Suppression Application of Copper Oxide Nanoparticles for Wilt-Inducing *Fusarium Equiseti* in Wheat

Tarek Abdelghany (✉ tabdelghany@yahoo.com)

Al-Azhar University <https://orcid.org/0000-0001-6418-5890>

Asmaa A. Alharbi

2Biology Department, Faculty of Science, Jazan University, Jazan, Saudi Arabia

Aisha M. H. Al-Rajhi

3Biology Department, Faculty of Science, Princess Nourah Bent Abdulrahman University

Research

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Abstract

Background: Recently, nanoparticles has proposed as a different and ecofriendly tools for solving many problems in agricultural field.

Aim: The current investigation is an attempt to assess the antifungal activity of Copper oxide nanoparticles (CuONPs) against wheat wilt disease.

Results: Isolates of *Fusarium sp.*, *Alternaria sp.* and *Rhizoctonia sp.* species were recovered from wheat roots showed wilt symptoms. Highest frequency (77.12%) was associated to *Fusarium sp.* *Fusarium sp.* isolates from pathogenicity test indicated that isolate (F1) out the highest frequency isolates caused high percentage of wilt reached to 65.1%. Molecular identification of F1 isolate was performed using ITS rRNA gene, and confirmed that it belong to *F. equiseti*. Inhibition (%) of *F. equiseti* growth was 3.45 and 16.66% at 100 ppm, and it was 74.14 and 78.21% at 400 ppm of CuONPs at 5 and 8 days, respectively. The action mechanism of CuONPs on *F. equiseti* inhibition was elucidated by transmission electron microscopy. CuONPs induced morphological changes and damage of the cell membranes of the *F. equiseti*. Healthy wheat plant without *F. equiseti* infection exhibited 11.24 ug. g⁻¹ dry weight of total phenol content (TPC), while infected plant showed highest content 14.25 ug. g⁻¹ dry weight. CuONPs at 200ppm induced TPC of infected plant by *F. equiseti* to 16.67 ug. g⁻¹ dry weight. Increment in Peroxidase, polyphenol oxidase, superoxide dismutase activities in wheat plant cultivated in soil inoculated by *F. equiseti* and amended with 400 ppm of CuONPs were recorded comparatively to wheat plant cultivated in soli inoculated by *F. equiseti* but without CuONPs.

Conclusion: The inhibitory effects of CuONPs are very actual management strategies for the control wheat wilt disease caused by *F. equiseti* in respective to present and future studies.

Background

Most of Human beings depend on Wheat (*Triticum aestivum* L.) as a source of food. Therefore it considered the most vital cereal crop in many countries after rice. Phytopathogenic fungi play a critical role in destroying and reducing wheat yield as well as other plants. Several fungi colonize and invade in the root of wheat plants causing root rots (Bentley et al., 2006). Wheat plant at vegetative growth and other development of growth such as flowering process may infect by various diseases via numerous species of fungi. Therefore many studies mentioned that leaves, stems, basal stem tissues, seedlings, seed and roots of wheat were attacked by a large number of fungi (Wiese, 1987, Moya-Elizondo et al., 2011). A main problems associated with plant cultivation and crops production are fungal diseases particularly *Fusarium* diseases. Deterioration of significant crops was recorded as a result of *Fusarium* diseases such as potato dry rot, beans decline, wheat crown rot, wheat head blight, rice bakanae and wheat wilting. According to earlier literature studies, the greatest frequently identified *Fusarium* spp. related to crown and root rot are *F. graminearum*, *F. culmorum* and *F. avenaceum* (Cook 1980, Kane et

al.,1987; Specht and Rush1988). Vascular wilt of cumin affected by *Fusarium equiseti* was reported in 2012 (Suthar and Bhatt 2012).

