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ANTIMYCOTIC ACTIVITY OF LEAD OXIDE (PbO) NANOPARTICLES

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ABSTRACT: Antimycotic activity of Lead oxide (PbO) nanoparticles prepared by the bio-safe method was evaluated for rot causing fungi. 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. The highest inhibition in the spore germination was found against *Aspergillus niger* and it varies from 74.26% to 37.63% in different concentrations of nano Pb, whereas the least reduction of spore germination was found against *P. expansum* and it ranges from 52.44% to 20.47% in different concentrations of nanoparticles respectively, as compared to untreated control. Similarly, inhibition in spore germination of all other tested fungi also varies significantly with the increase in the concentrations of Pb nanoparticles, and the highest concentration caused the highest reduction in the spore germination followed by lower concentrations of Pb nanoparticles, respectively. However, the maximum inhibition in the fungal growth was observed in the case of *Alternaria alternata* with the zone of inhibition of 11.00 mm, 12.00 mm, and 13.33 mm at 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml of Pb nanoparticles, respectively. The inhibition in the zone of fungal growth due to Pb nanoparticle against *Aspergillus niger* and *Penicillium expansum* was 09.66 mm, 10.66 mm, 12.66 mm, and 9.00 mm, 10.00 mm, 11.33 mm at 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml concentration respectively. The activity index was recorded highest against *A. niger* (0.90) and lowest against *P. chrysogenum* in the case of PbO at 0.5 mg/ml concentration.

INTRODUCTION: Nanotechnology involves the study, control, and manipulation of materials at the nanoscales, typically having dimensions less than 100 nm [1-2]. In recent years, the synthesis of nanomaterials is important to research in the various scientific and industrial fields [3-4]. The properties of such materials are novel and can be engineered by controlling the dimensions of these building blocks and their assembly via physical, chemical, or biological methods [5].

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Nanostructured materials have unique chemical and physical properties, but their physical application in many fields has stimulated the search for a new synthetic method for the material. The lead element has a lot of oxide forms, including PbO, Pb_2O_3, and PbO2. Lead oxide (PbO), is an important industrial material due to its unique electronic, mechanical and optical properties and its potential applications in nanodevices and functionalized materials [6].

Nanoparticles have great potential in the management of different diseases; they have great antimicrobial properties and are stable under harsh process conditions [7]. The use of nanoparticles as antifungal and antibacterial agents may have potential as a substitute for fungicides for the management of fungal rot of vegetables with less hazardous effect on plant and environmental health.

Therefore, in this study, an attempt was made to use an alternate eco-friendly management strategy in terms of the use of synthesized nanoparticles.

MATERIALS AND METHODS:

Antifungal Assay:

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.

Spore Germination Assay: To evaluate the efficacy of Lead 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 Lead oxide nanoparticles was prepared from the precipitated sample. Spore suspension with 1×10^3 conidia/ml was prepared in sterilized distilled water. An 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 the case of control, spore suspension was mixed with an 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 [8].

Percent spore germination = Number of spores germinated / Total number of spores examined × 100

Inhibition of spore germination (%) = Gc – Gt / Gc × 100

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

Agar well Diffusion Assay: The antifungal activity of the Lead oxide nanoparticles was determined by the agar well diffusion method as adopted by [9]. 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 Sabourad 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 the laminar air flow 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 Lead 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 a standard scale [10].

Assessment of Activity Index: The assessment of the activity index was calculated by comparing the inhibition zones of nanoparticles with the standard using the formula given by[11].

Activity Index = Inhibition zone by the NP sample / Inhibition zone by the standard

Statistical Analysis: Statistical analysis was carried out using SPSS statistical software (version 16.0). Data were analyzed by one-way analysis of variance (ANOVA), and a comparison of the means was done by Duncan multiple comparison tests at $P \leq 0.05$.

