Impact of Green Synthesized Zinc Oxide Nanoparticles for Treating Dry Rot in Potato Tubers

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Potato (*Solanum tuberosum*) crops have experienced a 22% reduction due to attacks from microbial pathogens and pests. Dry rot disease occurred from risks in El-Minya governorate, Egypt, where potato is the major cultivated crop. Potato cultivar 'Cara' samples were collected from the markets of six regions in El-Minya for isolation. They were also tested for the application of biosynthesized zinc oxide nanoparticles (ZnONPs) for disease management. The ability of *Exserohilum rostratum* to synthesize ZnONPs was documented *via* UV-visible, X-ray diffraction, Fourier transform infrared spectrum, and transmission and scanning electron microscopy. Spherical shape and crystallite small size (51.0 ± 3.0 nm) were attributed to the created ZnONPs. The ZnONPs were applied in the bio-control of the causative agent (*Fusarium nygamai*) of dry rot disease and large economic loss of potato cv. 'Cara' productivity. The mycosynthesized ZnONPs by *E. rostratum* at 100 µg/ mL (3 mM) showed antifungal activity against *F. nygamai* with higher reducing value of mycelium growth diameter 2.0 ± 0.14 cm compared to Revanol 50%, traditional product with a diameter of 3.85 ± 0.10 cm. Overall, ZnONPs are an excellent agent that can be applied to repress the most common fungal disease of potato.

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Keywords: Mycosynthesis; Potatoes disease; Zinc nanoparticles; Fusarium nygamai; Exserohilum rostratum

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INTRODUCTION

Potatoes (*Solanum tuberosum* L.) represent the fourth most important food crop after wheat, rice, and corn in the world and Egypt. Furthermore, Egypt contributed 6.25% to the value of global exports in 2020, worth $217.55 million (Khatab and El-Mouhamdy 2022). Due to the harmful effects of diseases caused by microbes and biotic pests, more than 22% of the potato has been lost, harming the crop's economy (Kulabhusan *et al*. 2022). Abiotic stress factors such as drought, toxins, water logging, and salinity can be tolerated by some potato cultivars (Swain *et al*. 2023). Multiple diseases that affect potatoes include dry rot, early blight, late blight, potato viruses, blackleg, scab, wilt, and black scurf. These diseases can spread through the air, soil, potato seed tubers, and significantly reduce yields across the world. Among these diseases, *Fusarium* species-caused dry rot severely harms the potatoes yield and causes significant damage to the processing companies and consumers (Dangi 2022).

The potato tubers and roots are attacked by *Fusarium* spp., which causes root rot, wilt disease, and damping-off. Several *Fusarium* isolates were isolated from infected potato tubers, including *F. nygamai*, *F. solani*, *F. acutatum*, *F. subglutinans*, *F. proliferatum*, and *F. oxysporum* (Tiwari *et al.* 2020). The potato cultivar 'Cara' is highly susceptible to the disease known as dry rot (Awad *et al*. 2022).

New innovative materials can be produced from enhanced biological components using nanobiotechnology. Some advantages of zinc oxide nanoparticles (ZnONPs) include being nontoxic, eco-friendly, biocompatible, and relatively simple to formulate. ZnONPs are promising candidates for biological applications (Kamal *et al*. 2023). The FDA designated zinc oxide as a "GRAS" chemical (generally recognized as safe). There are many applications for ZnONPs, which are more versatile and less harmful compared to NPs of other metal oxide (Anjum *et al*. 2021).

Several NPs have been created from various fungi, such as *Trichothecium* sp., *Trichoderma asperellum*, *Fusarium oxysporum*, *Colletotrichum* sp. (Kamal *et al.* 2023), *T. viride* (Meena *et al*. 2021), *Phanerochaete chrysosporium*, *Aspergillus niger* (Rashad *et al*. 2023), *F. semitectum*, *A. fumigatus* (Rai *et al*. 2021), *Penicillium brevicompactum*, *Coriolus versicolor*, and *Phoma glomerata* (Rehman *et al*. 2021). Selection of fungi for NPs synthesis was based on special properties, such as their resistance to metals and capacity to accumulate (Yang *et al*. 2023).

