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Antifungal applications of biosynthesized silicon and copper nanoparticles against molecularly identified root rot fungi of *Phoenix dactylifera* L.

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Abstract The date palm (*Phoenix dactylifera* L.) is the most significant crop in Egypt and the world. The study highlighted pharmaceutical importance of the biocontrol agents used, as they contain bioactive compounds with antimicrobial properties. The synthesis and application of nanoparticles as antifungal and antibacterial agents provide an eco-friendly and sustainable alternative to conventional chemical fungicides, promoting plant health and resistance against pathogenic infections. The fungal root rot disease was discovered to be present in date palm offshoots gathered from different regions and governorates, including Beheira, Giza, Fayoum, and Minia. The most virulent fungi found were *Rhizoctonia solani, Macrophomina phaseolina, Fusarium oxysporium, F. solani,* and *Lasiodiplodia theobromae* of the examined date palm cultivars, namely Barhey, Zaghloul, and Siwey. The primary fungal pathogen was identified using polymerase chain reaction (PCR) amplification. The nitrogen base sequences were then analyzed using the BLAST (the basics Local Sequence Analysis Tools) tool, which revealed that *L. theobromae* (PV594471.1) is the primary pathogen. The antifungal activity of nano silicon and copper produced biologically from *Paenibacillus polymyxa* (PbGK1) and chemically were tested against pathogenic fungi. Nano silicon and copper structural properties, such as small particle size and appropriate shape, contributed to its strong antifungal activity against fungal pathogens *i.e. L. thoebromae, M.phaseolina* and *F.solani at* concentration of 250 ppm. Silicon nanoparticles generated biologically from *P. polymyxa* showed the greatest increase in growth reduction percentages when compared to the other and control groups. Greenhouse trials revealed that date palm seedlings treated with silicon and copper nanoparticles generated biologically from *P. polymyxa* and chemically provide very positive results, with a low disease incidence and severity at 250 ppm concentration. Silicon nanoparticles created *P.polymyxa* was discovered to be more effective in compared with fungicide (Topsin M70%) in suppressing root rot disease in date palm offshoots both before and after infection. Comparing it to the untreated control, it simultaneously increases chitinase, peroxidase, and polyphenol oxidase. Our research shows that employing nano silicon as a biological alternative to fungicides can effectively manage date palm root rot disease.

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Keywords: Date palm, Nano silicon, Nano copper, Biologically and chemically nanoparticles, Root rot disease

Introduction

In the Arab world, especially in Egypt, date palms (*Phoenix dactylifera* L.) are an important and economical tree crop (Ibrahim and Abdel-Rahman., 2021). Date palm has multiple uses and products beyond its fruit. Its wastes are known to be high in nutrients that are beneficial to human health. The pulp of the fruit can be used to produce alcohol and antibacterial gel (Mahomoodally *et al.,* 2023). Date seed can produce high-quality oil for cosmetic, antimicrobial, antibiofilm, antiviral properties and pharmaceutical applications (Alahyane *et al.,* 2022 and Gomaa *et al.,* 2024). The nutritional and medicinal properties of different parts of the date palm have been extensively studied, highlighting their potential as functional foods and nutraceuticals (Achour *et al.,* 2022). Date palms are grown on 72395 hectares in Egypt, and production is expected to reach 1,867,064 tons (18% of global production) in 2023, with an average of 25789.1 kg/ha (FAO, 2023).

Numerous soil-borne pathogenic fungi, such as *Lasiodiplodia theobromae*, can cause major losses in mass production in date palm offshoots (Baraka *et al*., 2011). At a rate of 9.22% and 7.80%, respectively, *Fusarium solani* and *Lasiodiplodia theobromae* were isolated from date palm plants (Mansour *et al.,* 2023). In a greenhouse, pathogenic assays were performed on date palm seedlings at the same time that *Macrophomina phaseolina, Rhizoctonia solani,* and *Fusarium* species were isolated from date palm seedlings and offshoots (Ahmed, 2018).

Isolation attempts of naturally infected date palm root rot revealed four fungal species: *Fusarium solani, F. moniliforme, F. semitectum,* and *Lasiodiplodia theobromae*, pathogenicity tests revealed that all identified fungus might cause root rot on date palm seedlings (Shehata and Hassan, 2023). Disease symptoms typically appear in infected seedlings as a decrease in vegetative development. The outer leaves begin to dry first. The youngest leaves were shorter than usual and occasionally malformed.

Nanotechnology applications have developed dramatically in recent years (Haggag and Eid, 2022). Different nanomaterials are currently being employed in agriculture, resulting in the emergence of a new sector known as nanoagriculture (Haggag *et al.*, 2023 and Kutawa *et al*., 2021). Nanotechnology is gaining appeal as a safe and environmentally friendly alternative to pesticides and chemical fertilizers (Haggag *et al*., 2023). Silica (SiO2) is an essential mineral for monocot plants and has been shown to promote biotic and abiotic stress resistance. Silicon strengthens the plant's defenses by combining with nitrogen, phosphorus, and potassium, even though it is not required for normal plant growth or output. Applying soluble silicon in different forms and sizes helps plants acquire systemic

resistance (Murali-Baskaran *et al*., 2021 and Farhat *et al*., 2018). The silica-mediated defensive mechanism against fungi increases the accumulation of phenolic compounds and response enzymes such chitinases, peroxidase, and polyphenol oxidases (PPOs) (Murali-Baskaran *et al.,* 2021). To substantiate the effect of silica nanoparticles (SNPs) in inducing plant resistance, enzymes and phenols must be estimated. Previous research has shown that the antifungal action of copper nanoparticles is dependent on their form, size, and concentration, which may change depending on the fungus species (Geiser *et al*., 2021). Cu-NPs could be regarded a highly efficient alternative with improved antifungal characteristics (Ibarra-Laclette *et al*., 2022) that used topically restrict the growth of the *Colletotrichum gloeoesporioides*, *Fusarium solani*, *Fusarium kuroshium* and *Botrytis cinerea* and *Fusarium oxysporum* (Ibarra-Laclette *et al*., 2022 and Oussou-Azo *et al*., 2020).

This study aimed to examine and test the efficacy of nano silicon and copper synthesized biological and chemical approaches in reducing and management of *Lasiodiplodia theobromae, Fusarium solani* and *Macrophomina phaseolina* that induce date palm root rot of offshoots or seedlings under greenhouse conditions.

Materials and methods

Survey disease of date palm root rot

Survey disease of date palm root rot of offshoots and or seedling were assessed during March-April 2020/2021 growing season, at 8 different locations within various region/governorates *i.e.* Beheira (Alkhatatba & Imam Malik), Giza (Kerdasa & Giza) and Fayoum (Fayoum & Sinnuris) and Minia (Maghagha & Samalout), at three time *i.e.* early season (Es) (45 d after transplanting offshoots or seedlings), middle seasons (Ms) (90 d after transplanting offshoots or seedlings) and late seasons (Ls) (120 d after transplanting offshoots or seedlings). Survey of root rot disease was carried out at randomized fields using standard methods for surveying plant diseases (Large, 1966). Root rot disease incidence and severity percentage on the leaf and roots, at each various orchards and commercial nurseries were recorded. Root rot disease of date palm offshoots exhibiting on leaf system *i.e.* yellowing, wilting and leaf base rot. Syndromes of root system and leaf base *i.e.* necrotic lesions, maceration and discoloration of root system and leaf base under soil ground.

Evaluation of the disease caused by root rot in date palm offshoots

The survey was conducted by calculating as follow:-

Disease incidence: Infection prevalence can be assessed by enumerating the diseased and non-diseased seedlings or offshoots within various orchards or nurseries.

$$\text{Infection \%} = \frac{\text{Number of plants with diseases}}{\text{The total numbers of plant}} \text{ x } 100$$

Disease severity: A visual assessment technique of root-rot was dependent on infection severity rating from 0 to 4 adapted by author from the scale published by Ahmed (2018).

$$\text{Disease severity} = \frac{\sum D}{D\,\max \times n} \times 100$$

As follow:

n = Number of infected offshoots or seedlings of each grade.

N = The total count of the seedlings or offshoots examined

4 = Maximum disease severity grade.

v = Each grade's numerical value as follows:

0 = indicates a healthy seedling or branch (no yellowing of the leaves or discoloration of the roots).

1 = more than 1% to less than 25% of one yellow leaf or root discoloration.