In numerous fields of Montana, Moya-Elizondo et al., (2011) studied the associated pathogens of wheat. Where % incidence was 15%,13% and 8% for *Bipolaris sorokiniana*, *F. culmorum* and *F. pseudograminearum*, respectively. Many reports suggest that the diversity of *Fusarium* community has dependent on plant rotation (Luque et al., 2005; Wakelin et al., 2008). According to Goswami et al. (2008), tropical and sub-tropical areas are a common habitat of *F. equiseti*. The pathogenicity of *F. equiseti* was recorded on a varied range of plants and it has recorded recently by Hami et al., (2021) as a causal fungus of wilt in chilli wilt in Kashmir valley at Northern Himalayas. Numerous attempts from agronomists to create varieties of wheat resistant to phytopathogens (Harlapur et al., 1993, Ahmed et al., 2009) but these varieties not continue for long time and become sensitive to most diseases. Therefore the research for novel agent for controlling fungal diseases are necessary to solve these problem.

Currently, the nanoformulations attracted the scientific researchers for its applications in many fields as well as agricultural field not only for controlling the occurrence diseases but also for enhancing the physiological behavior in plants, productivity of crops and development of vegetative growth (Adamovskaya et al., 2005; Servin et al., 2015; Lopez-Lima et al., 2021). Numerous studied the lowest toxicity of metals such as silver, copper, zinc, and iron in nanoforms compared with its salts (Abdel Ghany et al., 2018), nanoparticles of copper, Zinc and iron are 7,30 and 40 times less poisonous as compared to their corresponding sulfate salts of copper (Panyuta et al., 2016).

Copper nanoparticles (CuNPs) was investigated as a fungicide against four phytopathogens by Kanhed et al. (2014), who found that CuNPs inhibit the mycelia development of *F. oxysporum*, *Alternaria alternata*, *Curvularia lunata* and *Phoma destructive*. Large surface area to volume ratio considered a promising and critical factor for efficacy the suppressing action of CuNPs (Abdel Ghany et al., 2018). Copper oxide nanoparticles (CuONPs) also were applied without any physiological toxicity for suppressed gray mold disease caused by *Botrytis cinerea* (Elmer and White 2016). Elmer et al. (2018) recorded less disease caused by *F. oxysporum* and *Verticillium dahliae* in Tomato and eggplant treated by CuONPs. The studies of *Fusarium* wilt of numerous plants are well advanced in some regions of the world, but are still narrow elsewhere in wheat. Therefore, the current research focused on controlling the causative agent of wheat wilt by CuONPs with studying the their effects on ultrastructure of pathogen and some biochemical changes in wheat plant.

Methods

Isolation and identification of wheat wilt accompanying fungi

Wheat (*Triticum aestvium* L.; cultivar Giza 171) plants showing symptoms of wilt (Fig. 1) were selected from Six different fields in Menofia Governorate, Egypt. The roots of plant were surface sterilized for 5

min in 0.1% sodium hypochlorite, then washed by triple sterile distilled water and dried in air in a laminar flow bench. A root was aseptically chopped transversely and longitudinally into 1 cm, then the segments were placed on potato dextrose agar (PDA) in petri plates supplemented with 0.4% streptomycin sulfate for inhibiting the bacterial growth, followed by incubation for 6 days at 25°C. The developed fungal colonies were purified via a hyphal tip technique (Dhingra and Sinclair 1985) and then identified according to mentioned identification keys including macro- and micro-scopic examination including observation of growth rate, colony color, presence or absence of macroconidia, microconidia, chlamydospores (Nelson et al 1983; Barnett and Hunter 1986; 2006, Leslie and Summerell, 2006). The purified isolates were kept on slants containing PDA and preserved in refrigerator for further tests.