Advances in nanotechnology have prompted a significant rise in demand for mycosynthesis of NPs (fungi mediated), which could be important for agricultural development and controlling plant diseases. Numerous proteins and enzymes produced by fungi serve as reducing mediators and are widely employed in the synthesis of metallic NPs (Sonawane *et al*. 2022). Agriculture is making an abundance of the application of nanotechnology-based nano growth boosters, nano pesticides, nano fungicides, and nano herbicides (Al Jabri *et al*. 2022). Crop enhancement alternatives that are safe, environmentally friendly, and green can be suggested using the nanotechnology method. It is possible to successfully apply NPs use in agriculture to increase soil nutrients and boost productivity (Sonawane *et al*. 2022). Recent studies have demonstrated that the usage of ZnONPs includes hazards and benefits that vary depending on the concentration, manufacturing method, and object under test. The observed concentrations of ZnONPs in consumer products and the levels required for achieving their antifungal and antibacterial actions are both high enough to be harmful to living organisms. The present investigation evaluated the fungal diseases in potato cv. 'Cara' in El-Minya Governorate, with control of the pathogens by ZnONPs compared to the traditional product Revanol 50%.

EXPERIMENTAL

Materials Used

Sampling collection for pathogen isolation

A commercial market evaluation of fungal diseases was conducted at six regions of El-Minya city (25º46'29''N;14º55'36''E), including Samalout (23º37'18''N;12º52'22''E), Matay (22º38'21''N;13º45'33''E), Beni-Mazar (20º32'21''N;11º22'18''E), Maghagha (21º44'25''N;10º46'35''E), and El-Edwa (27º41'23''N;17º50'32''E). In each market, cv. 'Cara' potato tubers were examined, and three samples (60 to 70 tubers/sample/region) from the variety were collected randomly and visually examined for dry rot disease in the market in a similar manner to another study (Awad *et al*. 2020). The potato tubers exhibiting the same disease symptoms were taken and enclosed in paper bags and placed in a cooling box that was kept at 7 °C, and relative humidity ranged from 85 to 90% for further assessment. The pathogen was isolated from the collected samples.

Samples preparation

The infected potato tubers cv. ‘Cara’ surfaces were sterilized in 70% ethanol for 2 min, washed using sterilized distilled water three times, sterilized using Clorox (20% v/v), washed with sterilized distilled water three times, and placed on sterilized filter paper until used (Muthuvel *et al*. 2020).

Screening and Pathogenicity Test of the Common Fungal Isolates

The samples of the infected potato tubers characterized with similar symptoms to dry rot disease were selected as a source of fungal phytopathogenic. Furthermore, one fungus of the phytopathogenic isolates was taken from the most common isolates to estimate the pathogenicity test and was identified by macroscopic and molecular characterization. Disease-free tubers of cv. ‘Cara’ (6 tubers) were inoculated with the isolated fungal to assess the pathogenicity test. The infected potato tubers were washed using sodium hypochlorite solution (1.5%) for 3 min and dipped with sterilized distilled water 3 times, and then placed on Whatman filter paper no.1 for drying. A 10 mm diameter cork-borer was used to puncture the inside of the tubers toward a depth of 10 mm. A 10 mm agar plug, made from the edge of a 7 day isolate culture was then placed into the tuber whole and covered with the same tissue. The inoculated tubers were kept in a cardboard box and incubated in the dark at 28 °C for 20 days (Hadizadeh *et al*. 2019).

Isolation, Purification, and Identification of *F. nygamai*

The infected potato tubers were washed with tap water, sterilized for 3 min with sodium hypochlorite solution (1%), and rinsed 3 times using sterilized distilled water. Afterward, small portions were cut from the area between healthy and diseased tissues, transferred in the petri plates containing growth medium of potato dextrose-agar (PDA) supplemented with ampicillin, and incubated at 28 °C for 3 to 7 days (Dang *et al*. 2023). The developed fungi (all fungal pathogens infected potato tubers during isolation process and morphological similar to the isolate as more common and caused dry rot disease), were examined macroscopically and microscopically. The developed hyphae, which are typical of *Fusarium* spp., were cultured on Czapek agar medium plate and slants. The isolate was identified morphologically and with molecular identification techniques.

Mycosynthesis and Characterization of ZnONPs by *E. rostratum*

First, (10 g) fungus mycelia (*E. rostratum*) was washed 3 times by sterilized distilled water and added to 100 mL of distilled water in sterilized dark bottle. The solution was removed by filtration. Then, the solids were dissolved with zinc acetate salt. The reaction mixture was heated at 100 °C for 6 h using a hot plate with a magnetic stirrer set (250 rpm). The color change of the reaction mixture to white was recorded. The reaction mixture was centrifuged for 15 min at 15,000 rpm. The supernatant was discarded, and the precipitated nanoparticles were washed and transferred into a glass plate to be dried at 300 °C using a hot plate. The formed dry particles were kept at room temperature (25 °C) for further characterization (Kumari *et al*. 2023). The created ZnONPs were characterized by UV-Vis spectrophotometry (spectrophotometer Cary E 500, Japan) at a wavelength ranging from 200 to 800 nm. ZnONPs were subjected to structural characterization and particle size analysis using transmission electron microscopy (TEM) (Model JEM-1230, JEOL, Akishima, Japan), scanning electron microscopy (SEM) (Model TM-1000, Hitachi, Japan), energy dispersive X-ray supplemented with Fourier transform infrared spectroscopy (EDX-FTIR) (Bruker, Germany) (Huang *et al*. 2017), and X-ray diffraction (XRD) utilizing CuKo radiation and an XPert PRO diffractometer. The prepared ZnONPs were analyzed for size and size distribution in terms of diameters of the average volume and index of polydispersity using photon correlation spectroscopy at a fixed angle of 173 °C at 25 °C utilizing a particle size analyzer Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, United Kingdom).

