

Original Research Article

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## Evaluation of Antimicrobial Activity of Silver Nanoparticles against Pathogens causing Rhizome Rot of Ginger

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### ABSTRACT

Nanotechnology can offer green and eco-friendly alternatives for plant disease management. In this study, the investigation was carried out for the antifungal activity of silver nanoparticles against the pathogens involved in the rhizome rot complex such as *Fusarium oxysporum*, *Pythium aphanidermatum*, *Sclerotium rolfsii*, and *Ralstonia solanacearum*. Metal nanoparticles of different concentrations such as 1 ppm, 5ppm, 10 ppm, 15 ppm, 20 ppm, 40 ppm, 50 ppm, and 100 ppm were treated against the pathogen complex by poison food technique as well as agar well method to determine the antimicrobial activities *in vitro*. Observations were recorded 12 hr, 24 hr, 48 hr, and 72 hr of pathogen inoculation. Application of 100 ppm concentration of silver nanoparticles produced maximum inhibition of the growth of the fungal as well as bacteria. However, 15 ppm, 20 ppm, 40 ppm, 50 ppm were also effective as compared to control. Microscopic observation revealed that nanoparticles caused damage effects on fungal hyphae and conidia. The study also revealed that the antifungal effects of silver nanoparticles are positively correlated to the concentration of nanoparticles as well as the time of exposure. Further, it can be exploited as an alternative for chemical management in the future.

#### Keywords

Nanoparticles,  
Antifungal,  
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### Introduction

Ginger (*Zingiber officinale* Rosc.) belongs to the family *Zingiberaceae* is one of the earliest known oriental spices and is being cultivated in India for both as fresh vegetable and as a dried spice since time immemorial. It is an herbaceous perennial, the rhizomes of which are used as a spice. Ginger is cultivated in most of the states in India. However, states namely Karnataka, Kerala, Orissa, Sikkim, Assam, Meghalaya, Arunachal Pradesh, and

Gujarat are the major ginger growing states in India.

The crop is affected by a variety of diseases like soft rot or rhizome rot, leaf spot, and bacterial wilt diseases among which soft rot or rhizome rot cause severe damage. Among the major constraints of ginger production, rhizome rot is very important because of severe crop losses. It occurs in several parts of India wherever the crop is grown. The term rhizome rot is commonly used for all the

diseases affecting the rhizome irrespective of pathogens involved since the ultimate result is the partial or total loss of rhizome. The pathogens involved decide the nature of the damage and also symptoms expression.

Rhizome rot disease has now become a major threat to all ginger growing areas causing huge economical losses. There are no curative effective methods for the management of rhizome rot and also preventive options are cumbersome and are not fully encouraging with any chemicals and biological.

There is still a need to develop alternative management strategies by using innovative techniques such as the use of elicitors to induce defense response in plants, use of nanoparticles to find the efficacy against the pathogen complex to ensure a more economical, feasible, and effective disease management schedule.

Nanotechnology offers an eco-friendly alternative for plant disease management and has many advantages over conventional chemical methods. Nanomaterials in agriculture aim to reduce the amount of sprayed chemical products by smart delivery of active ingredients. Silver nanoparticles are antibacterial, antifungal, and nematicidal in nature which can be effectively used as an alternative management strategy against the complex disease.

## Materials and Methods

### Isolation of pathogens involved in the disease complex

Rhizomes showing typical symptoms of water-soaked areas were cut into small pieces of 2 mm along with some healthy portions. The pieces were surface sterilized with 0.1 percent mercuric chloride solution for 20-30 sec, followed by subsequent washing with

sterile distilled water for three times. Six to seven pieces of infected rhizomes bits (5 mm) were blot dried on sterile filter paper and placed on cornmeal agar (CMA) supplemented with Pimaricin for the isolation of *Pythium* sp, PDA media supplemented with PCNB used for isolation of *Fusarium* spp. Specific TZC agar medium used for the isolation of virulent colonies of *Ralstonia solanacearum*

### Evaluation of silver nanoparticles against pathogens under *In vitro* conditions

An *In vitro* study was carried out to evaluate the antifungal activity of silver and silver nanoparticles against the four pathogens *R. solani*, *Pythium aphanidermatum*, *F. oxysporum*, and *S. rolfsii* causing rhizome rot of ginger. Efficacy of silver nanoparticles was tested at different concentrations such as 1 ppm, 5 ppm, 10 ppm, 20 ppm, 40 ppm, 50 ppm, 100 ppm by food poison technique.

The comparison was made with the recommended dose of a chemical at different concentrations. All the treatments were replicated three times. Control (only pathogen) was maintained without silver and silver nanoparticles and chemical. All the plates were incubated at  $28 \pm 1$  °C for 10 days. Observation on colony diameter was measured at 24 hr, 48 hr, and 72 hr interval until the control reached its maximum growth. The following formula is used for the calculation of the inhibition rate (%).

$$\text{Inhibition rate (\%)} = [(R-r) / R] \times 100$$

Where,

R is the radial growth of fungal mycelia on the control plate and r is the radial growth of fungal mycelia on the plate treated with silver and silver nanoparticles.

