

Research Article

Masudulla Khan, Zaki A. Siddiqui*, Aiman Parveen, Azmat Ali Khan*, Il Soo Moon, and Mahboob Alam*

Elucidating the role of silicon dioxide and titanium dioxide nanoparticles in mitigating the disease of the eggplant caused by *Phomopsis vexans*, *Ralstonia solanacearum*, and root-knot nematode *Meloidogyne incognita*

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Abstract: Nanoparticles (NPs) have a critical function in mitigating the disease of fruits and vegetables. In the present investigation, the effects of three levels of concentrations (0.05, 0.10, and 0.20 mg/mL) of titanium dioxide NPs (TiO₂-NPs) and silicon dioxide NPs (SiO₂-NPs) were investigated against fungus *Phomopsis vexans*, bacterium *Ralstonia solanacearum*, and *Meloidogyne incognita* (root-knot nematode). The present investigation's findings found that the application of SiO₂-NPs was more efficient against test pathogens in comparison to TiO₂-NPs. The best result produced by SiO₂-NPs against pathogenic strain was used in the molecular docking investigation with the protein of *R. solanacearum* to better understand the interaction of active amino acids

with SiO₂-NPs. The obtained results revealed that the administration of 0.20 mg/mL foliar spray of SiO₂-NPs in plants with *M. incognita* improves up to 37.92% of shoot dry weight and increases 70.42% of chlorophyll content. *P. vexans* growth was suppressed by 41.2% with 0.62 mm of inhibition zone when SiO₂-NPs were given at a dosage of 0.20 mg/mL. The reductions in egg hatching and *M. incognita* (J₂) mortality were greater in SiO₂-NPs than in TiO₂-NPs. The results of scanning electron microscopy confirmed that the application of both NPs harmed test pathogens. The confocal study also showed the penetration of NPs among test pathogens.

Keywords: SiO₂ and TiO₂ nanoparticles, *Ralstonia solanacearum*, *Phomopsis vexans*, *Meloidogyne incognita*, docking simulation

1 Introduction

In the last decade, the human population is increasing day by day at the global level; it is expected that will be populated by 10 billion by 2050 and 800 million people will be facing a hunger situation by the year 2030 [1]. Thus, with the increasing population, it is needed to increase the production of the agriculture sector worldwide to achieve zero hunger. Around 4,100 plant-parasitic nematode species are responsible to cause diseases in agriculturally important plants and cause yield loss of around \$US157 billion per year worldwide [2,3]. Generally, to control these diseases in plants, chemical pesticides are used on a large scale in the agriculture sector. The use of chemical pesticides can cause risks to human health and the environment through bioaccumulation and eutrophication. To overcome this problem, there is

* **Corresponding author: Zaki A. Siddiqui**, Department of Botany, Aligarh Muslim University, Aligarh 202002, India, e-mail: zaki_63@yahoo.co.in

* **Corresponding author: Azmat Ali Khan**, Pharmaceutical Biotechnology Laboratory, Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, 11451, Saudi Arabia, e-mail: azkhan@ksu.edu.sa

* **Corresponding author: Mahboob Alam**, Department of Safety Engineering, Dongguk University, 123 Dongdae-ro, Gyeongju, Gyeongbuk, 780714, Republic of Korea, e-mail: mahboobchem@gmail.com

Masudulla Khan: Botany Section, Women's College, Aligarh Muslim University, Aligarh 202002, India

Aiman Parveen: Department of Botany, Aligarh Muslim University, Aligarh 202002, India

Il Soo Moon: Department of Anatomy, Dongguk University College of Medicine, Gyeongju, 38066, Republic of Korea

a need to find out alternatives to chemical pesticides for disease management in plants.

Recently, the demands for the use of nanotechnology in the agriculture sector have been rapidly increased to increase food production by avoiding the use of chemical pesticides for plant disease management.

