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The Potent Effect of Selenium Nanoparticles (Se-NPs) against Fusarium Wilt Disease of Lupine, Insight into: Antifungal, Physiological and Ultrastructural Studies

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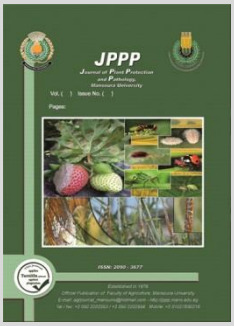

ABSTRACT

This study aimed to assess the efficacy of selenium nanoparticles (Se-NPs) in managing Fusarium wilt disease in lupine induced by *Fusarium oxysporum* f.sp. *lupini*. The study employed HR-TEM to characterize selenium nanoparticles, revealing a diverse morphology, uniform size distribution, and a mixture of crystalline and amorphous phases. The antifungal efficacy of Se-NPs (with an average particle size of 12-68 nm) at 5, 10, 15, 20, and 25 ppm was evaluated against the aggressive *F. oxysporum* isolate (Fox-Giza.1, accession No. PP230953.1). *In vitro* tests indicated that the Se-NPs at 25 ppm displayed the most significant inhibitory impact on mycelial growth, achieving a 100% decrease relative to the control. Anatomically, cross-sections of primary roots seen by scanning electron microscopy demonstrated that combining seed soaking and foliar spraying with Se-NPs treatment protected lupine plants against detrimental structural alterations caused by pathogens in the vascular cylinder structure. *In vivo*, testing performed in pots and fields demonstrated efficacy in combining seed soaking and foliar spray of Se-NPs treatment at 15 ppm for both, which was second only to the Topsin M-70® fungicide without significant differences, resulting in survived plants of 90.70%. In physiological studies, photosynthetic pigments, levels of peroxidase, polyphenol oxidase, chitinase, antioxidants capacity, total phenol, flavonoids, growth and yield metrics were increased in plants subjected to the successful combination treatment. In conclusion, the combination of seed soaking and foliar application of Se-NPs treatment were more effective than using each of them individually as a fungicide against Fusarium wilt disease in lupine.

Keywords: lupine; wilt; Fusarium; selenium; nanoparticles

INTRODUCTION

Lupine **(***Lupinus albus* L.**)** is planting for many purposes such as food, medical and industrial as well as ornamental plants. Lupine seeds a like other legumes are a good dietary source of minerals, protein. It also contains oil as well as cholesterol, in addition to, occasionally employed as stomachic atomic due to it contains alkaloids i.e. lupulin, sparatein, lupul and luponine (Smith *et al.*, 2004 and Abd El-Hai *et al*., 2016). Lupine may be utilized to create a diverse array of sweet and savory culinary items encompassing daily meals, traditional fermented products, baked goods, and sauces (Zian *et al.*, 2013). Lupine beans can be ground into flour or bran to increase fiber, texture, and protein in food production (Villarino *et al*., 2016). Lupins are used as recovery plants in degraded areas and for nitrogen fixation which leads to increased plant productivity and reduced agricultural operating costs. (Tassia *et al*., 2018).

Various soil-borne fungi, such as *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, and *Sclerotium rolfsii*, are known to affect lupine seeds, roots and stems occurring serious economic losses in germination and growth leading to negative significant effects and great reduction in productivity (Ali *et al*., 2009; Zain *et al*., 2013; Abd El-Hai *et al*., 2016 and Zian *et al*., 2019). Herein, Fusarium wilt occurs at all plant stages of development. Symptoms of Fusarium wilt include discoloration of vascular tissues, it appears orang-brown or red to black with the discoloration and rotting of secondary roots (Lv *et al*., 2021).The water conducting vessels are blocked after Fusarium infection therefore, plant wilting (Kuptsov *et al*., 2004).

The vascular wilt disease, induced by *F. oxysporum*, penetrates the roots and enters the water-conducting xylem arteries, leading to fungal growth that impedes the movement of water and minerals. The injury to xylem vessels, treachery elements impairs the transport of water and minerals which absorbs by the roots to photosynthetic organs. It consequently the leaves turn yellow and finally wilt leading to plant death. According to Yadeta and Thomma (2013), vascular wilt pathogens reside deep within their host plants, which often make chemical management strategies unsuccessful. Furthermore, these pathogens are capable of infecting a variety of host plants (Markakis *et al*., 2008).

There are two types of plant defense that occurs in the xylem tissues against vascular wilt pathogens, first is a physical or structural defensive which prevents pathogens from spreading into xylem vessels and, second is the chemical defense that kills or inhibits pathogens growth (Yadeta and Thomma, 2013). Tyloses are one of the most important effective structural plant defense responses in resistant plants in the formation of physic-chemical barriers in the xylem tissues (Kashyap *et al*., 2021).They are ingrowths of the axial and the ray parenchyma cells enter treachery cells when these become inactive or the xylem tissue is injured. Thereby,

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protecting the remaining xylem from pathogens damages (Lesniewska *et al*., 2017). Also, tyloses are accompany by gels or gums and are occlusions of xylem conductive tissues (De Micco *et al.*, 2016).

The excessive application of fungicides has caused considerable harm to human health due to environmental contamination and the development of physiological strains of pathogens resistant to fungicides. Herein, the pathogens resistance to fungicides reduces the effectiveness of these fungicides, accordingly, the need to use other fungicides or alternatives that differ in their mode of action an urgent. Recently, attempts are being made to find safe alternatives for humans and environment. One strategy now widely used is to use materials in nano scale form, known as nanotechnology. Nanoparticles have a unique physical and chemical property due to its small size (from 1 to 100 nm), large surface area to volume ratio leading to high reactivity and changed molecular interaction. So, it is more sufficient and effective comparing with its molecular and macro- scale counterpart (Wang *et al*., 2016). Control of soil-borne pathogens using nanoparticles was studied by some workers such as, Park *et al*. (2006) using silica silver against various plant pathogens, Dimkpa *et al*. (2013) using ZnO against *F. graminearum*, Hashem *et al*. (2021) using selenium synthesis by *Bacillus megaterium* against *R. solani* and Lazcano-Ramirez *et al*. (2023) using selenium nanoparticles obtained by plant-mediated synthesis against *F. oxysporum*.

This work aims to evaluate the efficiency of selenium nanoparticles (Se-NPs) within safe doses on management of lupine wilt disease caused by *F. oxysporium* f.sp. *lupini* and on some physiological characters and productivity *in vitro* and *in vivo*. Also, the alterations of root anatomical structure under artificial infestation were studies using scanning electron microscope.

MATERIALS AND METHODS

Source of seeds and Pathogen:

Lupine cultivar Giza-2 seeds were obtained from the Field Crop Research Institute and the Legume Crop Research Department at the Agriculture Research Center in Giza, Egypt.

An aggressive isolate of *F. oxysporum* was selected among seven isolates based on pathogenicity tests conducted by the Leguminous and Forage Crop Diseases Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

Molecular identification of pathogen isolate:

The extraction of pathogen DNA was molecularly identified using the protocol developed by Innis *et al*. (2012). DNA extraction was obtained from mycelia of *F. oxysporum* 14-day old cultures to get genomic DNA using Lee and Taylor method (1990). The resulting DNA pellets were washed with ethanol, dissolved in distilled water, and stored at -20 °C for further sequence analysis.

