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Antifungal activity of Zinc nitrate derived nano Zno fungicide synthesized from *Trachyspermum ammi* to control fruit rot disease of grapefruit

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ABSTRACT

Grapefruit (*Citrus paradisi*) is a widely grown citrus and its fruit is affected by a variety of biotic and abiotic stress. Keeping in view the hazardous effects of synthetic fungicides, the recent trend is shifting towards safer and eco-friendly control of fruit diseases. The present study was aimed to diagnose the fruit rot disease of grapefruit and its control by using zinc oxide green nanoparticles (ZnO NPs). Fruit rot symptoms were observed in various grapefruit growing sites of Pakistan. Diseased samples were collected, and the disease-causing pathogen was isolated. Following Koch's postulates, the isolated pathogen was identified as *Rhizoctonia solani*. For eco-friendly control of this disease, ZnO NPs were prepared in the seed extract of *Trachyspermum ammi* and characterized. Fourier transform infrared spectroscopy (FTIR) of these NPs described the presence of stabilizing and reducing compounds such as phenols, aldehyde and vinyl ether, especially thymol (phenol). X-ray diffraction (XRD) analysis revealed their crystalline nature and size (48.52 nm). Energy dispersive X-ray (EDX) analysis elaborated the presence of major elements in the samples, while scanning electron microscopy (SEM) confirmed the morphology of bio fabricated NPs. ZnO NPs exhibited very good anti-fungal activity and the most significant fungal growth inhibition was observed at 1.0 mg/ml concentration of green NPs, in vitro and in vivo. These findings described that the bioactive constituents of *T. ammi* seed extract can effectively reduce and stabilize ZnO NPs. It is a cost-effective method to successfully control the fruit rot disease of grapefruit.

1. Introduction

Citrus fruit has the highest global production compared to any other fruit genus, with an estimated production of 95 million metric tonnes in 2019–20, including grapefruit, lemon, oranges, and tangerines (Costa et al., 2020). Citrus is susceptible to various pathogens (Rasool et al., 2014) and these fruit losses are mostly caused by fungal pathogens (El-Otmani et al., 20011). Fungal diseases are a growing concern throughout the world, with a serious but poorly analysed effect on public health (Zhou et al., 2020). Food insecurity and malnutrition are the major problems in both developing and industrialised countries of the world (Friedmann, 1993; Koffi et al., 2017; Saleem et al., 2020a;

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Saleem et al., 2020b). Fungi have been reported to cause huge yield losses of economically important fruits. Due to poor pre and post-harvest management strategies, developing countries observe 20–25% fungal damage of total crop production (Dukare et al., 2019). Under unfavourable environmental conditions, this yield loss may raise to 50% or more (Carmona-Hernandez et al., 2019; Saleem et al., 2020c, 2020d, 2020e). Fungal pathogens can damage all kinds of plant tissues, at every stage of plant growth (Fernández-Acero et al., 2007). *Rhizoctonia solani, Phythophora* spp. and *Fusarium* spp. can infect the aerial and underground parts of plants (Bashir et al., 2018). Due to their perishable nature, fruits are very easily attacked and rotten by a variety of pathogenic fungi (Chohan et al., 2015). The yield losses of fruits by fungal pathogens have been estimated to be 50% of the total fruit production (Zhang et al., 2017).

The use of pesticides in crop production is important for the control of diseases (Zhang et al., 2011). Nonetheless, these agricultural activities may be detrimental to humans and the environment, and their prospective benefits should be balanced against these harms (Donley, 2019). Chemical fungicides (pesticides) are extensively used on different crops and they are being used as a key element of current farming (Dara, 2019). Excessive and unsafe use of these chemical fungicides has been reported to damage the fertility of the soil (Joko et al., 2017). Contaminated soil ultimately leads to the loss of growth, yield, and symbiotic attributes (Garg et al., 2017). Due to their detrimental effects, people have started finding alternatives to chemical fungicides. Scientists are emphasizing biologically active natural resources and most of the higher plants and their constituents are effective in managing plant diseases. Due to environmental and health-related concerns, plant scientists are interested in identifying cheaper and eco-friendly biological compounds to control plant diseases (Cherkupally et al., 2017).

Nanotechnology is an emerging research domain that has been applied in the fields of chemical, physical, biological, pharmaceutical and material sciences (Fahimmunisha et al., 2020; Govindarajan and Benelli, 2016). Nanotechnology is being applied in the field of plant pathology and it is exhibiting tremendous efficacy in the treatment of various diseases (Park et al., 2006). The use of nanoparticles as antifungal and antibacterial agents has been proposed as a cost-effective and environmentally safe alternative strategy for the treatment of pathogenic microbes (Alam et al., 2019). The promising application of nanotechnology in agriculture has opened new possibilities and perspectives. Nanomaterials are used in a variety of applications, ranging from plant defence to nutrition and farm management activities, due to their small scale, high surface to volume ratio and unique optical properties (Shang et al., 2019).