Nanotechnology is an innovative scientific approach to developing materials at the nanoscale to take forward the agro-food sector with promising new tools and technology to increase food safety and security by protecting plants from pests and microbes. Nano, a Greek word that means dwarf, and materials with a range of 1–100 nm or 1.0×10^{-9} m are known as nanoparticles (NPs) and they have unique properties, which are absent in bulk material [4,5]. Nanotechnology could provide a diverse way in agriculture, such as the production of nano-fertilizers, nano-pesticides, nano-nutrients, nanocides, and nano-herbicides, and it starts a new era as agro-nanotechnology [6,7]. They have the potential to boost the plant metabolism, could stimulate seed germination, and induce defense against pests and pathogens [8,9]. NP, such as silicon (Si), is generally known for its beneficial effects on plants' growth and physiological activities. It has the potential to mitigate the biotic stress in plants and induce the defense in plants against pathogens, such as fungi, bacteria, viruses, and nematodes, by reducing pathogen colonization and by inducing resistance [10,11]. The antimicrobial efficiency of the Si can be due to its induction of defense gene expression, which is related to pathogen-related proteins, hypersensitivity responses, and antimicrobial compound synthesis [12,13]. The nano-SiO₂ is also used in the agriculture sector to develop the nano-fertilizers to improve the seed efficiency for germination with protection against several types of pathogens in plants [14]. Moreover, the titanium dioxide NPs (TiO₂-NPs) are also used in the agriculture sector as an alternative to chemical pesticides for plant disease management and promote plant growth due to increasing antimicrobial activity against plant pathogens and reducing ecological toxicity [15,16]. This might be possible due to the strong oxidation reaction of titanium dioxide (TiO₂) against organic compounds. However, the field application of NPs in plant disease management has not been properly investigated yet. The current investigation aimed to examine the effects of three levels of concentrations (0.05, 0.10, and 0.20 mg/mL) of TiO₂-NPs and silicon dioxide NPs (SiO₂-NPs) in the disease management of eggplants. The study also explores the antimicrobial

activity of NPs against microbial strains, i.e., fungus *Phomopsis vexans*, bacterium *Ralstonia solanacearum*, and *Meloidogyne incognita* (root-knot nematode) of eggplants. Furthermore, molecular docking of SiO₂-NPs against *R. solanacearum* protein was performed to determine amino acid interactions with NPs to better understand the mechanistic approach to control the propagation of pathogenic strain.

2 Materials and methods

2.1 Experimental setup

Eggplant seeds (CV. Navkiran) were surface sterilized (3.5 kg of soil in clay pots with 30-cm diameter) as described in our previous study [17]. In this experiment, sandy loam soil was used and gathered from the field of the Botany Department, Aligarh Muslim University, India. Inoculation of pathogens and sterilization of soil were also done as explained in our previous study [17]. After being inoculated, jars were kept at 30°C on a glass-house bench. Five replicates for each treatment were used. Every day, 200 mL of water was added to each pot.

2.2 NPs' preparation

The SiO₂ nanopowder (particle size 5–15 nm, spherical, porous, product number 637246-50G) and TiO₂-NPs (product number 700347-25G, a mixture of rutile and anatase, particle size <150 nm) were bought from Sigma Aldrich (USA). Spraying of SiO₂-NPs/TiO₂-NPs with three levels of strengths (0.05, 0.10, and 0.20 mg/mL) each was done on 10-day-old seedlings. Suspension of 0.05, 0.10, and 0.20 mg of these NPs is prepared by dissolving NPs in 1 mL of distilled water and sonicated for 25–30 min for equal distribution of NPs in water.

2.3 Confocal microscopy and scanning electron microscopic study

Nanoparticles effect on *R. solanacearum* cells, *M. incognita* and *P. vexans* hyphae morphological was investigated

using Scanning Electron Microscopic (SEM). The SEM was performed after the second-stage juveniles of *M. incognita* were treated with SiO₂- and TiO₂-NP suspensions. After being exposed to NPs, J₂s were fixed with glutaraldehyde at 40°C for 12 h. After fixing, J₂s were dehydrated using an ethanol series treatment for 15 min for each concentration (10, 20, 40, 60, 80, 90, and 100%). Thereafter, J₂s were mounted on gold-coated stubs and, finally, J₂s were observed under SEM. The *R. solanacearum* cells were centrifuged at 5,000 rpm for 15 min at 4°C and the pellets were collected and rinsed with potassium phosphate-buffered saline (PBS) at pH 7. After that, the sample was fixed for 5–6 h with 2.5% glutaraldehyde. An ethanol series (10, 20, 40, 60, 80, 90, and 100%) was used to dehydrate the samples for 15 min each. Following that, the samples were subjected to SEM analysis (JEOL, Tokyo, Japan). The mycelium of *P. vexans* was fixed for 3–4 h with 2.5% glutaraldehyde and then washed with potassium phosphate buffer. The prepared samples were mounted on SEM stubs and observations were recorded. Confocal microscopic images of penetration of SiO₂-NPs in the mycelia and conidia of *P. vexans*, SiO₂-NPs attached with cells of *R. solanacearum*, and penetration of SiO₂-NPs in *M. incognita* J₂ were also taken.

