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Application of Selenium and Silica Nanoparticles on Pepper Seedlings for Controlling Root Rot Disease caused by *Rhizoctonia solani*

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Graphical abstract

Abstract

There is an urgent need to find sustainable alternatives to chemical pesticides in order to protect the environment, ensure healthy food production and improve seedling quality. Therefore, the current study is an attempt to find nano-alternatives to chemical fungicides for protecting pepper seedlings (*Capsicum annuum* L.) production from the phytopathogen of *Rhizoctonia solani* as well as to enhance their growth and quality by individually applying nanosilica and nano-selenium (n-SiO_2, n-Se), and their combination under nursery conditions. Single and combined applied doses of n-SiO_2 (200 mg L^{-1}) and n-Se (100 mg L^{-1}) were treated pepper seedlings for controlling the root rot disease of *R. solani*. Seedlings treated with nanosilica (200 mg L^{-1}) produced the highest values in the studied parameters (vegetative growth of pepper seedlings, enzymatic antioxidants and the photosynthetic parameters under the fungal infection). The proposed mechanism of nano silica in promoting the growth of pepper seedlings against *R. solani* was supported by the Scanning Electronic Microscope (SEM) images, which compared infected seedlings (control) with nanosilica-treated ones. A similar trend was observed for enzymatic antioxidants responses after nanosilica treatment, indicated enhanced resistance of pepper seedlings against studied fungal pathogen. This superior performance was followed by nano-Se treatment and then by combined application of both nanoparticles. This study provides a novel insight into the potential use of nanofungicides against the studied fungal pathogen (*R. solani*) to reduce the chemical of fungicides application and improve the growth and quality of pepper seedlings. Further research is recommended to investigate additional nanoparticles and new phytopathogens.

Keywords: Antioxidants, Disease severity, Pathogenicity, Phytopathogen, Scanning Electronic Microscope

1. Introduction

Pepper (*Capsicum annuum* L.) is as an important vegetable crop worldwide cultivated under both greenhouse and open field. In Egypt, the cultivated area of pepper covered approximately 57,683 ha with a total production of about 18.47 Mg ha^{-1} (FAOSTAT, 2025). The use of high-quality transplants is a common commercial practice in widespread production of peppers and most of vegetables for fast-sustained establishment joined with improvement of identical maturity, early and total yields beside quality properties (Hoffmann and Poorter, 2002). Pepper seedlings production faces several challenges due to climate change including abiotic and biotic stresses. Several studies have confirmed abiotic stresses can cause drastic, rapid and negative impacts on seedling growth and productivity such as metal stress (Altaf *et al.* 2022; Yu *et al.* 2025), low temperature (Li *et al.* 2023; Song X *et al.* 2023; Pu *et al.* 2025), heat stress (Jang *et al.* 2025), drought (Lu *et al.* 2023), and salinity stress (Yuce *et al.* 2025). Regarding biotic stresses, various microorganisms including fungi, bacteria, fungi-like viruses, and nematodes are able to cause diseases during the growth of pepper seedlings alongside soil borne pathogens (Wu *et* *al.* 2019; González-Hernández *et al.* 2021; El-Kazzaz *et al.* 2022; Hernández-Huerta *et al.* 2023; Xiao *et al.* 2025).

Soil-borne fungal diseases caused by many microbial genera such as *Rhizoctonia, Fusarium, Pythium*, and *Verticillium*), are among the most destructive pathogens affecting vegetable seedlings (Shalaby *et al.* 2022). Several studies reported that the controlling of such phyto-pathogen using environmentally friendly alternatives to chemical pesticides to protect both the environment and human health (He *et al.* 2023; Paz-Trejo *et al.* 2024; Yadav *et al.* 2025). In general, these alternatives are considered biological control agents having sustainable approaches, and eco-friendly approaches such as microbial inoculants (Hernández-Huerta *et al.* 2023; Xiao *et al.* 2025), nano-pesticides (Qiao *et al.* 2024; Zainab *et al.* 2024), nanomaterials (Kashyap *et al.* 2024; Vijayreddy *et al.* 2025), and plant growth regulators like paclobutrazol (Shalaby *et al.* 2022a, 2022b). Among soil-borne fungi *Rhizoctonia solani* is one of the most an important pathogen, posing serious threats and causing yield losses in various crops (Huang *et al.* 2025) such as rice (Senapati *et al.* 2022), quinoa (Chethan *et al.* 2025), tomato (Kirupanatha-Rajan *et al.* 2025), faba bean (Osman *et al.* 2025), pigeon pea (Gao *et al.* 2025), and pepper (Rodríguez *et al.* 2023).

Nano selenium (n-Se) and nano-silica (n-SiO_2) are considered promising nanoparticles (NPs) due to their anti-stress properties potential applications in agriculture (Gao and Tuda 2024). These NPs act as nano-fungicides capable of supporting plant growth under stress by stimulating biochemical, morphological, and molecular defenses mechanisms (Taha *et al.* 2023). The significance of such NPs was reported in numerous studies according to their low dose requirement; targeted supply improved bioavailability and organized release; low dose-dependent toxicity; more solubility and penetrability (Periakaruppan *et al.* 2023). The individual effects of n-Se and n-SiO_2 under biotic stress have been discussed in several studies (Goswami *et al.* 2022; Kang *et al.* 2023; Sulaiman *et al.* 2023; Shahbaz *et al.* 2023; Song J *et al.* 2023; Batista *et al.* 2025; Choudhary *et al.* 2025; Imran *et al.* 2025). However, few studies have examined the combined use of nano-Se and nano-silica to alleviate stress such as drought in strawberry (Zahedi *et al.* 2020), salinity in rice (Badawy *et al.* 2021), and *Alternaria* leaf spot disease in common bean (Taha *et al.* 2023).