In comparison to organic-based disinfectants and antimicrobial agents, ZnO is a metal oxide that is much more durable and has a longer existence. The phytochemical preparation of ZnO nanoparticles imparts significant antimicrobial properties (Khalil et al., 2018). The green synthesized ZnO is a biocompatible and naturally well-disposed material, which makes it attractive for biomedical applications (Petkova et al., 2016). As compared to other metal oxides, ZnO is considered to be more stable and secure (Kim et al., 2020). Approximately 550 tonnes of ZnO NPs are manufactured each year for a variety of applications, around the world (Bondarenko et al., 2013). ZnO NPs have been reported to be useful in improving soil fertility, plant productivity and zinc availability (Rossi et al., 2019; Esper-Neto et al., 2020). The US Food and Drug Administration has classified ZnO, along with four other zinc compounds, as generally recognised as a safe (GRAS) product (FDA Food and Drug Administration, 2015). ZnO nanoparticles are not toxic, and many studies have described their protective roles (Roselli et al., 2003). Extracts of different medicinal plants including *Pelargonium zonale, Punica granatum, Aegle marmelos, Olea ferruginea, Ruta graveolen, Hibiscus subdariffa, Passiflora caerulea* and *Berberis vulgaris* have been used to synthesize ZnO NPs (Anzabi, 2018; Hussain et al., 2020; Vahidi et al., 2019).

Trachyspermum ammi is known for its therapeutic properties and antimicrobial activities (Sharifzadeh et al., 2015). Seed of *T. ammi* is used in traditional medicine and it is famous for its aphrodisiac properties. Its seed contains thymol, which is used in the treatment of gastro-intestinal ailments and bronchial problems. Its roots are diuretic in nature and its oil exhibits antifungal and antimicrobial properties (Singh and Singh, 2000).

The present study was designed to use nanotechnology for the synthesis of ZnO nanoparticles from the seed extract of *T. ammi* to control fruit rot disease of grapefruit.

2. Materials and methods

2.1. Collection of diseased samples from citrus fruit core areas of Pakistan

During 2018–19, a severe fruit rot was observed in the orchards of four citrus-growing areas of Pakistan (Supplementary 1). Citrus orchard of National Agricultural Research Centre (NARC), Islamabad (33°40'12.4"N 73°07'34.0"E) was the first region/site for the collection of diseased fruit samples of grapefruit. From the second region of District Sargodha, diseased grapefruit samples were collected from three different sites i.e., Bhulwal (32°16'57.4"N 72°54'14.4"E), Kot Momin (31°57'39.6"N 73°06'38.8"E) and Siyal Mor (31°58'58.2"N 73°06'54.8"E) (Fig. 1). The infected rotten grapefruit were collected from random trees, which were at about 10 m, at least. All samples were collected from November 2018 to January 2019, kept in protective polythene bags and labelled, separately. Collected samples were taken to the laboratory and stored at 4 °C, for further study.

2.2. Isolation of pathogens from diseased samples

For the isolation of the disease-causing pathogen, diseased fruits were surface sterilized with 2% sodium hypochlorite solution and washed with distilled water. For the isolation and growth of disease-causing pathogens, diseased Sections (4 to 6 mm) were excised and placed on potato dextrose agar (PDA) media. Inoculated Petri dishes were sealed with parafilm and kept in an incubator at 26 ± 2 °C for 5 days.

2.3. Pathogenicity test

To confirm the pathogenicity of each isolated fungus, Koch's postulates were followed. About 4 mm fungal discs from 7-day-old isolated fungi were transferred to Czapek broth media and shaken for 3–4 days. The culture was filtered and a particular conidial suspension (10^6 conidia ml^{-1}) was maintained. A total of 12 healthy grapefruits were drilled with a sterile needle (5 mm deep). Out of these, six healthy fruits were inoculated with 5 μL conidial suspension and the remaining six were provided with 5 μL distilled water (control). The inoculated fruit was covered with muslin cloth and kept at 25 °C. Disease symptoms were observed and compared with the symptoms of field samples. The disease-causing pathogen was re-isolated on PDA media from these inoculated fruits and compared with initially isolated fungi.

2.4. Identification of isolated fungus by microscopy

Microscopy was performed to study the morphological characters of each isolated fungus. Key structural features like hyphae (septate / non-septate) and reproductive structure (sporangia and conidia) of fungi were observed under a compound microscope (James and Natalie, 2001). For this purpose, a glass slide of fungal mycelia was prepared. Using mounting needles, fragments of young mycelium from the fresh fungal culture margins were placed on the slide with one or two drops of lactophenol blue. Air bubbles were avoided by carefully placing the coverslip. The slides were examined under the microscope at 10 × and 40 × magnifications. Photographs were taken with the help of a mounting camera.