2.4 In vitro study

In vitro studies of the effects of SiO₂-NPs and TiO₂-NPs on *P. vexans* fungus were investigated. Separately, 0.20 mg/mL of SiO₂- and TiO₂-NP suspensions were prepared, and 10 mL was added to 500 mL of properly sterilized potato dextrose agar (PDA) medium. Medium-containing Petri dishes inoculated with fungus *P. vexans* were kept for 2 weeks to screen the effect of both NPs on the fungal growth. To screen the effect of SiO₂-NPs and TiO₂-NPs on bacterium *R. solanacearum*, we used a paper disk of 7 mm diameter dipped it in 0.20 mg/mL of NP suspension, dried it for 30 min, and placed it on nutrient agar Petri plate inoculated with *R. solanacearum*. The antibacterial activity of both NPs was determined by the presence or absence of an inhibitory zone surrounding the Petri dish after 24 h of incubation. To examine the effect of SiO₂ NPs/TiO₂-NPs on the nematode *M. incognita*, 50 mL of distilled water was mixed with 0.20 mg/mL suspension (5 mL) and 50 mL of distilled water was placed in each petri-dish. For hatching, ten washed egg masses were placed in each Petri plate. The number of hatched juveniles from eggs was observed under the microscope.

2.5 Statistical analysis

Using the software R (3.6.1) of statistics, a two-way analysis of variance (package library, agricolae) was used to statistically evaluate the data. Duncan's test was performed to evaluate whether the mean values were significantly different ($p = 0.05$).

2.6 Molecular docking

A silica NP model made up of (SiO₂)₇₂ cluster was used in the docking investigation. The NP framework was built directly using Cartesian atom coordinates derived from the literature [18]. PatchDock and FireDock software were used to conduct a molecular docking investigation [19,20]. Visualization of the best-docked pose was performed using the BIOVIA Discovery Studio Visualizer.

3 Results

3.1 Biological effects of SiO₂-NPs and TiO₂-NPs on bacteria, fungi, and nematodes

The inhibitory effect of SiO₂-NPs was found higher on *R. solanacearum* than that of TiO₂-NPs (Table 1;

Table 1: Impacts of SiO₂-NPs and TiO₂-NPs on fungus and bacteria *in vitro*, as well as effects of both NPs on *M. incognita* hatching and mortality

Treatments		Inhibition zone (mm)
SiO ₂ 0.20 mg/mL	<i>R. solanacearum</i>	0.62a
TiO ₂ 0.20 mg/mL	<i>R. solanacearum</i>	0.51b
Treatment		Antifungal activity (%)
SiO ₂ 0.20 mg/mL	<i>P. vexans</i>	41.2a
TiO ₂ 0.20 mg/mL	<i>P. vexans</i>	32.9b
Treatments	After 48 h, J ₂ of <i>M. incognita</i> hatching	After 48 h, mortality of J ₂
Distilled H ₂ O	476a	3c
SiO ₂ 0.20 mg/mL	196c	27a
TiO ₂ 0.20 mg/mL	313b	15b

At $p \leq 0.05$, data analysis of significance was done by Duncan's multiple range test. Within a column, the same letters are not significantly different.

