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Efficacy of nanoparticle zinc oxide in the resistance of fungus *Rhizoctonia solani* causing black scurf disease in local potatoes

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ABSTRACT

This study was conducted to isolate and purify the causative agent of Black Scurf disease on potato crop and to test the efficiency of three concentrations (1, 2 and 3%) of zinc oxide nanoparticles (ZnO-PNs) and zinc oxide (ZnO) in controlling the fungus *Rhizoctonia solani*. The results of morphology and then molecular diagnosis by PCR technology showed that isolate R22 represents the fungus *Rhizoctonia solani*, which is the cause of potato black scurf disease. The isolate was registered in the gene bank under accession number OM83978. According to the results of laboratory inhibition exhibited that 3% nano-zinc oxide leads to the highest inhibition rate (85.9%) compared to the control group. Also, 3% zinc oxide showed inhibition rate of 43.71% compared to the control. The results of the pot treatments test showed that all of them had an effect in reducing the infection rate (%) and the severity of infection with the fungus R22. So that, the nano-zinc oxide treatment recorded the lowest rate (%) and severity of infection (11% and 4.73% respectively), followed by the fungicide (Ethidium bromide), which scored 22.22% and 18.33% respectively, while in the case of zinc oxide, we recorded 33.33% and 19.03%, respectively, compared to the control group (plant with R22). The results of the study of growth parameters (plant height, fresh weight, dry weight and number of leaves) also exhibited that the nano-zinc oxide treatment recorded a positive increase in growth parameters that amounted to 54, 24.33, 7.33 and 99.33% compared to the control group.

Keyword: Nanoparticle, Zinc oxide, *Rhizoctonia solani*, Potatoes, plants.
Article type: Research Article.

INTRODUCTION

Potato, *Solanum tuberosum* L. belongs to the Aubergine family and is classified as a tuberous crop. It has been cultivated for 2000 years in the Mountains of South America, and ranks fourth worldwide after rice, wheat and maize (Rahul *et al.* 2016). Potatoes is very important from a nutritional point of view, as it is rich in vitamins B_1, B_2, B_6 and minerals such as potassium, phosphorous and magnesium, and also contains several acids such as pantothenic acid and riboflavin, while containing 60-80% starch. The moisture content in potato tubers is estimated at 79 % and 21% dry matter (Siddique *et al.* 2020). Soil and tuber-borne diseases such as wilt caused by *Fusarium* spp. and root rot caused by *Pythium* and *Phytophthora*, the most famous is the black scurf disease caused by *Rhizoctonia solani*, which is among the most important causes that hinder production. In addition, stem and root rot diseases are one of the most common diseases transmitted by tubers and soil and spread everywhere in the world (Larkin & Griffin 2007; Muhsen *et al.* 2015; Al-Dulaimi & Hussein 2019). Black scurf disease on potatoes causes severe damage to plant parts, different stages of growth and tubers and causes plant death, as well as damage to stem tissues from the crown area, deformation of tubers, poor growth and formation of black scurf on the surface of tubers, which affects the quality of tubers (Juber & Hasson 2012; Betancourth *et al.* 2021). Potato