Antifungal Activity of ZnONPs against *F. nygamai*

The antifungal activity of ZnONPs against *F. nygamai* was assessed *via* measuring the colony diameter. The activity of mycosynthesized ZnONPs at 100 µg/mL (3 mM) was compared with various treatments including, control (precursor of Zn acetate salt), fungicide (Revanol 50%), Revanol 50% with mycosynthesized ZnONPs, purchased chemically prepared ZnONPs, and Revanol 50% with purchased chemically prepared ZnONPs. Three replicates of the concentration of each treatment were added to PDA media individually, and *F. nygamai* was injected with a 5 mm disc in the center of the PDA media plates under sterile conditions. The fungus was treated with varying concentrations of ZnONPs on distinct PDA medium plates. The positive control plates were those that did not contain any ZnONPs treatment. The plates containing PDA were incubated for 7 days at 28 °C. The fungal colony growth were recorded (Abbas *et al*. 2022) *via* Eq. 3,

$$\text{Inhibition of colony growth \%} = (UTC - TC)/UTC \times 100 \qquad (3)$$

where UTC = Untreated colony (control), and TC = treated colony.

Statistical Study

The results were calculated to record mean ± standard deviation and standard errors (n = 3). All statistical analysis was completed using SPSS program version 20.0.

RESULTS AND DISCUSSION

Evaluation of the Fungal Disease of Potato Tubers

During the potato harvest in the wholesale market of six regions in El-Minya governorate, Egypt, the potato cultivars cv. ‘Cara’ that had symptoms of fungal disease were examined, and results are shown in Table 1. The data showed that from the six regions, the Samalout region recorded the highest tuber infection of fungal disease from all obtained samples, followed by Matay region (4 samples appeared to have fungal diseases). The remaining regions (including El-Minya, Beni-mazar, and Maghagha) showed the same tuber infections (3 samples) by fungal disease from the obtained total samples. The obtained data revealed that Samalout region had the highest tuber infection by fungal disease. The results may be due to the grower’s repeated potato culture in the same soil that increased the soil infestation and incidence of progeny tubers. These results are in harmony with previous reports (Jeng and Swanson 2006). Thus, the findings in Table 1 revealed that the disease incidence of fungal diseases is significantly different among the tested six regions. The fungal diseases recorded from the Samalout region had the highest disease incidence (6.6 ± 0.25 cm), while the lowest disease incidence (2.0 ± 0.47 cm) was the Beni-Mazar region. The repeated potato culture in the same soil without an agricultural cycle led to increasing soil infestation and subsequently increased disease incidence. The results were similar those of (Han *et al*. 2023), in that the causal agent of dry rot disease (*Fusarium* spp.) survives for several years in soil or as a colonizers of growing plants.

Table 1. Fungal Disease Evaluation on Potato cv. 'Cara' and Disease Incidence (DI) of 6 regions at El-Minya governorate, Egypt

Isolate	Regions																	
	El-Minya			Samalout			Matay			Beni-mazar			Maghagha			El-Edwa		
Fungal isolate	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3	S 1	S 2	S 3
	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-	-	-	+
Total isolate	1			3			2			1			1			1		
Disease Incidence (DI)	2.8±0.18			6.6±0.25			2.8±0.12			2.0±0.47			2.4±0.36			3.2±0.38		

*(+) positive & (-) negative, Data are mean ± standard errors (n = 3)

Preliminary and Molecular Identification of Fungal Isolate

Two isolates including pathogen of potato tubers and creator of ZnONPs were identified. The soil fungus isolate was recognized initially based on macroscopic features including the color of the colony and shape of mycelium, hyphae growth within the medium (Fig. 1). More fungal isolates were examined on the basis of colony development, morphological, and microscopic characteristics. The creator fungus of ZnONPs was isolated from agricultural soil that inhibited the pathogenic fungus isolated from infected cv. 'Cara' potato tubers (Figs. 1A and 1B). All isolates identified by traditional, phenotypic approaches were confirmed using molecular approaches. Moreover, phylogenetic analyses were performed *via* ITS sequences of the fungal isolate. A comparison was completed between the nearest neighboring organism from phylogenetic trees and the GenBank search results for each isolate having high-quality ITS sequencing data (Fig. 1C, 1D). GenBank accession numbers were assigned to all nucleotide sequences obtained using primers ITS1 and ITS4, as well as the corresponding sequences to which they exhibited the strongest match. Good-quality ITS sequences from two isolates were searched using BLAST against the GenBank sequence database. Most of the sequences produced BLAST responses with high sequence identity (99.17, 99.80% to 100%) and zero E values. When an identified species' BLAST result for an ITS sequence was the greatest sequence identity, it was possible to identify the related isolate with confidence using only the results of a GenBank search (Shehata *et al*. 2023).