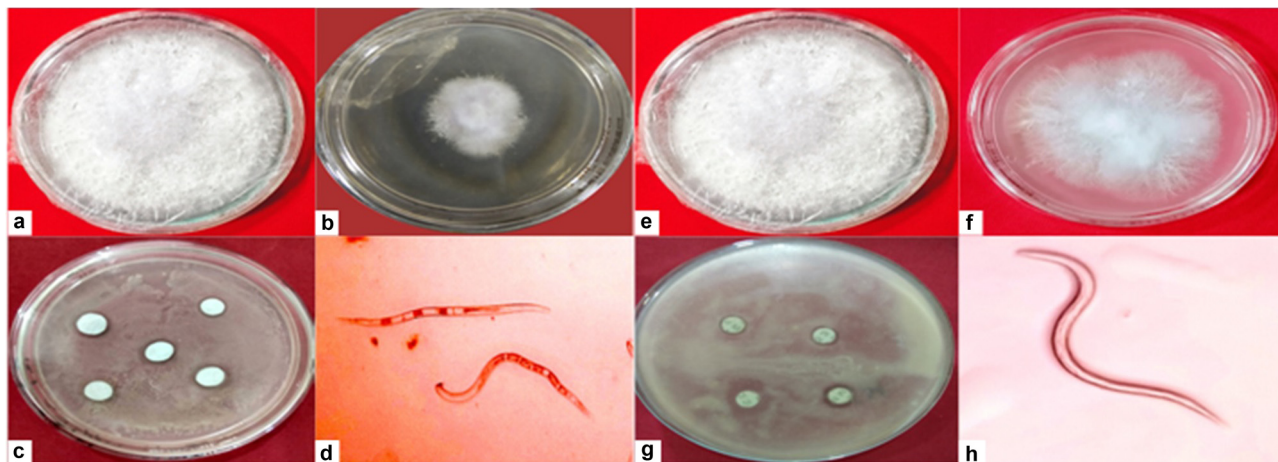


Figure 1: (a and e) *P. vexans* growth on PDA medium. (b) *P. vexans* growth on PDA medium with 0.20 mg/mL suspension of SiO₂-NPs. (f) *P. vexans* growth on PDA medium with 0.20 mg/mL solution of TiO₂-NPs. (c) Inhibition zone present around a paper disk dipped in 0.20 mg/mL suspension of SiO₂-NPs inoculated with *R. solanacearum*. (g) Inhibition zone formed around a paper disk dipped in 0.20 mg/mL suspension of TiO₂-NPs. (d) Treated J₂ of *M. incognita* with 0.20 mg/mL suspension of SiO₂-NPs for 24 h. (h) Treated J₂ of *M. incognita* with 0.20 mg/mL TiO₂-NPs suspension for 24 h.

Figure 1c and g). An inhibition zone of 0.20 mg/mL of SiO₂-NP and TiO₂-NP was recorded at 0.62 and 0.51 mm, respectively, after 48 h of incubation (Table 1).

Both SiO₂-NPs and TiO₂-NPs showed antifungal activity against *P. vexans* (Table 1; Figure 1b and f). SiO₂-NPs are more effective at inhibiting *P. vexans* than TiO₂-NPs. A concentration of 0.20 mg/mL of SiO₂-NPs resulted in a 41.2% reduction in the *P. vexans* growth, while 0.20 mg/mL of TiO₂-NPs resulted in a 32.9% reduction in the fungal growth (Table 1).

A concentration of 0.20 mg/mL of both SiO₂-NPs and TiO₂-NPs was used to determine the influence on

M. incognita mortality and hatching after 48 h of experiment setting (Table 1). SiO₂-NPs reduce hatching higher than by TiO₂-NPs. SiO₂-NPs caused 58.82% inhibition in hatching over control while TiO₂-NPs caused 34.24% inhibition in hatching. The mortality of the second-stage juveniles of *M. incognita* in SiO₂-NPs was reported to be 81.0% while the mortality was 45.0% in TiO₂-NPs after 48 h. It was observed that both NPs harm J₂ of *M. incognita* (Figure 1d and h).

Scanning electron micrograph of *P. vexans* treated with SiO₂-NPs and TiO₂-NPs shows disturbed and fragmented mycelium and conidia (Figure 2). Deformed cells