Therefore, the present study was designated to investigate the individual and combined roles of n-Se and n-SiO_2 in controlling damping-off disease caused by *Rhizoctonia solani* in pepper seedlings. The effects of these nano-fungicides on the growth and quality of pepper seedlings were comparing with the commercial fungicide and control treatments. This study also highlights the isolation and purification of the studied pathogen, *in vitro* and greenhouse experiments to produce pepper seedlings under disease stress, including chlorophyll content, and antioxidant enzymatic activities in pepper seedling leaves.

2. Materials and Methods

2.1. Pathogen isolation, purification and pathogenicity tests

Three isolates of *Rhizoctonia solani* were collected from infected pepper seedlings showing damping-off sympotoms at commercial nurseries in the Delta region, Egypt (Latitude 31°06'25.20" N Longitude: 30°56'26.99" E), (**Figure 1**). In brief, the infected pepper seedlings with damping off disease were collected and the pathogen was isolated, and purified following the method described by Dhingra and Sinclair (1985). The morphological traits and microscopic features of the isolates were confirmed as *R. solani* according to Leslie and Summerell (2006). For the pathogenicity test, the three isolates were evaluated for their ability to cause disease on pepper seedlings (California Wonder, cv.), which were obtained from the common vegetable nurseries under greenhouse conditions. Detailed procedures are described in Shalaby *et al.* (2022). The trials were organized in four replications, each one included three Styrofoam trays for each isolate alongside the non-infected control without the pathogen. Pre- and post-emergence damping-off was recorded 20 and 40 days after sowing, respectively, using pepper seedlings grown in Styrofoam trays (209 cells. The following equation describes the calculation of the pre- and post-emergence damping-off:

$$\text{Damping-off } (\%) = (\text{No. of infected plants/total No. of plants}) \times 100$$

Among the three isolates, the isolate Rs2 exhibited the highest pathogenicity based on symptoms severity and damping-off incidence. The identified isolates fungus was known as the pathogen of *R. solani* at the Mycology and Disease Survey Research Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

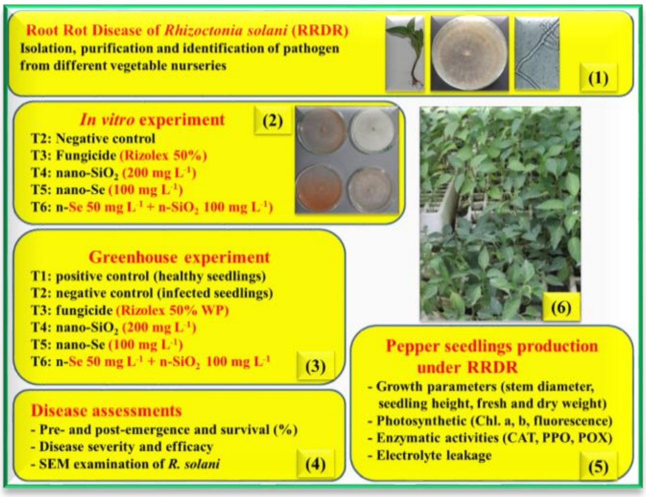

Figure 1. An outline on the design of the present study including various steps: photo (1) the isolation and purification of the studied pathogen, (2) *in vitro* experiment, (3) greenhouse experiment, (4) disease assessments, (5) production of pepper seedlings under disease stress, and (6) the main target of this study "healthy pepper seedlings".

2.2. Preparing nanoparticles

The nanoparticles were provided by from Department of Agricultural Microbiology Research, A.R.C., Giza, Egypt. Nano selenium (n-Se) was synthesized biologically using *Bacillus cereus* TAH strain, with particle sizes ranged from 41 to 102 nm (Ghazi *et al.* 2022). The transmission electron microscopy (TEM) was used to measure the magnitude of n-Se, with high-resolution (HR-TEM, Tecnai G20, FEI, The Netherlands), at the Central Laboratory of Nanotechnology and Advanced Material, ARC, whereas n-SiO_2 was prepared by a physical method. Its main characterization includes an average diameter of 10 nm for, pH 4–4.5, and specific surface area ranges from 260 to 320 $m^2\ g^{-1}$.

2.3. Antifungal activity of studied nanoparticles

Three isolates of *Rhizoctonia solani* with different degree of pathogenicity were evaluated. The most aggressive isolate (Rs2) was used for subsequent *in vitro* tests. The applied dose of n-Se was 100 mg L^{-1}, whereas n-SiO_2 was 200 mg L^{-1}. The combined treatment of both included using ½ of the used doses (i.e., 50 mg L^{-1} n-Se + 100 mg L^{-1} n-SiO_2). All used concentrations of the NPs were mixed with the PDA media (100 ml). Every dosage had five replications in Petri plates. 7 days after fungal growth, 5 mm in diameter agar masses from the actively growing edge of *R. solani* were placed at the center of Petri dishes containing the doses of nanoparticles. Plates were incubated at 25°C for 7 days. Colony diameters were measured, and the reduction in mycelial growth was calculated using the formula described by Ferreira *et al.* (1991):

$$R_e = \left[\left(R_L / a_{eq}\right) - \left(RHf_{ad}R_{ad}\right)\right] / \left[\left(\left(1-RH\right)/a_{kin}\right) + RH\left(1-f_{ad}\right)\right]$$

Where (R) is the radial growth of fungi in control and (r) is radial growth of fungi in treated plates.

Table 1. Details of applied treatments on pepper seedlings during both growing seasons.

