 15.66 mm at 0.1mg/ml, 0.25mg/ml and 0.5 mg/ml concentrations respectively. While as moderate inhibitory activity of FeO nanoparticles was found against *Aspergillus niger* and *Penicillium chrysogenum* with the zone of inhibition of 10.33 mm, 11.66 mm, 12.66 mm and 10.66 mm, 11.00 mm, 12.00 mm at 0.1mg/ml, 0.25mg/ml and 0.5 mg/ml concentration respectively. Likewise the least inhibition in zone of fungal growth due to FeO nanoparticle against *Penicillium expansum* and *Mucor plumbeus* was 09.00 mm, 10.66 mm, 12.33 mm and 09.00 mm, 9.66 mm, 11.00 mm at 0.1mg/ml, 0.25mg/ml and 0.5 mg/ml concentrations respectively. The results were compared with hexacanozole as control.

Table 4: Effect of FeO, nanoparticles on the spore germination of rot causing fungi

Concentration / Fungal Pathogens	Spore germination (%)			
	0.1mg/ml	0.25mg/ml	0.5 mg/ml	Control
Penicillium expansum	54.34± 0.02^{b}	47.84± 0.02^{c}	21.37± 0.01^{d}	91.24 ± 0.015^{a}
Aspergillus niger	76.33±0.015^{b}	64.34±0.015^{c}	40.76± 0.01^{d}	95.43 ± 0.015^{a}
Alternaria alternata	64.75±0.015^{b}	59.74± 0.02^{c}	22.32± 0.02^{d}	91.47 ± 0.01^{a}
Mucor plumbeus	65.72± 0.02^{b}	55.63± 0.03^{c}	30.57± 0.01^{d}	89.34 ± 0.015^{a}
Penicillium chrysogenum	56.41±0.015^{b}	49.85± 0.01^{c}	23.46± 0.01^{d}	93.36± 0.015^{a}
Trichothecium roseum	58.54± 0.02^{b}	51.63±0.015^{c}	24.35± 0.02^{d}	95.56 ± 0.015^{a}
Rhizoctonia solani	60.27±0.015^{b}	53.73±0.015^{c}	27.37±0.015^{d}	94.36± 0.01^{a}

Each value is mean of 3 replicates ± SD

Mean values followed by different superscript in a column are significantly different (p≤ 0.05)

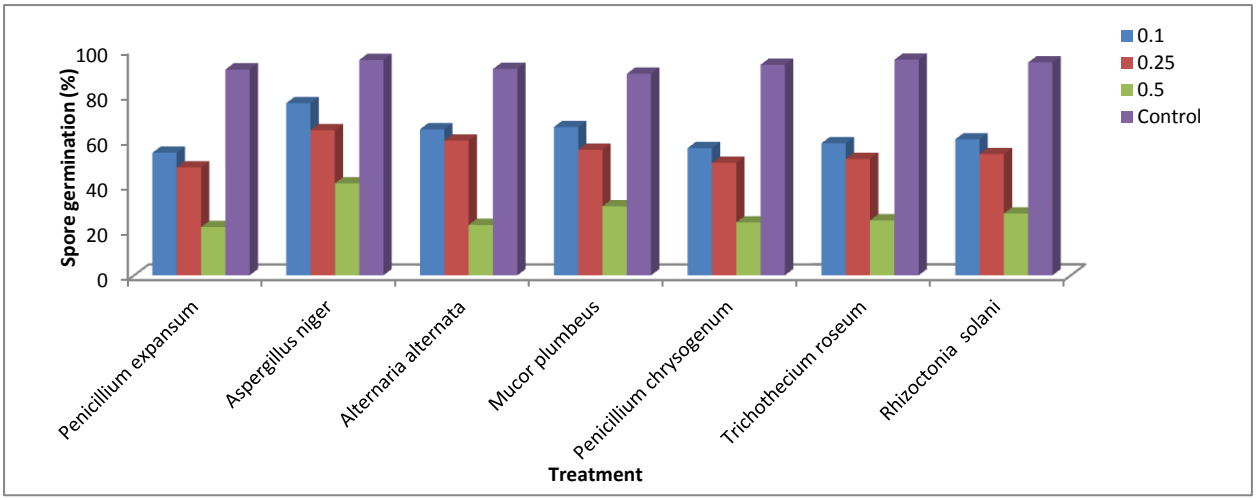

Figure 3: Effect of FeO nanoparticles on the spore germination of rot causing fungi.