2 = more than one yellow leaf or root discoloration between 26% and 50%.

3 = more than one wilted leaf or root discoloration ranging from 51% to 75%.

4 = dead seedlings and/or offshoots.

Isolation and morphological identification of the causal organisms

In several regions and governorates, including Beheira (Alkhatatba & Imam Malik), Giza (Kerdasa & Giza), Fayoum (Fayoum & Sinnuris), and Minia (Maghagha & Samalout), samples of sick date palm branch roots were gathered. Samples of root rot were carefully cleaned under running water and then sliced into 1-cm pieces. There were 15 milliliters of potato dextrose agar (PDA) medium on each plate. At 28°C, plates were incubated for five to seven days. To cultivate a pure culture of the isolated organisms, PDA medium was utilized. Choi *et al.* (1999) explained that all of the isolated fungus were purified using hyphal tips and single spores and then stored at 40°C until they were employed in later experiments. The microscopical and culture morphological features provided by (Padwick, 1945; Booth, 1971; Sutton, 1980; Nelson *et al.,* 1983 and Barnett and Hunter, 2006) in the NRC Plant Pathology Department were used to identify the isolated fungi.

Frequency of the fungal isolates was calculated according to Hassan *et al*. (2021) using following formula:

$$\text{Frequency of fungi \%} = \frac{\text{Number of isolated fungus}}{\text{Total number of isolated fungi}} \text{ x } 100$$

Molecular identification of main pathogen

Extraction of DNA

After being cultivated on PD broth medium using the, i-genomic BYF DNA Purification Mini Kit (iNtRON Biotech. Inc., Korean) in accordance with the instructions provided by the manufacturer, genomic DNA was extracted from a pure culture of highly pathogenic isolates of *Lasiodiplodia theobromae* (LdGG1), which were isolated from diseased date palm offshoots roots of various cultivars that were collected from Giza (Giza) (Sambrook *et al.,* 1989).

Internal transcribed spacer (ITS) partial amplification and sequencing using PCR

The initials ITS were used in molecular genetic research to identify the fungal isolates. A method based on (Boekhout *et al.,* 1994) was used to acquire partial sequences of the isolate 2S rDNA. Two distinct primers were used for amplifying the diverging domain of the gene: 5 ' TCCTCCGCTTATTGATATGC -3 ' for the initially used primer (ITS1) and 5 ' TCCGTAGGTGAACCTGCGG -3 ' for the following primer (ITS4).

These primers were provided by the Netherlands-based Operation Technology Company. The purified DNA sample was added to each polymerase chain reaction (PCR) bead along with 40 ng of the employed primer. The amplifying reaction's total volume was reached at 25 µl with the use of sterile distilled water. The reaction at 95°C for fifteen minutes was the amplification protocol. Each of the 35 cycles involved the subsequent segments: primer annealing at 55°C for 2 minutes, incubation at 72°C for 2 minutes for DNA the polymerization process and denaturing at 95°C for 1 minute. The reaction mixture was then maintained at 4ºC till analysis. Using a 1.0% agarose gel and 1X TBE (Tris-borate-EDTA) buffer, the DNA that was amplified products were electrophoresed for approximately two hours at a constant 100 V. The Gel Documentation System with UV Trans illuminator was used to take pictures of the separated bands after they were identified using the 100 bp DNA Ladder Prepared to Load (Solis Bio Dyne, Estonia) and stained with 0.5 µg/ml ethidium bromide.

Purification and sequencing of fungal DNA

Using the Gene JETTM PCR Purified Kits (Thermal K0701), the PCR product was cleaned. ITS primers were used to sequence the extracted PCR products' DNA using an ABI 3730xl DNA sequencing (GATC the company, Germany). The ITS nucleotide sequences for isolate no. (LdGG1) were compared to those in the National Center for Biotechnology Information (NCBI; www.ncbi.nih.gov) public domain databases using the Basic Alignment Search Tool for Nucleotide Sequences (BLASTN). The

ITS DNA sequences were aligned using the Clustal W program. A phylogenetic tree based on UPGMA (unweighted pair group technique for arithmetic analysis) was created using CLC Sequence Viewer Version 6.3. The branching's confidence level was assessed using bootstrap analysis (Fan *et al.,* 2015).

Synthesis of nanoparticles

Silicon (potassium hexa fluorosilicate K_2SiF_6) and copper (copper sulfate pentahydrate $CuSO_4 \cdot 5H_2O$) were synthesis of nanoparticles using biological and chemical methods.

Nanoparticle biosynthesis

The effectiveness of *Paenibacillus polymyxa* (PbGK1) in producing various silicon and copper nanoparticles was assessed.

Copper and silicon nanoparticle biosynthesis

The techniques outlined by Singh *et al.* (2008), Thakker *et al.* (2012) and Farhat *et al*. (2018) were used to anchor external production of silicon (SiNPs) and copper nanoparticles (CuNPs).

Every bacteria and fungus isolate's pre-inoculum was placed in to 90 mL of nutrient broth media (NB) and potatoes with dextrose broth (PDB), subsequently, and the mixture was shaken at 200 rpm for 72 hours at 31 °C. The bacterial and fungal biomass was collected during the log phase of its life cycle and cleaned under aseptic conditions using autoclave sterilized water. The collected bacterial and fungal tissue (5 gm wet weight or 1 gm dry weight) was then reconstituted in 100 mL of an aqueous solution of either 1 mM of copper sulfate pentahydrate CuSO4·5H2O or 10-3 M potassium hexafluorosilicate (K2SiF6). It was then maintained at 31oC in an anaerobic environment on a shaker (200 rpm). A 48-hour response that involved bacteria and fungus biomass and SiF62- or CuSO4 was conducted. Following the removal of the bacterial and fungal cells from the reactivity media by centrifugation at 5000 rpm for 10 minutes or filtration via the reaction products were gathered (Whatman paper No. 1). The collected bacteria biomass was reconstituted in autoclaved deionized water without K2SiF6 or CuSO4·5H2O in a control experiment, and the resulting product was examined for any indication of Cu/CuO2 or Si/SiO2 nano-composites.

The synthesis of chemicals

Creation of nanoparticles of silicon

Ammonia solution 33% extra pure and sodium silicate solution with a pH of 11.0–11.5 were made. Equal volumes of ammonia and ethanol

(A/E), or 30 milliliters each, were used to create a combination. A dropwise addition of 0.5 ml sodium silicate solution in 7 ml of distilled water was made to the A/E combination as a silica precursor media. It was centrifuged, cleaned with distilled water, and then dried after a one-hour aging period to produce silica nanoparticles. Six further A/E mixes with varying ammonia to ethanol ratios underwent the same process, as indicated in Table 1.

Table 1. Quantities of the materials used for the synthesis of silica nanoparticles

Ammonia/ ethanol ratio	Sodium silicate solution (ml)	Water (ml)	Ammonia (ml)	Ethanol (ml)
1:1	0.5	7.0	30	30
1.5:1	0.5	7.0	45	30
2:1	0.5	7.0	60	30
3:1	0.5	7.0	90	30
1:1.5	0.5	7.0	30	45
1:2	0.5	7.0	30	60
1:3	0.5	7.0	30	90

Copper nanoparticle synthesis

As part of the experiment, a 0.02 M ascorbic acid solution was made in deionized water. A 0.01 M copper sulfate pentahydrate (CuSO4·5H2O) solution was made separately in deionized water and added to the ascorbic acid solution while being continuously stirred by a magnetic stirrer. A 1 M NaOH solution in deionized water was added to change the pH. A 0.1 M solution of NaBH4 in de-ionized water was added while being continuously stirred for 30 minutes at room temperature. To finish the reaction, stirring was maintained in the ambient air for fifteen minutes. The original reactivity mixture's blue hue changed to a brownish-red hue.

TEM characterization of nanoparticles

The technique of transmission electron microscopy (TEM) was used to investigate and describe the bio-transformed compounds that were produced. The separated and reconstituted solution was drop-coated onto carbon-coated copper grids to create nanoparticles. At the electron microscopy unit of the National Research Center in Egypt, TEM measurements were carried out using a JEOL model 1200EX apparatus running at a voltage of acceleration of 120 kV (Woehrle *et al.,* 2006; Jain *et al.,* 2011 and Prakasham *et al.,* 2012).