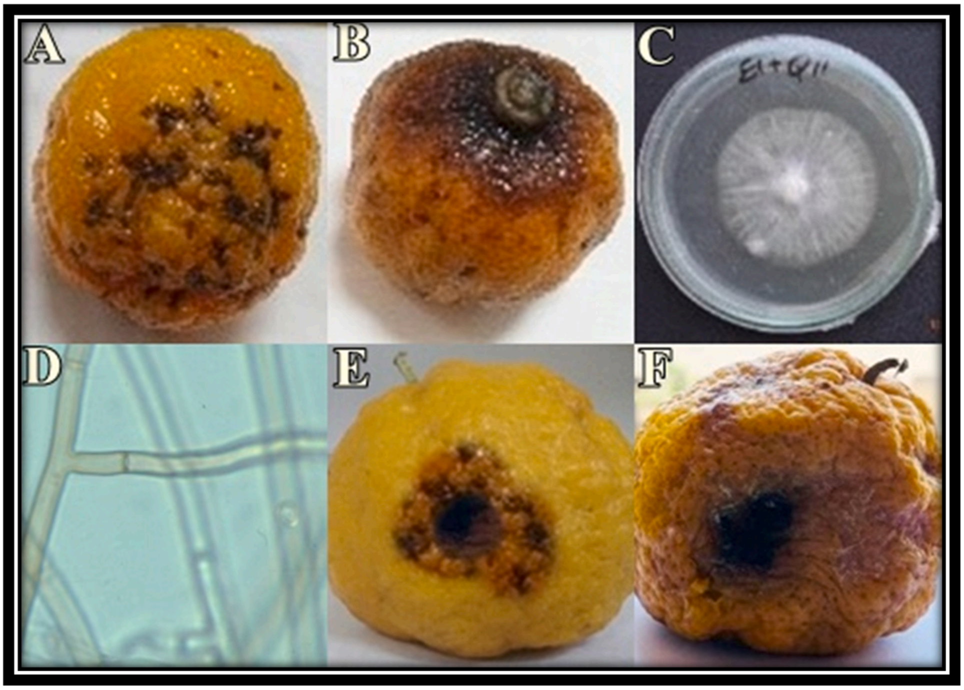


Fig. 1. Initially, symptoms appeared in the form of spot (A), progressed further into complete fruit necrosis (B). The fungus was isolated from diseased fruit on SDA media (C). Fungus mycelia were observed under a microscope at 100 × magnification power (D). After inoculation of healthy fruit, disease symptoms appeared slowly in 1st week (E) but progressed quickly in 2nd resulted in necrosis till 3rd week (F).

2.5. Molecular identification of the fungus

The isolated fungus was also identified by the sequence analysis of the 18 S ribosomal RNA gene. The DNA of the fungus was extracted using the CTAB technique (White et al., 1990). Nanodrop® was used to assess the quality and quantity of extracted DNA. For the amplification of the rRNA gene, universal forward and reverse primers were used. The PCR reaction mixture of 20 μL was comprised of 0.5 μL of each primer, 1 μL dNTP, 1 μL *Taq* DNA polymerase, 0.5 μL genomic DNA and 2 μL of 10 × polymerase buffer. The PCR reaction was carried out at 94 °C for 4 min, followed by 35 rounds of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 10 min. The final product was sequenced using Sequence Navigator (version 1.0.1, Applied Biosystems) and explored in the database of NCBI (https://www.ncbi.nlm.nih.gov/).

2.6. Preparation of plant extract

Extracts of six different plants including *Chenopodium album, Callistemon citrius, Magnifera indica, Lawsonia inermis, Cassia fistula* and *Trachyspermum ammi* were prepared for their antifungal activity analysis. Seeds of *T. ammi* and leaves of the other five plants were thoroughly rinsed in running water and shade dried in the control environment. Dried leaves and seeds were crushed into powder and 100 g powder of each sample was mixed in 1000 ml of autoclaved double distilled water. These mixtures were placed in a shaking incubator at 24 °C for 48 h and boiled for five minutes before being incubated for 15 min in a water bath at 50 °C. The mixture was allowed to cool and strained through muslin cloth and Whatman filter paper no. 1. All the filtrates were labelled and stored at 4 °C, till further use.

2.7. In vitro antifungal activity analysis of plant extracts

Using the poisoned food technique, the antifungal activity of six plant extracts was evaluated, in vitro. For this purpose, PDA media was autoclaved, and warm culture media was mixed with 5 μL of each plant extracts, separately and allowed to solidify. PDA media with no plant extract served as control. Each Petri plate was inoculated with an inoculum disc (5 mm) of isolated fungi and incubated at 25 °C. After seven days of incubation, mycelial growth inhibition was measured by the following formula:

Fungus growth inhibition %= (C-T) / 100

Where C = Average fungal mycelial growth in the positive control, T = Average fungal mycelial growth in treated Petri dishes.