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black scurf disease is one of the most important diseases globally, as it has a great economic impact, and is considered as a serious problem in the potato tuber production system and a lack of quality. Infection begins through vaccinations transmitted by tubers, soil, or the remains of infected plants. As soon as appropriate conditions are available, low temperatures and high soil moisture begin to grow and attack the stems and crown area, causing tissue damage, as they are in the form of spots and ulcers on the nearby leg from the surface of the soil. Once the infection progresses, these spots are brown and then black, and the source of the primary infection is to employ infected seeds, which is characterized by the presence of sclerotia covering the outer crust of the tubers, the distinguishing signs of infection with black scurf disease (Ferrucho *et al.* 2012). Using nutrients and chemical pesticides was not efficient in filling the food shortage and keeping pace with the growing population, in addition to the fact that the plant only benefited from a few manufactured fertilizers, especially the basic ones such as nitrogen, phosphorous and potassium, and the rest goes away from the target areas due to different conditions, including photolysis, hydrolysis, microbial filtration and stabilization (Subramanian *et al.* 2015). Modern technologies increase agricultural production in proportion to population growth and without affecting the environment, the most important of which is nanotechnology, which works to improve agricultural systems (Xu *et al.* 2021). Research related to nanoparticles (NPs) has gained interest among agricultural researchers in recent years to produce new sources of fertilizers, nutrients and nanopesticides to increase global food production (Seleiman *et al.* 2020; Zahmatkesh *et al.* 2020; AL-Isawi 2022; Haider & Hussein 2022; Kamali Omidi *et al.* 2022). Recent studies showed using nanoparticles as an effective and superior agent against a group of fungal pathogens (Parizi *et al.* 2014). Arciniegas-Grijalba *et al*. (2017) reported that zinc oxide nanoparticles (ZnO-NPs) are an inhibitor of fungal growth as well as deformation of fungal cells, which causes liquefaction of the fungal cell cytoplasm and makes it less dense and causes the presence of a number of vacuoles leading to a large separation from the fungal cell wall and thus the effect on vital functions. Zinc nanoparticles (ZnO-NPs) bind to fungal cell walls and internal organelles, causing damage to cell membranes and internal organelles. In addition, ZnO-NPs have an anti-fungal effect for many plant diseases, such as *R. solani*, *Alternaria alternate*, *Botrytis cinerea* and *Pythium aphanidermatum* (Hussein *et al*. 2017; Abd-Elsalam *et al.* 2018).

MATERIAL AND METHODS

Potato

The plant samples (stem, tubers, roots) exhibiting symptoms and signs of black crust disease were taken, washed well, sterilized and dried on sterile filter paper, then the affected parts were placed in petri dishes containing PDA medium (Potato Dextrose Agar). Afterward, the plants were distributed as 3-4 pieces per plate for each sample individually and then incubated at 2 ± 25 °C. The plant was examined under the compound microscope and diagnosed morphologically based on the characteristics of the colony and the nature of the mycelium and the structures using taxonomic keys (Samson *et al.* 1984; Pitt & Ailsa 2009), then was confirmed based on the molecular diagnosis by PCR technology.

Laboratory experiment

The efficiencies of ordinary zinc oxide and zinc oxide nanoparticles were examined against pathogenic isolates of *Rhizoctonia solani* as follows:

1- Zinc oxide (ZnO) in three concentrations (1, 2 and 3%).

2- Zinc oxide nanoparticles (ZnO-NPs) with three concentrations (1, 2 and 3%).

3. Evaluating the efficiency of ZnO-NPs in inhibiting the growth of *R. solani* isolate in laboratory:

The efficiency of ZNO-NPS nanoparticles was tested against the isolate of the fungus *R. solani* using three concentrations (1, 2 and 3%) for each fungus. The weights (1, 2 and 3 g) of ZNO-NPS were added to 99, 98 and 97 mL of deionized water for sterilized ions, respectively. Then each concentration was individually mixed with an ultrasonic homogenizer for a period of 4 min. It is a device that emits ultrasonic waves (KHz20, w250) for the purpose of maintaining the nanoparticle size as well as a homogeneous distribution (Reddy *et al.* 2016). Then 4 g of PDA nutrient medium was added to each concentration, then the media was sterilized in the osmosis at a temperature of 121 °C and a pressure of 1.5 kg cm^2 for 20 min. Afterward, the media was poured into petri dishes, and a part of the fungus with a diameter of 0.5 mm was taken from the edge of the aged fungal colony 6 days, with three replications for each concentration. In addition to preparing three dishes for isolates of fungi on PDA culture media without ZnO-NPs for comparison, all petri dishes were incubated at 25 ± 2 °C until the growth of

the control colony. Once the fungi reached the edge of the petri dishes, they were also prepared with the same number and replicates of ZnO-NPs, with the same three concentrations and for the same comparison. Thereafter, they were left in the incubator at a temperature of 25 °C and the dishes were followed until the growth of the control plates (PDA culture medium without zinc oxide) was completed. The percentage of inhibition was calculated according to the following equation according to Muhsen (2011):

Inhibition rate (%) = (average control colony diameter - treatment diameter average)/(average control colony diameter) × 100

