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Research Article

THE POTENTIAL OF NANOPARTICLES TO CONTROL BLACK SCURF DISEASE OF POTATO CAUSED BY *RHIZOCTONIA SOLANI* UNDER *IN VITRO* CONDITIONS

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Abstract: Nanoparticles have been known to have strong anti-microbial properties and therefore, these days, they are being used to control plant pathogenic diseases efficiently. In this context present study, we have assessed the effect of three different nanoparticles, *i.e.*, AgNPs, CuONPs and MgONPs of different concentrations like 25, 50, 75, and 100 ppm against the growth of against *Rhizoctonia solani* under *in vitro* conditions. Findings reveal that all the three nanoparticles showed inhibitory effects on mycelial growth and sclerotium production. However, increasing the dose of such nanoparticles shows more inhibition on mycelial growth along with sclerotium production as well. Hence, it is concluded that for controlling the plant disease by using nanoparticles is one of the eco-friendly approaches as compared to using the expensive chemicals.

Keywords: *Rhizoctonia solani, Nanoparticles*

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Introduction

Rhizoctonia solani is an important plant pathogenic fungi which is soil-borne in nature and has a global distribution with a wide host range also most widely recognized species of *Rhizoctonia* which was originally described by Julius Kühn on potato in 1858 [1]. The pathogen (*R. solani*) is well known to cause "Black scurf" disease of potato and can survive up to years without a host. A key structure called sclerotia helps the fungus to survive in an adverse condition; sclerotia is a compact mass of hardened structure which is about 2.48±0.32 mm in diameter [2]. The broad host range of the fungus makes it very difficult to control. *R. solani* can cause different diseases in different crops like "damping off" disease.

Nanoparticles

Nanotechnology is a field of science that includes the synthesis of several nanomaterials. Nanoparticles have unique physicochemical, biological and optical characteristics, and are used as antimicrobials in different fields. They also have many applications in [3] optical devices,[4] catalytic processes, [5] biological labelling, [6] and electronics and [7] may suppress the expression of proteins associated with adenosine triphosphate production.

Silver nanoparticles

Silver nanoparticles (AgNPs) range from 1 nm to 100 nm in size have the great potential to suppress the black scurf disease of potato and are also used in different industries due to their anti-microbial properties [8]. AgNPs are commonly used in textile and electronic industries to control microbes [9]. Nanoparticles can impart a negative effect on pathogen by penetrating the cells [10], sticking to cell surface[11], changing cell membrane properties which finally disrupts the DNA structure that results in death[12], interrupts cellular metabolism and respiration processes[13], produces reactive oxygen species (ROS), particularly superoxide hydroxyl and radical, that damage the cell[14].

Copper nanoparticles

A copper nanoparticle is a copper-associated nanoparticle that has 1 to 100 nm in size [15]. Copper nanoparticles show unique property including antifungal/antibacterial and catalytic properties. Firstly, CuONPs confirm a very effective catalytic action, a feature that can be linked with their catalytic surface area, used as reagents in controlling fungal growth, tight interaction with microbial cell membranes, produce toxic hydroxyl free radicals against the lipid membrane. As a result, intracellular bodies flow out from the cells; after which the cells are not able to provide fundamental biochemical processes. All these changes inside the cell generate free radicals which eventually lead to cell death [16].

Magnesium oxide nanoparticles

Magnesium nanoparticles are spherical dark black with high surface area particles typically 25-65 nm in size with a particular surface area extending from 30 to 80 m^2/g [17]. These nanoparticles are also prepared in ultra-high purity with coated, and scattered forms. MgONP is a metal-based antimicrobial nanoparticle that can be used as an antimicrobe in various plant pathogenic diseases. MgONPs exerts inhibitory, bactericidal, and fungicidal effects against prevalent bacteria, fungi, and yeasts [18]. Many research studies have investigated that the nanoparticles have the inheritance properties to inhibit the microbes including bacteria, fungi, yeast etc., efficiently without harming the crop health [19].