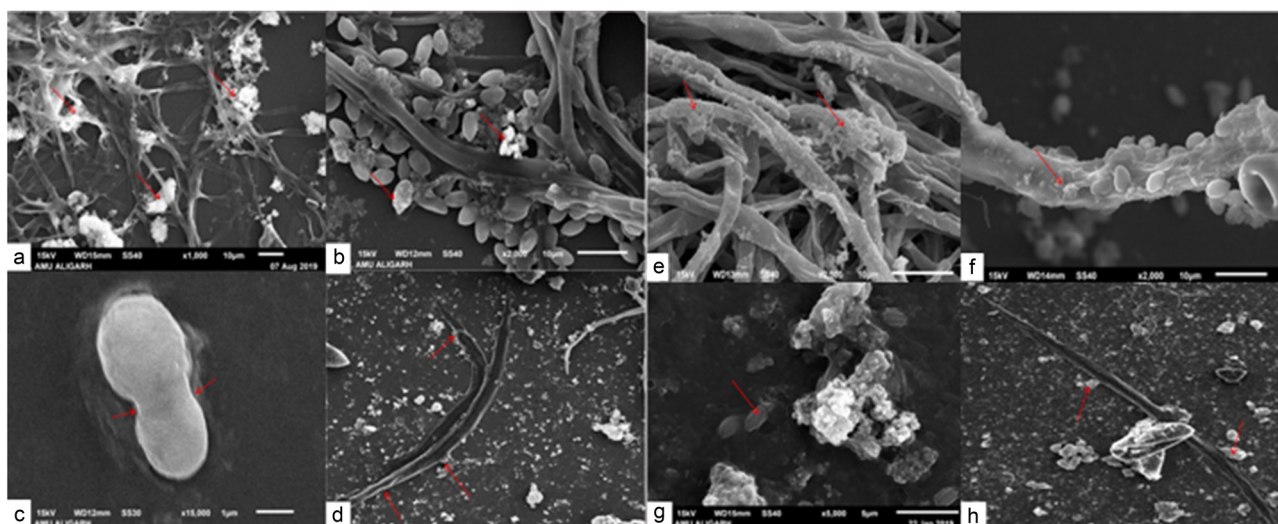


Figure 2: SEM images of test pathogens treated with SiO₂-NPs and TiO₂-NPs. (a and b) Mycelium and conidia of *P. vexans* sprayed with 0.20 mg/mL SiO₂-NPs. (c) *R. solanacearum* handled with 0.20 mg/mL SiO₂-NPs. (d) Nematode *M. incognita* handled with 0.20 mg/mL SiO₂-NPs. (e and f) Mycelium and conidia of *P. vexans* handled with 0.20 mg/mL TiO₂-NPs. (g) *R. solanacearum* treated with 0.20 mg/mL TiO₂-NPs. (h) *M. incognita* J₂ treated with 0.20 mg/mL TiO₂-NPs.

of *R. solanacearum* were also observed when treated with SiO₂-NPs and TiO₂-NPs. A disturbed cuticle layer of *M. incognita*'s second-stage juvenile was observed when added to the solution of SiO₂-NPs and TiO₂-NPs for hatching. Confocal microscopic images show the penetration of SiO₂-NPs in mycelium and conidia of *P. vexans* (Figure 3). SiO₂-NPs were also found attached to cells of *R. solanacearum*. Penetration of SiO₂-NPs was also observed in the *M. incognita* (J₂ juvenile) (Figure 3).

3.2 Impact of SiO₂-NPs and TiO₂-NPs on plants with single test pathogen

Plant fresh weight, plant length, shoot dry weight, root dry weight, leaf chlorophyll, and carotenoid were significantly affected by NPs and pathogens ($p = 0.05$).

3.2.1 Effect on the dry weight of shoot

Inoculation of test pathogens (*i.e.*, *P. vexans*, *R. solanacearum*, and *M. incognita*) resulted in a considerable reduction in plant growth attributes. Inoculation of *R. solanacearum* caused the highest reduction in the plant growth followed by *P. vexans* and *M. incognita* (Table 2). A significant increase in plant growth attributes occurs after the foliar spray of TiO₂/SiO₂-NPs at all concentrations, *i.e.*, 0.05, 0.10, and 0.20 mg/mL in comparison to the control one (Table 3). The highest increase in the plant growth was reported at 0.20 mg/mL SiO₂-NPs followed by 0.10 mg/mL SiO₂-NPs, 0.20 mg/mL TiO₂, 0.05 mg/mL SiO₂-NPs, and 0.10 mg/mL TiO₂. Spray with 0.05 mg/mL TiO₂-NPs was

the least efficient for the management of pathogens (Table 2).

Spraying TiO₂/SiO₂-NPs at all concentrations on plants without pathogens resulted in a considerable increase in shoot dry weight compared to the uninoculated control (Table 3). The plants inoculated with *P. vexans* or *M. incognita* had similar shoot dry weight after spraying 0.20 mg/mL SiO₂-NPs. The spraying of SiO₂-NPs at 0.20 mg/mL to plants with *M. incognita* or *P. vexans*/*R. solanacearum* improves shoot dry weight more than caused by SiO₂ 0.10 mg/mL. The spraying of 0.10 mg/mL SiO₂-NPs to plants with *M. incognita*/*R. solanacearum* improves shoot dry weight more than plants sprayed with 0.20 mg/mL TiO₂ (Table 3).

Spraying 0.20 mg/mL TiO₂-NPs to plants with *M. incognita* increased up to 22.89% shoot dry weight while a spray of plants with *P. vexans* and *R. solanacearum* caused 28.36 and 23.48% increases over their respective controls. Similarly, a foliar spray of 0.20 mg/mL SiO₂-NPs to plants with *M. incognita* caused a 37.92% increase in shoot dry weight, while a spray of plants with *P. vexans* and *R. solanacearum* caused 43.16 and 42.57% increases over their respective controls (Table 3).