Out of six plant extracts, seed extract of *T. ammi* showed the best results and it was further used for the synthesis of nanoparticles.

2.8. Green preparation of Zinc oxide nanoparticles (ZnO NPs)

Following the methodology of Matinise et al. (2017), ZnO NPs were synthesized in the seed extract of *T. ammi.* The seed extract was heated at 60–80 °C and 2 mg of Zinc Nitrate Hexahydrate [$Zn(NO_3)2.6\ H_2O$] was added. The mixture was boiled until the formation of deep yellow paste. This paste was placed in a ceramic crucible cup and subjected to intensive heat at 500 °C for 3 h, in a hot furnace. Due to this intensive heating, the light-yellow powder was obtained, which indicated the formation of ZnO NPs (Elumalai and Velmurugan, 2015). These NPs were characterized further, before their anti-fungal activity analysis.

2.9. Characterization of ZnO NPs

The following parameters were used to analyse the size, shape, and composition of NPs.

2.9.1. Four transform infrared (FTIR) spectroscopy

The type of associated functional groups of plant extract with nanoparticles was determined by FTIR spectroscopy. Using the KBr pellet method, 10 mg of nanoparticle powder was encapsulated in 100 mg of KBr pellet and analysed in an FTIR spectroscope with a resolution of 4 cm^{-1} and a scan range of 400–4000 cm^{-1}.

2.9.2. X-ray diffraction (XRD)

The crystalline nature of newly fabricated green nanoparticles was investigated using an X-ray diffractometer. The findings were derived from the atomic structure of powder samples and solid crystals, as well as the angles at which diffraction occurred. The size of the nanoparticles was determined using the following Scherrer's formula (2018):

$$D = 0.9\lambda/\beta\cos\theta$$

D = average crystalline domain size perpendicular to reflecting planes, K = shape factor, λ = X-Ray wavelength, β = FWHM (fullWidth at the Half Maximum), and θ = the diffraction angle.

2.9.3. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX)

SEM and EDX analyses were used to determine the shape, scale, and chemical composition of the prepared green nanoparticles. To make a suspension of zinc oxide green nanoparticles in distilled water, the samples were sonicated for five minutes. A drop of the suspension was mounted on conductive tape with a double carbon coating and allowed to dry under the lamp. On the VEGA3 TESCAN instrument, SEM and EDX analyses were performed.

2.10. Antifungal activity analysis of ZnO NPs, in vitro

For in vitro antifungal activity analysis, ZnO NPs were added in PDA media in different concentrations. With the help of a cork-borer, 4 mm inoculum discs of isolated fungi were placed at the centre of the PDA petri dish. The culture media without green nanoparticles served as a positive control. Inoculated Petri plates were placed in an incubator at 25 ± 1 °C and the fungi were allowed to grow. After one week, the growth inhibition in each Petri plate was measured by the following formula:

Fungus growth inhibition %= (C-T) *100

Where C= Average fungal mycelial growth in positive control and T = Average fungal mycelial growth in treated Petri dishes.

2.11. Comparative in vivo antifungal activity analysis of ZnO NPs and plant extracts

The antifungal activity of ZnO NPs and six plant extracts was also tested, in vivo. Selected healthy fruit of grapefruit was inoculated with isolated fungi, following a standard "wound inoculation method". For this purpose, six healthy fruit were wounded with a needle and inoculated with 10 μL of conidial suspension (10^6 conidia/ml) of fungus. The pathogen was allowed to penetrate and cause infection and after two days of inoculation, the above-mentioned nano-fungicides were sprayed (till runoff) on three randomly selected fruit. Three fruit were left untreated (control). All fruit was covered with an autoclaved muslin cloth to avoid any contamination. Following similar methodology, 10 μL of each selected six plant extracts were sprayed (till runoff) on three randomly selected fruit to control fruit rot disease, replacing nano-fungicide. After seven days of inoculation, the diseased area of each fruit was measured to assess the performance of individual fungicides.

3. Results

3.1. Isolation and characterization of the disease-causing pathogen from grapefruits

Disease symptoms were observed on fruit samples, in the field (Fig. 1A and B). The pathogen was successfully isolated and off-white mycelial colonies were observed on PDA (Fig. 1C). Microscopic observations helped us to see fungal morphology and long and rod-like fungal mycelia could be observed, easily (Fig. 1D). All these features identified this pathogen to be *Rhizoctonia solani* (Parmeter, 1970). The pathogenicity of isolated *R. solani* was carried out effectively. After four days of inoculation, the initial symptoms could be observed in the form of small dark brown spots (Fig. 1E). These spots progressed quickly in the 2nd week and resulted in complete necrosis till the 3rd week (Fig. 1F). The fungus was again isolated from these diseased fruits and found similar to an inoculated fungus. These findings confirmed the virulence and involvement of this fungus in the fruit rot of grapefruit. Successful sequencing of amplified 18 S rDNA region of isolated pathogen also confirmed its identification. BLAST analysis of resultant sequence revealed > 99% similarity with *R. solani* (HG934415.1).