RESULTS:

Effect of PbO Nanoparticles on the Spore Germination and Mycelial Growth of Rot Causing Fungi: It was observed from the results **Table 1, Fig. 1** that different concentrations of Pb nanoparticle caused inhibition in the spore germination of all the tested fungi such as *Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicillium chrysogenum*, *Trichothecium roseum*, and *Rhizoctonia solani*. The inhibition in spore germination increased with the increase in the concentration of nanoparticles. However, the maximum inhibition in the spore germination was

found at the highest concentration of 0.5 ml.

It was followed by 0.2 ml and 0.1 ml concentrations of nanoparticles. The highest inhibition in the spore germination was found against *Aspergillus niger*, and it varies from 74.26% to 37.63% in different concentrations of nano Pb, whereas the least reduction of spore germination was found against *P. expansum* and it ranges from 52.44% to 20.47% in different concentrations of nanoparticles respectively, as compared to untreated control. Similarly, inhibition in spore germination of all other tested fungi also varies significantly with the increase in the concentrations of Pb nanoparticles. The highest concentration caused the highest reduction in the spore germination, followed by lower concentrations of Pb nanoparticles, respectively.

TABLE 1: EFFECT OF PbO NANOPARTICLES ON THE SPORE GERMINATION OF ROT CAUSING FUNGI

Concentration Fungal Pathogens	Spore Germination (%)			
	0.1 mg/ml	0.25 mg/ml	0.5 mg/ml	Control
Penicillium expansum	52.44±0.015^{b}	45.77± 0.01^{c}	20.47± 0.01^{d}	89.64± 0.015^{a}
Aspergillus niger	74.26±0.015^{b}	63.45±0.015^{c}	37.63±0.015^{d}	93.36± 0.01^{a}
Alternaria alternata	62.37± 0.01^{b}	54.34± 0.02^{c}	21.44±0.015^{d}	87.46± 0.015^{a}
Mucor plumbeus	63.44± 0.02^{b}	52.62±0.015^{c}	28.37± 0.01^{d}	88.64± 0.02^{a}
Penicilliumchrysogenum	53.46±0.015^{b}	46.36±0.015^{c}	21.58± 0.01^{d}	91.47± 0.01^{a}
Trichothecium roseum	55.37± 0.01^{b}	47.76±0.015^{c}	22.34± 0.02^{d}	93.35± 0.015^{a}
Rhizoctonia solani	57.36±0.03^{b}	45.36± 0.01^{c}	26.36±0.015^{d}	92.26± 0.015^{a}

Each value is mean of 3 replicates ± SD Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$)

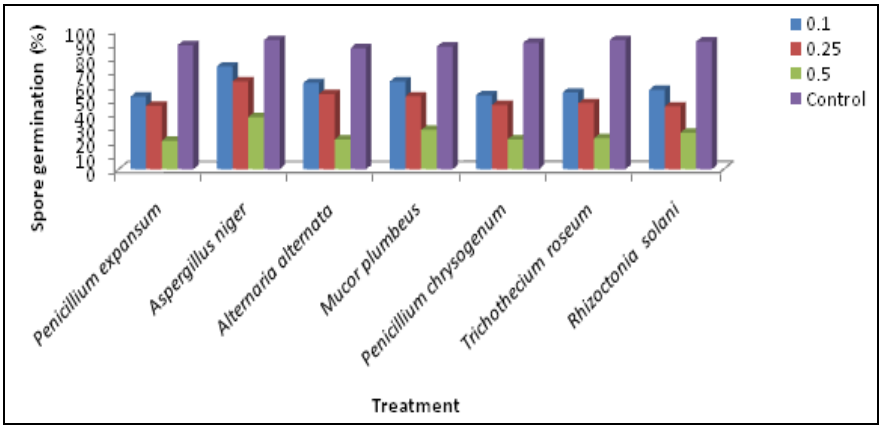

FIG. 1: EFFECT OF PbO NANOPARTICLES ON THE SPORE GERMINATION OF ROT CAUSING FUNGI

Further, it was observed from the results **Table 2, Fig. 2** that Pb nanoparticles caused inhibition in mycelial growth of all the tested fungi at different concentrations, but the highest concentrations proved more effective than lower concentrations in reducing the mycelial growth of fungi.