3.2.2 Effect on chlorophyll and carotenoid contents

A significant reduction in the chlorophyll and carotenoid content was found with *R. solanacearum* followed by *P. vexans* and *M. incognita* (Table 2). A significant increase in chlorophyll and carotenoid contents occurs after foliar spray at all concentrations of TiO₂/SiO₂-NPs in comparison to control (Table 2). The highest increase in total chlorophyll contents was reported at 0.20 mg/mL SiO₂-NPs. Spraying

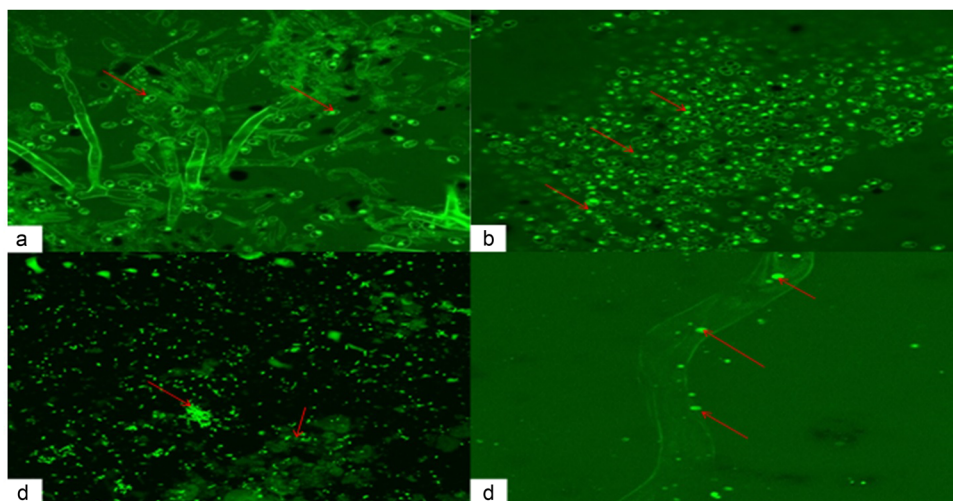


Figure 3: Confocal microscopic images of test pathogens: (a and b) penetration of SiO₂-NPs in the mycelia and conidia of *P. vexans*; (c) SiO₂-NPs attached with cells of *R. solanacearum*; and (d) penetration of SiO₂-NPs in *M. incognita* J₂.

Table 2: Effect of SiO₂-NPs, TiO₂-NPs, *P. vexans*, *M. incognita*, including *R. solanacearum* on eggplant plant growth and photosynthetic pigments

Treatment	Plant-length (cm)	Plant-fresh wt (g)	Shoot-dry wt (g)	Root-dry wt (g)	Chlorophyll in fresh leaves (mg/g)	Carotenoid in fresh leaves (mg/g)	Wilt/blight index
Control	71.74a	55.67a	11.05a	2.12a	1.764a	0.0595a	—
<i>M. incognita</i>	62.30b	47.92b	10.35b	1.78b	1.500b	0.0515b	—
<i>P. vexans</i>	59.45c	45.09c	9.48c	1.69c	1.435c	0.0490c	2
<i>R. solanacearum</i>	57.52d	42.35d	8.48d	1.51d	1.355d	0.0443d	2
Least mean square	62.75	47.75	9.84	1.77	1.513	0.0510	2
LSDP = 0.05	0.918	0.953	0.200	0.042	0.016	0.001	
Control	56.98g	42.75f	8.35g	1.13g	0.949g	0.0432g	3
TiO ₂ -0.05 mg/mL	59.72f	44.50e	8.95f	1.38f	1.207f	0.0465f	2
TiO ₂ -0.10 mg/mL	61.37e	46.16d	9.50e	1.63e	1.436e	0.0501d	2
TiO ₂ -0.20 mg/mL	63.90c	48.64c	10.27c	1.97c	1.584c	0.0528c	2
SiO ₂ -0.05 mg/mL	62.67d	47.74c	9.77d	1.82d	1.532d	0.0498e	2
SiO ₂ -0.10 mg/mL	65.69b	50.59b	10.60b	2.12b	1.824b	0.0557b	2
SiO ₂ -0.20 mg/mL	68.86a	53.92a	11.44a	2.39a	2.063a	0.0592a	1
Least mean square	62.74	47.75	9.84	1.77	1.513	0.0510	2
LSDP = 0.05	1.214	1.261	0.265	0.056	0.021	0.001	—

Data analysis of significance was done with Duncan's multiple range test at $p \leq 0.05$. The same letters within a column are not significantly different.

with 0.05 mg/mL TiO₂-NPs was found least effective in increasing chlorophyll and carotenoid contents (Table 2).