3.2. Antifungal activity analysis and selection of plant extract for the synthesis of ZnO NPs

Among six tested indigenous medicinal plants, the seed extract of *T. ammi* exhibited the best antifungal activity (Table 1). Based on these results, the seed extracts of *T. ammi* were used to synthesis ZnO NPs.

3.3. Characterization of ZnO NPs synthesized in the seed extract of T. ammi

The following parameters significantly helped us to characterize ZnO NPs and suggested their further application for antifungal activity analyses.

3.3.1. FT-IR spectroscopy of green ZnO NPs

FTIR analysis of ZnO NPs, prepared in *T. ammi* extract showed a characteristic peak at 1738.69 cm^{-1}, which indicated the presence of Aldehydes group (C=O stretching) on NPs (Fig. 2). The spectrum of this sample also showed some specific bands at 1366.16.34 cm^{-1} (O-H bending) and 1216.90 cm^{-1} (C-O stretching), indicating organic components of extracts that are responsible for the successful preparation of ZnO NPs. Prepared NPs were found to contain biomolecules such as phenols (thymol), aldehyde and vinyl ether. Thymol has been reported to be an important reducing and capping agent in the biosynthesis of NPs (Manukumar et al., 2017).

3.3.2. XRD analysis of synthesized ZnO NPs

X-Ray Diffraction pattern of ZnO NPs showed noticeable peaks at 31.8^0, 34.32^0, 47.44^0, 56.44^0, 62.80^0, 66.25^0, 67.80^0, 68.93^0, 72.51^0 and 76.86^0 corresponding to peaks values (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) (Fig. 3). The planes of XRD patterns had an excellent agreement with JCPDS No. 01-079-0207. The prepared NP sample was indexed to a single-phase Hexagonal structure with P63mc space group number: 186, zinc oxide. The average particle size (48.52 nm) was calculated by the Debye Scherrer formula, which was in agreement with previous findings (Saravanakkumar et al., 2016).

3.3.3. SEM and EDX analysis of ZnO NPs

EDX spectra of NPs exhibited the dominant presence of zinc (43.72%), carbon (30.12%) and oxygen (26.12%) (Fig. 4A). The EDX analysis also showed optical absorption peaks of NPs which indicate their surface plasmon resonance effect. The origin of these elements lies

Table 1
In vitro antifungal activity of six indigenous medicinal plant extracts *R. solani*.

Name of plant	Mycelial growth inhibition (%)
Trachyspermum ammi	50.9 ± 0.0
Chenopodium album	17.4 ± 0.6
Callistemon citruns	15.7 ± 0.6
Magnifera indica	24.3 ± 0.0
Lawsonia inermis	19.6 ± 0.6
Cassia fistula	20.0 ± 0.6

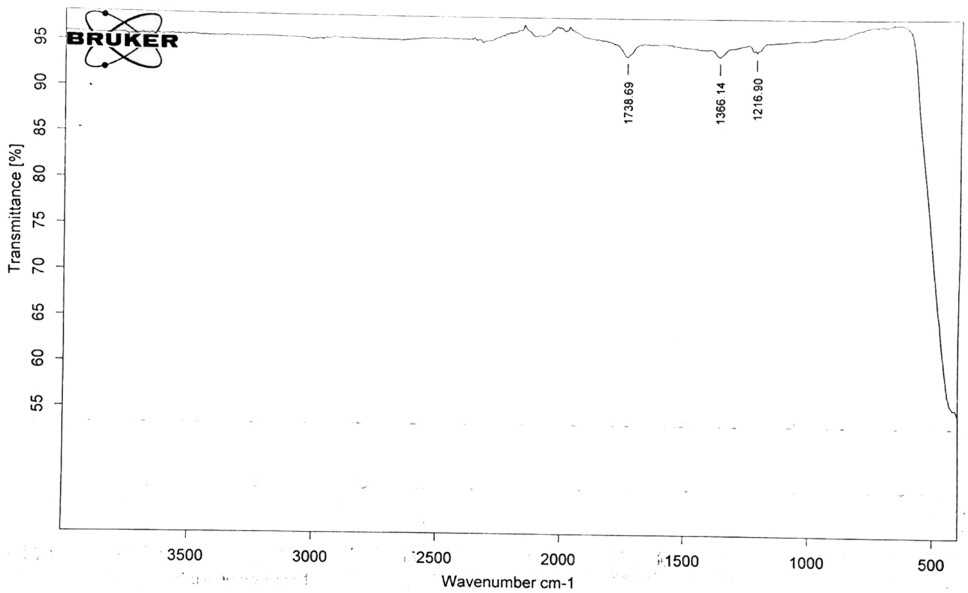


Fig. 2. FTIR spectrum showing sharp peaks of ZnO NPs synthesized in *T. ammi*.