PCR conditions, sequencing and multiple sequence alignment

White *et al.* (1990) reported the amplification of the ITS region of rDNA utilising the primers ITS 1 (5′ TCC GTA GGT GAA CCT GCGG 3′) and ITS 4 (5′ TCC TCC GCT TAT TGA TATGC 3′). The thermocycler protocol for amplifying the ITS region comprised an initial phase at 95 °C for around 3 minutes, succeeded by 40 cycles of 95 °C for 30 seconds, 68 °C for 45 seconds, and 72 °C for 90 seconds. The concluding extension occurred at 72 °C for 8 minutes.

The DNA sequences were obtained from amplified PCR products using the ABI Prism 3130xl Genetic Analyzer. Sequencing was performed bidirectionally with the same primers, ITS 1 and ITS 4. This sequence was made by Macrogen Corporation in Korea. The resulting DNA sequences were submitted to the GenBank NCBI database. For multiple sequence alignment, the CLUSTALW technique was used, and the reconstruction of the phylogenetic tree study was performed using the "build" feature of ETE3 v3.1.1 (Huerta-Cepas *et al.*, 2016), which is available on the Genome Net platform (https://www.genome.jp/tools/ete/).

Synthesis of selenium nanoparticles (Se-NPs)

Selenium nanoparticles (Se-NPs) were synthesized according to Shakweer *et al.* (2023), by reducing selenium dioxide (SeO_2, Merck Schuchardt OHG, Germany) with ascorbic acid (El-Nasr Pharmaceutical Chemicals Company). Briefly, 100 mg of selenium dioxide were dissolved in 100 mL of distilled water, then 10 mL of ascorbic acid (56.7 mM) was added dropwise to the selenium dioxide solution with vigorous stirring. Synthesized selenium nanoparticles (Se-NPs) can be observed from the change in the color of the resulting solution from colorless to red. Finally, centrifugation at 12000 rpm was used to separate Se-NPs from the solution.

Characterization of selenium nanoparticles

A high-resolution transmission electron microscope (HR-TEM) was employed to examine selenium nanoparticles using Thermo Scientific Talos F200i at the Chemical Warfare Administration of the Egyptian Ministry of Defence in Cairo, Egypt.

Fungicide:

Topsin- M70® WP (containing the active substances Thiophanate Methyl) purchased from Sigma Company was used as a commercial fungicide product.

***In vitro* studies:**

Preparation of Se-NPs concentrations:

Concentrations of Se-NPs (ranging from 5 to 25 ppm in 5 ppm intervals) were generated at ambient temperature by diluting the original stock solution, which had a dose of 1000 ppm, with sterile distilled water. For the check treatment, only sterile distilled water was used. The solutions were stored at 4°C until they were needed for future experiments. Prior to being added to sterilized fungal growth media or used for direct application on seeds by soaking, using the Transonic Type 420 Sonicator from Elma, Germany, the nanoparticles were sonicated for 30 minutes.

Antifungal activity of Se-NPs against pathogenic fungal growth:

The antifungal activity of Se-NPs at 5, 10, 15, 20, and 25 ppm was assessed against the linear growth of *Fusarium oxysporum* compared to Topsin-M70® Wp. Different concentrations of nanoparticles and Topsin-M70® Wp (at 200 ppm) were added to potato dextrose agar (PDA) before solidification. This mixture was poured into sterilized Petri dishes (9 cm in diameter). Once solidified, the plates were inoculated in the center with a fungal disc of the pathogen and incubated at 26 °C. Four plates were meticulously prepared for each treatment and four control plates contained only the pathogen without additional treatments. The fungal growth of the fungus was measured carefully when the control plates showed total growth. The percentage of fungal growth

suppression compared to the control was determined using the method established by Mohana and Raveesha (2007):

$$\textbf{Inhibition \%} = \frac{C-T}{C}\textbf{X100}$$

Where, **C = average fungal growth in the check**
T = average growth of pathogen with each treatment.

***In vivo* studies:**

Pots experiment

Inoculum Preparation:

The *F. oxysporum* fungal inoculum was cultivated on potato dextrose agar plates and incubated at 25°C for 5 days. Afterward, mycelial plugs were inoculated into a sterile medium composed of sorghum, coarse sand, and water in a 2:1:2 v/v ratio. The components of the medium were carefully combined, bottled, and sterilized for 20 minutes at 1.5 atmospheres of pressure. Following sterilization, the mixture was incubated at 25°C for 15 days in preparation for use.

Se-NPs *vis* lupine wilt pathogen:

The best concentration of Se-NPs was selected for further studies with neglecting other concentrations, according to laboratory results. Each pot was filled with 6 kg of antiseptic soil, made up of a mixture of clay and sand in a 2:1 volumetric ratio. The soil was inoculated with a previously prepared pathogen at a concentration of 3% (w/w), using the method established by Zian *et al*. (2013). The pots were rinsed regularly with tap water and left to rest for 7 days to promote the growth and propagation of the fungal inoculum. Sterilized lupine seeds by 2% sodium hypochlorite for 2 min were soaked in Se-NPs at 15 ppm while, Topsin-M70® Wp fungicide was used as seed coating at 3 g/kg seeds for 3 hours then planted in inoculated pots at 5 seeds pot^{-1}. Untreated seeds were planted in sterile soil without fungal inoculum as a healthy control, also untreated seeds were sown in inoculated soil as an infested control. Three replicates were used in this experiment, five pots for each replicate. The same concentrate of Se-NPs was used as a foliar spraying at 30 days from planting. Complete randomize design was used, based on this, the following six treatments were conducted under pots experiment:

1- Healthy Control (sterilized seeds immersed in distilled water and planted on sterilized soil "without any treatment"),
2- Infested control (planting the sterilized seeds immersed in distilled water in inoculated soil with *F. oxysporium*),
3- Fungicide; (planting the sterilized seeds coated in Topsin-70® Wp in inoculated soil with *F. oxysporium*),
4- Soaking treatment (Soaking the sterilized seeds in Se-NPs and planting in inoculated soil with *F. oxysporium*),
5- Spraying treatment (spraying the plants in Se-NPs and planting in inoculated soil with *F. oxysporium*),
6- Soaking + spraying treatment (Soaking the sterilized seeds in Se-NPs and planting in inoculated soil with *F. oxysporium*, then spraying the plants of Se-NPs after 30 days from sowing.

Disease assessment:

The percentage of wilt disease was determined by evaluating early and late wilt at 40 days and 90 days after planting, respectively. Additionally, the number of surviving plants (both healthy and infected plants) was recorded at 120 days following planting. Infected plants were examined by longitudinally cutting the stem and root, while healthy plants showed no visible signs of disease. The severity of visual wilt signs and any discoloration of the internal tissue was documented using a modified scale developed by Ishikawa *et al.* (2005), which ranges from 0 to 4 based on the proportion of internal browning observed in the stem and root: hence
0 = healthy, 1 = 25 % or less browning, 2 =26-50 % browning,
3 = 51-75 % browning and
4 =76-100 % browning.
The percentage of disease severity was determined using the formula below:
Disease severity% = €NPC×CR/NIP×MSC×100
Where: NPC= Number of plants in each class rate, CR= class rate, NIP= Number of infected plants and MSC=Maximum severity.