Materials and Methods

Synthesis of Nanoparticles

$AgNO_3$ was utilized as the initial reactant for Silver nanoparticle synthesis and 1% Trisodium citrate (TSC) and Ascorbic acid as reducing agents and stabilizers, respectively. About 80 ml of laboratory prepared $AgNO_3$ was added to 20 ml of 1 % of TSC and put on ice and added ascorbic acid step by step up to a stage where solution become colorless after constant stirring [20,35]. When the solution becomes colorless, it is stored at room temperature and was characterized by

Monochromatic laser light scattering and UV-Vis Spectrophotometer (300-700nm wavelength).

MgONPs: requires 5 grams of Magnesium sulphate ($MgSO_4$) which was liquified in 100 ml of sterilized distilled water and 5 grams of sodium hydroxide (NaOH) was dissolved in 100 ml of sterilized distilled water separately. Then after 50 ml of Magnesium sulfate solution and 50 ml of Sodium hydroxide solution were mixed [Fig-1]. The solution was continuously stirred with a magnetic stirrer for about 2 hrs after which Magnesium hydroxide was precipitated. The solution obtained after the precipitation was heated at 100°C for 2 hours in a hot air oven. The precursor, Magnesium hydroxide (MgONPs) synthesized. The precursor was then put in a muffle furnace at 300°C for 3 hrs after which MgONPs were produced and characterized by monochromatic laser light scattering and UV-Vis Spectrophotometer (200–900 nm wavelength) [19, 36].

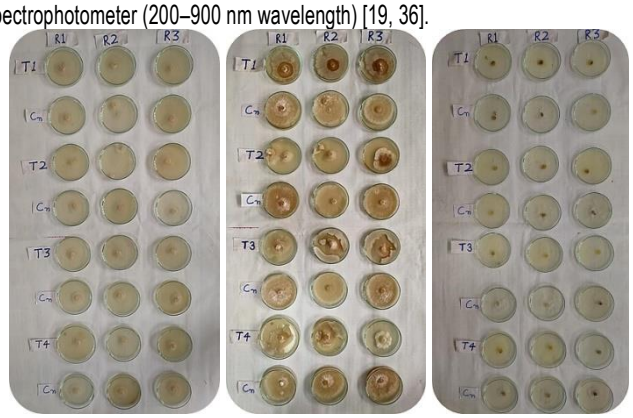

Fig-1 Efficacy of nanoparticles (Ag NPs, CuO NPs and MgO NPs) against *R. solani*
Notes: T_1= 25 ppm, T_2= 50 ppm, T_3= 75 ppm, T_4= 100 ppm and Cn= Control

CuONPs: obtained were synthesized by chemical reduction means using copper sulfate ($CuSO_4$) as precursor and starch was used as a capping agent. The preparation procedure begins with the addition of 0.1 M copper sulfate solution into 120 mL of starch solution (1.2 %) by using magnetic stirrer for 30 minutes. In the next step, 50 mL of 0.2 M ascorbic acid solution was added to the synthesis solution which was also mixed for the next 30 minutes by using a magnetic stirrer. Subsequently, 30 mL of 1 M Sodium hydroxide solution was gradually added to the prepared solution by continuous stirring and heating at 80°C for 2 hrs. The color intensity of the solution turned from yellow to ocher. After the end of the reaction, the solution was obtained from the heat and left to settle overnight and the supernatant solution was then discarded carefully. The precipitates were isolated from the solution by filtration and rinsed with deionized water and ethanol three times to get out the unnecessary starch coated with the nanoparticles. Ocher color precipitates obtained were dried at room temperature. After drying, nanoparticles were collected and stored in a glass vessel for further interpretation and characterized by monochromatic laser light scattering and UV-Vis Spectrophotometer (190–800 nm wavelength) [15,16, 37].

