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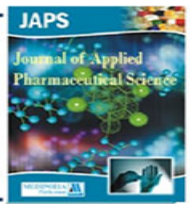

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A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi

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ABSTRACT

The antifungal activity of zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles prepared by bio-safe method was evaluated for *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus*. It was observed from the study that all the nanoparticles at different concentrations brought about significant inhibition in the germination of spores of *Alternaria alternata, Fusarium oxysporum, Rhizopus stolonifer and Mucor plumbeus*. However, the highest inhibition in the germination of all the test fungi was observed at higher concentrations followed by lower concentrations of nanoparticles. The nanoparticles of MgO at highest concentration was found most effective in reducing the spore germination followed by nanoparticles of ZnO at the same concentration.

Keywords: Nanoparticles; anti fungal activity; spore germination

INTRODUCTION

Nanosciences has reached within the last decade the status of a leading science with fundamental and applied research prospects in all basic cognitive sciences such as physical, life and earth sciences: from physics and chemistry, biology and medicines, to engineering and agriculture. Nanotechnology is the next industrial revolution and all most all industries will be radically transformed by it in few years and this technology would directly benefit a common man when it comes to commercial use. The emerging field of nanosciences and nanotechnology is leading to a technological revolution in the world (Shah and Towkeer, 2010). Nanotechnology has the potential to revolutionize the agriculture and food industry too with new tools for molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients (Patolsky *et al*., 2006). Nanoscale devices could be used to identify plant health issues before these become visible to the former. Smart devices could be used to deliver chemicals in a controlled and targeted manner in the same way as nano-medicine has implications for drug delivery in humans. Technologies such as encapsulation and controlled release methods have revolutionized the use of pesticides and herbicides (Scrins and Lyons, 2006). Besides, plants and/or their extracts provide a biological synthesis route of several metallic nanoparticles which is more eco-friendly and allows a controlled synthesis with well defined size and shape (Bar *et al*., 2009).

Plants are often attacked by various pathogens such as fungi, bacteria and viruses which results in great loss to farmers (Esfahani, 2006). Several conventional methods have been used for the control of these pathogens and each of these methods has one or other limitations. Some of these methods such as use of pesticides cause hazardous effect on the environment and human health. Thus, use of nanoparticles has been considered an alternate and effective approach which is eco-friendly and cost effective for the control of pathogenic microbes (Kumar and Yadav, 2009). These nanoparticles have a great potential in the management of plant diseases as compared to synthetic fungicides (Park *et al*., 2006). Zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles are an effective antibacterial and anti-odor agent Shah and Towkeer, 2010). The increased ease in dispensability, optical transparence and smoothness make ZnO and MgO nanostructures an attractive antibacterial ingredient in many products. Both have also been proposed as an anti-microbial preservative for wood or food products (Aruoja *et al*., 2009; Huang *et al*., 2006; Sharma *et al*., 2009). Properly functionalized nanocapsules provide better penetration through cuticle and allow slow and controlled release of active ingredients on reaching the target weed. The use of such nano-biopesticide is more acceptable since they are safe for plants and cause less environmental pollution in comparison to conventional chemical pesticides (Bark *et al*., 2008). In this communication, the antimycotic effects of ZnO and MgO nanoparticles having average size of ~30 ± 10nm and ~50 ±10nms respectively are tested on some pathogenic fungi such as *Alternaria alternate, Fusarium oxysporum, Rhizopus stolonifer and Mucor plumbeus***.** The nanoparticles of MgO at highest concentration were found most effective in reducing the spore germination followed by nanoparticles of ZnO at the same concentration. To the best of our knowledge and belief, the use of nanostructures prepared without organics or toxic solvents has not been used for in-vitro studies so far.

MATERIALS AND METHODS

Preparation of nanoparticles and Fungal culture

The method employed for preparation is same as described earlier (Al-Harbi *et al*., 2011; Shah and Quarshi, 2009). In a typical synthesis of ZnO and MgO nanoparticles, 3 mg of zinc and magnesium powder was taken in a vial containing 30 ml of de-ionized water separately and was well sonicated for 10 minutes each before placing at desired temperature in a Teflon bomb. The reaction mixture was transferred to teflon-lined stainless steel chamber and has been kept at 110^{o}C in an oven for 6h. After the desired time, the system was naturally cooled to room temperature. The reaction mixture was centrifuged to reclaim the precipitated sample and was washed with distilled water. After drying in air, synthesized nanoparticles were characterized by various techniques. The average particle size of ZnO and MgO nanoparticles is ~ 30 ± 10nm and 50 ±10nms respectively (Figure 1 and 2). The Fungal culture was prepared in Plant Pathology Laboratory, Department of Botany, University of Kashmir on Potato dextrose Agar medium PDA. Fungal species of *Alternaria alternate, Fusarium oxysporum* and *Rhizopus stolonifer.and Mucor plumbeus* were isolated from rotten samples of tomato and bringal, cultured on PDA and identified following Koch's postulates.