Pathogenicity test

Conidial suspensions of three fungal isolates belonging to *Fusarium* sp. (F1, F2 and F3) were used as inoculums for soil cultivated by wheat plants. Spores suspension was centrifuged at 3000 rpm per 5 min, then the spores pellet was washed, followed by repeating centrifugation. The source of used wheat grains (*Triticum aestivum* L.; cultivar Giza 171) was Agricultural Research Center, Giza, Egypt. The wheat grains were surface sterilized by HgCl₂ (0.1%), followed by rinsing with distilled water. The grains were cultivated in 20cm diameters Plastic pots filled with 2 kg of autoclaved soil/ pot, then the soil in pots inoculated at level 2x10⁴ spores/ml (200 ml) suspensions that calculate using a hemocytometer. The pots were irrigated each 5 days and the developed seedlings were viewed and disease assessment was documented through 25 days (Alon et al., 1973). The causative agent of wilting disease of wheat from F1, F2 and F3 that confirmed by pathogenicity test was used for further study.

$$\text{Pathogenicity} = \frac{\text{Number of wilting plants}}{\text{Total number of plants}} \times 100$$

(1)

Molecular identification of pathogen

From pathogenicity test, the potent isolate of *Fusarium* sp. was inoculated on plate containing PDA plates, followed by incubation at 25 °C for 5 days for molecular identification (Hami et al., 2021). DNA was extracted from mycelia growth using Microprep Kit of quick-DNA fungi/bacteria (Zymo research; D6007) via the manufacturer's protocol of Sigma Scientific Services Company, Egypt. PCR was achieved via Maxima Hot Start PCR Master Mix (Thermo; K1051). ITS1-F (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4-R (5'- TCCTCCGCTTATTGATATGC-3') were used as forward and reverse primers, respectively for the amplification of pathogen DNA. Thermal Cycler Condition: One cycle for initial denaturation 94°C for 6 min., 35 cycles of denaturation, annealing and Extension for 45,45 and 60 s at 94,56 and 72°C, respectively. One cycle for final extension for 5min at 72°C. Pathogen DNA was examined using gel electrophoresis. BLAST analysis was applied for sequences homologous sequences at NCBI (<http://ncbi.nlm.nih.gov/BLAST>). Neighbor joining method was used for reconstruction the evolutionary tree.

Copper oxide nanoparticles

Copper oxide nanoparticles (CuONPs) less than 100 nm were provided in powder form from Nawah Scientific company, Egypt .

Antifungal activity assessment of CuONPs on radial growth and ultra-structures of pathogen

Potato dextrose agar (PDA) medium supplemented with different concentrations of CuONPs (100, 200, 400 ppm) was applied for deterring the inhibitory action of CuONPs against pathogen (Abdel Ghany et al. 2020). Disc (0.5 mm) of actively growing colony of pathogen at 5 days incubation period was inoculated in the center of plat containing growth medium, incubated at 25°C for 7 days, followed by measuring the colony radius and recording the inhibition % according to the following equation.

$$\text{Inhibition\%} = \frac{(\text{RGC} - \text{RGT})}{\text{RGC}} \times 100 \quad (2)$$

Where RGC, radial growth of the control and RGT, radial growth of treatment. Medium without any concentration of was used as control.

Transmission electron microscope (TEM) studies were carried out as following (Abdel Ghany and Tayel 2009), fungal pathogen was inoculated on cellophane membrane located on CYA petri dish amended with different concentrations of CuONPs. Tips of fungal hypha were cuted under the light microscope from the margin of colony, then fixed in 5% gluteraldehyde for 24 h. followed by washing using phosphate buffer (pH 7.2) for three times. The buffer was removed, then the sections were dipped for 2 h in aqueous solution of 1% osmium tetroxide. Osmium solution was removed from hyphae sections, then it dehydrated using a series of different doses of ethanol (50–96%). After removing the absolute ethanol, propylene oxide was added to the hyphae sections for 60min. The hyphae sections were placed in propylene oxide and Epon 812 resin (2:1), followed by placed in pure resin for 12h, then placed at 60°C in an oven for 48 h. Using ultra microtome, blocks (50 nm)were sectioned, then stained using uranyl acetate-lead citrate 500A and examined by TEM (C Joel Jem- 1200 EX II. Acc. Voltage 120 KV. MAG- medium) at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Pot experiment and effect of CuONPs on plant pathogenic fungus