## Results and Discussion

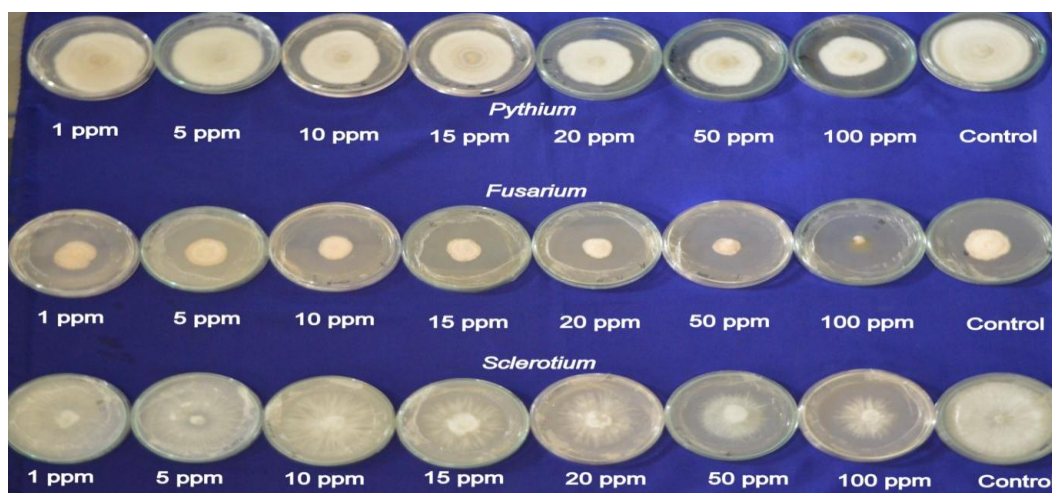
The silver nanoparticles were found very effective against all the pathogens. An increase in the concentration of nanoparticles effectively inhibited pathogen growth (Table 1). 50 ppm, 100 ppm concentrations of silver nanoparticles showed 36.94, 38.95 percent inhibition of the *Pythium aphanidermatum* after 24 hrs of inoculation. The control

treatment without any silver nanoparticles showed zero inhibition. The inhibition rate increased per concentration and time of exposure. After 72 hrs of pathogen incubation, *Pythium* showed 48.073 percent inhibition at 100 ppm concentration. In the case of *Fusarium oxysporum*, silver nanoparticles showed 69.16 percent inhibition at 100 ppm concentration after 24 hrs of incubation.

**Table.1** *In vitro* evaluation of silver nanoparticles against pathogens

Percent Inhibition over control									
Incubation period	Control	Silver nanoparticles against <i>Pythium aphanidermatum</i>							
		1 ppm	5 ppm	10 ppm	15 ppm	20 ppm	40 ppm	50 ppm	100 ppm
24 hr	0.00	6.82	9.63	13.65	20.08	28.11	34.53	36.94	38.95
48 hr	0.00	10.00	12.22	13.89	19.26	22.22	29.26	34.44	43.33
72 hrs	0.00	12.85	15.96	17.037	24.443	31.667	36.11	39.447	48.073
Silver nanoparticles against <i>Fusarium oxysporum</i>									
24 hr	0.00	40.00	45.33	47.50	52.50	55.00	58.33	61.66	69.16
48 hr	0.00	42.18	48.86	50.24	54.53	57.08	62.20	68.94	71.68
72 hrs	0.00	45.00	49.91	51.66	57.16	63.46	66.91	70.00	75.25
Silver nanoparticles particles against <i>Sclerotium</i>									
24 hr	0.00	1.23	12.37	14.45	23.52	28.08	34.28	38.82	42.56
48 hr	0.00	10.74	16.67	17.04	28.89	33.33	34.44	45.18	48.89
72 hr	0.00	13.89	20.00	20.55	23.89	25.00	42.96	46.11	50.89

**Fig.1** *In vitro* evaluation of Silvers against *Pythium*, *Fusarium* and *Sclerotium*



Percent inhibition of the pathogen increased over the period time and at 72 hrs pathogen showed 71.68 and 75.25 percent inhibition at 50 ppm, 100 ppm concentration. As compared to *Pythium aphanidermatum*, *Fusarium oxysporum* showed more inhibition. In the case of *Sclerotium rolfsii*, increased concentration and time of exposing increased the inhibition of the pathogen. The control samples showed zero inhibition at all the periods. 50 ppm, 100 ppm concentrations of silver nanoparticles showed 38.82 and 42.56 percent inhibition of the *Sclerotium rolfsii* after 24 hrs of inoculation. After 72 hrs nanoparticles showed 46.11 and 50.89 percent inhibition against *Sclerotium rolfsii* at 50 and 100 ppm concentration (Fig. 1).

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