Table 5: Effect of FeO, nanoparticles on the mycelial growth of rot causing fungi

Concentration / Fungal Pathogens	Zone of mycelial Inhibition (mm)			
	0.1mg/ml	0.25mg/ml	0.5 mg/ml	Control
Penicillium expansum	09.00 ± 1.00^{c}	10.66 ± 1.15^{b}	12.33 ±0.57^{a}	13.33 ± 0.57^{a}
Aspergillus niger	10.33 ± 1.52^{b}	11.66 ± 1.52^{ab}	12.66 ±1.15^{ab}	14.00 ± 1.00^{a}
Alternaria alternata	12.33 ± 0.57^{b}	13.00 ± 1.00^{b}	15.66 ± 0.57^{a}	16.66 ± 0.57^{a}
Mucor plumbeus	09.00 ± 1.00^{b}	9.66 ± 0.57^{ab}	11.00 ±1.00^{ab}	11.66 ± 1.52^{a}
Penicillium chrysogenum	10.66 ± 0.57^{b}	11.00 ± 1.00^{b}	12.00 ± 1.00^{b}	14.00 ± 1.00^{a}
Trichothecium roseum	12.00 ± 1.00^{b}	13.33 ± 0.57^{b}	16.00 ± 1.00^{a}	16.33 ± 0.57^{a}
Rhizoctonia solani	12.00 ± 1.00^{d}	14.33 ± 0.57^{c}	16.33 ± 0.57^{b}	18.00 ± 1.00^{a}

Each value is mean of 3 replicates ± SD

Mean values followed by different superscript in a column are significantly different (p≤ 0.05)

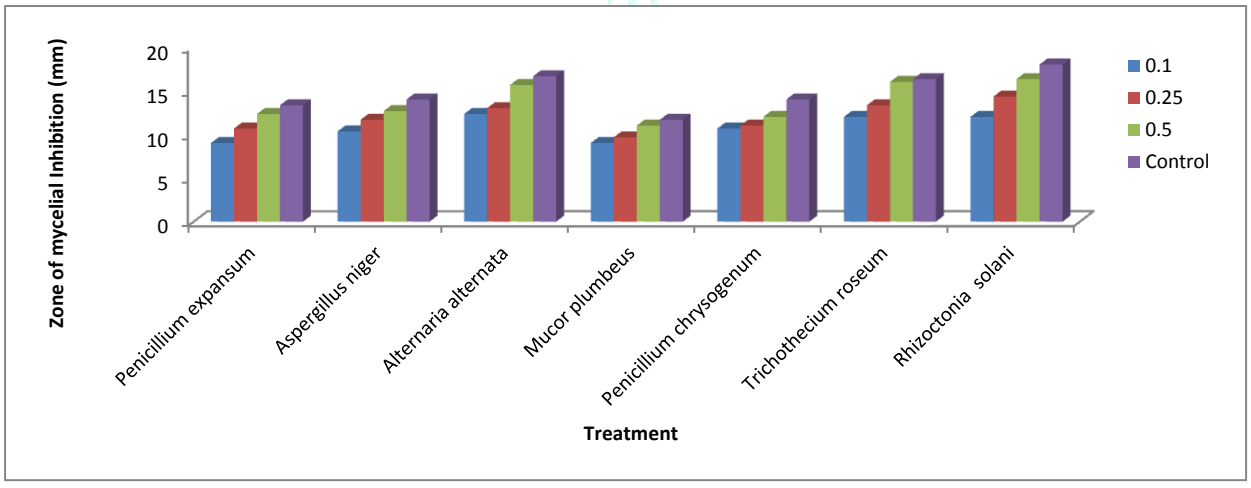

Figure 4: Effect of FeO nanoparticles on the mycelial growth of rot causing fungi.