From the pathogenicity test, the identified isolate of pathogen was re-subcultured on the autoclaved PD broth medium under aseptic conditions, then incubated for 7 days at 25°C. After incubation period, the fungus spores were taken in for spores suspensions preparation in sterile water and used for inoculation of plants at level 2×10^4 spores/ml suspensions that calculate using a hemocytometer. The source of used wheat grains (*Triticum aestvium* L.; Cultivar Giza 171) was Agricultural Research Center, Giza, Egypt. The wheat grains were surface sterilized by HgCl_2 (0.1%), followed by rinsing with distilled water. The

grains were cultivated in 20cm diameters Plastic pots filled with 2 kg of autoclaved soil/ pot and then divided into groups for treatments as following, without inoculation, inoculated with phytopathogen, inoculated with phytopathogen + 100ppm CuONPs, inoculated with phytopathogen + 200ppm CuONPs, inoculated with phytopathogen + 400ppm CuONPs, without inoculation + 100ppm CuONPs, without inoculation + 200ppm CuONPs and without inoculation + 400ppm CuONPs. The number of grains in each pot was 6 in triplicate, irrigated with sterilized water each 5 days, incubated for 20 days at approximately $24 \pm 2^{\circ}\text{C}$ under natural photoperiod under greenhouse conditions. During seedling growth, the symptoms of wilting disease were observed, and the plants were collected for biochemical analysis and further study (Abdel Ghany et al., 2015).

Total phenolic content determination in shoot extracts of wheat plants

According to Kaur and Kapoor (2002), total phenolic content in shoot extract of wheat plants was determined. The reaction mixture for 5min contained 0.5ml of wheat plant extract, 2.5ml distilled water and 0.25ml of 2N Folin-Ciocalteu reagent. Followed by the addition of 0.75ml of Na_2CO_3 (20% w/v) beside 0.95ml distilled water. Then the reaction mixture was kept at 37°C in without exposure to light for 1 h. At 765nm, the absorbance was recorded to determine total phenolic content compared to Gallic (GA) acid as standard and expressed as mg GA g⁻¹ dry weight.

Antioxidant activity

The treated plant seedlings were collected for measuring the antioxidant activity using 5g of fresh leaves and stems via homogenization in cold phosphate buffer (0.05 M, with pH 6.5). The homogenized extract was centrifuged for 10 min at 4000g. The obtained supernatant was applied as a source enzymes after rising to 5 ml. The formed precipitate from centrifugation process was washed with distilled water, then re centrifuged, the collected residues were immersed for 60 min in 10 ml NaCl (1 M) to release cell wall-bound peroxidase (POX), followed by centrifugation for 10 min at 4000g. The obtained supernatant was applied as a source of wall-bound POX (Saroop et al. 2002). According to Beyer and Fridovich (1987), nitro blue tetrazolium (NBT) reduction method was used superoxide dismutase (SOD) activity, while soluble and cell wall-bound (POX) and polyphenol oxidase (PPO) were determined according to Saroop et al. (2002) and Kar and Mishra (1976) respectively. U mg⁻¹ protein min⁻¹ was applied for expression of enzymes activity.

Statistical analysis

With using SPSS software (version 14), all results were analyzed and it was expressed as mean $\pm$ SD.

Results

Isolation and pathogenicity

Fungal isolates belonging to *Fusarium sp.*, *Alternaria sp.*, *Rhizoctonia sp.* were isolated from wilted wheat plants (Fig. 2). *Fusarium sp.* showed the highest frequency (77.12%) followed by *Alternaria sp.* (15.84%), followed *Rhizoctonia sp.* (7.04%). Pathogenicity test was performed using three the highest frequency isolates of *Fusarium sp.* (Fig. 3). The highest percentage of wilt was recorded by isolates F1 (65.1%) followed by isolates F2(30.5%) and F3 (22.4%). For confirmed, the pathogenicity of isolates F1, it was re-isolated from diseased wheat plants that artificially inoculated with pathogen.