Estimated of defense enzymes activity:

Forty-eight hours after treatment, the activities of the enzymes polyphenol oxidase, peroxidase, and chitinase, which are involved in plant defense, were measured in lupine leaves collected 32 days after planting. The leaf tissue was ground using specific buffers: sodium phosphate for polyphenol oxidase, potassium phosphate for peroxidase, and citrate-phosphate for chitinase. Following this, the mixture was filtered and centrifuged. The enzyme activities were then determined using established methods: Esterbauer *et al.* (1977) for polyphenol oxidase, Kato and Shimizu (1987) for peroxidase, and Monreal and Reese (1969) for chitinase. The results were quantified as mg of catechol equivalent per gram of fresh weight for polyphenol oxidase, mg of pyrogallol equivalent per gram of fresh weight for peroxidase, and grams of glucose per gram of fresh weight for chitinase.

Antioxidants activity:

The reducing power of lupine leaf extracts was measured colorimetrically at 700 nm, 32 days after sowing (Li *et al*., 2015), with higher absorbance indicating greater reducing power. Two methods were used to determine the antioxidant capability of the leaf extracts:
a) ABTS+ cation radical scavenging (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), a chemical compound used to examine the reaction kinetics of specific enzymes.
b) DPPH· cation radical scavenging (2,2-diphenyl-1-picrylhydrazyl), which serves as a monitor for chemical reactions involving radicals and is a widely used antioxidant assay (Sharma and Bhat, 2009), following the procedures proposed by Christodouleas *et al*. (2014).

The absorbance of the resultant greenish-blue solution was quantified at 734 nm using the ABTS+ technique. Conversely, the absorbance of the DPPH· technique was measured at 517 nm. The inhibition percentage was determined by quantifying the decrease in absorbance by the subsequent formula:

$$\textbf{Inhibition \%} = \frac{A-A1}{A}\textbf{X100}$$

Where, **A = the absorbance of a control**
A1 = = the absorbance of the sample treatment.

Anatomical structure of lupine root seen using Scanning Electron Microscope (SEM):

The anatomical structure of lupine roots was examined using a Scanning Electron Microscope (SEM) at 35 days post-planting. This study investigated the anatomical changes in lupine roots in response to the pathogen *F. oxysporum*, as well as the effects of Se-NPs and fungicide treatments. The methodology followed the approach outlined by Denise and Anjali (2019).

Physiological Activities:

At 60 days post-planting, the physiological activities were assessed as follows:

Photosynthetic pigments:

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content were determined in the third leaf from the lupine tip using a spectrophotometric method (Lichtenthaler and Buschmann, 2001; Robinson and Britz, 2000; Mackinney, 1941). Pigments were extracted in 90% methanol with sodium carbonate, and absorbance was measured at 452.5 nm, 650 nm, and 665 nm. Results are reported as mg/g fresh weight.

The total phenolics compound:

Fresh shoots were analyzed for total phenolics using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965). The quantification was carried out spectrophotometrically at 650 nm after 60 minutes, with catechol used as a reference, following the method described by Blainski *et al.* (2013).

Total flavonoids content:

The total flavonoid content in fresh leaves was quantified using a spectrophotometric method described by Li *et al*. (2015).

Field experiments:

These experiments aims to study the study the possible role of Se-NPs to impact wilt disease comparing with fungicide Topsin- M70® Wp, as well as enhance growth of lupine plants under natural infection. Two field trials were performed in two different locations, with one experiment in each locality; Faculty of Agriculture Farm, Mansoura University Dakahlia Province and Giza Research Station, Giza Province. Lupine seeds were soaked in Se-NPs at 15 ppm while, Topsin-M70® WP fungicide was applied as a seed coating at 3 g/kg seeds for 3 hours. Lupine seeds (cv Giza 2) were sowed on November 10th in both locations and subjected to natural infection. A foliar spray treatment was administered 30 days post-planting. A complete randomized block design with three replicates was used in the field trials. The occurrences of early and late wilt at 40 and 90 days after sowing were recorded using the same method as in the pot experiment.

Morphological characters and yield components:

During the harvest stage, samples were collected to evaluate growth metrics, including plant height, the number of branches per plant. Additionally, specific yield components were assessed, such as the number of pods per plant, the total weight of plant yield (g), and the weight of 100 seeds (g) and Seed yield weight (Kg).

Statistical analysis:

The gathered data was subjected to statistical analysis by analysis of variance (ANOVA) for a completely randomized design, conducted with CoStat software (version 4.6). Mean comparisons were made at a significance level of 1% for laboratory experiments and 5% for pot experiments. For field experiments, data were organized into one-way randomized complete blocks. The least significant difference (LSD) method was used to assess differences between treatment means at a 5% probability level. The Duncan test was employed for all experiments to evaluate the comparisons further.

RESULTS AND DISCUSSION

Results

Characterization of selenium nanoparticles:

Transmission Electron Microscopy (TEM)

The HR-TEM micrograph (Figure 1) was employed to visualize and characterize the microstructure of the nanomaterial. This technique allows for high-resolution imaging of individual atoms and their arrangement within the material, providing valuable insights into its crystal structure, defects, and other microstructural features. The nanoparticles exhibit a variety of shapes, including spherical, rod-like, and irregular. This diversity might be attributed to variations in nucleation and growth conditions during the synthesis process. There seems to be a relatively uniform size distribution (12-68 nm) among the nanoparticles, with some variation. The relatively uniform size distribution suggests consistent nucleation and growth rates, potentially influenced by factors like reactant concentrations and temperature. Some degree of aggregation is evident, with nanoparticles clustering together. The contrast in the image is good, allowing for clear visualization of the nanoparticles and their boundaries. The nanoparticles exhibit a crystalline structure, as evidenced by the lattice fringes observed within some of them. This suggests that the selenium is likely present in a crystalline phase. The lattice spacing of the crystalline regions seems to vary, indicating the presence of different crystallographic orientations or phases. This suggests that the nanomaterial has a well-defined atomic arrangement (Wu *et al.*, 2012 and Moreau *et al.*, 2013). The varying lattice spacing indicates the presence of different crystallographic orientations or phases within the nanoparticles. This could be attributed to factors like strain, defects, or the coexistence of multiple polymorphs (Esposito and Castelli, 2020). Some regions of the nanoparticles may appear amorphous or less crystalline, suggesting the presence of disordered or non-crystalline phases. The morphology and size distribution of the nanoparticles may be influenced by the synthesis conditions used, such as temperature, concentration, and reducing agent. The aggregation observed might be due to the growth of nanoparticles during the synthesis process or sample preparation (Li and Kaner, 2006). The crystalline nature of the nanoparticles suggests that the selenium has crystallized during or after the synthesis process.