The spray of SiO₂-NPs and TiO₂-NPs at all three concentrations to uninoculated plants caused significant ($p < 0.05$) increases in chlorophyll and carotenoid contents over control (Table 3). Inoculation of either of the test pathogens caused a significant reduction in chlorophyll and carotenoid contents. The spray of SiO₂-NPs at 0.20 mg/mL caused the highest increase in chlorophyll and carotenoid contents and the spray of 0.05 TiO₂-NPs was found least effective. The chlorophyll content in plants with *M. incognita* increased 27.98 and 51.80% after the spray of 0.05 and 0.20 mg/mL TiO₂-NPs, respectively, while plants with *P. vexans* and *R. solanacearum* caused a 72.92 and 98.32% increase over their respective controls after spraying 0.20 mg/mL TiO₂-NPs. The spray of 0.05 TiO₂-NPs to plants with *P. vexans* and *R. solanacearum* caused a 30.69 and 34.26% increase over their respective controls (Table 3). Similarly, the use of 0.20 and 0.05 mg/mL SiO₂-NPs to plants with *M. incognita* caused a 70.42 and 43.38% increase in the chlorophyll content, respectively, while a spray of plants with *P. vexans* and *R. solanacearum* caused 79.78 and 96.65% increase over their respective controls after spraying with 0.05 TiO₂-NPs. Foliar spray of 0.20 mg/mL SiO₂-NPs to plants with *P. vexans* and *R. solanacearum* caused a 146.33 and 180.36% increase over their respective controls (Table 3).

Foliar spray of 0.20 mg/mL TiO₂-NPs to plants with *M. incognita* caused a 21.99% increase in the carotenoid

content while a spray of plants with *P. vexans* and *R. solanacearum* caused a 19.63 and 32.09% increase over their respective controls. Similarly, the use of 0.05 mg/mL SiO₂-NPs to plants with *M. incognita* caused a 15.19% increase in the carotenoid content while a spray of plants with *P. vexans* and *R. solanacearum* caused a 9.23 and 30.37% increase over their respective controls. Spraying 0.20 mg/mL SiO₂-NPs to plants with *M. incognita* caused a 33.11% increase in the carotenoid content while a spray of plants with *P. vexans* and *R. solanacearum* increased the carotenoid content by 28.18 and 49.28% over their respective controls (Table 3).

3.2.3 Effect on nematode population and galling

A significant population reduction and galling of *M. incognita* occur after foliar spray of TiO₂/SiO₂-NPs in all three concentrations (Figures 4 and 5). The significant ($p < 0.05$) highest reduction in nematode population and galling occur after the spray of plants with *M. incognita* at 0.20 mg/mL SiO₂-NP treatment. *R. solanacearum* and *P. vexans* also harmed the galling and population of *M. incognita* (Figures 4 and 5).

3.2.4 Disease indices

Blight and wilt indices caused by *P. vexans* and *R. solanacearum*, respectively, were 3 (Table 2). Disease indices

Table 3: Effect of SiO₂-NPs and TiO₂-NPs on the plant growth and photosynthetic pigments of eggplants inoculated with test pathogens, i.e., *M. incognita*, *P. vexans*, and *R. solanacearum* and uninoculated