Field experiment

We evaluated the efficiency of ZnO and ZnO-PNs in the control of the fungus R22 in pots: An experiment was carried out in 2-kg pots to test the best concentration of ZnO and ZnO-PNs obtained in the laboratory. The soil was mixed with peatmoss at a ratio of 2:1 successively, then the mixture was sterilized, placed in 2-kg pots, and earlier isolates of fungi were prepared. R22 was transferred on the PDA medium until it reached the age of 6 days, then contaminated the sterilized potting soil manually and directly by half a plate for each anvil of 2 kg capacity. Afterward, we moistened the soil with water and closed the pots with polyethylene bags for 4 days to provide a suitable time and environment for fungi growth. Local potato tubers of medium sizes suitable for planting in pots were prepared, superficially sterilized with sodium hypochlorite solution, then planted in pots by 21 pots, watered well and each pot individually covered with polyethylene bags in order to stimulate growth and activity. After 3 days of planting, treatments were treated with ZnO, ZnO-PNs and a fungicide (Ethidium bromide). The experiment was conducted with 3 replicates (3 pots) for each treatment. The experiment included the following treatments:

1 - Control treatment 1 (plant only)
2- Control treatment 2 (plant + R22).
3- Treatment 3 (plant + R22 + fungicide); adding the pesticide three days after planting.
4- Treatment 4 (plant + R22 + ZnO 3%); adding the oxide with planting the tubers directly.
5- Treatment 5 (plant + ZnO); adding after three days from the date of planting.
6- Treatment 6 (plant + R22 + ZnO-PNs 3%); oxide was added directly with cultivation.
7- Treatment (plant + ZnO-PNs 3%); oxide was added after three days from the date of planting.

The pots were followed up for watering and periodic follow-up starting from the day the seedlings appeared to record the most important readings related to the percentage of germination, symptoms and signs every 3 days until the last day of the crop's life. Then, we calculated the severity of the infection and also the growth parameters, wet weight, dry weight and total length. The rate (%) of disease severity on the stems was estimated based on the following pathological evidence:

0 = the plant is healthy, there are no symptoms of infection on the roots or on the aerial parts.
1 = one spot with a diameter of less than 25 mm
2 = one spot with a diameter of more than 25-50 mm
3= The presence of a single spot with a diameter of more than 50 mm or a group of spots with a diameter of more than 50 mm that does not change the circumference of the stem.
4 = The presence of one spot with a diameter of less than 25 mm that completely surrounds the stem.
5 = The presence of spots more than 25 mm in diameter surrounding the stem completely (Hall *et al.* 2000).

The percentage of infection severity was calculated based on Mckinney's (1923) equation as shown below:

Infection severity (%) = (Total number of plants of degree zero × zero + ...+ number of plants of degree 4 × 4) ÷ (Total of plants examined × 4).

The germination rate (%) was calculated according to the following equation:

Germination rate (%) = (Number of germinated seeds)/(Total number of seeds sown) × 100
Infection rate (%) = (The number of infected plants)/(Examined for the total number of plants) × 100.

Measuring the concentration and purity of the extracted DNA

A Nanodrop spectrophotometer was used to measure the concentration and purity of DNA extracted from samples, by reading the absorbance with a wavelength ranging between 260-280 nm. The extracted DNA purity was measured by the following equation:

DNA purity = OD260 / OD280.

Where OD = optical density

Statistical analysis

The SAS (2012) program, which adopts a complete randomized design (CRD) was used in the implementation of both the laboratory study experiments and the pots experiment.

RESULTS AND DISCUSION

Morphology diagnosis

The results of the morphology diagnosis showed the fungus *R. solani* (Fig. 1), which is represented by the shape and colour of the mycelium, ranging from white to white-brown, as well as the formation of sclerotia on the surface of the tubers (Fig. 2). These are consistent with those reported by Hassan *et al.* (2012) and Misawa & Kurose (2019).