Impact of nanoparticles on the in vitro development of pathogen mycelia

Three highly pathogenic fungal isolates that cause root-rot disease in date palm offshoots or seedlings—*Lasiodiplodia theobromae*, *Fusarium*

solani, and *Macrophomina phaseolina*—were tested to ward nano silicon and nano copper at rates of 0, 100.0, 200.0, and 250.0 ppm (made from biological nanoparticles) using the dilution method and chemical methods as previously mentioned. After adding each concentration of nano silicon and nano copper individually to heated PDA, the mixture was transferred into petri dishes (10 ml/plate). Discs (5 mm) taken from the edges of seven-day-old cultures of each of the previously indicated pathogenic fungal isolates were used to inoculate the middle of each of these treated plates independently. Positive control treatment consisted of plates injected with pathogens alone without nanoparticles, whereas the opposite control included of plates that were not treated. The inoculation plates were incubated for five days at 27± 2°C. For every treatment, three plates were utilized as replication, and three plates served as checks. When the mycelial matting filled the PDA surfaces in the untreated control plates, the experiment was over. Each plate's growth rate was recorded, average diameters were computed, and the percentage of fungal decrease was computed using the following a formula:

Reduction %= (C-T/C) x 100.

C is the control hyphal growth's diameter.

T is the treated hyphal growth's diameter.

Experiments in greenhouses

The Plant Pathology Department at the National Research Center in Giza, Egypt, carried out tests in a greenhouse setting to assess the most promising effective nanoparticles at twice the lethal concentration to prevent pathogenic fungal pathogens and the insecticide Topsin (M-70%) on the incidence of root-rot in date palm seedlings of the highly susceptible cultivar (cv. Zaghloul) during the 2022–2023 growing season. The entire block of the greenhouse trial was selected. The trial was conducted again.

Fungal pathogenic inoculum preparation

For the highly pathogenic isolates of *L. theobromae* (LdGG1), *F. solani* (FsBI2), and *M. phaseolina* (MpFF2), inoculum was made by culturing each isolate produced on (PDB) medium for 15 days at 25±2°C. The developing upper solid layers of each isolate were then washed and blended in sterile water before being filtered through multiple shreds. A hemocytometer slide was used for adjusting the spore counts to $4X10^6$ spores/ ml.

Preparation of nanoparticles suspensions

Fresh stock suspension of different nanoparticles prepared by dissolving nanoparticles in sterilized distilled water. Biological and chemical methods of the most effective concentrations of silicon and cooper nanoparticles were tested at 250 ppm.

Fungicide (Thiophanate Methyl) Topsin M 70 % WP (produced by Sumitomo Corporation Company, Cairo, Egypt), at concentration of 1.0 g / L was applied as Soil treatment and foliar spray for comparison purposes.

Treatment of soil

The author modified the method described by Baraka *et al*., (2011) by surface-disinfesting date palm seeds (cv. Zaghloul) for 10 minutes in a sodium hypochlorite solution NaOCl (1.5% available chlorine), soaking them under tap water for 24 hours, and then treating them with dry heat at 45°C for two hours. The seeds were then placed in petri dishes within two players of wet cotton to provide humidity, and they were incubated at 27±2°C to activate their germination. Pots with dimensions ranging of 25 cm and one seed each were filled with a sterilized combination of equal parts (v/v) soil, sand, and clay (3 kilogram / pot) once the seeds had germinated. The seedlings were left to grow for six months or until they reached the two to three leaf stage. After that, they were soaked in 500 milliliters, depending on the field capacity, of new stock solutions of various nanoparticles made by chemical, biological, and fungicide procedures using Topsin (M-70%). Before seven days of soil infestation, treatments were carried out as soil treatment and soil treatment with a subsequent foliar spray with the same doses. Five pots were utilized as replicates, while five pots that had been infected with the pathogens (after seven days) and left free were employed as control treatments.

Infestation of soil

According to El-Zawahry *et al.,* (2000) the soil of date palm seedlings was infested individually by highly pathogenic isolates of *Lasiodiplodia theobromae* (LdGG1), *Fusarium solani* (FsBI2), and *Macrophomina phaseolina* (MpFF2) after a week of soil treatment (and soil treatments + foliar spray) by various nanoparticles prepared by biological, chemical, and fungicide Topsin (M-70%). To simulate each pathogenic fungus, 100 ml of spore suspension ($4X10^6$/ml) or hyphal suspension was added to each pot. To guarantee that the tested fungus was distributed, the pots were watered every three to four days. To maintain high humidity for 48 hours following inoculation, each plant was covered separately with plastic bags. For each treatment, five pots served as duplicates. Ninety days following inoculation, the severity of the disease was assessed, and the previously described disease assessment was conducted.

Assay of biochemical changes associated with treatments

Samples of date palm roots (Cv. Zaghloul) at experimental end 90 day were used for determined enzymes activities of oxidative enzymes *i.e.* chitinase, peroxidase and polyphenol oxidase.

Effect of treatments on some enzymatic activities

The process of enzyme extraction and analysis involved homogenizing 200 mg of root samples (at the conclusion of the 90-day trial) with 10 ml of buffered phosphate pH 6.8 (0.1 M). The samples were then centrifuged at 2 ^{0}C for 15 minutes at 17,000 × g in a chilled centrifuge. Enzyme activity was assessed using the clear supernatant (Kar and Mishra, 1976).

Assay for Peroxidase (PO)

Five milliliters (125 µmoles of phosphate buffer (pH 6.8), fifty µmoles of pyrogallol, fifty µmoles of H2O2, and one milliliter of enzyme extract were utilized in the assay combination for peroxidase activity. The mixture was then incubated for five minutes at 25°C. The reaction was stopped by adding 0.5 ml of 5% (v/v) H2SO4. Using a Spectrophotometer (Spectronic 20-D), PO activity was evaluated as the increase over one minute at 25°C, as determined by absorption at 420 nm per gram of fresh weight (Kar and Mishra, 1976).

Assay for polyphenol oxidase (PPO)

A spectrophotometric method that monitored the initial rate of absorbance increase at 420 nm was used to evaluate the PPO activity (Soliva-Fortuny *et al.,* 2001) Within a quartz cuvette with a path length of 1 cm, activity was carried out in a 3 mL reaction mixture that contained 1 mL of substrate (0.02 M pyrogallol and distilled water), 100 µL of enzyme extract, and 1.9 mL of phosphate buffer (pH 6.5). With a spectrophotometer (Spectronic 20-D), PPO activity was measured as the increase in absorbance at 420 nm per gram fresh weight at 25°C for five minutes.

Assay for Chitinase

According to the methods described by (Ishaaya and Casida, 1974) chitinase activity was assessed using the 3, 5-dinitrosalicylic acid reagent to quantify the free aldehydic groups of hexosaminase produced during chitin breakdown. 0.3 ml of 0.5% colloidal chitin (Skujins *et al*., 1965) 0.12 ml of 0.2M phosphate buffer (pH 6.6), and 0.18 ml of sample homogenate made up the reaction mixture. Enzyme activity was stopped by adding 1.2 ml of 3, 5-dinitrosalicylic acid reagent (DNSA) after 60 minutes of incubation at 37°C. After being heated to 100°C for five minutes, the reaction mixture was cooled in an ice bath and diluted with 1.2 milliliters of distilled water. A Spectrophotometer (Spectronic 20-D) was used to detect the absorbance of the supernatant at 550 nm after centrifugation for 15 min. at 6000 rpm. A linear correlation between absorbance and NAGA quantity is produced by the direct reaction of N-acetylglucosamine (NAGA) with DNSA reagent under conditions similar to those of the enzymatic reaction. According to the Excel software (2010), 1 mg of NAGA yields an absorbance value of 0.78.

$$y = 0.108x - 0.0597 \quad R^2 = 0.9929$$

Chitinase activity is quantified as µg of N-acetylglucosamine released per sample per hour.

Statistical analysis

All data were statistically analyzed using three replicates and analysis of variance (ANOVA), with Duncan's multiple range tests used to compare the means at significance levels of $P < 0.05$ (Duncan, 1955) Tukey test software was used for multiple mean comparisons, and the data were adjusted to provide the normal distribution needed for statistical analysis (Neler *et al.,* 1985).