Food poisoning method

R. solani investigated in the present study was isolated from the infected potato disease and cultured on PDA for the future usage. To evaluate the *in vitro* antifungal actions of nanoparticles against *R. solani*, four different concentrations of nanoparticles had been taken (25, 50, 75, and 100 ppm) to petri dishes. *R. solani* plug with a diameter of 10 mm were inoculated simultaneously at the centre of each Petri dish containing nanoparticles. Nanoparticles *i.e.*, AgNPs, CuONPs, MgONPs were incorporated at different ppm on PDA media separately at 25, 50, 75 & 100 ppm [Table-1]. Sterile media containing AgNPs were swirled thoroughly before being plated on to petri dishes. Once the media had solidified, a 10 mm disc of 7-day-old *R. solani* culture was placed in the centre and incubated at 25+l° C for 3 days. The growth of *R. solani* was determined as colony area after 3 days of incubation and compared with the control plate. The colony growth area was measured. Control was prepared without nanoparticles solution. Percent growth inhibition was measured by the Vincent's (1947) formula as follows:

Mycelial growth inhibition (I %) = = C–T/C × 100

Where, I= is the mycelial growth inhibition (%), T= diameter of the fungal colony on the treated plate and C= diameter of the fungal colony on the control plate. Average of three replications of each test is taken for calculations.

Table-1 *Calculation on Nanoparticles for application on PDA Medium*

PPM	Nanoparticles Solution (ml)	PDA Medium(ml)	Net volume(ml)
25	1.5	58.5	60
50	3.0	57.0	60
75	4.5	55.5	60
100	6.0	54.0	60

Statistical analysis

All data presented are the mean values of three replicates. Values are expressed as means of three replicates ± standard error (S.E) in each group. All statistical analyses were performed using One-way Analysis of Variance (ANOVA).

Results and Discussion

Efficacy of nanoparticles

Ag nanoparticles

Silver nanoparticles (AgNPs) are known to have anti-microbial properties and therefore have the potency to control fungal plant pathogens including *R. solani*. In this study, we investigated the growth of *R. solani* in the presence of AgNPs. The effect of AgNPs at different ppm concentration (25, 50, 75 and 100) on mycelial growth in *R. solani* was studied. The results showed that at 25, 50, 75 and 100 ppm concentration AgNPs have suppressed *R. solani* mycelial growth along production of sclerotia.

In addition, greater than 60% (64.86%) inhibition was observed against *R. solani* when treated with a 100 ppm AgNPs on PDA medium. The lowest level of inhibition was observed against *R. solani* when treated with a 25 ppms concentration *i.e.*, 38.89 percent concentration of AgNPs on PDA [Table- 2]. The results symbolize that AgNPs have the potential to manage *R. solani* growth and the development of plant disease symptoms. The presence of AgNPs in growth media limited the growth rate of *R. solani*.

Antifungal activity of biogenically produced silver and gold nanoparticles against *Rhizoctonia solani*, the cause of rice sheath blight [22]. Both nanoparticles were evaluated against the pathogen at concentrations of 1, 5, 10, 50, 100, and 200 ppm. Silver nanoparticle (Ag NP) at 200 ppm showed the highest inhibition (73.39%) in the radial growth of *R. solani*, while gold nanoparticle (AuNPs) at the same concentration inhibited the growth of the pathogen by up to 60.83%. AgNPs at 200 and 100 ppm caused complete inhibition of sclerotial germination of *R. solani*. In a pot experiment to test the efficiency of Ag NP at 200 ppm against rice sheath blight, it was discovered that treatment of Ag NP boosted plant growth characteristics compared to control, with a lower percentage of disease incidence (20.00%) than inoculated control *R. solani* (88.00 %). Secondary metabolites such as phenols, flavonoids, terpenoids, and total soluble sugars were also elevated when Ag NP was applied [21].

Oktarina *et al.* [23] earlier showed that AgNPs, at 75 and 100 ppm reduced the mycelial area of *R. solani* significantly when compared to controls (*i.e.*, no AgNPs). A previous study had stated that very low AgNPs concentration (2-6 mg) suppress hyphal growth of *R. solani*. Similarly, the study uses six different strains of *R. solani* that are sensitive to low levels of AgNPs. These findings imply that sensitivity of *R. solani* by silver is likely varied between different fungal strains of the same species but this should be examined further using comparable growth conditions.