Symptoms of Fungal Disease on Potato Tubers Visually, Macroscopic & Microscopic Examinations

The symptoms showed during the cross-section are exhibited in Fig. 2. The potato tubers infected by isolated fungal exhibited dry rot disease (Fig. 2A and B) and became converted into mummified shape (Fig. 2C and D). The macroscopic photo shows the presence of yellow pigment as a reverse color in the Petri dish (Fig. 2E) and the mycelium growth of isolated fungal as cotton shape on the Petri dish (Fig. 2F). The microscope also indicated that the characters of mycelium appeared as elongated and separated (Fig. 2G) but the spore showed an oval shape (Fig. 2H).

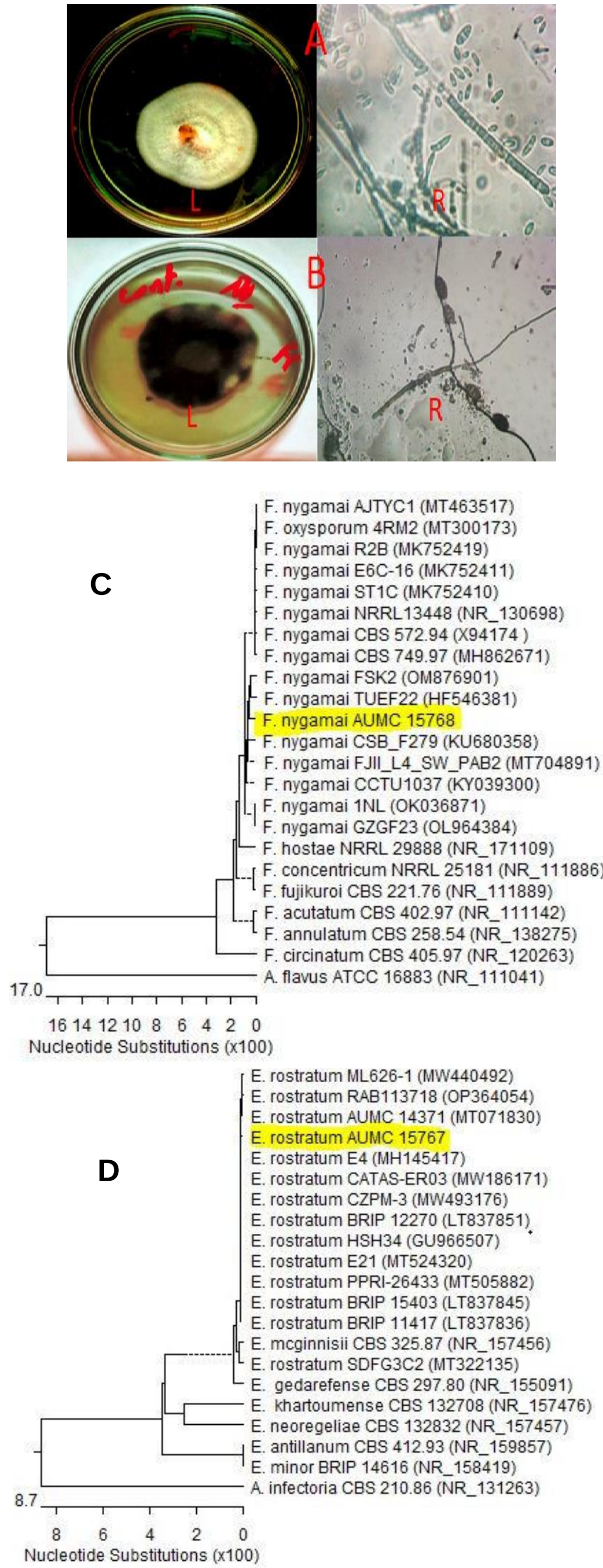

Fig. 1. Macroscopic showing mycelium shape (L) and microscopic showing spore formation (R) of fungal isolates, *Fusarium nygamai* (A) & *Exserohilum rostratum* (B). Phylogenetic tree based on sequencing ITS region of rDNA of *F. nygamai* AUMC 15768 (C), *E. rostratum* AUMC 15767 (D) (arrowed)