Results

Survey

Root-rot severity and incidence rate were surveyed at three times, *i.e.* ES (early season), MS (middle seasons) and LS (late seasons), 45, 90 and 120 d after transplanting offshoots or seedlings, respectively in four Governorates, *i.e.* Minia, Fayoum, Beheira and Giza at growing season 2020-2021 are shown on Figure 1). According to the data in Figure 1, the highest average percentages of the root rot disease incidence and severity of date palm offshoots were found in Beheira (30.0, 39.5 and 53.0 in early, middle and late seasons) % and (19.5, 30.3 and 39.5 in early, middle and late seasons) %, Giza Governorate (23.0, 27.0 and 36.0 in early, middle and late seasons) % and (12.4, 16.6 and 22.3 in early, middle and late seasons) %, respectively. Fayoum Governorate had the highest percentages of (15.0, 17.5 and 29.0 in early, middle and late seasons) % and (8.9, 12.1 and 18.0 in early, middle and late seasons) %, respectively. While the grown offshoots in Minia Governorate showed the least percentages of (12.5, 18.5 and 22.5 in early, middle and late seasons) % and (5.5, 8.5 and 11.5 in early, middle and late seasons) %, respectively.

Frequency of fungi

He findings of the prevalence and frequency percentage of date palm root-rot disease of offshoots and its related fungal pathogens in four Egyptian governorates: Minia (Maghagha**,** Samalout), Fayoum (Fayoum, Sinuuirs), Beheira (Alkhatatba, Imam Malik) and Giza (Kerdasa, Giza) are shown in Table 1. The isolation results revealed that all the fungal isolates associated with this disease belonged to the *Rhizoctonia solani, Macrophomina phaseolina, Fusarium* spp. *Fusarium oxysporium, F. solani,* and *L. theobromae*. Data show that, the highest average percentages of fungal frequency which recorded on Beheira and Giza Governorate. The

most common fungi isolated date palm offshoots were *L. theobromae* followed by *F. solani* and *Fusarium oxysporium* on Beheira (25.35, 22.5 and 18.9), Giza (25.0, 27.5 and 17.5), Governorate, respectively. While the grown offshoots in Minia Governorate showed the least frequency percentage of disease and fungi *i.e. L. theobromae* followed by *F. solani* and *Fusarium oxysporium* (23.9, 22.25 and 18.4), respectively.

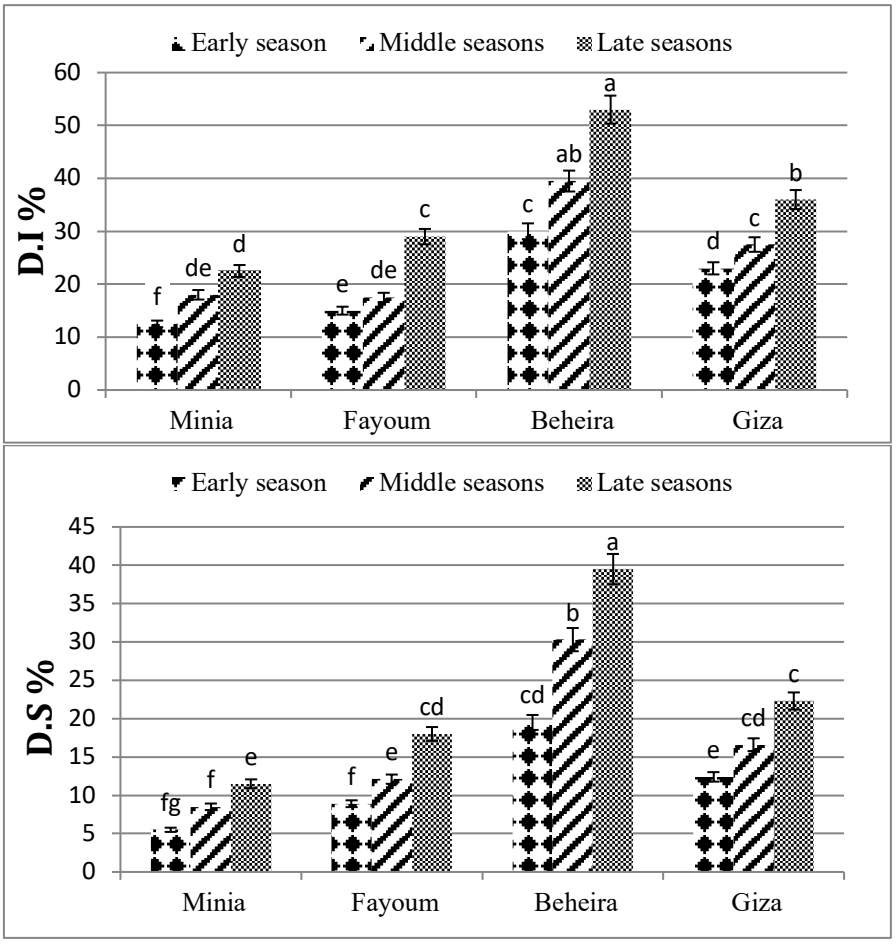

Figure 1. Survey of diseased date palm offshoots or seedlings under field conditions at four Governorates in Egypt, at three times after transplanting during 2020/2021 season

Note:- There was not significant difference between figures that have the same letters in the same column (P=0.05).

According to the author's modification of the scale outlined by Ahmed (2018), root rot was determined by the infection severity rate, which ranged from 0 to 4 Root-rot severity rate was scored at three times, *i.e.* ES (early season), MS (middle seasons) and LS (late seasons), 45, 90 and 120 d after transplanting offshoots or seedlings, respectively.

Table 1. Frequency (%) of fungi isolated from roots of date palm offshoots under field conditions at four governorates in Egypt

Governorates	locations	Frequency (%) of isolated fungi from date palm roots						
		F. oxysporium	*F. solani*	*F. spp*	*M. phaseolina*	*R. solani*	*L. thoebromae*	Others
Minia	Maghagha	15.4$^{e(1)}$	23.1^{c}	15.4^{a}	11.5^{c}	10.7^{b}	19.2^{d}	4.7^{a}
	Samalout	21.4^{b}	21.4^{c}	14.3^{b}	7.2^{d}	7.1^{c}	28.6^{b}	-
Mean$^{(2)}$		18.4^{ab}	22.25^{b}	14.85^{a}	9.35^{b}	8.9^{a}	23.9^{b}	2.35^{b}
Fayoum	Fayoum	21.1^{b}	15.8^{d}	12.5^{c}	15.8^{b}	5.3^{d}	26.3^{c}	3.3^{b}
	Sinuuirs	17.7^{d}	15.7^{d}	10.5^{d}	26.3^{a}	15.7^{a}	11.5^{e}	2.6^{c}
Mean		19.35^{a}	15.75^{c}	11.5^{b}	21.05^{a}	9.7^{a}	18.9^{c}	2.95^{a}
Giza	Kerdasa	25.0^{a}	25.0^{b}	10.0^{d}	10.0^{c}	5.0^{d}	20.0^{d}	5.0^{a}
	Giza	10.0^{f}	30.0^{a}	10.0^{d}	10.0^{c}	10.0^{b}	30.0^{b}	-
Mean		17.5^{b}	27.5^{a}	10.0^{c}	10.0^{b}	7.5.0^{b}	25.0^{a}	2.5.0^{b}
Beheira	Alkhatatba	18.4^{cd}	28.9^{a}	13.6^{b}	10.5^{c}	5.4^{d}	18.4^{d}	4.8^{a}
	Imam Malik	19.4^{c}	16.1^{d}	16.1^{a}	9.7^{c}	6.4^{c}	32.3^{a}	-
Mean		18.9^{a}	22.5^{b}	14.85^{a}	10.1^{b}	4.25^{c}	25.35^{a}	2.4^{b}

(1) There was not significant difference between figures with the same letters in the same column but no mean values (P=0.05).
(2) There was not significant difference between figures for mean values that have the same letters in the same column (P=0.05).

Identification of highly pathogenic isolates of Lasiodiplodia theobromae (LdGG$_1$) using molecular biology

The ITS genes of *Lasiodiplodia spp*., including the 5.8S ribosomal rRNA, were amplified and DNA sequences were determined. A novel pair of primers for the polymerase chain reaction was developed for the targeted amplification of DNA by sequence information comparison, and this primer pair successfully amplified a 700-bp DNA sequence 98–100% of the time. Results indicate that the NCBI alignment showed the percentage of identity (99.26%) of *Lasiodiplodia theobromae* (PV594471.1) between studied isolates and Gene bank isolate, whereas results in indicated that the phylogenic tree showed convergence between our isolates (yellow color) and Gene bank isolate (Figure 2). Our isolates are shown in separated cluster that means it had diversity.