Kim *et al.* [24] reported that silver nanoparticles (AgNPs) range from 1nm and 100 nm in size and are widely engaged in research studies due to their antimicrobial properties. Common applications involve the use of AgNPs for antimicrobial coats on particle silica to control bacteria and other microbes they may adhere to the cell surface, penetrate the cells, change cell membrane characteristics and ultimately result in DNA destruction due to the dissolution of Ag ions. AgNPs also produce reactive oxygen species (ROS), especially hydroxyl radical, and superoxide radical, that result in damage to the cell. The precise anti-microbial mechanism of AgNPs is however, not completely understood.

Table-2 *Mycelial growth inhibition of Rhizoctonia solani by different concentration of nanoparticles in potato dextrose agar media*

Nanoparticles	Concentration (in PPM)	Control Average	Mycelial growth average	Average of Mycelial growth inhibition
Silver nanoparticles (Ag NPs)	25	67.40	41.17	38.89
	50	58.44	33.13	43.33
	75	60.80	29.30	51.74
	100	67.53	23.73	64.86
Magnesium oxide (MgO NPs)	25	47.67	24.50	48.15
	50	47.50	20.83	56.09
	75	46.30	19.50	57.81
	100	48.50	18.50	61.82
Copper oxide nanoparticles (CuO NPs)	25	35.40	27.50	21.83
	50	40.50	26.50	34.50
	75	43.20	25.80	39.84
	100	59.40	31.50	46.95
C.D. (at 5 %)		3.25	4.76	13.08
SE(m)		1.11	1.62	4.46

The potential of AgNPs to suppress *R. solani* has been studied as the effects of silver nanoparticles on sclerotium-forming *R. solani* fungi.

AgNPs cause cell wall disintegration, surface protein damage, nucleic acid damage by production and accumulation of ROS and free radicals, and blockage of proton pumps. AgNPs are thought to cause a buildup of silver ions, which obstructs respiration by causing intracellular ion efflux, causing harm to the electron transport system. Antifungal action is linked to nanoparticles with a smaller size to big surface ratio. AgNPs with smaller size can penetrate easily through cell boundaries. The toxicity of AgNPs is partially attributed through production of reactive oxygen species (ROS), which leads to apoptosis [25].

The current study validates that AgNPs have the potency to suppress the mycelial growth of *R. solani*. A study had described that low AgNP concentrations (25-100 µL/L) inhibited mycelial growth of *R. solani* by causing an irregular shape of the hyphal walls which then failed to multiply. These findings insinuate that the sensitivity of silver is likely to inhibit the growth of *R. solani*.

CuO nanoparticles

Copper oxide nanoparticles (CuO NPs) were evaluated *in vitro* against *R. solani* at four different concentrations *i.e*., 25, 50, 75, and 100 ppm concentration. Results [Table-2] indicate that all evaluated concentrations of nanoparticles suppressed the mycelial growth of *R. solani* in comparison to control. However, copper oxide nanoparticles displayed the highest inhibition of mycelial growth of *R. solani* (46.95%) at concentration 100 ppm whereas, lowest inhibition of mycelial growth (21.83%) at 25 ppm concentration. Furthermore, CuONPs exhibited a clear percent reduction in fungal mycelial growth in the range of 21.83-46.95 at all concentration. The acquired results coincided with those examined by Kim [26] who asserted that the mycelial growth rate of *R. solani* was reduced typically by more than 90% at a 60 ppm the concentration of Nano sized copper and silica hybrid silver complex (NSS). Similar results were also studied, Kanhed [27] investigated CuNPs extraordinary inhibitory action against *A. alternata, F. oxysporum,* and *C. lunata*. The increased antifungal activity of CuNPs was due to their larger surface area to volume ratio.