Code	Treatments	Description of the treatment(s)
T1	Control (negative control)	Healthy seedlings treatment without any infection
T2	Control (positive control)	Seedlings infected with *Rhizoctonia solani*
T3	Fungicide	Seedlings infected by *R. solani* and applied commercial fungicide Rizolex 50% WP
T4	nano-silica (n-SiO_2)	Seedlings infected by *R. solani* and applied 200 mg L^{-1}
T5	nano-Se (n-Se)	Seedlings infected by *R. solani* and applied 100 mg L^{-1}
T6	½ (n-Se + n-SiO_2)	Seedlings infected by *R. solani* and applied 50% of T4 and T5 (i.e., n-SiO_2= 100 mg L^{-1} + n-Se= 50 mg L^{-1})

2.4. Greenhouse experiment

The greenhouse experiments were conducted at the Faculty of Agriculture, Kafrelsheikh University, during the two successive seasons of 2023 and 2024. This study aimed to evaluate the effeteness of n-SiO_2, n-Se and their combinations in controlling root rot caused by *R. solani* and improving pepper seedlings growth and quality under greenhouse conditions. The root rot-susceptible pepper cultivar (California Wonder) was used in all experiments. Seeds were sown in 209 cells trays containing 1:1 v/v mixture of coco peat and vermiculite. The trays were irrigated daily with equal amount of water to maintain field capacity and seedling were fertilized weekly after full germination with a compound fertilizer including 400 N, 400 P_2O_5, 400 K_2O at the rate of 1 g L^{-1}. The treatments are presented in **Table 1**.

Healthy pepper transplantings (uninfected by the pathogen of *R. solani*) were utilized as a negative control, while transplants infected by *R. solani* represented the positive control. To prepare the inoculated growing media, barley grains infected with *R. solani* pathogen were mixed individually with growing substrate at 1:1 (w/w) ratio, then placed directly inti trays. Based on a preliminary trial, concentration of 100 mg L^{-1} for n-Se and 200 mg L^{-1} for n-SiO_2 were selected for this study. The combined treatmants, n-Se and n-SiO_2 were applied at 50 and 100 mg L^{-1}, respectively. The fungicide (Rizolex 50% WP, which imported from Japan) produced in Japan by Company of Sumitomo Chemical was applied at the recommended rate of 2 g/Lto the growing media under the current pathogen (*R. solani*). Six treatments were applied by adding solutions directly prior to seeds planting. After planting, the trays were maintained under greenhouse conditions at 25 ± 2 °C for 40 days. The experiment arranged in four replications; each replication containing three trays per treatment besides untreated control treatment, which was trays with pathogen-free (non-diseased). All treatments were repeated again after 15 days from sowing, once seeds had fully germinated by sprinkling the solutions onto trays until the drops penetrated the growing media and reached the roots.

2.5. Disease assessments

The pre- and post- emergence percentages of pepper transplants were recorded after 20 and 40 days from sowing, as reported by Chandler and Santelman (1968). The disease severity was assessed after 40 days using the scale of 5-rating (Altier and Thies, 1995) (1 = vigorous and non-diseased, 2 = principal root end necrotic, 3= principal root end spineless and rotten, 4 = dead transplantings, emergence seeds with rotten essential roots and 5 = dead seed, un-germinated seed)**.** The following equation was applied to calculate disease severity percent:

$$\text{Disease severity, \%} = \frac{\left(\text{rating no.} \times \text{no. of seedlings in rating}\right)}{\left(\text{Total no. of seedlings} \times \text{highest rating}\right)} \times 100$$

2.6. Growth Assessments of Transplantings

After 40 days of sowing, vegetative growth parameters were measured; including seedling height (cm), stem diameter (mm), fresh and dry weights of seedlings (g/seedling), and dry weight was registered after drying at 70 °C for 48 h.

2.7. Chlorophyll determination

Leaf chlorophyll content was determined according to Nagata and Yamashita (1992) using a 645 and 663 nm wave length at spectrophotometer and calculating by relieving the analysis as follows:

$$\text{Chl.a} = 0.999 \times \text{A663} - 0.0989 \times \text{A645}$$
$$\text{Chl.b} = 0.328 \times \text{A663} + 1.77 \times \text{A645}$$

As reported by Maxwell and Johnson (2000), the highest efficacy of the photosystem (Fv/Fm) was assessed with a movable Optic-Science OS-30p + Fluorometer (Inc., Hudson NH, USA).

2.8. Antioxidant enzyme activities and electrolyte leakage

Fresh leaf tissue (0.5 g) was homogenized in 3 ml of 0.05 M Tris buffer (pH 7.8), comprising 0.001 M EDTA–Na_2 and 7.5% Polyvinylpyrrolidone at 0–4°C for enzymes activity. Homogenates were recorded by spectrophotometer (Shimadzu, Japan). Catalase (CAT), peroxidase (POX), and polyphenol oxidase enzyme (PPO) activities were determined according to Aebi (1984), Rathmell and Sequeira (1974), and Malik and Singh (1980), respectively. Electrolyte leakage was measured using EC meter or electrical conductivity meter (Lutts *et al.* 1996). All vegetative growth traits of pepper seedlings, chlorophyll content, chlorophyll florescence, electrolyte leakage, and antioxidant enzymes were measured 40 days after sowing in both seasons.

2.9. Microscopic investigation

The microscopic measurements were achieved using the fungal growth of the pathogen on the PDA media accompanied by n-SiO_2 application (microscope power of 200X by tacky bar touch process after 7 days' incubation). Scanning electron microscope (Model: SEM, JEOL JSM 6510 Iv, Japan) was performed at Nanotechnology Institute (Kafrelsheikh University, Egypt) to observe) the influence of the n-SiO_2 treatment at 200 mg L^{-1} on growth performance changes of *Rhizoctonia solani*.

2.10. Statistical analyses

The experiment was arranged in a completely randomized design (CRD) with four replications. Statistical tests were achieved by the CoStat program package (CoHort Software, CA, USA; Version 6.303) by use of ANOVA and then Duncan's multiple range tests at 5% level of possibility used to match significant variances among averages of the treatments (Snedecor and Cochran, 1989).