Synthesis nanoparticles

Paenibacillus polymyxa (PbGK$_1$) was used for biosynthesis of silicon (A) and copper (B), nanoparticles (Figure 3). Simultaneously, copper (B) and silicon (A) nanoparticles were created chemically (Figure 4). Analysis using transmission electron microscopy (TEM) shed more light on the size and shape of silicon and copper nanoparticles that were produced chemically and biosynthesized. A significant range in particle size was noted, with an average diameter. Transmission electron microscopy (TEM) analysis revealed that the silicon nanoparticles exhibited mainly a fine spherical morphology with an average diameter of about 2.9 -3.5 nm and copper to 6.15- 11.2 nm. The size distribution was relatively form, as shown in Figure 3. Scanning electron microscopy (SEM) images also confirmed the morphology of the silicon nanoparticles and provided insights into their surface properties. The nanoparticles exhibited a smooth surface. Transmission electron microscopy analysis also showed that the silicon and copper nanoparticles using chemical synthesis exhibited a mesospherical shape with an average diameter of about 16.0-26.0 and 3.7-63.70 nm, respectively.

Isolate Code	Name	**Accession number**	**Closest phylogenetic relative and accession number**	**Identity %**
Ld1	*Lasiodiplodia theobromae*	PV594471.1	*Lasiodiplodia theobromae* isolate BPPCA157 (MK530031.1)	99.26

NCBI Ref. / GenBank:

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  1 tttcgagctc cggctcgact ctcccaccct ttgtgaacgt acctctgttg ctttggcggc
 61 tccggccgcc aaaggacctt caaactccag tcagtaaacg cagacgtctg ataaacaagt
121 taataaacta aaactttcaa caacggatct cttggttctg gcatcgatga agaacgcagc
181 gaaatgcgat aagtaatgtg aattgcagaa ttcaaagaat catcgaatct ttgaacgcac
241 attgcgcccc ttggtattcc gggggggcat
```

Figure 2. The phylogenic tree demonstrated convergence between the Gene Bank isolate and our isolated shaded area. Since our isolates are displayed in a distinct cluster, diversity was present

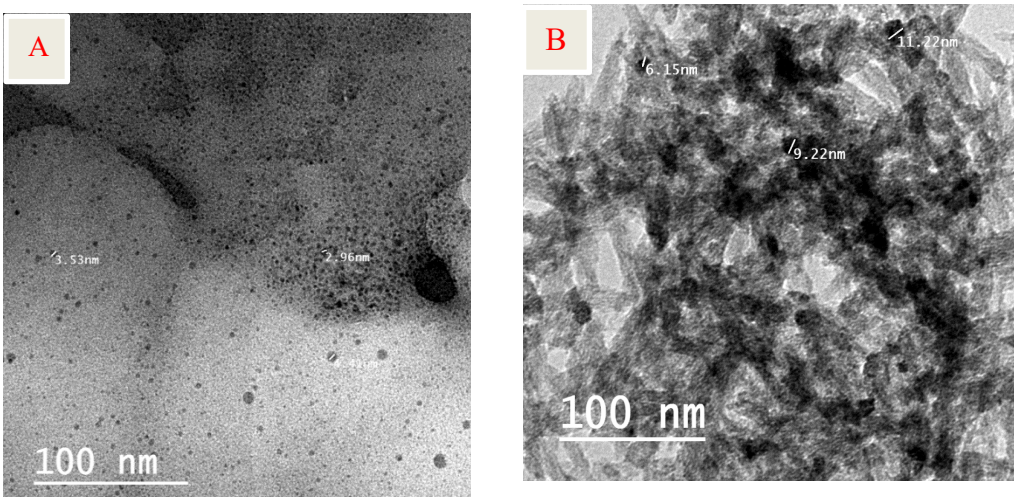

Figure 3. An illustration of the biogenesis of silicon (A) and copper (B) nanoparticles using transmission electron microscopy (TEM)

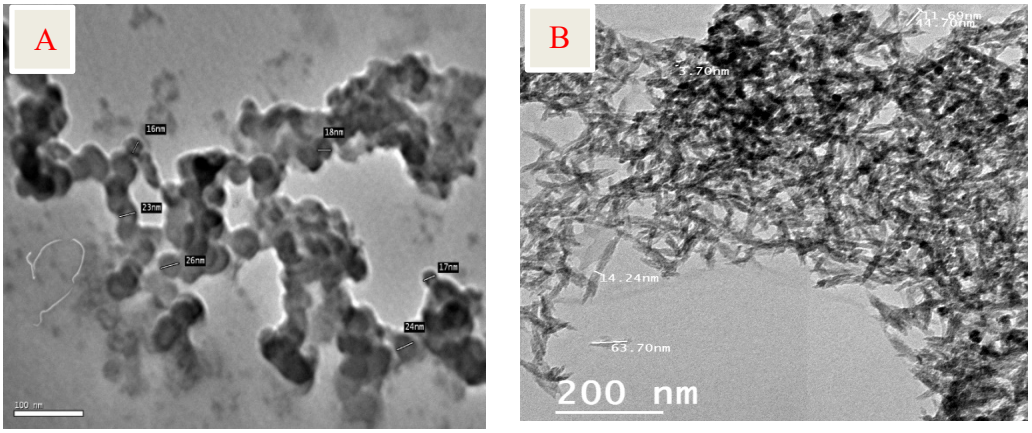

Figure 4. An illustration of the chemical of silicon (A) and copper (B) nanoparticles using transmission electron microscopy (TEM)

Effect of nanoparticles on pathogens mycelia growth in vitro

Three concentrations of copper and silicon nanoparticles, both chemically and biologically generated from *Paenibacillus polymyxa* (PbGK1), were tested against the growth of *L. thoebromae, M. phaseolina,* and *F. solani* fungi (Table 2 and Figure 5). All synthesized nanoparticles strongly inhibited the mycelial growth of all investigated harmful fungi, according to the results shown in Table 2.

Table 2. Effect of nano silicon and copper produced biologically from *Paenibacillus polymyxa* (PbGK1) and chemically on pathogens mycelia growth *in vitro*

Nanoparticles	Con. (ppm)	Linear fungal growth (mm)					
		L. thoebromae ($LdGG_1$)	Reduction (%)	*M. phaseolina* ($MpFF_2$)	Reduction (%)	*F. solani* ($FsBI_2$)	Reduction (%)
Nano silicon biology	100	62.3^{e}	30.8	41.7^{d}	53.7	48.0^{e}	46.7
	200	20.3^{e}	77.4	24.3^{e}	73.0	30.0^{g}	66.7
	250	11.7^{f}	87.0	10.7^{f}	88.1	13.3^{h}	85.2
Nano silicon chemical	100	76.7^{b}	14.8	84.3^{ab}	6.3	89.7^{a}	0.3
	200	64.0^{c}	28.9	72.7^{c}	19.2	84.0^{ab}	6.7
	250	36.7^{d}	59.2	37.3^{d}	58.6	64.7^{cd}	28.1
Nano cupper biology	100	88^{a}	2.2	89.3^{a}	0.8	70.7^{bc}	21.4
	200	86.7^{a}	3.7	86.7^{a}	3.7	58.0^{d}	35.6
	250	32.3^{d}	64.1	74.0^{c}	17.8	39.0^{f}	56.7
Nano cupper chemical	100	78.7^{b}	12.6	87.3^{a}	3.0	90.0^{a}	0.0
	200	66.3^{c}	26.3	78.3^{bc}	13.0	85.0^{a}	5.6
	250	37.0^{d}	58.9	42.3^{d}	53.0	67.0^{c}	25.6
Control		90^{a}	0.0	90^{a}	0.0	90^{a}	0.0

There was not significant difference between figures that have the same letters in the same column (P=0.05).

Silicon nanoparticles synthesized chemically and biologically were the most effective antagonist at all concentrations. Since, silicon nanoparticles generated biologically showed the greatest increase in growth reduction at concentration of 250 ppm, as it decreased linear growth to 11.7, 10.7, and 13.3 mm for *L. thoebromae*, *M. phaseolina* and *F. solani*, respectively, compared to the control (90 mm). Conversely, among all the fungi examined, nano silicon and copper nanoparticles produced chemically had the least impact being for nano silicon as 36.7, 37.3 and 64.7 and copper nanoparticles as 37, 42.3 and 67.0, respectively.

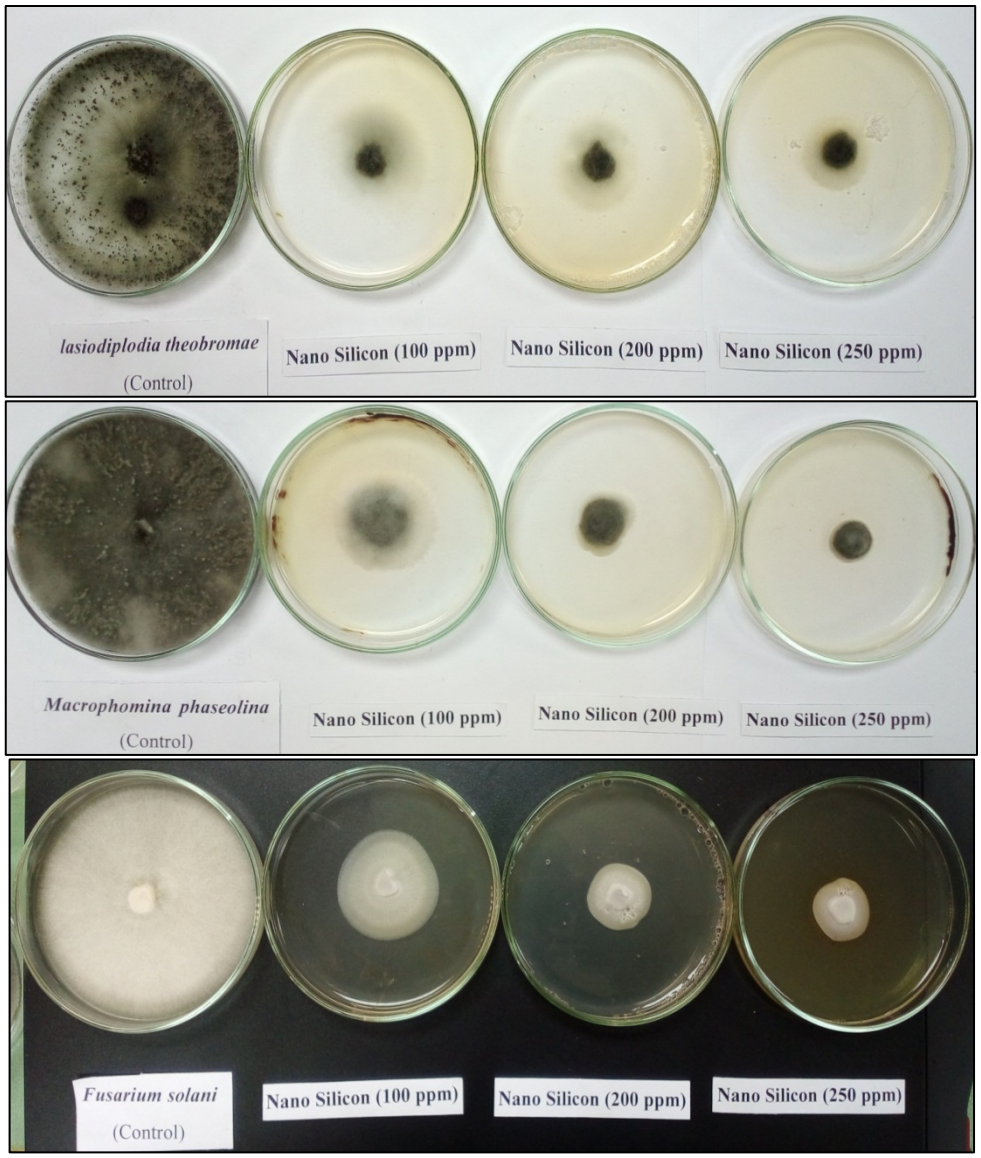

Figure 5. Efficiency of nano silicon at concentrations of 0, 100.0, 200.0 and 250.0 ppm produced biologically from *Paenibacillus polymyxa* (PbGK1) on linear growth of *Lasiodiplodia theobromae* ($LdGG_1$), *Macrophomina phaseolina* ($MpFF_2$) and *Fusarium solani* ($FsBI_2$) *in vitro*

Greenhouse experiments

The effectiveness of silicon nanoparticles (SiNPs) and copper nanoparticles (CuNPs), which were chemically and biologically synthesized from *Paenibacillus polymyxa* (PbGK1) at 250 ppm, in reducing the severity of root rot disease in date palm offshoots during the 2023 and 2024 seasons was assessed in greenhouse and artificially infested conditions through soil treatments and/or foliar spraying. In comparison to control and fungicide (Topsin M 70%) treatments, the results showed that all tested SiNPs and CuNPs manufactured chemically and biologically significantly reduced the severity of root rot disease of date palm offshoots (Table 3 and Figure 6).

Significantly, SiNPs synthesized biologically at a concentration 250 ppm exhibited higher effectiveness in reducing disease severity in artificially inoculated soil with *L. thoebromae*, *F. solani* and *M. phaseolina*, and foliar spraying were 28.0, 27.0 and 22.2% and foliar spraying were 22.0, 22.5 and 15.0% than synthesized chemically which were 45.0, 43.0 and 35.9), (36.0, 38.0 and 26.3) and fungicide treatments being (23.5, 22.4 and 20.0) and (17.2, 18.5 and 13.2), respectively. These findings highlight the considerable potential of SiNPs synthesized biologically in controlling date palm offshoots.

While CuNPs synthesized chemically treatments resulted in low reductions of disease severity in artificially infested soil and foliar spraying, being (47.2, 48.5, and 42.4) and (43.4, 47.5 and 37.4) in compared with untreated control, being (84.5, 81.2 and 69.4).

Assessment of treatment-related biochemical alterations

Silicon and copper nanoparticles at concentration of 250 ppm synthesized chemically and biologically were tested for their effect on some enzymatic activities *i.e.* polyphenol oxidase, peroxidase, and chitinase enzymes under artificial conditions.

Effect on peroxidase activity (PO)

All treatments were considerably raised the peroxidase activity in offshoots infected with *L. theobromae* ($LdGG_1$), *F. solani* ($FsBI_2$), and *M. phaseolina* ($MpFF_2$) isolates (Table 4). The most successful treatments were silicon nanoparticles (SiNPs) created biologically that enhanced the peroxidase activity by 648.1, 593.5, and 544.7%, and chemically (607.4, 416.1, and 460.5%), respectively, followed by copper nanoparticles (CuNPs) produced biologically increased the peroxidase activity by 314.8, 332.3 and 297.4 %,

respectively, as compared with untreated offshoots. Meanwhile, the activity of peroxidase in samples treated with CuNPs produced chemically increased the peroxidase activity by 237.0, 238.7 and 192.1 %, respectively, despite being lower. On the other hand, the results showed that the fungicide (Topsin M 70%) increased the peroxidase activity by 529.6, 467.7 and 410.5 %, respectively.

Effect on polyphenol oxidase activity (PPO)