The morphological identification of grown pathogen (Fig. 4) from pathogenicity test was confirmed using molecular characterization, which is based on ITS rDNA (Figs. 5A and B). Multiple sequence alignment on a database (BLAST) was used for studying the 18S rRNA sequence of the *F. equiseti* isolate. Result of alignment profile(Fig. 5B) demonstrated that *F. equiseti* strain SKASS_BRH16F1 rRNA amplicon closely exhibited harmonized to other isolates of *F. equiseti* (> 99%) as shown in phylogenetic tree(Fig. 5A).

Inhibition Of Pathogen By Cuonps

Colony diameter of *F. equiseti* was determined at the incubation periods of 5 days and 8 days at different concentrations of CuNPs. Results in table (1) exhibited increase in fungus diameter at low concentration of CuONPs as the incubation period increased but negligible increase in colony diameter was observed at high concentration of CuONPs particularly at 400 ppm. Therefore the inhibition (%) of fungal growth at 5 and 8 days was 3.45 and 16.66% at 100 ppm while it was 74.14 and 78.21% at 400 ppm, respectively. At the same time Sporulation (%) was accompanied to inhibition %. In contrast, the growth at control still grew clearly and their diameter increased normally.

TEM examination showed that *F. equiseti* generally displayed integrity of the internal organs including cell membrane, mitochondria, organized cytoplasm, and visible nuclei and with well-defined envelopes as well as cell wall (Fig. 5). On the other hand, ultrastructures in treated fungus with CuONPs reflected the presence of distortions inside fungal mycelia as well as conidia (Fig. 5), these alterations increment with the increasing CuONPs concentration. The Plasma membrane remained intact at 100 ppm of CuONPs but become more affected at 200 ppm of CuONPs. Clear collapsing in cell membrane out from cell wall with irregular shape was observed particularly at 200 and 400 ppm of CuONPs, and the cell approximately appeared empty (Fig. 5). Raised condensation and aggregation of cytoplasmic characterize the fungus cell exposed to CuONPs.

Total phenol content of wheat plant infected by *F. equiseti* was highest 14.25 ug. g⁻¹ dry weight than that detected in healthy plant without infection 11.24 ug. g⁻¹ dry (Table 2). Negligible variation in the total phenol content was observed with the addition of CuONPs to plant without infection, while its addition with *F. equiseti* increased the total phenol content to 16.67 ug. g⁻¹ dry weight at 200ppm of CuONPs (Table 2).

Table 1

The Colony diameter, Inhibition and Sporulation of *Fusarium* sp. according to incubation period at various CuONPs concentrations.

CuONPs Concentration (ppm)	Growth (cm)		Inhibition (%)		Sporulation (%)	
	5 days	8 days	5 days	8 days	5 days	8 days
Control	5.8 ± 0.52	7.8 ± 0.33	0.0	0.0	100	100
100	5.6 ± 0.45	6.5 ± 0.25	3.45	16.66	95	97
200	3.4 ± 0.06	3.8 ± 0.66	41.38	51.28	60	65
400	1.5 ± 0.25	1.7 ± 0.15	74.14	78.21	22	23
Chemical fungicide	1.1 ± 0.02	1.3 ± 0.22	81.03	83.33	15	16

Table 2

Total phenol content of wheat plant cultivated in soil inoculated by *F. equiseti* and amended by CuONPs

Treatment	*Total phenol (ug. g ⁻¹ dry weight)
Control	11.24 ± 0.7
100 ppm	10.55 ± 0.5
200 ppm	11.12 ± 0.4
400 ppm	11.55 ± 0.5
<i>F. equiseti</i>	14.25 ± 1.2
<i>F. equiseti</i> + 100 ppm	16.48 ± 1.5
<i>F. equiseti</i> + 200 ppm	16.67 ± 0.4
<i>F. equiseti</i> + 400 ppm	13.08 ± 0.8

Antioxidant Enzymes

In the current results, activities of superoxide dismutase (SOD), polyphenol oxidase (PPO) and Cell wall bound peroxidase (POX) increased in plant infected by pathogen (Table 3). Remarkable activities of oxidative enzymes were observed with the addition of CuONPs to soil inoculated by pathogen particularly at high concentration 400 ppm. Therefore, these enzymes protect the plant from the biological and non-biological stresses resulted from the influence of pathogen and metal, respectively.