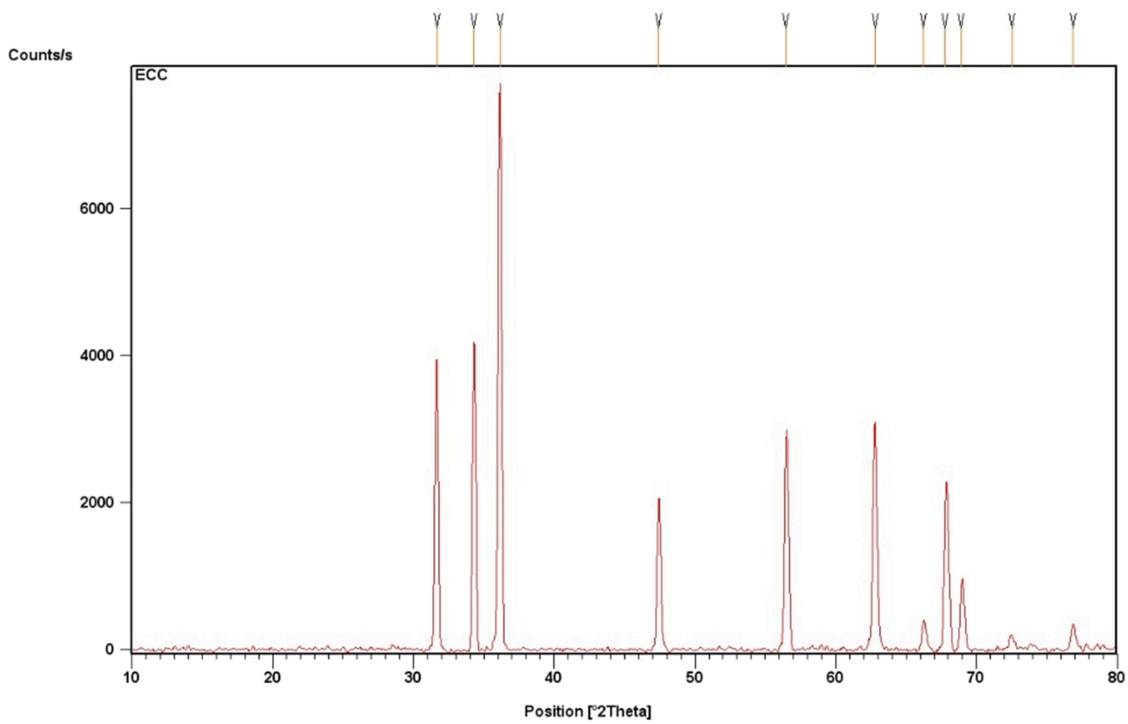


Fig. 3. XRD analysis of ZnO NPs synthesized in *T. ammi*.

in the components of phyto-extracts (Wei et al., 2009).

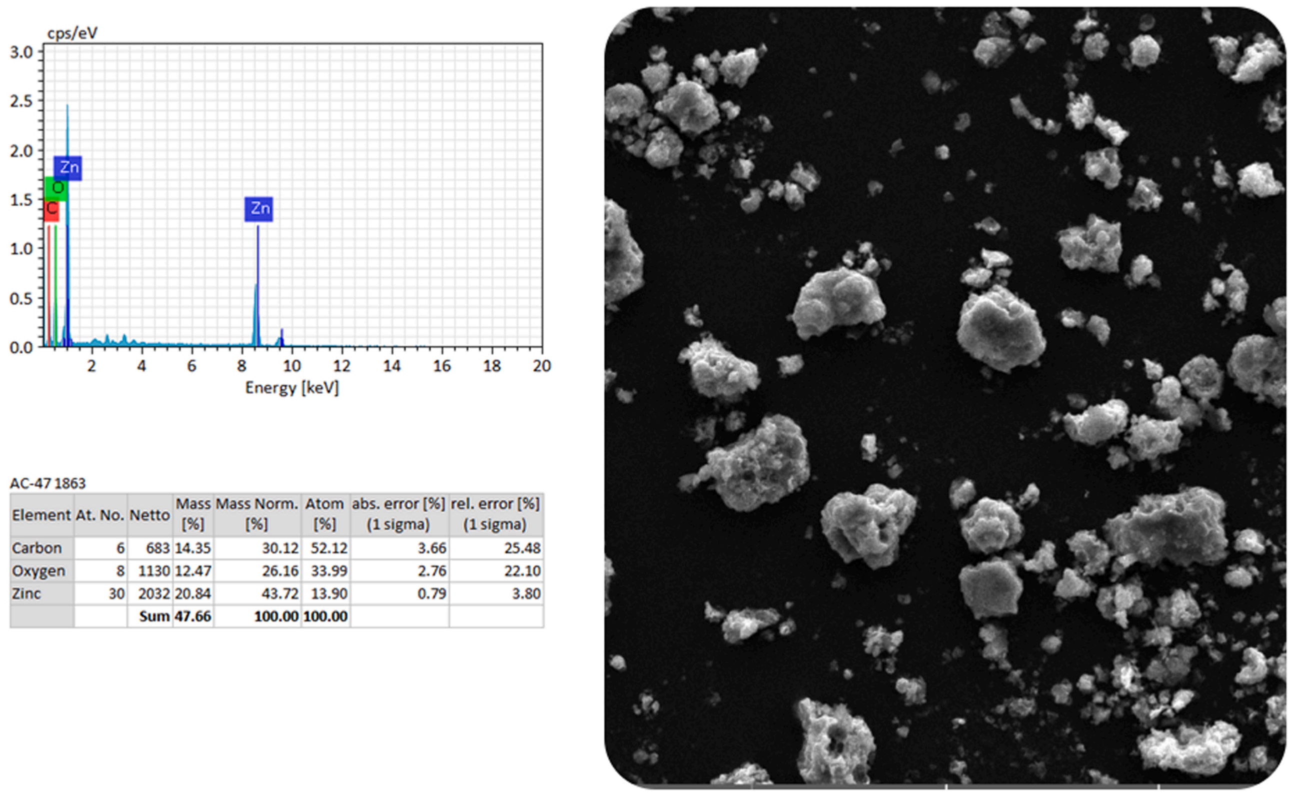
SEM analysis helped us to investigate the superficial morphology of ZnO NPs, prepared in the extract of *T. ammi*. SEM images showed the hexagonal shape of synthesized NPs, which was aligned upward to form an aggregation of bundles (Fig. 4B).