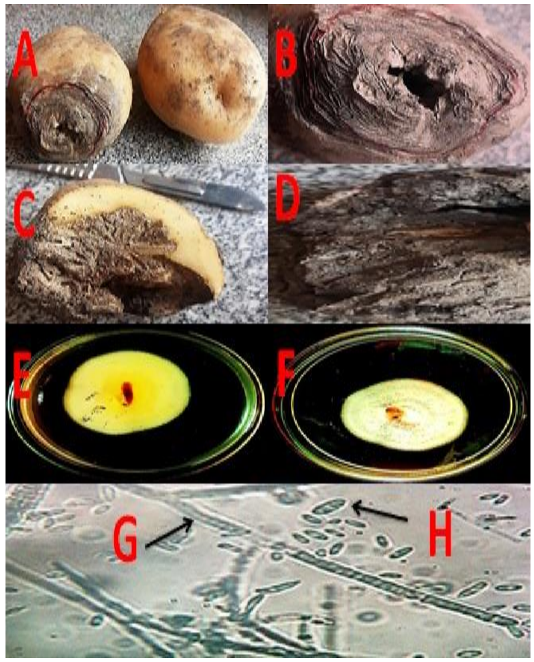

Fig. 2. Infected potato tubers, Mycelium growth, and Microscopic characters of fungal isolate. (A,B) potato dry rot symptoms, (C) cross section exhibiting dry rot, (D) Potato mummification, (E) mycelium growth, (F) pigment formation after 5 days, (G) mycelium shape, (H) spore formation

Pathogenicity Test of *F. nygamai*

After 20 days from the inoculation of potato cv. 'Cara' tubers with 7 day-old *F. nygamai* isolate at 28 °C in the dark, the mycelium growth appeared, developed, and led to inner tuber tissues being destroyed compared with the control treatment (Fig. 3). The symptoms appeared the same in all inoculated potato tubers as in dry rot disease, and the causal agent was *F. nygamai* AUMC 15768. This result is in harmony with other study (Ramadan and Haleem 2023). Thus, the tested potato tuber cv. 'Cara' was shown to be susceptible to the fungal pathogen.

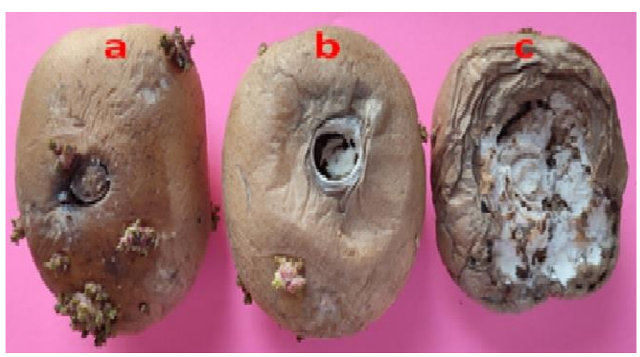

Fig. 3. Symptoms of dry rot *Fusarium nygamai* on cv. 'Cara' potato tubers showed (a) control treatment, (b, c) development of the isolated pathogen

Characterizations of ZnONPs by *E. rostratum*

Preparation and UV Analysis of ZnONPs

The synthesis of the ZnONPs by *E. rostratum* was detected *via* a UV-Vis spectrophotometer. The peak was centered at 281.5 nm, indicating that ZnONPs had been synthesized. According to published scientific papers, the spectra of UV visible exhibited peaks ranged from 260 and 290 nm of the synthesized ZnONPs (Tripathy *et al*. 2023).

TEM, SEM, and EDX analyses of ZnONPs

The cubic and hexagonal structure, besides the spherical shape of the synthesized ZnONPs, was documented *via* TEM micrographs (Fig. 4), which adapted to the image by SEM (Fig. 4A). The surface morphology of the created ZnONPs was examined using SEM microscopy (Fig. 4B). The SEM images in the present results, obtained for the mycosynthesized ZONPs were similarly as irregular in shape and matched to the previous findings (Manimegalai *et al*. 2023). The occurrence of zinc oxide element, which denotes the reduction of a zinc ion in the reaction mixture, was detected *via* EDX investigation. The great purity of the generated ZnONPs was validated *via* the analysis of elemental composition, which reflected the existence of Zn and O. Based on the concentrations of other elements and the presence of stoichiometric mass, the relative percentages of Zn and O in the created ZnONPs (47.92% and 38.71%, respectively) are comparable to hypothetical expectations (Fig. 5C). Additionally, this study's elemental percent of ZnONPs matches up to other research (Veselova *et al*. 2022).