All treatments were raised the polyphenol oxidase activity in offshoots infected with *L. theobromae* ($LdGG_1$), *F. solani* ($FsBI_2$), and *M. phaseolina* ($MpFF_2$), each of which is a highly pathogenic isolate. When compared to untreated offshoots, the highest polyphenol oxidase activity increased with silicon nanoparticles (SiNPs) produced biologically, which increased the polyphenol oxidase activity by 250.0, 233.3, and 200.0 percent, and chemically, by 212.5, 166.7, and 100.0 percent, respectively (Table 5). CuNPs which created biologically were increased the polyphenol oxidase activity by 137.5, 108.3, and 63.2 percent, respectively. However, the polyphenol oxidase activity in samples treated with CuNPs produced chemically were lower as 87.5, 58.3, and 21.1%, respectively. The fungicide Topsin M 70% raised the polyphenol oxidase activity by 162.5, 166.7, and 110.5%, respectively.

Effect on chitinase activity

All treatments increased the chitinase activity in offshoots infected with *L. theobromae* ($LdGG_1$), *F. solani* ($FsBI_2$), and *M. phaseolina* ($MpFF_2$). The chemically generated silicon nanoparticles (SiNPs), and biologically SiNPs showed the largest increase in chitinase activity, surpassing 222.2 percent, as compared to untreated offshoots. Other treatments showed a moderate activity. However, the fungicide Topsin M 70% enhanced the chitinase activity by 300.0, 214.3, and 188.9 percent, respectively.

Table 3. Effect of promising nanoparticles at 250 ppm on root rot severity of date palm offshoots in artificially infested soil after 90 days under greenhouse conditions

Nanoparticles Source	Root rot disease severity(2) %											
	Mean of Growing seasons (2023 and 2024)											
	L. thoebromae ($LdGG_1$)				*F. solani* ($FsBI_2$)				*M. phaseolina* ($MpFF_2$)			
	2023	2024	Men(3)	Eff. (%)	2023	2024	Men	Eff. (%)	2023	2024	Men	Eff. (%)
Soil treatment												
Nano silicon biology	(1) 30.0^{ef}	26.0^{d}	28.0^{de}	66.9	29.2^{ef}	24.8^{e}	27.0^{ef}	66.7	24.0^{e}	20.4^{ef}	22.2^{ef}	68.0
Nano silicon chemical	43.3^{bc}	46.7^{b}	45.0^{b}	46.7	44.0^{bc}	42.0^{bc}	43.0^{bc}	47.0	38.0^{c}	33.8^{c}	35.9^{c}	48.3
Nano cooper biology	37.5^{cd}	34.0^{c}	35.8^{c}	57.6	38.4^{cd}	33.5^{d}	36.0^{d}	55.7	31.2^{d}	27.4^{d}	29.3^{d}	57.8
Nano cooper chemical	48.4^{b}	45.9^{b}	47.2^{b}	44.1	50.0^{b}	47.0^{b}	48.5^{b}	40.3	44.8^{b}	40.0^{b}	42.4^{b}	38.9
Topsin (fungicide)	26.0^{f}	21.0^{de}	23.5^{def}	72.2	23.2^{fg}	21.6^{ef}	22.4^{fg}	72.4	21.7^{e}	18.3^{fg}	20.0^{fg}	71.2
Control	88.5^{a}	80.5^{a}	84.5^{a}	0.0	82.9^{a}	79.6^{a}	81.2^{a}	0.0	71.0^{a}	67.8^{a}	69.4^{a}	0.0
Soil treatments + foliar spraying												
Nano silicon biology	24.6f^{g}	19.4^{e}	22.0^{ef}	74.0	23.8^{fg}	21.2^{ef}	22.5^{fg}	72.3	16.0^{f}	14.0^{gh}	15.0^{gh}	78.4
Nano silicon chemical	37.6^{cd}	34.5^{c}	36.0^{c}	57.4	40.0^{cd}	36.0^{cd}	38.0^{cd}	53.2	27.1^{de}	25.5^{de}	26.3^{de}	62.1
Nano cooper biology	33.2^{de}	27.3^{d}	30.3^{cd}	64.1	34.0^{de}	31.8^{d}	32.9^{de}	59.5	27.1^{de}	24.5^{de}	25.8^{de}	62.8
Nano cooper chemical	42.5^{bc}	44.2^{b}	43.4^{b}	48.6	49.5^{b}	45.4^{b}	47.5^{b}	41.5	39.0^{c}	35.7^{bc}	37.4^{bc}	46.1
Topsin M 70% (fungicide)	18.6^{g}	15.8^{e}	17.2^{f}	79.6	19.5^{g}	17.5^{f}	18.5^{g}	77.2	14.4^{f}	12.0^{h}	13.2^{h}	81.0
Control	88.5^{a}	80.5^{a}	84.5^{a}	0.0	82.7^{a}	79.6^{a}	81.2^{a}	0.0	71.0^{a}	67.8^{a}	69.4^{a}	0.0

(1) There was not significant difference between figures that have the same letters in the same column (P=0.05).
(2) Root-rot was determined by the author using a modified version of the scale outlined by Ahmed (2018), which ranged from 0 to 4.
(3) After ninety days of inoculation, the severity rate of root rot was scored. Seedlings at six months or two to three leaves stage were treated with 250 ppm of nano silicon and cooper as a soil treatment and soil treatment with a subsequent foliar spray seven days before to pathogen inoculation.

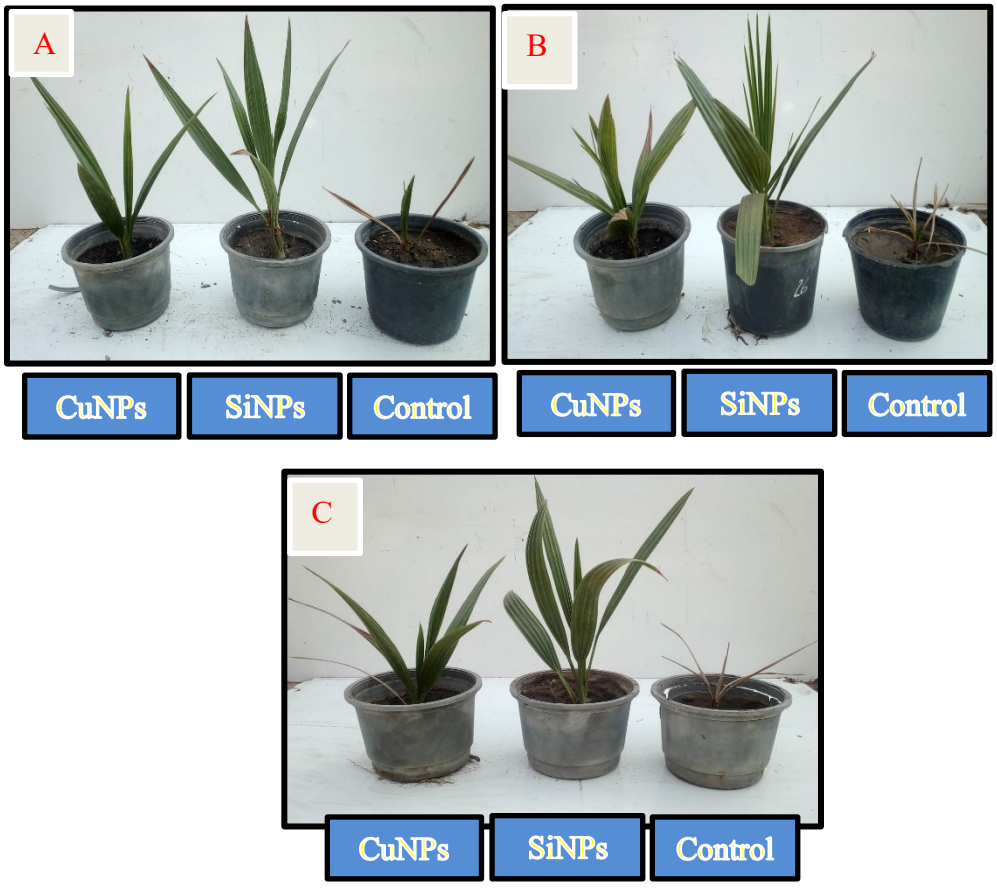

Figure 6. Effect of Nano silicon and copper at concentrations 250.0 ppm produced biologically from *Paenibacillus polymyxa* (PbGK1) on root rot severity of date palm offshoots in artificially infested soil after 90 days under greenhouse conditions. *Macrophomina phaseolina* (A), *Fusarium solani* (B) and *Lasiodiplodia theobromae* (C)

Table 4. Effect of silicon and copper nanoparticles on peroxidase activity of date palm offshoots (cv. Zaghloul)

Nanoparticles Source	Peroxidase activity (ΔO.D/1 min/g fresh weight)					
	L. thoebromae ($LdGG_1$)		*F. solani* ($FsBI_2$)		*M. phaseolina* ($MpFF_2$)	
	Activity	**Increase %**	**Activity**	**Increase %**	**Activity**	**Increase %**
SiNPs Biological	2.02[a]	648.1	2.15[a]	593.5	2.45[a]	544.7
SiNPs Chemical	1.91[ab]	607.4	1.60[b]	416.1	2.13[b]	460.5
CuNPs Biological	1.12[c]	314.8	1.34[c]	332.3	1.51[c]	297.4
CuNPs Chemical	0.91[d]	237.0	1.05[d]	238.7	1.11[d]	192.1
Topsin M 70% (Fungicide)	1.70[b]	529.6	1.76[b]	467.7	1.94[b]	410.5
Control	0.27[e]	0.0	0.31[e]	0.0	0.38[e]	0.0

(1) There was not significant difference between figures that have the same letters in the same column (P=0.05).
(2) Change in absorbance at 420 nm/one minute/gram fresh weight is a measure of peroxidase activity.
(3) Three replicates were used in the statistical analysis.

Table 5. Effect of silicon and copper nanoparticles on polyphenol oxidase (PPO) activity of date palm offshoots (cv. Zaghloul)

Nanoparticles Source	PPO activity (ΔO.D/5 min/g fresh weight)					
	L. thoebromae ($LdGG_1$)		*F. solani* ($FsBI_2$)		*M. phaseolina* ($MpFF_2$)	
	Activity	Increase %	Activity	Increase %	Activity	Increase %
SiNPs Biological	0.28[a]	250.0	0.40[a]	233.3	0.57[a]	200.0
SiNPs Chemical	0.26[b]	212.5	0.32[b]	166.7	0.38[b]	100.0
CuNPs Biological	0.19[d]	137.5	0.25[c]	108.3	0.31[c]	63.2
CuNPs Chemical	0.15[e]	87.5	0.19[d]	58.3	0.23[d]	21.1
Topsin M 70% (Fungicide)	0.21[c]	162.5	0.32[b]	166.7	0.40[b]	110.5
Control	0.08[f]	0.0	0.12[e]	0.0	0.19[e]	0.0

(1) There was not significant difference between figures that have the same letters in the same column (P=0.05).
(2) Change in absorbance at 420 nm/five minute/gram fresh weight is a measure of PPO activity.
(3) Three replicates were used in the statistical analysis.

Table 6. Effect of silicon and copper nanoparticles on chitinase activity of date palm offshoots (cv. Zaghloul)

Nanoparticles Source	Chitinase activity (µg NAGA/60min/g root)					
	L. thoebromae ($LdGG_1$)		*F. solani* ($FsBI_2$)		*M. phaseolina* ($MpFF_2$)	
	Activity	**Increase %**	**Activity**	**Increase %**	**Activity**	**Increase %**
SiNPs Biological	0.20^{a}	566.7	0.41^{a}	485.7	0.45^{a}	400.0
SiNPs Chemical	0.14^{bc}	366.7	0.25^{b}	257.1	0.29^{b}	222.2
CuNPs Biological	0.11^{d}	266.7	0.20^{c}	185.7	0.24^{c}	166.7
CuNPs Chemical	0.08^{e}	166.7	0.15^{d}	114.3	0.17^{d}	88.9
Topsin M 70% (Fungicide)	0.12^{cd}	300.0	0.22^{bc}	214.3	0.26^{bc}	188.9