However, the maximum inhibition in the fungal growth was observed in the case of *Alternaria alternata* with the zone of inhibition of 11.00 mm, 12.00 mm, and 13.33 mm at 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml of Pb nanoparticles respectively.

The inhibition in the zone of fungal growth due to Pb nanoparticle against *Rhizoctonia solani* and *Trichothecium roseum* was 10.66 mm, 11.66 mm, 13.33 mm, and 10.66 mm, 11.00 mm, 13.33 mm at 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml concentration respectively. While as moderate inhibitory activity of Pb nanoparticles was shown against *Mucor plumbeus* and *Penicillium chrysogenum* with the zone of inhibition of 10.00 mm, 11.33 mm, 13.66 mm and 7.66 mm, 11.00 mm, 12.33 mm at 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml concentration respectively.

The inhibition in the zone of fungal growth due to Pb nanoparticle against *Aspergillus niger* and *Penicillium expansum* was 09.66 mm, 10.66 mm, 12.66 mm, and 9.00 mm, 10.00 mm, 11.33 mm at 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml concentration respectively. The results were compared with hexaconozole as control.

TABLE 2 EFFECT OF PbO NANOPARTICLES ON THE MYCELIAL GROWTH OF ROT CAUSING FUNGI

Concentration Fungal Pathogens	Zone of Mycelial Inhibition (mm)			
	0.1 mg/ml	0.25 mg/ml	0.5 mg/ml	Control
Penicillium expansum	09.00 ± 1.00^{c}	10.00±1.00^{bc}	11.33 ±1.15^{b}	13.33 ± 0.57^{a}
Aspergillus niger	9.66 ± 0.57^{c}	10.66 ± 0.57^{c}	12.66 ± 0.57^{b}	14.00 ± 1.00^{a}
Alternaria alternata	11.00 ± 1.00^{c}	12.00±1.00^{bc}	13.33 ± 0.57^{b}	15.33 ± 0.57^{a}
Mucor plumbeus	10.00 ± 1.00^{c}	11.33± 1.00^{c}	13.66 ± 0.57^{b}	16.66 ± 0.57^{a}
Penicilliumchrysogenum	7.66 ± 1.52^{c}	11.00 ± 1.00^{b}	12.33 ± 0.57^{b}	20.00 ± 1.00^{a}
Trichothecium roseum	10.66 ± 0.57^{c}	11.00 ± 1.00^{c}	13.33 ± 0.57^{b}	15.00 ± 1.00^{a}
Rhizoctonia solani	10.66 ± 0.57^{c}	11.66 ± 0.57^{c}	13.33 ± 1.15^{b}	16.00 ± 1.00^{a}

Each value is mean of 3 replicates ± SD Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$)