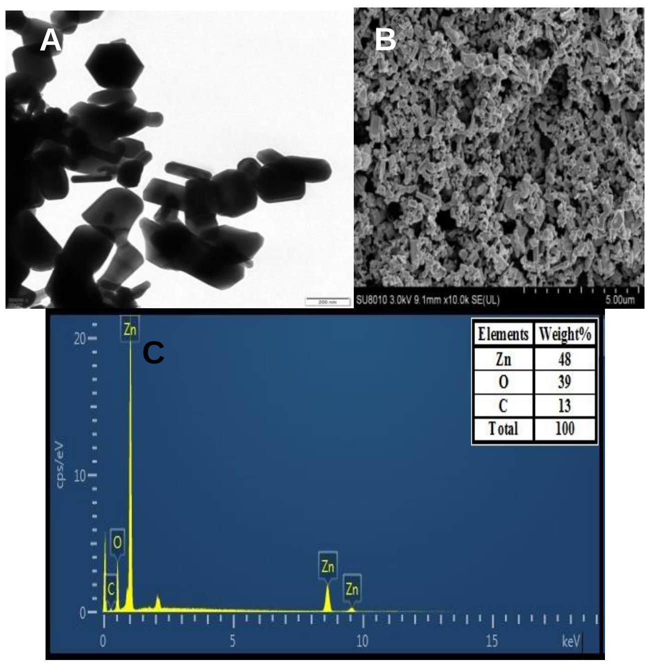

Fig. 4. ZnONPs synthesized by *E. rostratum*. (A) TEM analysis, (B) SEM analysis, and (C) Elemental composition EDX analysis

FTIR analysis of ZnONPs

The functional groups in the created ZnONPs were detected *via* FTIR spectra and were scanned from 400 cm^{-1} to 4000 cm^{-1}. Fourier-transform infrared spectrometry reflected several absorption bands indicating the presence of various functional groups (Fig. 5). The FTIR spectra of the mycosynthesized ZnONPs revealed the presence of the phenolic OH group, confirming a potential mechanism for ZnONPs synthesis. The peak at 3440.7 cm^{-1} indicated a high content of alcoholic O–H groups. The peak at 1631.6 cm^{-1} signified the N–O strong bond of the nitro compound. The band at 1409 cm^{-1} indicated the C–H group, which was associated to the alkane compound. The peak at 655.1 cm^{-1} was attributed to the existence of C–Br of halo compound (Misra *et al*. 2021).

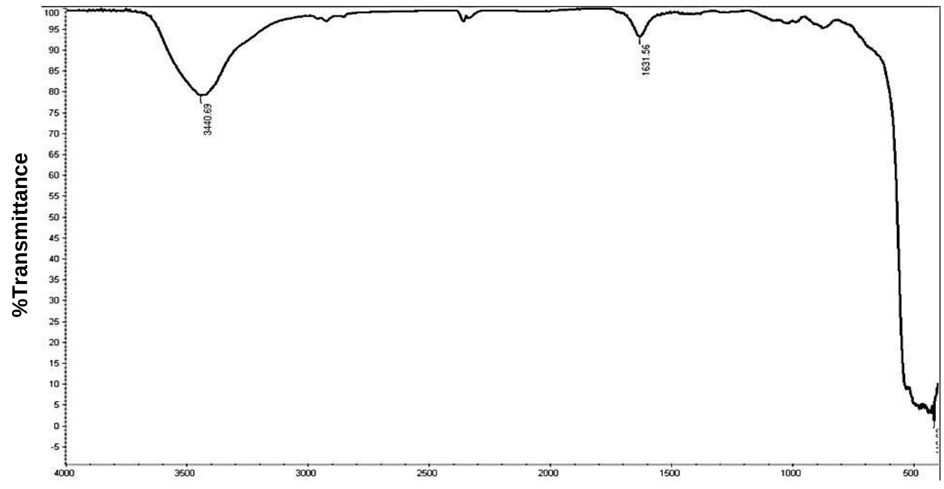

Fig. 5. FTIR analysis of ZnONPs synthesized by *E. rostratum*

XRD Spectrum

Using *E. rostratum*, the XRD analysis assessed the produced ZnONPs' crystallinity (Fig. 6). The XRD spectrum identified the structural characteristics of the mycosynthesized ZONPs. The atoms appeared with apparatus in the form of regular arrays and crystals. The ZnONPs possessed a hexagonal crystalline configuration and offered distinct diffraction in the spectra that were correspondent to peak values (Gakis *et al*. 2023).