Table 6: Activity index of FeO nanoparticles at different concentrations against different fungi

Fungal Pathogens	**Activity index**		
	0.1mg/ml	**0.25mg/ml**	**0.5 mg/ml**
Penicillium expansum	0.67	0.79	0.92
Aspergillus niger	0.73	0.83	0.90
Alternaria alternata	0.74	0.78	0.93
Mucor plumbeus	0.77	0.82	0.94
Penicillium chrysogenum	0.76	0.78	0.85
Trichothecium roseum	0.73	0.81	0.97
Rhizoctonia solani	0.66	0.79	0.90

4. DISCUSSION

In the present study nanoparticles of MgO and FeO were tested for their antimycotic activity. It was clear from the results that Magnesium oxide and iron oxide nanoparticles at different concentrations used in the present study caused considerable inhibition in the spore germination and mycelial growth of all the tested fungal pathogens. The highest concentration was found more effective followed by the lower concentrations. [10] reported that silver nanoparticles caused significant reduction in mycelial growth and spore germination. Antifungal effect of silver nanoparticles on some pathogenic fungi has also been reported by some workers[11, 12]. [13] evaluated the antifungal activity of Magnesium oxide nanoparticles on *Fusarium oxysporum* f. sp. lycopersici, pathogenic agent of tomato. Antimycotic activity of some nanoparticles of silver has also been reported on some fungi like wood rotting fungi, *Fusarium* species and on the phytopathogenic fungi. It is also observed that antifungal activity may be due to suppression of enzymes and toxins used by the fungal pathogens for pathogenesis[14, 15]. [16] also reported that the highest concentration of MgO, FeO and ZnO nanoparticles proved effective than the lower concentrations. [17] reported the antimycotic activity of MgO and ZnO nanoparticles against *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus*. [18] studied the antifungal activity of silver nanoparticles against *Cladosporium cladosporoides* and *Aspergillus niger* and reported that these NP's have effective biocidal properties even at low concentrations. [19, 20] reported the antifungal and antibacterial activity of iron oxide nanoparticles against *Aspergillus niger*, *Fusarium solani*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*. They also reported that iron oxide NP's can be used as effective antimicrobial agents. Activity index of iron oxide nanoparticles was also recorded, the concentration of the iron oxide which shows larger zone of inhibition shows highest activity index. The mechanism of the antimicrobial activity of nanoparticles is due to their small size and larger surface area to volume ratio which effectively covers the microorganism and reduce oxygen supply for respiration[21]. Thus, nanoparticles used in the present study showed significant antifungal activity and can be used as an alternate control measure against some other fungi causing diseases in the vegetables under storage.