Table 3
Antioxidant activity of wheat plant infected by *F. equiseti* and treated by CuONPs

Treatment	Antioxidant activity		
	SOD	PPO	Cell wall bound POX (U mg ⁻¹ protein min ⁻¹)
Control	11.25 ± 1.0	13.78 ± 0.5	0.87 ± 0.01
100 ppm	11.74 ± 0.8	13.91 ± 0.4	0.92 ± 0.02
200 ppm	11.89 ± 1.2	13.79 ± 1.5	1.62 ± 0.01
400 ppm	12.61 ± 1.3	13.85 ± 1.2	1.81 ± 0.05
<i>F. equiseti</i>	12.56 ± 0.6	15.56 ± 1.4	1.98 ± 0.04
<i>F. equiseti</i> + 100 ppm	13.54 ± 0.5	16.04 ± 1.1	2.50 ± 0.01
<i>F. equiseti</i> + 200 ppm	14.25 ± 0.4	16.72 ± 0.4	2.79 ± 0.02
<i>F. equiseti</i> + 400 ppm	21.41 ± 1.2	26.41 ± 1.8	10.2 ± 0.06

Discussion

The observed symptoms of wheat wilting disease initiate with partial yellowing of leaves, followed by complete yellowing of plant, during the growth of seedlings. The ability of fungal isolates from wilted seedlings to cause wilt symptoms was varied the percentage of infection and disease severity. These observation may related to isolate pathogenicity. The results of pathogenicity test confirmed that *F. equiseti* considered the causative agents of wheat will disease. Therefore, the identification was documented by molecular tool. According to some studies (Romanelli et al. 2010; Delgado-Serrano et al. 2016), ITS rDNA application for fungi identification at level of species is mainly depend on the variable nature of the ITS areas of DNA. In Nigeria, Chimbekujwo (2020) studied the wilt disease of cotton (*Gossypium hirsutum*), who found that the major (90%) isolated fungi belong to *Fusarium solani* and *F. equiseti*, these study documented that *F. equiseti* isolates were less virulent compared to *F. solani*. Some studies focused on the pathogenicity of *F. equiseti*, where numerous members of the leguminoseae beside some cereals were reported as a host range for *F. equiseti* causing root rot and damping-off diseases (Rubella et al., 2008). In Algeria,, Bencheikh et al. (2020) documented recently the first report on crown rot and damping-off on durum wheat caused by *Fusarium equiseti*. In China, the first report on cauliflower wilt by *F. equiseti* was mentioned previously in 2017 (Pan-Liang et al. 2017). Inhibition % of *F. equiseti* growth was promoted by high concentrations of CuONPs 200 and 400 ppm. It can be noted that the fungicidal potential of CuONPs depends on its concentrations as well as on fungal species as mentioned previously (Pariona et al., 2019). The inhibitory effect of CuONPs could be attributed to smallest nanoparticles size. The applied of CuONPs as fungicide was documented against many species of *Fusarium* including *F. oxysporum*, *F. equiseti* and *F. culmorum* which are the most common phytopathogens in Central India according to Bramhanwade et al. (2015). Inhibition of *F. equiseti* growth

at different concentrations of CuONPs at 5th and 8th days was recorded. Viet et al. (2016) observed that *Fusarium* sp. was not increased with increasing incubation period from 3 to 9 days at 450 ppm CuNPs. Toxicity of CuONPs in the current study may depends on the combination of numerous factors like as concentration and length of exposure.