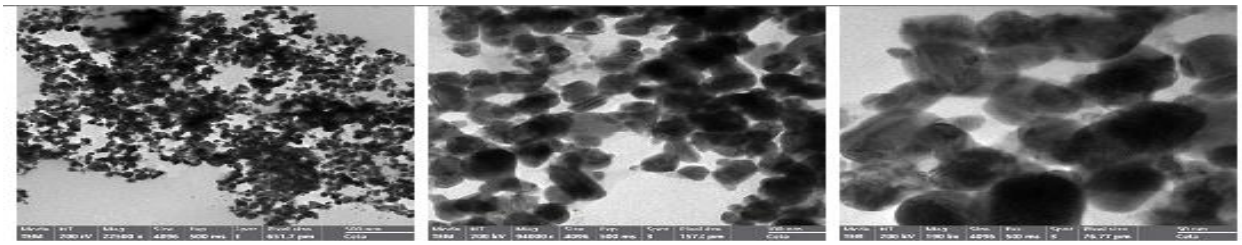

Figure 1. HR-TEM micrographs of selenium nanoparticles.

Molecular identification of the pathogenic fungus:

The resultant sequence was uploaded to the GenBank database under accession number : PP230953.1 for the examined *F. oxysporum* isolate Fox-Giza.1. The nucleotide sequences and BLAST evaluation show a high similarity of nucleotide sequence between the tested *F.* oxysporum isolate and other *F. oxysporum* isolates in the GenBank under accession numbers PQ358207.1, FJ985285.1 and FJ664911.1 (Figure 2).

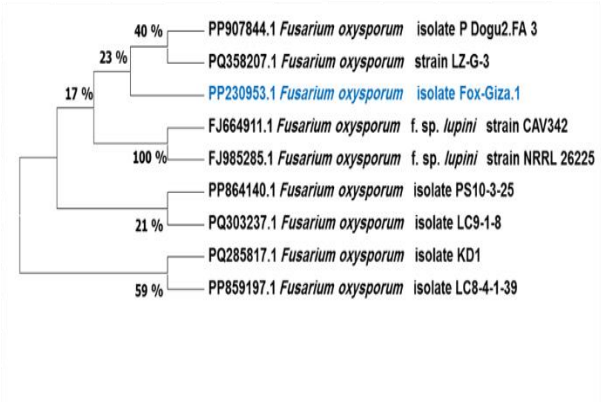

Figure 2. Phylogenetic analysis of *Fusarium oxysporum* isolate Fox-Giza.1 (GenBank accession No.: PP230953.1) was conducted in MEGA 11 utilizing the neighbor-joining technique with 1000 bootstrap repetitions.

***In vitro* studies:**

The antifungal activity of Se-NPs versus fungal growth of *F. oxysporum* f.sp. *lupini:*

The antifungal activity of Se-NPs was examined versus *F. oxysporum* f.sp. *lupini* at variable concentrations ranging from 5 to 25 ppm, as shown in Table 1. Results illustrate that all the tested concentrations had antifungal activity against the tested fungus. The reduction percentage showed a positive correlation with increasing Se-NP concentration. Moreover, 25 ppm concentration had the highest antifungal activity and gave a reduction percentage of 100 %, whereas 5 ppm had the lowest antifungal activity and gave a reduction percentage of 71.22%.

Table 1. The antifungal activity of Se-NPs versus fungal growth of *F. oxysporum* f.sp. *lupini*

Treatments	Fungal growth (mm)	Reduction%
Se-NPs at 25 ppm	0.00 ^{e}	100.0
Se-NPs at 20 ppm	5.60 ^{d}	93.80
Se-NPs at 15 ppm	7.95 ^{d}	91.17
Se-NPs at 10 ppm	12.54 ^{c}	86.07
Se-NPs at 5 ppm	25.91 ^{b}	71.22
Topsin M-70® at 200 ppm	0.00 ^{e}	100.0
Control	90.00 ^{a}	-

Different letters after means in each column indicated significant variances P≤0.01

In vivo studies:

Impacts of Se-NPs on Fusarium wilt disease of lupine under pots conditions:

The results presented in Table 2 indicate that the use of Se-NPs, applied through seed soaking, foliar spraying and a combination of both methods, on lupine planted in potted soil infested with *Fusarium oxysporum* f.sp. *lupini* showed significant effectiveness against the pathogen. The most effective treatment was the combined application of seed soaking and foliar spraying, which ranked just behind the Topsin M-70® fungicide without significant differences, resulting in survived plants of 90.70% compared to the infested control. Following that, the foliar spraying of seedlings achieved survived plants of 88%. In contrast, the seed soaking treatment exhibited the lowest effectiveness, with survived plants of 85.30% relative to the infested control

Table 2. Impact of Se-NPs on lupine Giza-2 cultivar grown in artificially infested soil with *Fusarium oxysporum* f.sp. lupini under pots conditions.

Treatments	Wilted plants %				Survived Plants %	Disease severity %
	Early wilt		Late wilt			
	Incidence %	Reduction %	Incidence %	Reduction %		
Healthy control (Non-infested soil)	1.40 ^{b}	-	0.00 ^{e}	-	98.60 ^{a}	2.40 ^{d}
Infested control (Infested soil)	13.40 ^{a}	-	20.00 ^{a}	-	66.60 ^{e}	47.00 ^{a}
Topsin M-70® Wp (Thiophanate methyl)	1.40 ^{b}	89.55	5.30 ^{d}	73.50	93.30 ^{b}	12.70 ^{c}
Soaking seeds in Se-NPs at 15ppm	2.70 ^{b}	79.85	12.00 ^{b}	40.00	85.30 ^{d}	18.40 ^{b}
Spraying seedlings with Se-NPs at 15 ppm	4.00 ^{b}	70.14	8.00 ^{c}	60.00	88.00 ^{cd}	15.70 ^{bc}
Soaking and spraying with Se-NPs at 15 ppm	4.00 ^{b}	70.14	5.30 ^{d}	73.50	90.70 ^{bc}	13.70 ^{bc}

Different letters after means in each column indicated significant variances P≤0.05

Defense-related enzymes activity:

From Table 3, it can deduce that soil infested with *F. oxysporum* as well as it's interacted with both fungicide and Se-NPs induced the activity of polyphenol oxidase (PPO) and peroxidase (PO) enzymes compare with healthy control. Soaking lupine seeds in Se-NPs with foliar spraying of grown plants after 30 days from sowing was more effective followed by spraying treatment then soaking treatment.

Table 3. Enzymatic activity as a response to Se-NPs in the lupine cultivar Giza-2 planted in soil artificially inoculated with *F. oxysporum* f.sp. *lupini* under pot conditions.

Treatments	polyphenol oxidase (mg/g.F.wt)	Peroxidase (mg/g.F.wt)	Chitinase (mg/g.F.wt)
Healthy control (Non-infested soil)	0.202 ^{f}	0.482 ^{f}	0.143 ^{d}
Infested control (Infested soil)	0.234 ^{e}	0.508 ^{e}	0.127 ^{e}
Topsin M-70® Wp (Thiophanate methyl)	0.287 ^{d}	0.667 ^{d}	0.134 ^{e}
Soaking seeds in Se-NPs at 15ppm	0.385 ^{c}	0.717 ^{c}	0.431 ^{c}
Spraying seedlings with Se-NPs at 15 ppm	0.404 ^{b}	0.824 ^{b}	0.547 ^{b}
Soaking and spraying with Se-NPs at 15 ppm	0.464 ^{a}	0.927 ^{a}	0.672 ^{a}

Different letters after means in each column indicated significant variances P≤0.01

While, the lowest values of PPO and PO activity was mentioned in healthy control then infested control. On the other side, the lowest chitinase activity was mentioned in infested control followed by fungicidal treatment then healthy

control. Whereas, all of Se-NPs treatments increased the activity of this enzyme, soaking + spraying treatment was more effective.