3. Results

3.1. Pathogenicity evaluation

To evaluate the pathogenic aptitude of *Rhizoctonia solani* isolates, three isolates of the pathogen were tested in a greenhouse experiment. The most aggressive isolate was identified as Rs2 (*Rhizoctonia solani*), while Rs3 exhibited the lowest one (**Table 2 and Figure 2**). Rs2 isolate was recorded the highest disease severity (78%) as well as the highest pre- and post-emergence damping-off rates (67.13 and 15.51 %, respectively). On the other hand, the lowest survival seedlings rate (17.63%) was belonged to the isolate Rs2. Therefore, the Rs2 isolate of *Rhizoctonia solani* was selected for subsequent experiments.

Table 2. Pathogenicity test of three isolates obtained from pepper seedlings under

Pathogen isolates	Pre-emergence (%)	Post-emergence (%)	Survival seedlings (%)	Disease severity (%)
Control (healthy)	2.50 d	0.001 c	97.50 a	0.001 d
Rhizoctonia solani (Rs1)	55.40 b	13.10 a	31.50 c	57.70 b
Rhizoctonia solani (Rs2)	67.13 a	15.51 a	17.63 d	78.00 a
Rhizoctonia solani (Rs3)	35.77 c	7.88 b	56.35 b	45.87 c
F. test	**	**	**	**

*** indicates highly significant treatment and values of means in each column followed by the same letter are not significantly at level of p < 0.05*

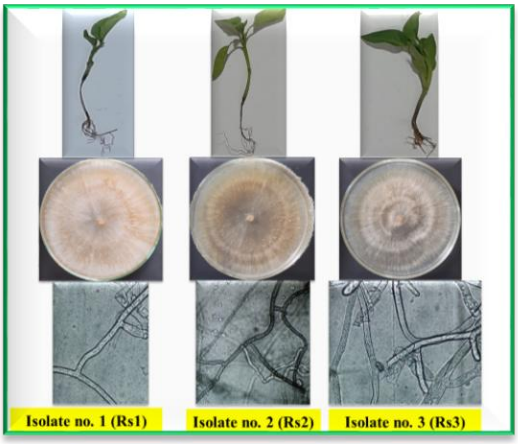

Figure 2. The three isolates (Rs1, Rs2, Rs3) of *Rhizoctonia solani* as investigated using Petri dishes and their morphological and microscopic parameters

3.2. In Vitro antifungal activity of selected nanoparticles

Individual and combined antifungal effects of n-Se and n-SiO_2 were examined *in vitro* experiment using isolate Rs2 of *R. solani*. The highest antagonistic result was found with fungicide application (i.e., synthetic control, which recorded reduction rate 94.44%), followed by n-SiO_2, and n-Se recording reduction rate of 81.11% and 66.67%, respectively. All used nanoparticles statistically reduced mycelia growth of *R. solani* compared with the control treatment (**Table 3; Figure 3**).

Table 3. Responses of mycelial growth of *Rhizoctonia solani* to different nanoparticles under *in vitro* conditions and the corresponding reduction of the pathogen

Treatments	Mycelial growth (cm)	Reduction (%)
(T2) Negative control	9.0 a	0.001 f
(T3) Fungicide (Rizolex 50% WP)	0.5 e	94.44 a
(T4) n-SiO_2 (200 mg L^{-1})	1.7 b	81.11 e
(T5) n-Se (100 mg L^{-1})	3.0 c	66.67 cd
(T6) 1/2 (n-SiO_2+ n-Se) (100+50 mg L^{-1}, respectively)	3.6 d	60.00 bc
F. test	**	**

*** indicates highly significant treatment and values of means in each column followed by the same letter are not significantly at level of $p < 0.05$ (±, SD =standard deviation; N= 5).*

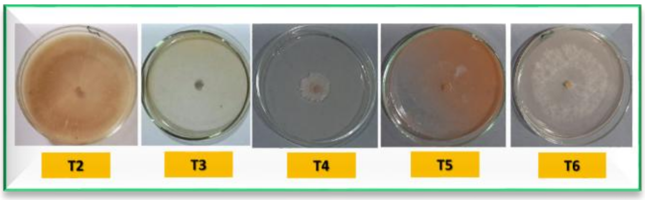

Figure 3. Images illustrating of the inhibition of *Rhizoctonia solani* mycelial growth due to applying of the treatments in the *in vitro* experiments. Where, T2: control (positive control), T3: fungicide, T4: n-SiO_2 (200 mg L^{-1}), T5: n-Se (100 mg L^{-1}), and T6: ½ (n-Se + n-SiO_2) (50 + 100 mg L^{-1}, respectively)

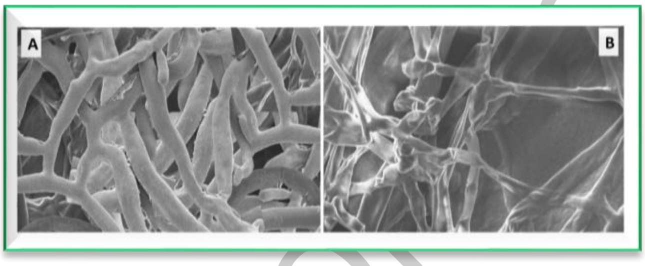

Figure 4. Scanning electron microscope (SEM) investigation of *Rhizoctonia solani* comprising with applied control (A) and with applied n-SiO_2 at dose of 200 ppm (B) on PDA media. Scale bars in both A and B are 20 µm with x 950

3.3. Microscopic Investigation of the R. solani

The Rhizoctonia mycelium was examined using scanning electronic microscope (SEM) under *in vitro* conditions. The obtained images were belonged the control and n-SiO_2 treatments, where a clear variation in the mycelium growth was observed for n-SiO_2 compared with the negative control treatment (**Figure 4**). Moreover, an occurrence of alterations, along with the variation in mycelia development was observed seven days after inoculation as observed by SEM. The application of n-SiO_2 (200 mg L^{-1}) induced notable morphological modifications in hyphae and mycelia growth. Mycelium and hyphae of the *R. solani* was severely damaged and shriveled in the presence of n-SiO_2 (**Figure 4B**) with the secondary mycelium appearing folded and damaged, as compared to negative control that was un-treated and showed typical mycelial structures with a smooth surface, while the growth of secondary mycelium was complete as typical fungus morphological features (**Figure 4A**).