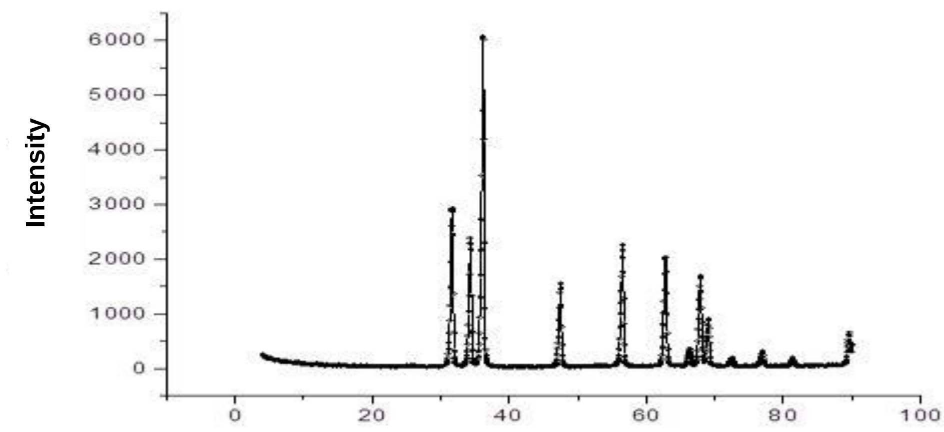

Fig. 6. XRD spectra (Y) showing the intensity a.u and (X) the 2*θ* in degrees of synthesized ZnONPs using *E. rostratum*

Zeta potential and particle size detection

Numerous factors such as sonification, coagulation, and stabilizer addition (*e.g.* sodium hexametaphosphate hexamethyl) during synthesis of NPs can affect the distribution of particle size. Results showed that the value of the zeta potential was + 35 mV (Fig. 7A), and the diameter size was approximately 50 nm (Fig. 7B). The obtained results were agreement with (Marsalek 2014).

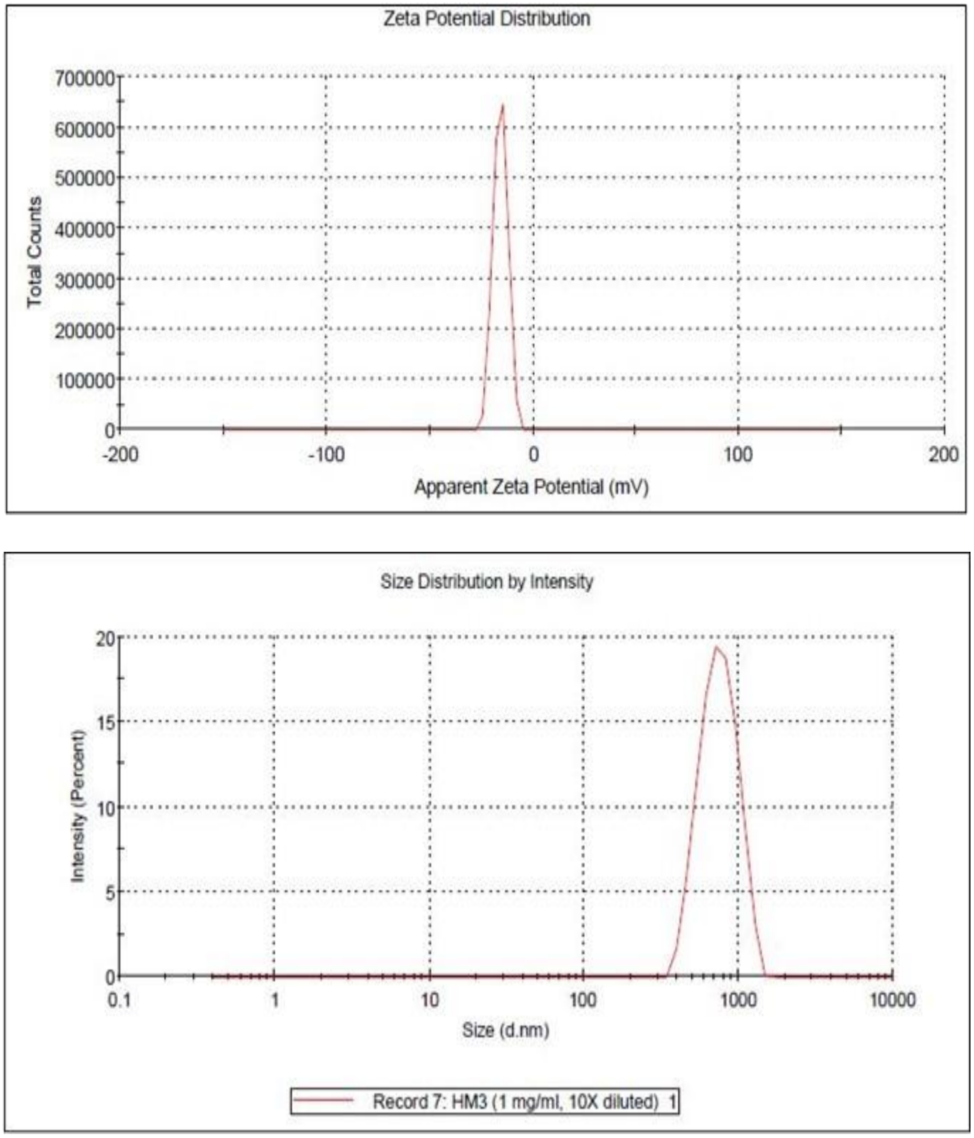

Fig. 7. Particle size and zeta potential assay of ZnONPs synthesized by *E. rostratum*