Antioxidants activity:

Fusarium oxysporum infestation of the soil resulted in decreased inhibition of DPPH and ABTS, as well as decreased power in lupine leaves as relative to the healthy control (Table 4). However, applying of fungicide or Se-NPs significantly increased ABTS and DPPH inhibition, as well as reduced power compared to both controls. The maximum antioxidants capacity was observed under fungicide treatment and also dual treatment of Se-NPs (seed soaking + foliar spraying) without non-significant differences between them. Foliar treatment came second followed by soaking treatment.

Table 4. The antioxidants capacity as a response to Se-NPs in lupine cultivar Giza-2 cultivated in soil artificially inoculated with *Fusarium oxysporum* f.sp. *lupini* under pots conditions.

Treatments	ABTS^{+} inhibition%	DPPH· inhibition %	Reducing power (at 700 nm)
Healthy control (Non-infested soil)	21.18 ^{d}	10.37 ^{d}	1.12 ^{d}
Infested control (Infested soil)	9.47 ^{e}	5.13 ^{e}	0.46 ^{e}
Topsin M-70® Wp (Thiophanate methyl)	29.27 ^{a}	17.71 ^{a}	1.81 ^{a}
Soaking seeds in Se-NPs at 15ppm	22.37 ^{c}	12.18 ^{c}	1.26 ^{c}
Spraying seedlings with Se-NPs at 15 ppm	24.25 ^{b}	14.47 ^{b}	1.54 ^{b}
Soaking and spraying With Se-NPs at 15 ppm	29.25 ^{a}	17.72 ^{a}	1.81 ^{a}

Different letters after means in each column indicated significant variances P≤0.01

Lupine root anatomical structure under SEM:

The normal structure of lupine root showed well-formed three main primary structures; a: epidermis, b: cortex and c: vascular cylinder. Root epidermis layer is typically consists of closely packed elongated cells with thin walls which is called uniseriate. Lupine root cortex is homogeneous and simple in structure where it contains one of cell type. It consists mainly of parenchyma cell which arranged in orderly radial rows or they may alternate with one another in the successive concentric layers with presence of intercellular spaces among parenchyma cells. The inner row of cortex which separate between cortex and vascular cylinder called endodermis. The root's central vascular cylinder contains the vascular system, parenchyma, and pith. The root's vascular system composed of alternating xylem and phloem strands, is well-defined. Xylem, primarily responsible for water transport, consists of vessels and tracheids. Phloem, which transports carbohydrates from photosynthesis, consists of sieve tubes, companion cells, and phloem parenchyma. The most abundant cell types in the sieve tubes are sieve elements and companion cells. Also found in the phloem, parenchyma and sclerenchyma cells which filling in extra gaps and offering support.

Many morphological symptoms are seen in lupine plants cultivated in soil artificially infested with *F. oxysporum* under pots experiment, as yellowing; then brown spots appear on the leaves. With advance in age, these brown spots enlarge and turn dark spots. In case of a severe infection, leaves become wilted and infected leaves fall off. Also seen necrotic lesions on all of vegetative growth, root rot, wilt and at the end plant.

There are a clear difference among healthy cross sections of lupine root plant under scanning electron microscope, (Fig. 3:A "1,2") and infected plant with *F. oxysporum* (Fig.3: B"1,2"). Cross-sections of infected roots showed major changes in the xylem, such as the formation of tylosis (multiple tyloses blocking vessels at the same time) and fungal hyphae at the edges of the xylem. Infected roots also exhibited altered xylem vessel dimensions, likely an adaptive response to reduced water uptake. Scanning electron microscopy showed plasmolysis and cell hydrolysis in parenchyma cells associated with xylem vessels, leading to vessel closure (Fig. 3: C"1,2"). However, seed soaking and foliar application of Se-NPs, particularly the combined treatment, mitigated these detrimental impacts of *F. oxysporum* on structure of roots (Fig. 3: D"1,2").

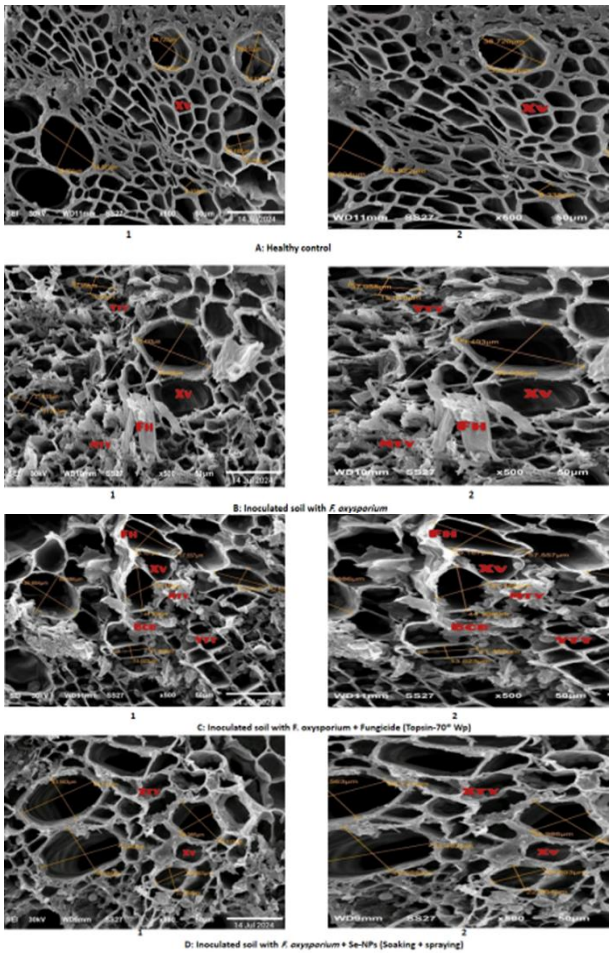

Fig. 3. SEM micrographs of lupine root cross-sections illustrating anatomical structural alterations resulting from *F. oxysporum* infection, fungicide application, and Se-NPs. Xy = xylem vessel mTy = multiple tylosis yTy = young tylosis Bcb = barenchyma cells blasmolized 1 = X500 2 = Zoom in to illustrate changes in xylem vessels

Physiological Activities:

A. Photosynthetic pigments:

All living organisms need energy, which they obtain from food stored in plants that perform photosynthesis. In turn, photosynthetic pigments that absorb light energy to

transform it into chemical energy within the plant are considered one of the most important indicators of plant health. As shown in Table 5, the infested control gave the lowest concentration of chlorophyll a and carotenoids in lupine leaves. It is worth noting that fungicide treatment led to increase in chlorophyll b over chlorophyll a, in contrast to the normal situation. In the same table, it was observed that, all SeO_2-NPs treatments whether seed soaking or foliar spraying or both together significantly increased these parameters as relative to other treatments. The dual treatment (soaking + spraying) was the best treatment then spraying treatment.