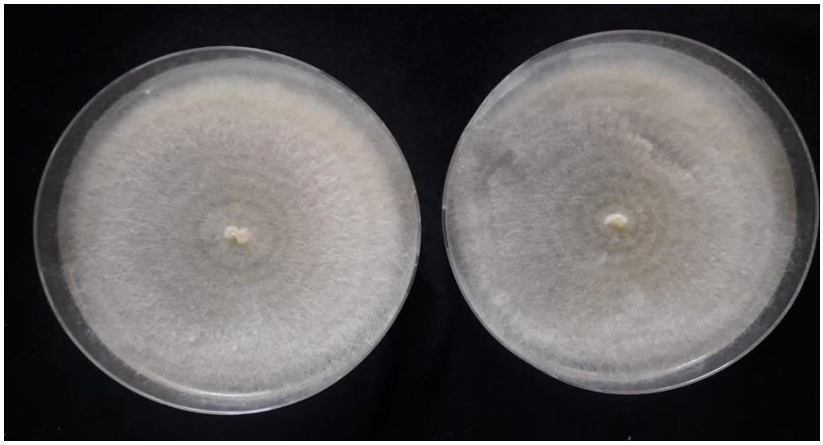

Fig. 1. Isolates of *Rhizoctonia solani* on PDA medium.

Fig. 2. Potato tubers with the sclerotia on them of the fungus that causes black scruf disease.

Molecular diagnosis of *R. solani* by PCR technique

The results of the molecular diagnosis showed the isolate *R. solani* which is the most pathogenic. The results of the electrophoresis (Fig. 3) on the agarose gel showed the presence of a bundle of molecular size (655). The results of nucleotide sequences exhibited *R. solani* isolate (R22) causing black scurf disease on potatoes, and the rate of match was 98% when compared to global isolates in the Gene Bank. The nucleotide sequence of the isolate was deposited in the Gene Bank under accession number OM839787.

Efficiency test of ZnO-PNs against the pathogenic fungus *R. solani* in laboratory

The results of Table 1 showed that all treatments recorded significant differences between the three concentrations (1, 2 and 3) of ZnO and ZnO-PNs, all of them recorded clear inhibition rates against *R. solani* with an inhibition

rate of 85.9% compared to the control (*R. solani* only) which displayed 0% inhibition. In the case of the treatments of ZnO-PNs with 2 and 3% compared to the control and other treatments, the inhibition rates were 8.84 and 9.85% respectively and exhibited significant differences with the rest of the concentrations in addition to their superiority over all oxide treatments. It was followed by the treatment of ZnO-PNs at 1%, revealing the inhibition rate of 58.5%.

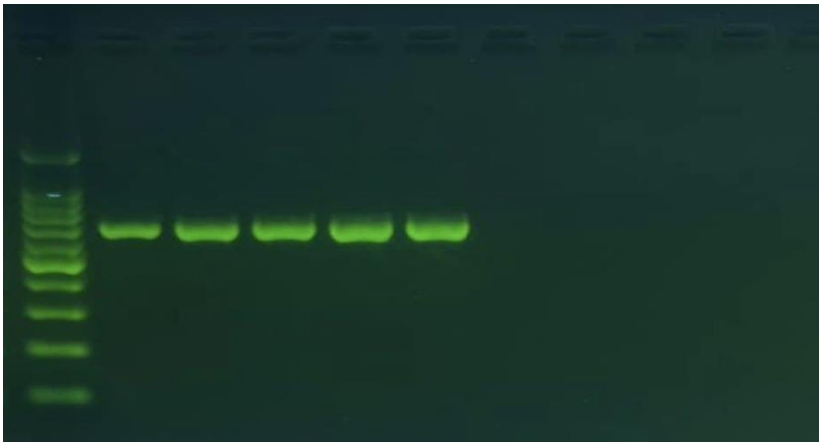

Fig. 3. Electrophoresis of isolates of *R. solani* (R22).

These results are in agreement with Akintelu &Folorunso (2020) stated, who confirmed that nano-zinc oxide is a good anti-pathogen, especially fungi, and is considered an effective pesticide in their resistance, as an antifungal for fungi and bacteria together, and the reason for inhibiting the fungus by nano zinc oxide is due to the effectiveness of its molecules that bind to the walls of fungal cells and internal organelles, which leads to damage to cell membranes and the destruction of internal organelles and thus not performing their vital functions, which leads to The death of mycelium. and this was confirmed by Al-Dhabaan *et al*. (2017), Abd-Elsalam *et al*. (2018), considered nano-zinc oxide to be an effective antifungal against fungi *R. solani* and *Alternaria alternate* causing leaf blight on cucurbits and *Botrytis cinerea* causing gray mold on vegetables. As for zinc oxide treatments, a concentration of 2 and 3% showed that it inhibited the fungus *R. solani* compared to the control (*R. solani* only), and significant differences were recorded, as the inhibition rate was recorded as 41.66% and 43.71% respectively, followed by the zinc oxide treatment at a concentration of 1% The inhibition rate was 30.9% compared with the control treatment which was 0% inhibition.