Figure 5. Pepper seedlings after 40 days from infection with *R. solani* at greenhouse and applying the treatments, where T1: negative control (healthy seedlings), T2: control (positive control), T3: fungicide, T4: n-SiO_2 (200 mg L^{-1}), T5: n-Se (100 mg L^{-1}), and T6: ½ (n-Se + n-SiO_2) (50 + 100 mg L^{-1}, respectively)

3.4. Disease Severity of pepper seedlings under Greenhouse Conditions

Under the greenhouse experiments, survival seedlings, disease severity and its efficacy along with pre-, and post-emergence percentage of pepper transplanting's infected with *R. solani* were studied compared to the control and healthy ones (**Table 4** and **Figure 5**). Disease severity was significantly affected by all applied treatments during both seasons. The highest disease severity values were recorded in the positive control (T2) treatment during the two growing seasons (76.70 and 80.06 %, respectively). The seedlings treated by nano-silica (T4) exhibited the lowest disease severity (15.93 and 13.93 %) in both seasons, respectively, which was nearly comparable to the results obtained with fungicide application. The pre- and post-emergence as well as the survival rate of seedlings, were measured as shown in **Table 4** and showed the same tendency in both seasons. On the other hand, the combined application of nanoparticles (n-Se+n-SiO_2) recorded a similar result of survival seedlings comparable to fungicide treatment under infection by tested fungus pathogen with no significant differences between them in most cases. The feature of pepper seedlings after 40 days of infection with *R. solani* under greenhouse conditions for each treatment is shown in **Figure 5.** It is clear that seedlings treated with nano-silica (T4) showed growth performance closely similar to the control under infection conditions.

Table 4. Survival seedlings, disease severity, pre-, and post-emergence of pepper seedlings infected with *R. solani* compared to the healthy ones and control in both seasons

Treatments	Pre-emergence (%)	Post-emergence (%)	Survival seedlings (%)	Disease severity (%)	(%)
			First season (2023)		
T1	3.71 d	00.001 e	96.29 a	00.001 c	00.001 d
T2	75.82 a	10.46 a	13.72 d	76.70 a	00.001 d
T3	16.99 c	3.92 d	79.08 b	10.24 d	86.65 a
T4	28.10 b	4.58 cd	75.82 b	15.93 c	79.23 b
T5	19.60 c	5.88 c	66.03 c	23.83 b	68.93 c
T6	31.37 b	7.84 b	60.79 c	26.70 b	65.19 c
F-test	**	*	**	**	**
			Second season (2024)		
T1	5.23 e	0.001 e	94.78 a	0.001 d	0.001 c
T2	77.78 a	9.80 a	12.42 d	80.06 a	0.001 c
T3	15.03 d	2.61 b	82.35 b	11.81 c	85.25 a
T4	24.84 c	5.23 d	79.08 b	13.93 c	82.60 a
T5	15.69 d	6.54 c	68.62 c	26.93 b	66.36 b
T6	30.29 b	8.49 b	61.22 c	28.59 b	64.29 b
F-test	**	*	*	***	**

*Note: * and ** indicate significant and highly significant, respectively*

Values of means in each column followed by the same letter are not significantly at $p < 0.05$

Where, T1: negative control (healthy seedlings), T2: control (positive control), T3: fungicide, T4: n-SiO_2 (200 mg L^{-1}), T5: n-Se (100 mg L^{-1}), and T6: ½ (n-Se + n-SiO_2) (50 + 100 mg L^{-1}, respectively)

Table 5. Chlorophyll content and fluorescence along with vegetative growth traits of pepper seedlings after 40 days after sowing during both seasons

Treatments	Seedling height (cm)	Stem diameter (mm)	Fresh weight (g/seedling)	Dry weight (g/seedling)	Chlorophyll Fluorescence (F_V/F_M)	Chl. a	Chl. b
						(mg g^{-1} FW)	
			First season (2023)				
T1	21.36 a	3.18 a	1.14 a	0.118 a	0.723 a	4.78 b	1.5 a
T2	5.18 e	1.85 c	0.46 d	0.056 d	0. 633 b	3.28 c	1.03 c
T3	19.16 b	2.99 a	1.12 a	0.097 b	0.719 a	4.55 b	1.5 a
T4	19.97 b	3.04 a	1.15 a	0.118 a	0.726 a	5.85 a	1.5 a
T5	14.74 c	2.85 a	0.85 b	0. 079 c	0.725 a	4.50 b	1.3 b
T6	10.94 d	2.64 b	0.69 c	0.066 cd	0.731 a	4.58 b	1.5 a
F-test	**	**	**	**	*	*	*
			Second season (2024)				
T1	23.14 a	3.34 a	1.17 a	0.125 a	0.742 a	4.66 c	1.5 b
T2	7.10 e	1.79 b	0.45 d	0.049 d	0.642 b	2.77 e	1.1 c
T3	18.55 bc	3.21 a	1.15 a	0.119 a	0.734 a	4.87 b	1.6 ab
T4	21.15 a	3.25 a	1.24 a	0.122 a	0.735 a	5.64 a	1.5 a
T5	16.45 c	3.06 a	1.02 b	0.085 b	0.731 a	5.15 a	1.4 a
T6	13.61 d	3.01 a	0.86 c	0.074 c	0.728 a	4.82 d	1.4 c
F-test	**	*	*	**	*	*	**