Table 5. In vivo, photosynthetic pigments as a response of Se-NPs in lupine cultivar Giza-2 planted in artificially inoculated soil with F. oxysporum f.sp. lupini under pots conditions.

Treatments	Chlorophyll a	Chlorophyll b	Carotenoids
Healthy control (Non-infested soil)	1.33 ^{c}	0.49 ^{cd}	0.417 ^{d}
Infested control (Infested soil)	0.51 ^{e}	0.57 ^{bc}	0.255 ^{f}
Topsin M-70® Wp (Thiophanate methyl)	0.85 ^{d}	0.42 ^{d}	0.292 ^{e}
Soaking seeds in Se-NPs at 15ppm	1.77 ^{b}	0.61 ^{b}	0.485 ^{c}
Spraying seedlings With Se-NPs at 15 ppm	1.82 ^{b}	0.73 ^{a}	0.528 ^{b}
Soaking and spraying with Se-NPs at 15 ppm	1.97 ^{a}	0.82 ^{a}	0.567 ^{a}

Different letters after means in each column indicated significant variances P≤0.01

B. Total phenols and flavonoids:

Results in Table 6, pointed out that total phenol content and total flavonoids considerably increased as a result to infection with *F. oxysporum* and the application of fungicide as well as Se-NPs whether seed soaking or foliar spraying or both together compared with healthy control. With regard to Se-NPs treatments, the dual treatment of Se-NPs (seed soaking + foliar spraying) gave the highly increment in both characters followed by foliar spraying treatment, while seed soaking treatment with Se-NPs came late.

Table 6. In vivo, total phenols and flavonoids as a response of Se-NPs in lupine cultivar Giza-2 planted in artificially inoculated soil with Fusarium oxysporum f.sp. lupini under pots conditions.

Treatments	Total phenols (mg/100g)	Flavonoids (mg QE/g fw)
Healthy control (Non-infested soil)	420.67 ^{e}	0.83 ^{f}
Infested control (Infested soil)	482.00 ^{a}	3.92 ^{a}
Topsin M-70® Wp (Thiophanate methyl)	470.33 ^{b}	3.25 ^{b}
Soaking seeds in Se-NPs at 15ppm	429.33 ^{d}	2.04 ^{e}
Spraying seedlings With Se-NPs at 15 ppm	450.33 ^{c}	2.52 ^{d}
Soaking and spraying with Se-NPs at 15 ppm	464.33 ^{b}	2.86 ^{c}

Different letters after means in each column indicate significant variances P≤0.01

Impact of Se-NPs on Fusarium wilt disease of lupine under field conditions:

Field trials were conducted to assess the effectiveness of Se-NPs at the Giza Agricultural Research Stations and the Faculty of Agriculture Farms at Mansoura University during the winter growing season of 2023/2024. The treatments included seed soaking, foliar spraying, and a combination of both, compared to the fungicide Topsin M-70®, to reduce Fusarium wilt disease in lupine plants. According to Table 7, all the examined treatments significantly decreased the incidence of Fusarium wilt. The fungicide Topsin M-70® achieved the highest survival rates of plants, at 92.60% and 90.30% at the two locations, resulting in the most notable reduction in disease occurrence. Following this, the combination of seed soaking and foliar spraying with Se-NPs also demonstrated substantial effectiveness, with survival rates of 90.00% and 88.70%. While, the foliar spraying of seedlings achieved survived plants of 87.60 and 86.30%. However, seed soaking with Se-NPs exhibited the least impact among the treatments, yielding lower reductions in disease incidence and survival rates of 84.30% and 85.30%, respectively.

Table 7. Impact of Se-NPs on Fusarium wilt disease of lupine under field conditions.

Treatments	Wilted plants %				Survived plants %	Increasing %
	Early wilt		Late wilt			
	Incidence%	Reduction%	Incidence%	Reduction%		
Giza Agricultural Research Station						
Control(Un-treated seeds)	10.40 ^{a}	-	17.30 ^{a}	-	72.30 ^{e}	-
Topsin M-70® Wp(Thiophanate methyl)	3.40 ^{d}	67.30	4.00 ^{d}	76.87	92.60 ^{a}	28.07
Soaking seeds in Se-NPs at 15ppm	6.40 ^{b}	38.46	9.30 ^{b}	46.24	84.30 ^{d}	16.59
Spraying seedlings with Se-NPs at 15 ppm	5.40 ^{bc}	48.07	7.00 ^{c}	59.53	87.60 ^{c}	21.16
Soaking and spraying with Se-NPs at 15 ppm	4.40 ^{cd}	57.69	5.60 ^{c}	67.63	90.00 ^{b}	24.48
Faculty of Agriculture Farm, Mansoura University						
Control (Un-treated seeds)	12.00 ^{a}	-	19.00 ^{a}	-	69.00 ^{d}	-
Topsin M-70® Wp (Thiophanate methyl)	4.40 ^{c}	63.40	5.30 ^{c}	72.10	90.30 ^{a}	30.86
Soaking seeds in Se-NPs at 15ppm	6.00 ^{b}	50.00	8.70 ^{b}	54.21	85.30 ^{c}	23.62
Spraying seedlings with Se-NPs at 15 ppm	5.40 ^{bc}	55.00	8.30 ^{b}	56.31	86.30 ^{bc}	25.07
Soaking and spraying with Se-NPs at 15 ppm	5.00 ^{bc}	58.40	6.30 ^{c}	66.84	88.70 ^{ab}	28.55

Different letters after means in each column indicated significant variances P≤0.05

Impact of Se-NPs on growth parameters of lupine under field conditions.

The data in Table 8, shows that both locations had similar outcomes, with a significant improvement in crop characteristics relative to the control (untreated plants). These characteristics include plant height (cm), number of shoots per plant, pod count per plant, seed weight per plant (g), 100-seed weight (g), and seed yield per feddan (kg). Among the

treatments, combining seed soaking and foliar spraying with Se-NPs resulted in the most significant enhancements in these crop characteristics, particularly in plant height and the number of branches, pods, and seeds per plant. Additionally, the foliar spraying and seed soaking with Se-NPs treatments had intermediate effects on crop characteristics at both locations. In contrast, the Topsin M-70® treatment showed the least improvement, resulting in lower enhancements in growth parameters. Overall, all methods of selenium application and their combinations significantly boosted yield parameters, including 100-seed weight, seed weight per plant (g), and seed yield per feddan (kg) compared to the control. Combining seed soaking and foliar spraying with Se-NPs was more effective than the individual treatments.

Table 8. Impact of Se-NPs on growth parameters of lupine under field conditions.