MgO nanoparticles

This investigation was carried out to test the efficiency of magnesium oxide nanoparticles (MgONPs) to control the plant pathological fungus *R. solani* in the culture media *i.e*., PDA. The result of the table showed an effect of MgO NPs in inhibition of the *R. solani* fungus at 25, 50, 75, and 100 ppm compared with the control sample, and the inhibition rates were 48.15, 56.09, 57.81, and 61.82 percent, respectively, where the MgO nanoparticles affected greatly and reached to 61.82 percent in the *R. solani* mycelial inhibition at 100 ppm. These results coincide with the results of multiple studies which referred to the high activity of nanoparticles and their oxides in inhibition many of the plant pathogenic fungi [28; 29; 30)]. Kim [31] also reported high effectiveness of AgONPs and MgONP at 100 ppm against plant pathogenic fungi on PDA media.

Ismail [32] found that antifungal effect of CuONPs and MgONPs on the mycelial growth of *R. solani in vitro*. All tested concentrations of the tested NPs reduced the mycelial growth of *R. solani* relative to control treatment. The treatment with MgONPs at 200 mg/L exhibited the greatest inhibitory effect in reducing the growth of *R. solani*, with inhibition reached 73.47 % followed by CuONPs at 200 mg/L, which recorded 62.02 % inhibition of the growth of *R. solani*. On the other hand, low concentrations of CuONPs had a weak effect on the growth of *R. solani*. Moreover, the inhibitory effect of CuONPs and MgONPs was decreased gradually with decreasing concentration. None of the tested NPs resulted in a complete inhibition of *R. solani* growth *in vitro*.

It was highlighted by Al-Kaise [33] that the great efficiency of MgO nanoparticles which gave up to 100% inhibition ratio at 2 % and 3% concentrations and 95.33% in 1 ppm concentration in *A. flavus* fungus inhibition in the collected seeds of corn. These measured concentrations were 1, 2, and 3 percent. The weights 1, 2, and 3 grams of MgO nanoparticles were used and to each one of these weights, 100 ml of distilled water and mixed to get 1, 2 and 3 percent concentrations, which results in mycelial inhibition of *R. solani* 96.33, 100 and 100 percent, respectively.

Silver leads to influence a broad range of biological processes in different microorganisms. Silver nanoparticles (AgNPs) can be used for slow oxidative dissolution of the cell membranes of microbes, by production of silver ions and eventually results in death of the cells. A broad spectrum of silver activity assures its use against a number of diverse pathogens including *R. solani*. Silver leads to influence a broad range of biological processes in microorganisms [34].

Besides, the application of copper and magnesium as micronutrients, they can increase the resistance against black scurf (*R. solani*). An integrated strategy of fungicides, organic amendment, crop rotation, and cultivation methods were also used. It was interesting in the development of plant protection by nanotechnology method such as application of different nanoparticles at 100 ppm have been reported to show approximately 70% growth inhibition of *R. solani*. Other nanoparticles that have been applied to crop protection are nano-forms of copper (Cu), iron (Fe), silica (Si), silver (Ag), and carbon (C).

Conclusion

Three nanoparticles (AgNPs, CuONPs, MgONPs) have been tested for suppression of the mycelial growth of *R. solani* under *in vitro* conditions. Nanoparticles were incorporated against *R. solani* to find out the antagonistic properties nanoparticels *i.e*., AgNPs, MgONPs and CuONPs and the highest mycelial growth inhibition of *R. solani* was observed in AgNPs *i.e*., 64.86 percent followed by MgONPs and CuONPs *i.e*., 61.82 and 46.95 percent, respectively. All these results were evaluated under *in vitro* conditions.

Application of research: Using nanoparticles is one of the eco-friendly approach as compared to using the expensive chemicals.

Research Category: Mycology and Plant Pathology

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Study area / Sample Collection: Institute of Agricultural Sciences, Varanasi, 221005, Uttar Pradesh, India

Cultivar / Variety / Breed name: Potato

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