Antifungal Activity of Mycosynthesized ZnONPs by *E. rostratum* against *F. nygamai*

The diameter of fungal mycelium growth of pathogen (*F. nygamai*) was recorded for various treatments (Fig. 8). For the control, mycosynthesized ZnONPs by *E. rostratum* at 100 µg/mL (3 mM) and purchased chemically prepared ZnONPs, the pathogen fungus growth was 5.75 ± 0.08 cm, 2.00 ± 0.14 cm, and 4.20 ± 0.12 cm, respectively. With the traditional fungicide (Revanol 50%), the pathogen fungus growth was 3.85 ± 0.10 cm. With the (Revanol + mycosynthesized ZnONPs) and (Revanol + purchased chemically prepared ZnONPs), the fungus growth was 2.95 ± 0.08 cm and 3.95 ± 0.10 cm, respectively (Fig. 8). These results were in agreement with (Dimkpa *et al*. 2013 and Ali *et al*. 2022), who reported that ZnONPs were more effective against pathogen fungus isolate than zinc acetate salt (Control).

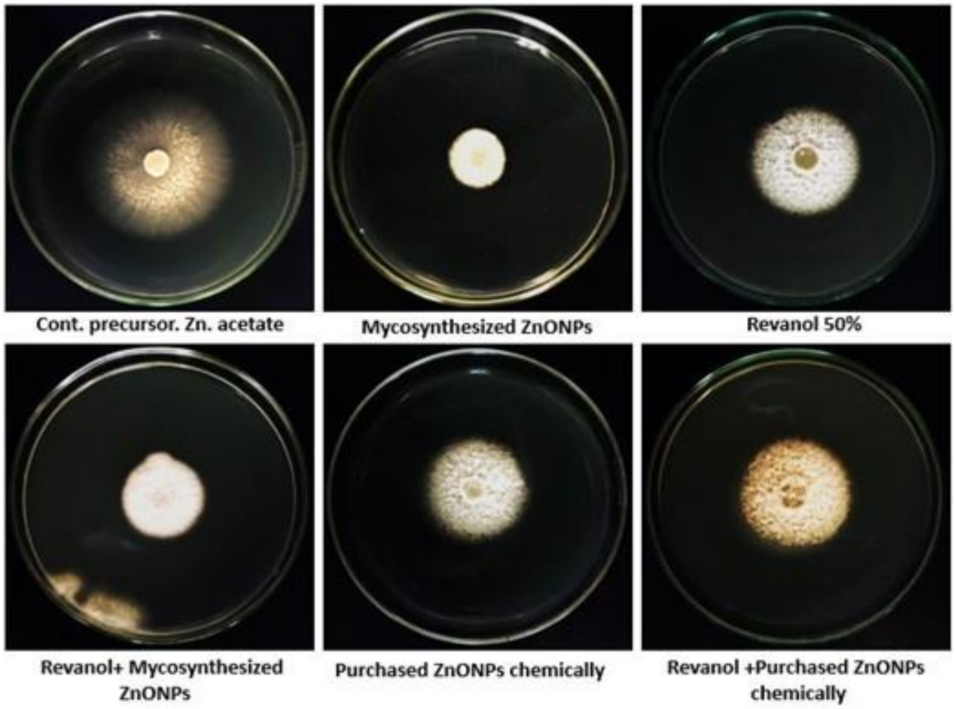

Fig. 8. Antifungal activity of mycosynthesized ZnONPs by *E. rostratum* with fungal pathogen growth in vitro

CONCLUSIONS

1. The most common disease organisms in potato tubers were fungal on the 'Cara' potato cultivar.
2. The Samalout region recorded the highest dry rot incidence, and Beni-Mazar recorded the lowest.
3. ZnONPs were successfully biosynthesized using isolated fungal from agricultural soil (*E. rostratum*).
4. The antifungal activity of the ZnONPs concentration 100 gm/mL (3 mM) was examined against *F. nygamai*. Results showed that the mycosynthesized ZnONPs by *E. rostratum* inhibited mycelium growth of *F. nygamai* as a pathogenic fungal.
5. The minimum inhibition of mycelium growth was completed with control Precursor Zn acetate treatment.

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