Treatments	Plant height (cm)	Branches Number /plant	Pods number / plant	Seed weight /plant (g)	100 seed Weight (g)	Seed yield weight (Kg/ feddan)
Giza Agricultural Research Station						
Control (Un-treated seeds)	70.50 ^{c}	7.40 ^{d}	16.40 ^{d}	15.30 ^{d}	23.44 ^{c}	636.50 ^{e}
Topsin M-70® Wp (Thiophanate methyl)	88.40 ^{b}	8.40 ^{d}	18.33 ^{c}	20.44 ^{c}	25.37 ^{c}	1134.10 ^{d}
Soaking seeds in Se-NPs at 15ppm	89.70 ^{b}	11.07 ^{c}	19.40 ^{c}	24.94 ^{b}	29.10 ^{b}	1300.17 ^{c}
Spraying seedlings with Se-NPs at 15 ppm	94.17 ^{b}	13.30 ^{b}	22.70 ^{b}	27.24 ^{b}	32.20 ^{b}	1466.00 ^{b}
Soaking and spraying with Se-NPs at 15 ppm	102.84 ^{a}	17.40 ^{a}	25.30 ^{a}	31.50 ^{a}	36.07 ^{a}	1720.24 ^{a}
Faculty of Agriculture Farm, Mansoura University						
Control (Un-treated seeds)	67.00 ^{e}	6.40 ^{c}	12.70 ^{d}	13.47 ^{c}	21.20 ^{d}	519.60 ^{e}
Topsin M-70® Wp (Thiophanate methyl)	74.00 ^{d}	7.70 ^{c}	16.70 ^{c}	18.96 ^{b}	24.27 ^{c}	1034.10 ^{d}
Soaking seeds in Se-NPs at 15ppm	84.50 ^{c}	10.64 ^{b}	18.60 ^{bc}	20.86 ^{b}	26.14 ^{c}	1082.20 ^{c}
Spraying seedlings with Se-NPs at 15 ppm	88.84 ^{b}	11.30 ^{b}	20.40 ^{b}	25.37 ^{a}	29.17 ^{b}	1333.40 ^{b}
Soaking and spraying with Se-NPs at 15 ppm	95.00 ^{a}	14.97 ^{a}	23.33 ^{a}	26.80 ^{a}	31.57 ^{a}	1475.24 ^{a}

Different letters after means in each column indicated significant variances P≤0.05

Discussion

Lupine (*Lupinus albus* L.) grown in Egypt for some purposes such as food, medical and industrial due to it contains many important chemical compounds. It is also used in animal nutrition and improves the soil fertility by supplementing with nitrogen fixation (Erbas *et al*., 2005 and Sulas *et al*., 2016). It is suffering from many destructive diseases. Fusarium wilt is a significant soil-borne disease that limiting lupine cultivation. *F. oxysporum* is causative agents of vascular wilt which infect the plant roots and proliferate in xylem vessels in turn obstruct the water and mineral translocation enter the plant. It is blocked the xylem vessels after infection by fungal hypheae in addition to secretion of mycotoxins such as fusaric acid (Yadeta and Thomma, 2013). Similar data obtained by Mahmoud *et al*. (2013) where found that the fungal enzymes led to seed rot, cotyledons rot and turn by damping-off. In this investigation, the effect of the fungus on the root anatomical structure using scanning electron microscope supports this, as it leads to the closure of xylem vessels and thus affects all physiological processes. Also, the dimensions of xylem vessels were increased in the vessels free of the pathogen for adaptation to compensate for the lack of water absorption. The observed results also showed that all physiological activities had significantly decreased in soil infested by *F. oxysporum*. The reduced ABTS and DPPH radical scavenging activity and diminished reducing power in infected plants indicate compromised antioxidant defense mechanisms. This suggests that the fungal infection has damaged plant tissues, weakening their ability to neutralize free radicals and leading to increased oxidative stress.

Fungal infections can have a detrimental impact on root anatomical structure due to the action of exo- and endo-cellular hydrolytic enzymes, which are a result of the fungus's ethylene generation. This leads to tissue softness and disintegration (El-Samra *et al*., 1994). The infection of faba bean by *F. solani* resulted in hydrolysis, separation, and ultimately full disruption of some xylem channels, as well as the appearance of dark regions in cross sections observed under SEM, attributed to the degradation and dissolution of certain cellular components, leading to cell death (El-Sayed, 2022). Abd El-Hai *et al*. (2010) similarly demonstrated that soil inoculated with *Macrophomina phaseolina, Rhizoctonia solani, F. oxysporum*, and *F. solani* causes deformation of the anatomical structures in the basal region of the soybean stem, resulting in complete disruption of epidermal cells and substantial plasmolysis in cortical cells. Fouda and Abdalla (2000) and Abd El-Hai and El-Saidy (2016) noted that fungal infections result in the fast breakdown of cell walls due to the action of cellulolytic and pectolytic enzymes generated by various pathogens, leading to the disintegration of the cell wall and hydrolysis of cellular components.

It is known chemical control is used as a fast and effective therapy against plant pathogens, so seed treatments are considered from one of the most important method for plant protection from seed and soil-borne pathogens. Moreover, in our study coating of lupine seeds with recommended dose of Topsin M-70 fungicide led to a highly significant decrease in Fusarium wilt under both *in vitro* and *in vivo* conditions comparing with other treatments. While, it adversely impacted all physiological components. This is may be due to the seeds require protection from pathogens during the germination period to help an adequate plant stand in the soil where this treatment accelerates the emergence of seedlings on the soil surface in turn reduces infection of seedlings diseases (Abd El-Hai and Ali, 2018). However, the adverse effects of fungicides are acknowledged; therefore, the application of fungicides in seed treatments poses persistent toxicity and presents risks to humans and animals through environmental pollution as well as reduction of beneficial microorganisms besides affecting on seed quality **(**Al-Kahal *et al*., 2009 and Walters *et al*., 2007). Therefore, in this research we tried to use an alternative control strategy to nullify the hazards of fungicide on environmental balance and public health.

Recently, agricultural nanotechnology become one of the most important strategies which reported as an activity in

plant diseases management as well as crop protection (Gogos *et al*., 2012). According to Kurtijak *et al.* (2017) who reported that inorganic nanoparticles are non-toxic, hydrophilic, environmentally friendly, and very stable. Nanoparticles have unique physicochemical properties, such as small size, a high surface area to volume ratio, increased reactivity, and modified molecular interactions, making them more effective than their molecular and macroscopic equivalents (Wang *et al*., 2016). Therefore, the main purpose of using Se-NPs in this investigation is reducing the fungicide damage as well as decrease the amount used compared to regular particles.

Here, the present results illustrated the role of Se-NPs as seed socking and/or foliar spraying in decreasing the infection of Fusarium wilt in lupine plants. Where showed the antifungal activity against the pathogen linear growth in vitro. It also decreases the injurious effects of *F. oxysporum* on root structure especially combined of soaking + foliar spray treatment. In addition to, it led to the protection of lupine plants from early and late wilt under artificially infested in greenhouse and under natural infection under field condition in both localities (Mansoura and Giza Province). The dual treatment (seed soaking + foliar spraying) was more effective. These results can be explained through chemical analysis of bean leaves where showed a highly significant increase of defense-related enzymes activity, antioxidant enzymes, carotenoids, phenols and flavonoids, which have a major role in pathogen resistance and scavenging free radical associated with pathogen infection (Cheynier *et al.,* 2013 and Abd El-Hai *et al*., 2019). Increased activity of polyphenol oxidase, peroxidase, and chitinase is associated with improved plant disease control. This enhancement is likely due to the degradation of pathogen cell walls and inhibition of their growth (Harman *et al*., 2004). Polyphenol oxidase aids in defense by producing toxic quinone compounds that limit pathogen access to cellular proteins (Li and Steffens, 2002; Raj *et al.*, 2006). Peroxidase also contributes to plant defense by modifying the plant cell wall, making it more resistant to microbial enzymes (Caruso *et al*., 2001; Nawar and Kuti, 2003). Additionally, both polyphenol oxidase and peroxidase play vital roles in scavenging reactive oxygen species, which are harmful byproducts that can damage plant cells (Hasanuzzaman and Fujita, 2011). Adams (2004) added that chitinase enzyme is crucial for inhibiting fungal growth.