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REFERENCES

1. Oza V. Pandey S. Shah V. Sharon M. Extracellular Fabrication of Silver Nanoparticles using *Pseudomonas aeruginosa* and its Antimicrobial Assay. Pelagia Research Library Advances in Applied Science Research. 2012. 3(3):1776-1783.
2. Mansoori GA. Principles of Nanotechnology – Molecular-Based Study of Condensed Matter in Small Systems, World Scientific Pub. Co., Hackensack, NJ. 2005.
3. Mansoori GA. George TF. Zhang G. Assoufid L. Molecular Building Blocks for Nanotechnology, Springer, New York, 2007.
4. Park HJ. Kim SH. Kim HJ. Choi SH. A new composition of nanosized silica silver for control of various plant diseases. *Plant Pathol. J*. 2006; 22:25-34.
5. Raghupatri RK. Koodali RT. Manna AC. Size dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. *Langmuir*. 2011; 27:4020-4028.
6. Kiraly Z. Klement Z. Solymosy F. Voros J. Methods in Plant Pathology with special reference to breeding for resistance to breeding for resistance, Elsevier publishing company, New York.1974. p. 212
7. Perez C. Pauli M. Bazerque P. An antibiotic assay by the well agar method. *Acta Biolog, Med. Experiment*. 1990; 15:113-115.
8. Norrel SA. Messley KE. Microbilogy Laboratory Manual Principles and Applications, Prentice Hall, Upper Saddle River. New Jersey. 1997. 85-90.
9. Singariya P. Kumar P. Mourya KK. Antimicrobial activity of friut coat (calyx) of *Withania somnifera* against some multi drug resistant microbes. *Int. J. Biol. Pharmaceut. Res*. 2012; 2:252-258.
10. Morones JK. Elechiguerra JL. Camacho A. Holt K. Kouri JB. Ramirez JT. Yacaman J. The bacterial effect of silver nanoparticles. *Nanotechnology*. 2005; 16(10):2346-2353.
11. Kim SW. Kim KS. Lamsal K. Kim YJ. Kim SB. Jung M. Sim SJ. Kim HS. Chang SJ. Kim JK. Lee YS. An *in vitro* study of the antifungal effect of silver nanoparticles on oak wilt pathogen, *Raffaelea* sp. *J. Microbiol. Biotechnol*. 2009; 19:760-764.
12. Jo YK. Kim BH. Jung G. Antifungal activity of silver ions and nanoparticles on Phytopathogenic fungi. *Plant Disease*. 2009; 03:1037-1043.
13. Parizi MA. Moradpour Y. Roostaei A. Khani M. Negahdari M. Rahimi G. Evaluation of the antifungal effect of magnesium oxide nanoparticles on *Fusarium oxysporum* F. Sp. lycopersici, pathogenic agent of tomato. *European Journal of Experimental Biology*, 2014; 4(3):151-156.
14. Bhainsa KC. D'Souza SF. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*. *Colloids Surf B Biointerfaces*. 2006; 47(2):160-164.
15. Vahabi K. Mansoori GA. Karimi S. Biosynthesis of silver nanoparticles by fungus *Trichoderma reesei. Inscience J*. 2011; 1:65-79.
16. Wani AH. Shah MA. A unique and profound effect of iron oxide and ZnO nanoparticles on some pathogenic fungi. *J. Appl. Pharmaceut. Sci*. 2012; 2:40-44.
17. Wani AH. Amin M. Shahnaz M. Shah MA. Antimycotic activities of MgO, FeO and ZnO on some pathogenic fungi. *Int. J. Manuf. Mater. Mech. Eng*. 2012; 2(4):59-70.
18. Pulit J. Banach M. Szczyglowska R. Bryk M. Nanosilver against fungi. Silver nanoparticles as an effective biocidal factor. *Acta Biochim. Pol*. 2013; 60(4):795-798.
19. Nehra P. Chauhan RP. Garg N. Verma K. Antibacterial and antifungal activity of chitosen coated iron oxide nanoparticles, *Br. J. Biomed. Sci*. 2017. https://doi.org/10.1080/09674845.2017.1347362.
20. Parveen S. Wani AH. Shah MA. Devi HS. Bhat MY. Koka JA. Preparation, Characterisation and antifungal activity of iron oxide nanoparticles. *Microbial Pathogenesis*. 2018; 115:287-292.
21. Abdeen S. Issac RR. Geo S. Somalekshmi S. Rose A. Praseetha PK. Evaluation of antimicrobial activity of biosynthesized iron and silver nanoparticles using the fungi *Fusarium oxysporum* and *Actinomyces* sp. on human pathogens. *Nano Biomed. Eng.* 2013; 5(1):39-45.