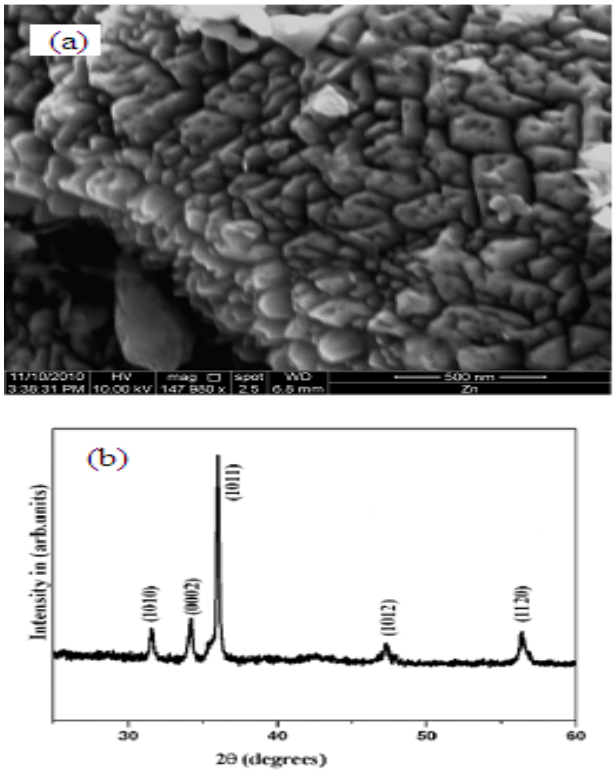

Fig. 1: (a) FESEM image of ZnO nanoparticles (b) XRD of the samples.

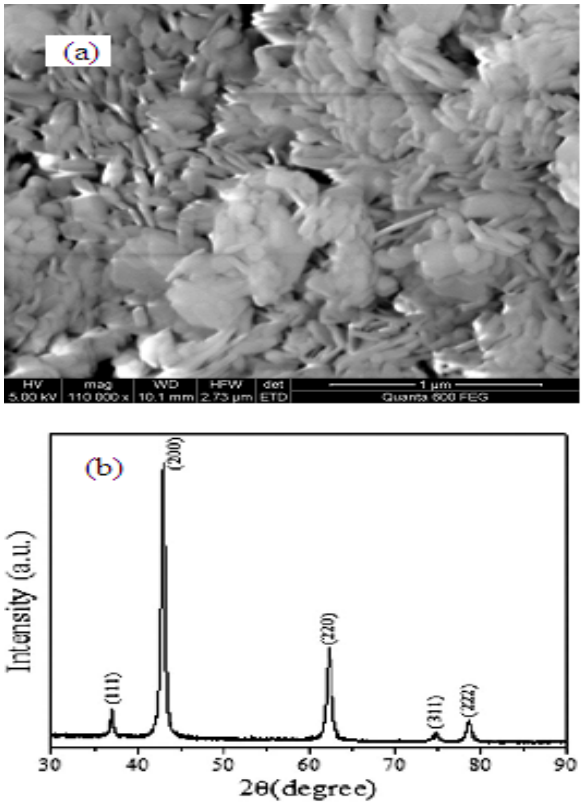

Fig. 2: (a) FESEM image of MgO nanoparticles (b) XRD of the samples.