Table 1. Efficiency test of oxide in inhibiting the fungus *Rhizoctonia solani.*

Oxide	Treatment	Colony diameter rate	Inhibition (%)
	control	90	0
	%1	37.33	58.5
nano zinc	%2	13.66	84.8
oxide	%3	12.66	85.9
LSD = 0.05		6.5738	5.11
	%1	62.16	30.9
	%2	52.66	41.66
zinc oxide	%3	50.66	43.71
LSD = 0.05		5.2544	3.8257

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Efficiency test of ZnO and ZnO-PNs in resistance to *R. solani* on potted potato

The test results in the pot experiment showed a decrease in the percentage of infection severity and the percentage of infection in potato with Black Scurf disease caused by *R. solani* fungus in all oxide and pesticide treatments compared with the control treatment (potato plant with *R. solani*),The nano zinc oxide treatment, the pesticide treatment and the zinc oxide treatment recorded the highest resistance to the pathogen compared to the control of the treatments and with significant differences, as it reduced the percentage of infection rate to the lowest level, which was (11, 22.22 and 33.33)%, respectively compared with control treatment, (potato plant with *R. solani*) which recorded a percentage of infection with 88.89%, as well as with regard to the percentage of infection severity, The same treatments recorded the lowest percentages of infection severity, as the infection severity was reduced to (4.73, 18.33, 19.03)% respectively compared to the control treatment, which reached the highest The percentage of infection severity, which is 69.40%. The results of this study also came in line with those of many researchers such as Sardella *et al*. (2018) and Malandrakis *et al*. (2019), who indicated that ZnO-PNs are among the best antifungals to resist a wide range of fungal diseases, including *R. solani.* The low rates of infection and severity of infection may be explained by the ability of ZnO-PNs to cause distortions in the walls of fungal cells and thus to distort the fungal hyphae and conidia. In addition, ZnO-PNs cause liquefaction in the cytoplasm of the free cell and makes it less density and lead to a large separation in the free cell walls due to the gaps caused by ZnO-PNs. In addition, they also affect the vital functions and also impact on the stock of sugars and proteins in the fungal cell as well as the total fat content (%) that is necessary for the continued growth and spread of the fungus due to the influence of the process.

Fungal cell respiration has been confirmed by Ouda (2014) and Arciniegas *et al*. (2017). Additionally, ZnO-PNs exhibit an inducing activity on the systemic resistance of plants. So, they stimulate the plant to produce phenolic compounds and phytotoxins, which are general inhibitors of the growth of fungi and prevent them from developing and multiplying. ZnO-PNs also reduce their spread within plant tissues. Perelshtein *et al.* (2009) and Ruffo Roberto *et al.* (2019) reported that all the nanocomposites proved to display a clear effect on the pathogen directly as an anti-fungal cell or indirectly by inducing systemic resistance to stimulate the plant to form phenolic compounds effective in resisting the fungus. Hence, these composites function as a safe alternative to control plant pathogens, unlike synthetic chemical compounds.

Table 2. Efficiency test of ZnO-PNs in resistance to *R. solani*, growth parameters ratios, rate and severity of infection.

N	Treatments	Plant length	Soft weight	Dry weight	Number of sclerotia	Number of leaves	Severity of infection (%)	Infection rate (%)
1	Plant control	52.333	22.667	6	0	97.67	0	0
2	Plant + *R. solani*	28.667	12.333	3.66	81.333	44.67	69.40	88.89
3	Plant + *R. solani* + Fungicide	39.333	14	2.333	6.667	76	18.33	22.22
4	Plant + *R. solani* + ZnO (%)	51.333	21.667	4.666	0	80.67	19.03	33.33
5	Plant + ZnO-PNs (%)	51.667	21.3	4.666	0	94.33	0	0
6	Plant + *R. solani* + ZnO-PNs (%)	52.333	23.333	4.666	0	98	4.73	11
7	Plant + ZnO-PNs (%)	54	24.333	7.333	0	99.33	0	0
	LSD = 0.05	7.1213	3.5332	1.662	16.694	23.119	22.444	29.667

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