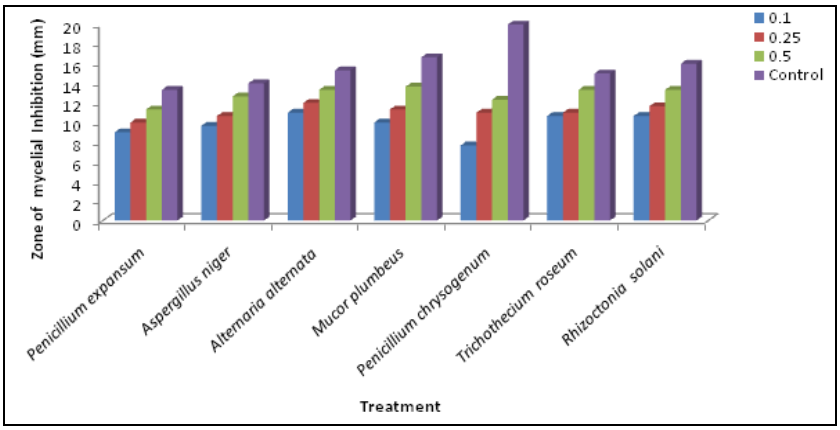

FIG. 2: EFFECT OF PbO NANOPARTICLES ON THE MYCELIAL GROWTH OF ROT CAUSING FUNGI

Assessment of Activity Index: The activity index of Lead oxide nanoparticles at different concentrations against all the selected fungi sgivenin **Table 3**. The activity index depends on the zone of inhibition. The activity index of Lead oxide NP was highest against *A. niger* (0.90) at 0.5 mg/ml concentration followed by *T. roseum* (0.88), *A. alternata* (0.86), *P. expansum* (0.84), *R. solani* (0.83), *M. plumbeus* (0.81) and least activity was shown against *P. chrysogenum* (0.61) at the same concentration. However, other concentrations also proved effective but to a lesser extent.

TABLE 3: ACTIVITY INDEX OF PbO, NANOPARTICLES AT DIFFERENT CONCENTRATIONS AGAINST DIFFERENT FUNGI

Fungal Pathogens	Activity Index		
	0.1 mg/ml	0.25 mg/ml	0.5 mg/ml
Penicillium expansum	0.67	0.75	0.84
Aspergillus niger	0.69	0.76	0.90
Alternaria alternata	0.71	0.78	0.86
Mucor plumbeus	0.60	0.68	0.81
Penicilliumchrysogenum	0.38	0.55	0.61
Trichothecium roseum	0.71	0.73	0.88
Rhizoctonia solani	0.66	0.72	0.83

DISCUSSION: In the present study, nanoparticles of PbO was tested for their antifungal activity. It was clear from the results that Lead oxide nanoparticle 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. These nanoparticles, which have been evaluated for the first time for their antifungal activity against rot causing fungi in Kashmir Valley, may help in a long way for screening other nanomaterials for their antifungal activity [12]. Observed a significant reduction in mycelial growth and spore germination incubated with silver nanoparticles [13]. Studied the antifungal activity of silver/chitosan nanoformulations against *Aspergillus flavus*, *Alternaria alternata*, and *Rhizoctonia solani* and reported that the silver/chitosan NF caused the highest inhibition against *Aspergillus flavus*

followed by *Alternaria alternata* and *Rhizoctonia solani* [14]. Also reported that the highest concentration of MgO, FeO, and ZnO nanoparticles proved effective than the lower concentrations [15] reported the antimycotic activity of MgO and ZnO nanoparticles against *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus* [16] evaluated the antifungal effect of copper and copper oxide nanoparticles against *Penicillium* on Orange Fruit [17]. Studied the antifungal activity of Iron oxide nanoparticles against *Trichothecium roseum*, *Cladosporium herbarum*, *Penicillium chryso-genum*, *Alternaria alternata*, and *Aspergillus niger* and reported that higher concentrations of iron oxide NP's proved more effective [18].

Studied the antifungal activity of leaf extract of *Platanus orientalis* against *Mucor piriformes* and *Aspergillus niger* and observed that higher concentrations of leaf extracts proved more effective than lower concentrations [19] evaluated the antifungal activity of Magnesium oxide and Iron oxide nanoparticles against the fungi causing rot diseases of tomato and brinjal [20] studied the antifungal activity of Tungtan oxide nanoparticles against *Fusarium oxysporum*, *Penicillium funiculosum*, *Candida albicans* and *Trichoderma resei* and observed that higher concentrations proved more effective than lower concentrations.

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 reduces 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.

CONCLUSION: It was concluded from the results that the nanoparticles at different concentration brought about significant inhibition in mycelial growth and spore germination of fungi causing fungal rot of tomato and brinjal. However the highest concentration of the nanoparticles caused maximum inhibition in mycelial growth and spore germination followed by lower concentrations. These nanoparticles thus may have potential as a substitute for fungicides for the management of fungal rot of vegetables with less hazardous effect on plant and environmental health. However, further research is needed to screen these nanoparticles for their antimycotic activity.

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CONFLICTS OF INTEREST: Authors declare that they have no conflict of interest.

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