** and ** indicate significant and highly significant, respectively*

Values of means in each column followed by the same letter are not significantly at $p < 0.05$

Where, T1: negative control (healthy seedlings), T2: control (positive control), T3: fungicide, T4: n-SiO_2 (200 mg L^{-1}), T5: n-Se (100 mg L^{-1}), and T6: ½ (n-Se + n-SiO_2) (50 + 100 mg L^{-1}, respectively)

3.5. Vegetative Growth and Photosynthetic Traits of pepper seedlings under biotic stress

After 40 days from seeds sowing, seedlings growth parameters, chlorophyll fluorescence and chlorophyll contents were measured (**Table 5**). In general, application of nano-silica recorded the highest value for almost all measured growth traits (seedling height, stem diameter, fresh and dry weights) compared with both fungicide treatment and untreated plants. a similar trend was observed during both growing seasons for the measuring photosynthetic parameters (chlorophyll fluorescence and content). The highest values of chlorophyll *a* and *b* were achieved by using single n-Se application or the combined n-Se and nano-silica treatment in both seasons. However, pepper seedlings infected only with *R. solani* exhibited the lowest values of all vegetative traits. Healthy (non-infected) pepper seedlings recorded the greatest height, diameter, fresh and dry weights of seedlings compared to all infected treatments with no-significant differences between T4 (nano-silica) and T3 (fungicide treatment) in both seasons. Therefore, the favorable seedlings quality traits as moderate height, thicker stem and greater dry mass and higher chlorophyll content was resulted from of individual application of nano-silica (T4) under the infection by *R. solani* compared with the other treatments.

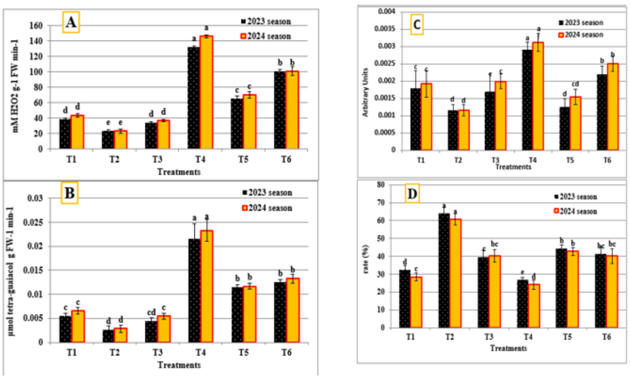

Figure 6. Total antioxidant activity including (A) peroxidase (POX), (B) catalase (CAT), (C) polyphenol oxidase (PPO), and (D) electrolyte leakage percentage measured 40 days after sowing during the both growing seasons. Results are given as mean values ± standard error bars

3.6. Antioxidant Activity and Electrolyte Leakage of pepper seedlings under biotic stress

The activities of three antioxidant inzymes including CAT, POX, and PPO were measured in pepper seedlings leaves 40 days after sowing, along with the percentage of electrolyte leakage under different treatments in both seasons (**Figure 6**). Generally, the highest enzyme activities were recorded with the application of n-SiO_2 (200 mg L^{-1}), followed by the combined n-Se (50 mg L^{-1}) and n-SiO_2 (100 mg L^{-1}), and both n-Se (100 mg L^{-1}) and chemical fungicide applications, whereas the lowest enzyme activities were recorded in the positive control (T2). In contrast, the infected seedlings (T2) had the highest electrolyte leakage values followed by treatments T5, T6 and T3, The n-SiO_2 treatment at 200 mg L^{-1} showed the lowest electrolyte leakage in both seasons. Best (lowest) values of electrolyte leakage and antioxidants were obtained with n-SiO_2 (200 mg L^{-1}) as confirmed by the earlier assessing enzymes, followed by the negative control and the combined n-Se (50 mg L^{-1}) and n-SiO_2 (100 mg L^{-1}). It is obvious that n-silica can enhance the producing of enzymes in 3-5 folds compared to the controls.

Where, T1: negative control (healthy seedlings), T2: control (positive control), T3: fungicide, T4: n-SiO_2 (200 mg L^{-1}), T5: n-Se (100 mg L^{-1}), and T6: ½ (n-Se + n-SiO_2) (50 + 100 mg L^{-1}, respectively)

4. Discussion

The quality of vegetable seedlings a critical factor in vegetable production, which mainly depends on the growing conditions. Effective management of seedling production requires several parameters should be kept in mind including the growing conditions, abiotic/biotic stress and cultivation facilities. Therefore, the present study was carried out to examine the potential of applied nanoparticles (nano selenium and nano silica) to boost pepper seedlings production and to control the root rot disease caused by *Rhizoctonia solani*. This work also explores the individual and combined effects of these nanoparticles in combating the pathogen during the production of pepper seedlings. To what extent can the applied NPs be effective for inhibiting studied pathogen, as compared to traditional fungicides? Concerning the most effective applied NPs (individually or in a combination), *in vitro* and under greenhouse conditions were achieved.