3.4. Antifungal activity of ZnO NPs, in vitro and in vivo

Synthesized ZnO NPs exhibited significant growth inhibition of *R. solani* at 1.0, 0.75 and 0.5 mg/ml concentrations (Table 2 and Supplementary 2). The lower concentration of NPs (0.25 and 0.1 mg/ml) showed google the least growth inhibition of tested fungus. In the in vivo treatment, the ZnO NPs nanofungicide exhibited good disease control (Table 3 and Supplementary 3). NPs controlled disease tremendously and the antifungal activity of these ZnO NPs at 1.0 mg/ml concentration was better than all plant extracts. Significant growth inhibition describes the importance and potential use of these NPs in disease control.

4. Discussion

This study has described the successful isolation and characterization of fruit rot fungi and their environment-friendly control by using nanoparticles. In this study, *R. solani* was found to be associated with the fruit rot of grapefruit in Pakistan. Control of these diseases is a matter of



Element	At. No.	Netto	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	rel. error [%] (1 sigma)
Carbon	6	683	14.35	30.12	52.12	3.66	25.48
Oxygen	8	1130	12.47	26.16	33.99	2.76	22.10
Zinc	30	2032	20.84	43.72	13.90	0.79	3.80
		Sum	47.66	100.00	100.00		

A B

Fig. 4. EDX spectrum (A) and SEM photograph (B) of ZnO NPs prepared in *T. ammi*.

Table 2
Growth inhibition of *R. solani* ZnO NPs at different mg/ml concentrations.

Treatment mg/ml	Growth inhibition (%)
1.0	70.2 ± 0.5
0.75	61.4 ± 0.5
0.5	48.5 ± 0.5
0.25	1.2 ± 0.5
0.1	0.6 ± 0.5
0	0 ± 0.5

Table 3
Diseased area on grapefruit after treatment with different concentrations of ZnO NPs.

Treatment	Diseased area (mm)
1.0 mg/ml ZnO NPs	3.1 ± 5.4
0.75 mg/ml ZnO NPs	5.3 ± 0.5
0.5 mg/ml ZnO NPs	7.1 ± 1.1
0.25 mg/ml ZnO NPs	17.9 ± 3.1
0.1 mg/ml ZnO NPs	48.1 ± 5.4
0 mg/ml ZnO NPs	60.9 ± 5.4
Trachyspermum ammi	12.4 ± 2.3
Chenopodium album	28.8 ± 3.3
Callistemon citruns	26.3 ± 5.1
Magnifera indica	17.9 ± 3.1
Lawsonia inermis	20.1 ± 4.1
Cassia fistula	18.8 ± 3.8

great concern for modern-day plant pathologists. For better human health, the use of chemical pesticides is being discouraged in the civilized world. Scientists are focusing on the use of natural antimicrobial products, especially those derived from plants.