Control	0.03^{f}	0.0	0.07^{e}	0.0	0.09^{e}	0.0

(1) There was not significant difference between figures that have the same letters in the same column (P=0.05).
(2) Chitinase activity expressed as µg N-acetyle glucose amine equivalent released / gram fresh weight / 60 minutes.
(3) Three replicates were used in the statistical analysis.

Discussion

Several pathogenic soil fungi attack the date palm's roots in Egypt and in the world, leading to damaging disease such root rot (Baraka *et al.,* 2011; Ahmed, 2018 and Jassim and Jaaafer, 2023). Date palm samples showed root rot disease were obtained from different governorates and sites in Egypt, including Minia, Fayoum, Giza, and Beheira. Different species of fungi were isolated from the obtained roots by isolation tests. *F. oxysporium, F. solani, M. phaseolina, R. solani, and L. thoebromae* were confirmed as fungi after purification. The biological interactions of microbes with soil types, NPK, biological fertilizers, and other environmental variables can account for the variation in the quantity of isolated fungi from various districts. *L. thoebromae* with accession number (PV594471.1) is the primary fungal pathogen of date palm offshoots of cvs, according to the percentage of disease incidence, severity on various date palm cultivars *i.e.* Siwey, Barhey, and Zaghloul to infection by various fungal pathogens under greenhouse circumstances as well as Following using Polymerase Chain Reaction (PCR) amplification to identify the primary fungal pathogen. According to Mansour *et al.* (2023) who isolated

Lasiodiplodia theobromae and *Fusarium solani* from date palm trees and obtained frequency percentages of 9.22% and 7.80%, respectively. One significant genus, *L. thoebromae*, contains widely distributed phytopathogenic species (Mansour *et al.,* 2023). Numerous methods, including fungicides, bio-elicitors and biological approaches, were validated to manage the isolated fungus (Haggag *et al*., 2017). Thus, the current work managed the fungal root decay disease of date palm offshoots using silicon and copper nanoparticles produced both chemically and biologically from *Paenibacillus polymyxa* (PbGK1). NSI and NSI concentrations were found to have an impact on several antifungal, development of plants, and antioxidant defense processes.

The antifungal properties of the silicon and cooper nanoparticles that were manufactured using both biological and chemical approaches were evaluated against three pathogen fungi that were isolated from date palm offshoots: *L. theobromae, F. solani,* and *M. phaseolina*. In compare to the chemical fungicide (Topsin M-70%), biologically produced silicon nanoparticles were the most effective antagonist at all concentrations. Conversely, among all the fungi examined, copper nanoparticles produced chemically had the least impact. The fact that these physicochemical characteristics differ from those of bulk materials has made nanomaterials especially significant. Moreover, the physicochemical properties of nanomaterials are determined by their dimensions, form, and composition (Maryam *et al*., 2024). Due to their outstanding antifungal properties, several nanomaterials are a good substitute for fighting phytopathogenic fungi (Kutawa *et al.,* 2021). Since nanoparticles have demonstrated strong antifungal action against a broad range of phytopathogenic fungi, their application represents a novel approach to managing phytopathogenic fungi in agriculture (Arciniegas-Grijalba *et al*., 2017).

Date palm seedlings treated with silicon and copper nanoparticles produced chemically and biologically exhibit remarkably encouraging results, with a minimal percentage of DS symptoms at 250 ppm. In terms of lowering the root rot disease of date palms both prior to and following soil infection, biologically generated silicon nanoparticles outperformed the fungicide Topsin M70%. These findings are consistent with those of Khandelwal *et al.* (2016), who reported that materials scientists have produced nanoparticles with particular properties, such as shape, size of pores, and surface area, which they can utilize to conjugate, encapsulate, or adsorb an active ingredient, such as a pesticide, for precise and specific application or as protective agents.

Increases in date palm oxidative enzymes were influenced by the nanoparticles' application time. The results of the study showed that oxidative enzymes, including polyphenol oxidase, peroxidase and chitinase, increased

more when exposed to nanoparticles (Haggag and Eid, 2022). Different fungi that cause root rot and various nanoparticle materials may be responsible for these outcomes. These results suggest that rather than being a preventative measure against the date palm root rot disease of offshoots, nanoparticles can be employed as a treatment. Because of their size effect and charge characteristic, SiNPs can immediately pass through plant barriers such the cell wall, plasma membrane, and epidermis in plants that are exposed to foliage or root application. Maryam *et al.* (2024) discovered that by reducing oxidative stress and stimulating the antioxidant enzyme system, application to the leaves of SiNPs can mitigate the detrimental effects of Pb on maize crops. Despite not being necessary for typical plant development or yield, silicon works in tandem with nitrogen, potassium and phosphorus to fortify the plant's defenses. The application of soluble silicon in a variety of sizes and shapes aids in the development of systemic resistance in plants (Namakka *et al*., 2023). Copper (Cu), one of the eight essential micro-nutrients, is essential for the synthesis of metalloproteinase and acts as a cofactor to regulate enzyme activity (Kaur *et al.,* 2023). It plays a key part in controlling the combination of several macromolecules that are essential to plant metabolism, such as respiration, photosynthesis, cell wall lignification, and other defensive mechanisms against abiotic stressors (Lodde *et al.,* 2021 and Irshad *et al.,* 2024).

The application of silicon (SiNPs) and copper (CuNPs) nanoparticles synthesized chemically and biologically from *Paenibacillus polymyxa* (PbGK1) in date palm offshoot or seedlings is demonstrated by the current findings. It was clear that using organically produced SiNPs nanoparticles improved plant growth, decreased disease incidence, and increased antioxidant defense.

Conflicts of interest

The authors declare no conflict of interest.

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