Furthermore, Mellersh *et al*. (2002) clarified that phenols serve as substrates for several antioxidant enzymes and scavengers of free radicals. Where, reported that phenolic compounds restrict fungal growth and prevent penetration of pathogens. The initial defensive response in plants involves a fast buildup of phenols at the infection site, functioning as an antioxidant, antibacterial agent, and photoreceptor, which restricts or inhibits pathogen growth (Lamba *et al*., 2008). There is a vital role of phenols in plant metabolic process regulation and lignin synthesis in turn the healthy plant growth and decrease plant disease infection (Martin-Tanguy, 2001). On the other side, De Melo *et al.* (2016) clear that the flavonoids had extended effect as antioxidants and anti-mutagenic as well as antiviral agent.

The present investigation revealed that, the application of Se-NPs whether soaking or foliar spray or both enhancement of growth and productivity. These results may be due to the role of selenium as antioxidant in protection the plant cell from oxidative damage by antioxidant defenses (Juárez-Maldonado *et al.,* 2019), increase the content of starch in chloroplast (Seppänen *et al.,* 2003). Selenium also is considered a beneficial element and has a bio-stimulant effect for growth, increase plant metabolism and crop quality and stress tolerance (Cittrarasu *et al.,* 2021), due to it is an essential element necessary for many enzymes activity and proteins (Davis *et al.,* 2012). Furthermore, Freeman *et al.* (2007) demonstrated that incorporating selenium in plants stimulates their defense against biotic and abiotic stressors. Selenium inhibiting the growth of fungi and bacteria through important antimicrobial properties (Hanson *et al*., 2003 and Alam *et al.,* 2016).While, El-Saadony *et al*. (2021) stated that lower concentrations of SeNPs had antifungal properties such as 50–150 µg/mL.

Moreover, Se-NPs treatment increased significantly photosynthetic pigments in lupine leaves. This effect beside to reduce the negative effect of Fusarium wilt disease, had a positive response in morphological characters and yield components of lupine plants. Generally, the response of photosynthetic pigments and other physiological aspects reflected on plant healthy and yield components. There was a positive relationship between the ability of photosynthetic pigments to capture of light energy and polysaccharides content such as pectin in plant organs, pectin is considered structurally compound in cell wall, it is acts as a first barrier against pathogen penetration (Hamideh *et al.,* 2013). On contrast, the infested soil with *F. oxysporum* had a negative impact on photosynthetic pigments due to reduce in the uptake of water and essential nutrients such as magnesium which required for chlorophyll synthesis and interfere with the photosynthesis reactions (Feng *et al*., 2015). The infection caused by the pathogen resulted in reduced photosynthetic activity, especially impacting photosystem II. This decline in photosynthesis is likely due to the loss of several thylakoid membrane proteins and a reduction in leaf-soluble proteins, including RuBisCO (Weintraub and Jones, 2010).

CONCLUSION

From the previous results, it may be concluded that using of Se-NPs at 15 ppm as seed soaking followed by foliar spraying after 30 days from planting could be applied for reducing Fusarium wilt disease in lupine plant. The HR-TEM analyses revealed the diverse morphology, size distribution, and crystalline structure of Se-NPs. In addition to induced the activation of defense-related enzymes activity, antioxidant enzymes, carotenoids, phenols and flavonoids which have a major role in pathogen resistance. The same treatment enhanced photosynthetic pigments content followed by improved plant growth and yield components. Thereby, Se-NPs can be used an alternative control strategy to nullify the hazards of fungicide on environmental balance and public health.

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التأثيرالقوي لجزيئات السيلينيوم النانوية ضد مرض الذبول الفيوزاريومي في الترمس، نظرة ثاقبة فى : الدراسات المضادة للفطريات، الفسيولوجية، والتركيبية الدقيقة.

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الملخص

تهدف هذه الدراسة إلى تقييم فعالية جزيئات السيلينيوم النانوية في مقاومة مرض الذبول الفيوزاريومي المتسبب عن فطر *Fusarium oxysporum* f.sp. *lupini*. في نباتات الترمس . وقد تم تقييم فعالية السيلينيوم النانوية المضادة للفطريات (بمتوسط حجم جزيئي قدره 12-68 نانومتر) عند تركيزات 5 و10 و15 و20 و25 جزء في المليون ضد العزلة الأكثر شراسة (Fox-Giza.1) *F. oxysporum*، والمسجلة برقم إيداع (PP230953.1) . حيث أظهرت الاختبارات المعملية أنه عند إستخدام جزيئات السيلينيوم النانوية عند تركيز 25 جزء في المليون كانت لها التأثير المثبط الاقوى على نمو الفطريات، محققة انخفاضاً بنسبة 100% مقارنةً بالكنترول. من الناحية التشريحية، أظهرت مقاطع عرضية من الجذور الاولية باستخدام الميكروسكوب الإلكتروني الماسح أن الجمع بين نقع البذور ورش الأوراق بمعاملة السيلينيوم النانوية وفر حماية لنباتات الترمس من التغيرات التركيبية الضارة التي يسببها الفطر الممرض في بنية الأسطوانة الوعائية. في التجارب الحية التي أجريت في الأصص والحقل، أظهرت فعالية الجمع بين نقع البذور ورش الأوراق بمعاملة السيلينيوم النانوية عند تركيز 15 جزء في المليون، والتي كانت تلي معاملة مبيد التوبسين أم-70 فى الترتيب دون اختلافات معنوية، مما أدى إلى بقاء 90.70% من النباتات مقارنةً بالكنترول. في الدراسات الفسيولوجية، زادت مستويات أصباغ التمثيل الضوئي، ومستويات البيروكسيداز، البوليفينول اوكسيديز، والكيتيناز، وقدرة مضادات الأكسدة، والفينولات الكلية، والفلافونويدات، ومعايير النمو والإنتاجية في النباتات التي خضعت للمعاملة المشتركة الناجحة. بإختصار، كانت المعاملة المشتركة ما بين نقع البذور والرش الورقى للنباتات بجزيئات السيلينيوم النانوية الأكثر فعالية من استخدام كل منهما منفرداً ضد مرض الذبول الفيوزاريومي في نباتات الترمس. يمكن استخدام هذه المعاملة كجزء من استراتيجية متكاملة لمكافحة الأمراض في المحاصيل الحقلية التي تتجنب استخدام المبيدات الفطرية.