Seedling growth and quality need a specific handling in the nursery of vegetables. There is an urgent need concerning the role of environmental factors and their impact the growth of pepper seedlings. To produce high-quality seedlings, various factors must be considered, particularly environmental stresses such as light intensity, temperature, nutrients availability and water), and biotic stress (Islam *et al.* 2024). Several fungal diseases can cause root rots, damping-off, and wilting in pepper seedlings. Numerous reports discussed different fungal phytopathogens causing root rot in pepper seedlings including *Rhizoctonia* spp. (Mannai *et al.* 2018; El-Kazzaz *et al.* 2022), and Oomycetes such as *Pythium* spp. (Ao *et al.* 2024), and *Fusarium* spp. (Hafez *et al.* 2025). **Table 6** summarizes important studies worldwide on fungal, bacterial, and viral infections affecting pepper plants. Due to susceptibility of pepper to many diseases; the control strategy of such pathogens mainly depends on the pathogen species (e.g., virus, bacteria, fungi, and nematode). Among various control strategies, nanomaterials are considered a promising agent against biotic stress besides the biological controls such as beneficial microorganisms (mainly Tricoderma, Arbuscular mycorrhizal fungi species), and chitosan (**Table 6**).

Table 6. A survey on the published studied on pepper diseases infected by bacterial, fungal, and viral pathogens along with the possible control methods

Country	Pepper variety	Studied pathogen	The common disease	Control strategy	Refs.
Egypt	Pepper seedlings (*Capsicum annuum* L.)	*Rhizoctonia solani* and *Fusarium oxysporum*	Pepper root rot and wilt diseases	*Paenibacillus polymyxa* and *Trichoderma longibrachiatum*	El-Kazzaz *et al.* (2022)

Egypt	Sweet pepper (cv. Golden plants)	Fungus (*Fusarium oxysporum*) and nematode (*Meloidogyne incognita*)	Root-knot nematodes & root rot fungus	Bio-ZnO-NPs & *Trichoderma longibrachiatum*	Ghareeb *et al.* (2024)
Egypt	Pepper seedlings	Fungus (*Fusarium Oxysporum*)	Wilt disease of pepper plants	Green Ag-NPs by *Aspergillus Ustus* ON076464	Yahia *et al.* (2023)
India	Pepper chilli (var. Arka Lohith)	Fungus (*Colletotrichum truncatum*)	Chilli anthracnose disease	Chitosan-silver nanocomposite (CS-AgNPs)	Gowda *et al.* (2023)
India	Pepper chilli	Fungus (*Colletotrichum capsici*)	Chilli fruit rot disease	Green copper nanoparticles	Iliger *et al.* (2021)
Pakistan	Pepper chili (*Capsicum annuum* L.)	Fungus (*Colletotrichum capsici*)	Chilli fruit rot disease	Green silver nanoparticles	Nawaz *et al.* (2024)
India	Pepper chili (*Capsicum annuum* L.)	Fungi (*Phomopsis vexans* and *Colletotrichum capsici*)	Blight and fruit rot in chili	*Trichoderma*-mediated ZnO-NPs	Kumar *et al.* (2024)
Mexico	Varieties of jalapeño pepper	Begomovirus	Pepper huasteco yellow vein virus	Chemical ZnO-NPs	Rivero-Montejo *et al.* (2023)
Mexico	Pepper (*Capsicum annuum L.*)	Virus disease	Tobacco mosaic virus	Titanium dioxide nanoparticles (TiO_2-NPs)	Acuña-Fuentes *et al.* (2022)
Egypt	Sweet pepper (cv. Sirtaki)	Tobamovirus	Pepper mild mottle virus	Chemical Ag-NPs	Elbeshehy *et al.* (2023)
Pakistan	Bell pepper (*Capsicum annuum* L.)	Bacterial leaf spot	*Xanthomonas campestris* pv. vesicatoria	Green Cu-Zn hybrid-NPs	Ali *et al.* (2024)
Pakistan	Chilli pepper (*Capsicum annuum* L.)	Bacterial leaf spot	*Xanthomonas campestris* pv. vesicatoria	Green Ag-NPs and Zn-NPs	Atiq *et al.* (2023)
Turkey	Pepper variety INAN-3363 F1	*Fusarium solani* and *F.* mix (*F. solani*, *F. oxysporum*; *F. oxysporum* f.sp. *vasinfectum*)	Root and crown rot pathogens	Arbuscular mycorrhizal fungi species	Bilgili (2025)
China	Pepper seedlings	Fungus (*Fusarium solani*)	Pepper root rot	*Bacillus subtilis* PTS-394 wettable powder	Qiao *et al.* (2023)
China	Pepper seedlings	Fungus (*Fusarium solani*)	Pepper root rot	*Bacillus velezensis* Yao	Pei *et al.* (2023)

As far as we know, this study is first report about using nanoparticles of selenium and silica to control root rot disease of pepper seedlings caused by *Rhizoctonia solani*. This section explains our choice of nanoparticles and the proposed mechanism. A variety of nanomaterials have previously been used against phytopathogens of pepper seedlings such as ZnO-NPs, Ag-NPs, CuO-NPs, TiO_2-NPs, Cu-Zn hybrid-NPs, and Zn-NPs. On the other hand, many pepper diseases were reported to be controlled by applying such nanomaterials or nanoparticles depending on microbial species such as virus, bacteria, fungi, and nematode (**Table 6**). Among fungal phytopathogens, *Rhizoctonia solani* is a devastating phytopathogen causing pepper root rot disease (Ao *et al.*, 2024).

The selected NPs (nano selenium and nano silica) have well-documented applications under abiotic and biotic stress due to their unique chemical, physical, and biological properties (Díaz-Parra *et al.* 2025). Numerous studies confirmed the potential of selected Se-NPs and

SiO_2-NPs under biotic stress individually (e.g., Bhat *et al.* 2021; Goswami *et al.* 2022; Naidu *et al.* 2023; Mahawar *et al.* 2023; Yan *et al.* 2024; Qin *et al.* 2025), whereas a few in combined application (Taha *et al.* 2023). The reasons behind selecting nanoparticles of selenium and silica because their well-known role in controlling biotic stress on plants through their inducing plant defense system and systemic resistance, activating the antioxidants system, and enhancing the plant growth of stressful plants (Qin *et al.* 2025; Díaz-Parra *et al.* 2025). As far as we know, this is first report on using nano—silica and nano-Se to control the pathogen *R. solani* in pepper seedlings.