TEM revealed the presence of many alterations in *F. equiseti* cells and spores exposed to CuONPs. Surprisingly, plasma membrane ruptured at 400 ppm of CuONPs. Abdel Ghany et al. (2020) studied the effect of different levels of CuONPs on the ultrastructure of *Aspergillus niger*, who revealed that CuNPs treatments stimulated the ultrastructures changes of the fungus. Highly changes were observed in the mycelium and cell membranes of many fungi including *Fusarium oxysporum*, *F. solani*, and *Neofusicoccum* sp. as a result of exposition to CuONPs (Pariona et al., 2019).

Synthesis of phenolic compounds was induced as a result of fungal infection. Cell wall degrading enzymes play a critical role during pathogenesis caused by fungi, therefore as mentioned previously, phenolic compounds suppressed the action of cell wall degrading enzymes produced by *F. oxysporum* (Modafar and Boustani, 2001). Recently, El-Sharkawy and El-Shora (2020) observed decreasing in pectinase, proteases, xylanase and cellulase activities by plant phenolic compounds. Addition of CuONPs at 200ppm increased the total phenol content of the infected wheat plant to 16.67 ug. g⁻¹ dry weight by *F. equiseti*. Plants possess numerous defense mechanisms against invasion of phytopathogens and play a critical role in plants cultivated under stress conditions. According to the physiological studies, enzymatic and non-enzymatic mechanisms were reported as a protectors to repress the progress the pathogen. Certain enzymes POX, PPO, and SOD recognized as antioxidant enzymes, and protect the plants through peroxidation prevention of membrane lipid and minimize oxidative stress resulting from pathogenicity. A defense reaction to the cellular injury caused by phytopathogen toxins was reported previously (Chandrakar et al. 2018) via stimulation of the antioxidant system in plant. The present study showed that high concentration of CuONPs (400 ppm) inhibit the growth of pathogen *in vitro* compartment to low concentration, however oxidative enzymes increased in plant infected by pathogen and treated with high concentration 400 ppm of CuONPs (Table 3). These observation confirmed that the essential role of antioxidant system against non-biostress condition. Choudhary et al. (2017) in another study evaluated the defense mechanism in maize plant infected by Curvularia leaf spot. Who recorded that SOD and PPO activities were significantly increased in plant treated by Cu-chitosan NPs. Degeneration and scavenging of reactive oxygen species may be due to oxidative enzymes activities that increased after CuONPs treatments. These processes protect the plant from oxidative stress throughout pathogen attack.

Conclusion

The effect of CuONPs on *F. equiseti* as a causative agent of wheat wilt was investigated in vitro and under greenhouse conditions. CuONPs treatments are very effective management strategies for the control of *F. equiseti* in respective to present and future studies. In pot experiment, CuONPs had an fungicide effect on *F. equiseti* and minimize the wilt infection in the seedlings of wheat. Addition of

CuONPs to inoculated soil by *F. equiseti* increased the defense mechanism represented by oxidative enzymes of wheat plant against pathogen.

Abbreviations

CuONPs

Copper oxide nanoparticles

CuNPs

Copper nanoparticles

NPs

Nanoparticles

SOD

Superoxide dismutase

PPO

Polyphenol oxidase

POX

Cell wall bound peroxidase

RGC

Radial growth of the control

RGT

Radial growth of treatment

PDA

Potato dextrose agar

TEM

Transmission electron microscope

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

All authors have read and approved the manuscript

ATM: Designed, Performed some experiments; Analyzed and interpreted the data; Wrote the paper.

AAA: Designed some experiments; Analyzed and interpreted the data

AMHA: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Figures

Figure 1

Isolation of wheat wilt accompanying fungi. Red arrows showing plant wilt symptoms, Blue arrows health plant.