Preparation of spore suspension

Different concentration of nanoparticles of ZnO and MgO were evaluated for their effect on the spore germination of *Alternaria alternate, Fusarium oxysporum* and *Rhizopus stolonifer.and Mucor plumbeus.* The fungal inoculates were prepared on potato dextrose agar (PDA) media (a common microbial media for culturing fungus) at 28°C in Petri-plates. Spore suspension of each isolate of fungi containing at least 20-30 spores per microscopic field was prepared from 10 days old fungal culture. One drop about 0.1ml of spore suspension was put in a cavity glass slide containing a drop (about 0.1ml) of different concentration of nanoparticles. These slides were kept in moist chamber prepared by putting two folds of filter paper in both sides of Petri-plates. These Petri plates were incubated at 24±2°C for 24 hours. Each treatment was replicated five times. The percent spore germination was recorded using formula as:

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

Statistical analysis

The data collected during these investigations were subjected to appropriate statistical analysis using Minitab software. The data wherever needed was subjected to appropriate transformation before statistical analysis. The method given by Freiner (2007) was also used for statistical analysis of the data.

RESULTS AND DISCUSSION

Effect of MgO and ZnO nanoparticles on the spore germination of *Alternaria alternate*

It was revealed from the study (Table 1) that the different concentrations of nanoparticles of ZnO and MgO caused inhibition in the spore germination. However, the maximum inhibition in the spore germination was found at highest concentration '0.5ml'.

Table 1: Effect of MgO and ZnO nanoparticles on the spore germination of *Alternaria alternate.*

Concentration (ml)/ Treatment	Spore germination (%)				
	0.0	**0.1**	**0.2**	**0.3**	**0.5**
Nano-MgO	91.50 (72.06)	44.94 (41.52)	33.60 (34.82)	22.29 (27.48)	9.80 (18.20
Nano-ZnO	93.57 (74.19)	70.39 (56.41)	59.61 (49.96)	40.26 (38.80)	21.44 (26.88)
	SE. diff.	CD. (P = 0.05)		CD. (P = 0.01)	
Fungicides	1.80	1.61		2.09	
Concentration	0.93	1.86		2.42	
Fungicide x conc.	1.61	3.22		4.19	

Mean of five replicates; **Figures in parentheses are arc Sin√%age transformed value and are statistically identical.

It was followed by 0.3ml, 0.2ml, and 0.1ml concentrations of nanoparticles. The nano-MgO at highest concentration was found most effective in reducing the spore germination followed by other concentration of nano-ZnO. The inhibition in spore germination varies from 44.94% to 9.80% in different concentrations of nano-MgO.

Effect of MgO and ZnO nanoparticles on the spore germination of *Rhizopus stolonifer*

It was revealed from the study that (Table 2) that different concentration of of nanoparticles of MgO and ZnO caused inhibition in the spore germination of *Rhizopus stolonifer*. However, inhibition in spore germination increases with the increase in the concentration of nanoparticles. The maximum inhibition in the spore germination was found at highest concentration '0,5ml'. It was followed by 0.4ml, and 0.1ml concentrations of nanoparticles. The nano-Mgo at highest concentration was found most effective in reducing the spore germination followed nano-Zno.

Table 2: Effect of Mg and ZnO on the spore germination of *Rhizopus stolonifer*

Concentration(ml)/ Treatment	Spore germination (%)				
	0.0	**0.1**	**0.2**	**0.3**	**0.5**
Nano-MgO	88.78 (69.54)	43.60 (40.75)	26.21 (30.14)	18.17 (24.48)	9.15 (17.60)
Nano-ZnO	92.11 (72.66)	56.34 (48.07)	48.95 (43.83)	36.24 (36.42)	23.57 (28.37)
	SE. diff.	CD. (P = 0.05)		CD. (P = 0.01)	
Fungicides	0.80	1.58		2.09	
Concentration	0.92	1.83		2.41	
Fungicide x conc.	1.60	3.17		4.18	

Mean of five replicates; ** Figures in parentheses are arc Sin√%age transformed value and are statistically identical.

Effect of MgO and ZnO nanoparticles on the spore germination of *Fusarium oxysoprum*

It was revealed from the study (Table 3) that different concentration of nanoparticles of MgO and ZnO caused inhibition in the spore germination of *Fusarium oxysporum*. But inhibition in spore germination increased with the increase in the concentration of nanoparticles. However, the maximum inhibition in the spore germination was found at highest concentration '0.5ml'.

Table 3: Effect of MgO, and ZnO nanoparticles on the spore germination of *Fusarium oxysporum.*

Concentration (ml) Treatment	Spore germination (%)				
	0.0	**0.1**	**0.2**	**0.3**	**0.5**
Nano-MgO	93.92 (74.54)	66.28 (53.90)	43.46 (41.20)	36.06 (36.31)	12.96 (20.24)
Nano-ZnO	94.43 (74.04)	78.00 (60.67)	65.96 (53.11)	51.28 (44.59)	42.61 (39.59)
	SE. diff.	CD. (P = 0.05)		CD. (P = 0.01)	
Fungicides	1.25	2.25		3.01	
Concentration	1.29	2.61		3.48	
Fungicide x conc.	2.25	4.52		6.03	

Mean of five replicates; ** Figures in parentheses are arc Sin√%age transformed value and are statistically identical.