Our current findings confirmed thatnano-sillica (n-SiO_2) was the most effective treatment against *Rhizoctonia solani* (**Table 3**). *In vitro*, n-SiO_2 achieved an 81.1% reduction in mycelial growth compared to other NPs. Concerning the survival seedlings percentage under greenhouse conditions, there is no significant difference between chemical fungicide (82.3 and 79.4%) and n-SiO_2 (79.0 and 75.8%) in both seasons, respectively. These results suggested that n-SiO_2 at 200 mg L^{-1} is a promising alternative to chemical fungicide (Rizolex 50% WP) for controlling *Rhizoctonia solani* in pepper seedlings. Similar results were reported by Awad-Allah *et al.* (2021) who recognized the effect of n-SiO_2 at 150 mg L^{-1} against bacterial leaf spot (*Xanthomonas vesicatoria*) due to the stimulating of n-SiO_2 to release salicylic acid which activates the plant defense and immune system along with protecting plants against pathogen attacks. In this concern, it is reported that n-silica improved the biochemical, physiological response and growth in plants against stress conditions (Hasan *et al.* 2024).

Furthermore, n-silica (200 mg L^{-1}) treatment induced the highest values of vegetative growth parameters and the enzymatic antioxidants (CAT, POX, PPO) in pepper seedlings. It is found that applied n-silica (150 mg L^{-1}) induced pepper resistance to bacterial pathogen by stimulating activity of PPO (Awad-Allah *et al.* 2021). These results were also confirmed on infected plants with *R. solani* after application of n-silica (100 mg L^{-1}) on wheat plants (*Triticum aestivum* L.) by Abdelrhim *et al.* (2021), on carrot (*Daucus carota* L.) by Ahamad and Siddiqui (2021), on oat (*Avena sativa* L.) by Ahmad *et al.* (2023), and on rice (*Oryza sativa* L.) by Tan *et al.* (2025). Why applied n-silica record the best results in controlling *R. solani* in the current study? To answer the previous question, three levels of the explanations are being needed including the vegetative growth of pepper seedlings and the biochemical measurements of enzymatic antioxidants along with the photosynthetic parameters.

Concerning the vegetative growth parameters of pepper seedlings, all studied vegetative parameters Stem diameter, seedling height, fresh and dry weights of seedlings were produced from n-silica treatment (Table 5). The suggested mechanism of n-silica to strengthen the vegetative parameters against the pathogen may go back to the deposition of n-SiO_2 in pepper root tissues, which improved plant defense against the studied pathogen as confirmed by the SEM investigation (**Figure 4**). The suggested defense mechanisms may go back to (1) forming physical barriers by accumulation of Si in cell walls and below the cuticle, preventing the infection pegs of *R. solani* appressoria (Ahammed and Yang 2021), and (2) forming Si-enriched layer through uniform distribution of Si-aggregates and preventing the fungal ingress (Abdelrhim *et al.* 2021).

Along with the vegetative parameters of pepper seedlings, the highest values of chlorophyll fluorescence, Chl. a, and Chl. b were achieved with n-silica application. Under *R. solani* stress, it is expected reducing the photosynthesis process through inhibiting photosynthetic activities by causing damage to the structural along with stomatal closures of the seedlings (Taha *et al.* 2023). N-silica is known to protect chloroplasts from oxidative damage, increase chlorophyll content, and act as a cofactor in many enzymatic reactions (Zahedi *et al.* 2020; Naidu *et al.* 2023). This enhanced efficiency of photosynthesis likely contributes to strong seedlings (Ahamad and Siddiqui 2021).

Regarding the enzymatic antioxidants, pepper seedlings treated with n-silica showed the highest activities of antioxidant enzymes (CAT, POX, and PPO) compared to other treatments. This result is in agreement with the suggested defense mechanism, which confirm the role of Si in producing defense non-enzymatic compounds (i.e., anthocyanins, flavonoids and phenolics) and enzymatic activities leading to the development of physical barriers against phyto-pathogens (Ahanger *et al.* 2020). The opposite behavior can be noticed for the electrolyte leakage, which recorded the lowest values upon n-silica compared to control and other treatments.

5. Conclusions

This study presents noval insight into alternatives to chemical fungicides through the use of nanofungicides. As far as we know this is first report on applying n-silica and n-Se in controlling *R. solani* on pepper seedlings. Among the treatments n-silica at 200 mg L^{-1} recorded the highest values in all studied vegetative parameters, photosynthetic attributes, and antioxidants enzyme activities (CAT, POX, PPO), as compared to other treatments. It could be recommended that application of n-silica (200 mg L^{-1}) is a promising and effective alternative to the chemical fungicide (Rizolex 50% WP) against root rot disease caused by *R. solani*. Moreover, nano- silica) is better than n-Se and/or the combined of n-Se + n-silica. While current findings enhance our understanding of the antifungal potential of n-Se and/or n-silica nanofungicides, further research is wanted arrented to explore their interactions and efficacy across different crops before broad agriculture application.

CRediT authorship contribution statement

Conceptualization and visualization, Y.A.B. and N.A.T.; methodology, N.A.T. and Y.A.B.; software, M.S.A, A.A.K.; validation, M.S.A. and S. M. A.; formal analysis, T.A.S., H.S.E. and Y.A.B.; investigation, N.A.T.; resources, H.E.R.;

data curation, Y.A.B.; writing—original draft preparation, H.E.R. and T.A.S.; writing, review and editing, all authors; visualization, A.A.K, S.H. and H.E.R.; supervision, Y.A.B. and T.A.S.; project administration, H.E.R. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

Authors declare no conflict of interest.

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Available on request.

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