Figure 2

Frequency (%) of fungal isolates

Figure 3

Wilt (%) of wheat infected by Fusarium sp. isolates

Figure 4

Colony of *F. equiseti* on Petri dish containing PDA

A

Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
Fusarium equiseti isolate SKASS_BRH16F1 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	833	833	100%	0.0	100.00%	MK968015.1
Fusarium chlamydosporum isolate SKASS_BRH16F2 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed	833	833	100%	0.0	100.00%	MK968002.1
Fusarium equiseti isolate SKASS_STP7 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, cor	833	833	100%	0.0	100.00%	MK967999.1
Fusarium solani isolate SKASS_STP9 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, com	833	833	100%	0.0	100.00%	MK967998.1
Fusarium chlamydosporum strain Fusv05 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	833	833	100%	0.0	100.00%	MT555603.1
Fusarium incarnatum strain JL5-2 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	833	833	100%	0.0	100.00%	MT563420.1
Fusarium incarnatum strain JL3-4-1 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and i	833	833	100%	0.0	100.00%	MT563419.1
Fusarium incarnatum strain CBB-2 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	833	833	100%	0.0	100.00%	MT563418.1
Fusarium incarnatum strain JL3-3 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	833	833	100%	0.0	100.00%	MT563417.1
Fusarium incarnatum strain CBA-3 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	833	833	100%	0.0	100.00%	MT563408.1
Fusarium incarnatum strain CBB-3 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, comple	833	833	100%	0.0	100.00%	MT560229.1
Fusarium incarnatum strain CBB-1 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	833	833	100%	0.0	100.00%	MT560226.1
Fusarium incarnatum strain CBA-2 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	833	833	100%	0.0	100.00%	MT560219.1
Fusarium equiseti strain CB33-2 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	833	833	100%	0.0	100.00%	MT558602.1
Fusarium equiseti strain DH02-4-1 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	833	833	100%	0.0	100.00%	MT558603.1
Fusarium equiseti strain TH01-3 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	833	833	100%	0.0	100.00%	MT558598.1
Fusarium equiseti strain CB33-1 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	833	833	100%	0.0	100.00%	MT558596.1
Fusarium equiseti strain DH02-6 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	833	833	100%	0.0	100.00%	MT558586.1
Fusarium sp. isolate RL521 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequ	833	833	100%	0.0	100.00%	MT557352.1

Query	1	GGAGGGATCATTACCGAGTTTACAACCTCCCAAACCCCTGTGAACATACCTATACGTTGCC	60
Sbjct	19	GGAGGGATCATTACCGAGTTTACAACCTCCCAAACCCCTGTGAACATACCTATACGTTGCC	78
Query	61	TCGGCGGATCAGCCCGCGCCCGTAAACGGGACGGCCCGCCCGAGGACCCCTAAACTCT	120
Sbjct	79	TCGGCGGATCAGCCCGCGCCCGTAAACGGGACGGCCCGCCCGAGGACCCCTAAACTCT	138
Query	121	GTTTTTAGTGGAACCTTCTGAGTAAACAAACAATAAATCAAACCTTTCAACAACGGATC	180
Sbjct	139	GTTTTTAGTGGAACCTTCTGAGTAAACAAACAATAAATCAAACCTTTCAACAACGGATC	198
Query	181	TCCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGA	240
Sbjct	199	TCCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGA	258
Query	241	ATTCACTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT	300
Sbjct	259	ATTCACTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT	318
Query	301	GCCTGTTTCGAGCGTCATTTC AACCTCAAGCTCAGCTTGGTGTGGGACTCGCGGTAACC	360
Sbjct	319	GCCTGTTTCGAGCGTCATTTC AACCTCAAGCTCAGCTTGGTGTGGGACTCGCGGTAACC	378
Query	361	CGCGTTCCCCAAATCGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAATCATACACC	420
Sbjct	379	CGCGTTCCCCAAATCGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAATCATACACC	438
Query	421	TCGTTACTGGTA	432
Sbjct	439	TCGTTACTGGTA	450

B

Figure 5

(A) Phylogenetic tree among the *Fusarium equiseti* strain SKASS_BRH16F1 and the ITS sequences of closely related fungal strains retrieved from the NCBI GenBank database; and (B) Cluster analysis of *Fusarium equiseti*

Figure 6

Ultrastructures of *F. equiseti* at different concentrations of CuONPs (100, 200 and 400ppm)

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