In this study, we used six-well reputed and well-documented indigenous plants to check their antifungal potential. Out of these, *T. ammi* seed extract with the best antifungal activity was used for the green synthesis of NPs. The involvement of alkaloids and flavonoids, which are known to be active bio compounds against fungi and bacteria, could indicate the antifungal effectiveness of *T. ammi* (Avita, 2013). Individually or in combination, the bioactive polyphenol compounds found in seed extracts interfere with the life cycle of fungi by binding with their protein molecules, acting as chelating agents, altering structural component synthesis, weakening, or destroying the cell membrane permeability barrier, and altering the cell physiological status (Rongai et al., 2015). Previous studies have also proved the antifungal potential of *T. ammi* against a variety of fungal strains due to the presence of many antifungal compounds in the seed extract of *T. ammi* (Jyoti et al., 2019). FTIR spectra of prepared NPs showed the presence of thymol, which is a famous antimicrobial phenolic compound (Khan, 2017). Previous studies also describe that the bioactive compounds of plant extracts act as capping agents and reduce the metal ions to produce NPs. This combination becomes more lethal for pathogen and control disease, more efficiently (Chouhan and Meena, 2015). *T. ammi* leaf extracts have also been used to fabricate green nanoparticles of copper, silver, nickel and magnesium (Jagana et al., 2017).

In this study, the green synthesis of ZnO NPs was executed successfully by using seed extract. Studies describe that bioactive compounds present in seed extracts of *T. ammi* could be adsorbed on the surface of metal nanoparticles by possible interaction of functional groups and these compounds acting as reducing and stabilizing agents, during the formation of NPs. Plants are rich in compounds like amino acids, polyphenols, nitrogen bases and reducing sugars. Such types of chemicals serve as reduction and stabilisation agents, during magnetite nanoparticle synthesis (López and Antuch, 2020). In this study the prepared green ZnO NPs shown enhanced antifungal because of their small size and stability. Smaller size increases the dispersion and penetration of the intracellular matrix and interferes with intracellular absorption of Ca^{2+}

and induces cell damage (Srihasam et al., 2020). Attachment of the NPs with the microbial cell membrane results in damage to the cell membrane and intracellular organelles (Basak et al., 2014).

ZnO NPs are environmentally friendly, non-toxic, bio-safe, and biocompatible (Mohammad et al., 2010). The US Food and Drug Administration has listed ZnO, along with four other zinc compounds, as a "generally recognised as safe (GRAS)" substance (FDA Food and Drug Administration, 2015). Previous studies have described that ZnO are not toxic, if used at low concentration. ZnO NPs may sometime adversely affect the living system if liver cell is exposed to 14–20 mg/ml concentration for 12 h (Siddiqi et al., 2018). It also induced DNA damage by oxidative stress. Antimicrobials made of zinc oxide nanoparticles (ZnO NPs) have emerged as a new class. ZnO NPs first encounter bacterial cells and adhere to the plasma membrane's outer surface. The structure of the plasma membrane is disrupted, and its permeability is affected because of this interaction. Disruption of membrane structure and subsequent accumulation of ZnO NPs in the cytoplasm obstruct basic cell growth processes (Zhang et al., 2017). ZnO NPs mediates hydrogen peroxide, which is one of the most significant antibacterial effectors (Sawai et al., 1998). In addition, ZnO NPs contain other reactive oxygen species, such as hydroxyl radicals and singlet oxygen, which stimulate cell death.

5. Conclusion and prospects

The use of plant extracts to prepare NPs is an easy and cost-effective method. These NPs are environmentally friendly and non-toxic. Plant biomolecules such as proteins (enzymes), amino acids, polysaccharides, alkaloids, alcoholic compounds and vitamins can play a role in the reduction, formation and stabilisation of NPs. The growth of pathogenic fungi can be inhibited by small ZnO NPs. Nanotechnology can have agricultural solutions and can revolutionize current disease management systems. Material scientists and biologists need to work together to gain a deeper understanding of the fundamental mechanisms of interaction in a complex bio-nano system. A detailed understanding of the structural properties of nanoparticles, such as morphology, scale, functional groups and active adsorption/loading capability, can provide a useful guide as a starting point for the rational selection of suitable nanoparticles. It is also important to select a robust and reproducible system for conducting biocompatibility and efficacy studies at the ecosystem levels of cells, organisms and pests, to achieve as close-to-field conditions as possible.

CRediT authorship contribution statement

Musrat Ali, **Urooj Haroon:** Conception and design of study. **Hassan Javed Chaudhary**, **Musrat Ali:** Acquisition of data. **Farooq Hussain Munis**, **Asif Kama**l: Analysis and/or interpretation of data. **Kamal Usman**, **Aishah Alatawi**, **Muhammad Hamzah Saleem:** Drafting the manuscript. **Shafaqat Ali**, **Qurban Ali**: revising the manuscript critically for important intellectual content. **Musrat Ali**, **Xiukang Wang**, **Urooj Haroon**, **Hassan Javed Chaudhary**, **Asif Kamal**, **Qurban Ali**, **Muhammad Hamzah Saleem**, **Kamal Usman**, **Aishah Alatawi**, **Muhammad Farooq Hussain Munis**, **Shafaqat Ali:** Approval of the version of the manuscript to be published.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113311.

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