The nano-Mgo at highest concentration was found most effective in inhibiting the spore germination followed by standard concentration of nanoparticles of MgO, and ZnO. The inhibition in spore germination varies from 66.28% to 12.96% in different concentrations of nano-MgO. Similarly in nano-ZnO, the inhibition in spore germination ranges from 78.00% to 42.61% in its different different concentrations compared to untreated control which showed least inhibition in spore germination.

Effect of MgO, and ZnO nanoparticles on spore the germination of *Mucor plumbeus*

It was revealed from the study (Table 4) that different concentrations of MgO, and ZnO caused inhibition in the spore germination but inhibition in spore germination increased with the increase in the concentration of nanaoparticles. However, the maximum reduction in the spore germination was found at highest concentration 0.5ml. It was followed by 0.3 ml, 0.2 ml, and 0.1 ml concentrations of nanoparticles. The nano MgO at highest concentration was found most effective in reducing the spore germination followed by standard concentration ZnO. The decrease in spore germination varies from 41.54% to 6.40% in different concentrations of nano MgO, whereas in nano ZnO, the reduction of spore germination ranges from 68.66% to 31.64% in different concentration of nanoparticles respectively, as compared to untreated control which shows least reduction in spore germination.

Table. 4: Effect of of MgO and ZnO nanoparticles on the spore germination of *Mucor plumbeus* .

Concentration (ml)/ Treatments	Spore germination (%) 0.0	0.1	0.2	0.3	0.5
Nano-MgO	89.59 (70.26)	41.54 (39.55)	24.22 (28.81)	16.22 (22.97)	6.40 (14.55)
Nano-Zno	90.45 (71.05)	68.66 (55.96)	59.64 (49.98)	43.95 (41.53)	31.64 (33.61)

	SE. diff.	CD. (P = 0.05)	CD. (P = 0.01)
Fungicides	0.87	1.70	1.26
Concentration	1.00	1.98	2.62
Fungicide x conc.	1.74	3.43	4.53

Mean of five replicates; ** Figures in parentheses are arc Sin√%age transformed value and are statistically identical.

The present study revealed the efficacy of different concentrations different concentration of nanoparticles of MgO, and ZnO on the spore germination of fungi such as *Alternaria alternate, Fusarium oxysporum, Rhizopus stolonifer and Mucor plumbeus,*. It was clear from the results, that different concentration of MgO and ZnO nanoparticles caused significant inhibition in the spore germination as compared to control. The highest inhibition was observed at highest concentration of nanoparticles followed by lower concentration of nanoparticles repetitively. The highest inhibition in the spore germination of all the test fungi was observed in MgO nanoparticles at all its concentrations followed by different concentrations of zinc nanoparticles respectively. The effect of nanoparticles of magnesium and zinc oxide on the inhibition of spore germination may be due to their fungicidal effect on the all the test fungi and such study have been carried out for the first time in Kashmir.

Antifungal effect of silver nanoparticles on some pathogenic fungi has also been reported by some workers (Kim *et al*., 2009; Jo *et al*., 2009). It is observed a significant reduction in mycelial growth and spore germination incubated with silver nanoparticles (Morones *et al*., 2005). Inhibitory effect was found to be dependant over incubation and the concentration of silver nanoparticles as indicated in the present study. In the present study nanoparticles of Mgo and nano ZnO were tested for antimycotic activity. The inhibitory effect of nanoparticles may be due to release of extracellular enzymes and metabolites that serve as an agent for their own survival when exposed to stress from toxic materials and temperature variations as have been demonstrated in in case of fungus *Trichoderma reesei* and other fungi (Pere-de-Luque and Diego, 2009). Antimycotic activity of some nanoparticles of silver have also been reported on some fungi like wood rotting fungi, *Fusarium* species and othe phytopathogenic fungi. It is also observed that antifungal activity of may be due to suppression of enzymes and toxins used by the fungal pathogens for pathogenesis (Bhainsa, and D'Souza, 2006; Vahabi *et al*., 2011).

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