Treatment	Pathogens	Plant length (cm)	Plant fresh wt (g)	Shoot dry wt (g)	Root dry wt (g)	Chlorophyll in fresh leaves (mg/g)	Carotenoid in fresh leaves (mg/g)	Wilt/blight index
Control	C ^A	67.72de	52.17de	9.71jk	1.32o	1.192l	0.0508ijk	—
	M	56.93lmn	42.88lmn	8.65mnop	1.20p	1.058m	0.0441qr	—
	P	52.41op	39.40op	7.97q	1.13p	0.831o	0.0433r	3
	R	50.89q	36.58q	7.07r	0.87q	0.718p	0.0349t	3
TiO ₂ -0.05 mg/mL	C ^B	69.76cd	53.22cde	10.13ij	1.61klm	1.427j	0.0527gh	—
	M	59.55jk	44.81jkl	9.33kl	1.42o	1.354k	0.0479mn	—
	P	55.63mno	41.49mno	8.56nop	1.38o	1.086m	0.0461op	2
	R	53.97op	38.50pq	7.81q	1.11p	0.964n	0.0395s	2
TiO ₂ -0.10 mg/mL	C ^B	71.55bc	54.94cd	11.04defg	1.92ghi	1.698g	0.0576d	—
	M	61.11hij	46.43ghijk	9.89j	1.63klm	1.519i	0.0504jkl	—
	P	57.62klmn	43.15lm	8.98lmn	1.54mn	1.318k	0.0492klm	2
	R	55.21no	40.15nop	8.09pq	1.44no	1.211l	0.0432r	2
TiO ₂ -0.20 mg/mL	C ^B	72.12bc	55.89bc	11.49bcd	2.28cd	1.869d	0.0598c	—
	M	64.51g	48.52fgh	10.63ghi	1.96gh	1.606h	0.0538fg	—
	P	60.75ij	46.29ghijk	10.23hij	1.92ghi	1.437j	0.0518hij	2
	R	58.22kl	43.87klm	8.73mno	1.73jk	1.424j	0.0461op	2
SiO ₂ -0.05 mg/mL	C ^C	70.94bc	54.77cd	10.89efg	2.21de	1.708fg	0.0559e	—
	M	62.29ghi	47.34ghij	10.75fgh	1.81ij	1.517i	0.0508ijk	—
	P	59.63jk	45.98hijk	9.20klm	1.69kl	1.494i	0.0473no	2
	R	57.85klm	42.87lmn	8.27opq	1.57lm	1.412j	0.0455pq	2
SiO ₂ -0.10 mg/mL	C ^C	73.25b	57.76b	11.76bc	2.59b	2.067b	0.0691b	—
	M	64.41g	50.97ef	11.32cdef	2.11ef	1.647h	0.0549ef	—
	P	63.22gh	48.10ghi	10.01j	1.91ghi	1.838de	0.0502jkl	2
	R	61.91hij	45.55ijkl	9.33kl	1.88hi	1.744f	0.0488lmn	2
SiO ₂ -0.20 mg/mL	C ^C	76.87a	60.94a	12.34a	2.91a	2.391a	0.0707a	—
	M	67.31e	54.54cd	11.93ab	2.35c	1.803e	0.0587cd	—
	P	66.95ef	51.23ef	11.41bcde	2.28cd	2.047bc	0.0555e	1
	R	64.62fg	48.97fg	10.08ij	2.02fg	2.013c	0.0521hi	1
L.S.D. NPs × pathogens		2.428	2.521	0.530	0.112	0.041	0.002	

Data analysis was done by using Duncan's multiple range test at $p \leq 0.05$. The same letters within a column are not significantly different. M = *M. incognita*; P = *P. vexans*; R = *R. solanacearum*; AC = control without pathogen without SiO₂-NPs and TiO₂-NPs; BC = control without pathogen with TiO₂-NPs; C^C = control without pathogen with SiO₂-NPs.

were reduced to 2 when TiO₂-NPs at all concentrations and SiO₂-NPs in two concentrations (0.05 mg or 0.10 mg/mL) were sprayed on plants with either of the test pathogens. Spraying with 0.20 mg/mL SiO₂-NPs reduces disease indices to 1 when inoculated with either of the test pathogens (Table 3).

3.3 Effects of SiO₂-NPs and TiO₂-NPs on plants inoculated with two or three test pathogens

3.3.1 Effect on shoot dry weight

Inoculation of all three test pathogens together caused a significant ($p < 0.05$) reduction in shoot dry weight over

the un-inoculated control (Table 4). Inoculation of all the three pathogens together caused the highest reduction in shoot dry weight followed by the inoculation of *M. incognita* plus *R. solanacearum*, *M. incognita* with *P. vexans* while *R. solanacearum* plus *P. vexans* caused the least reduction in shoot dry weight. Inoculation of SiO₂-NPs or TiO₂-NPs in all three concentrations caused a significant increase in shoot dry weight over the un-inoculated control. Application of 0.20 mg/mL SiO₂-NPs has the potential to increase shoot dry weight followed by 0.10 mg/mL SiO₂-NPs, 0.20 mg/mL TiO₂-NPs, 0.05 mg/mL SiO₂-NPs, and 0.10 mg/mL TiO₂-NPs. Spraying with 0.05 mg/mL TiO₂-NPs was found to be least effective. Inoculation of *P. vexans* plus *M. incognita*/*M. incognita* plus *R. solanacearum*/*P. vexans* plus *R. solanacearum* or all the three

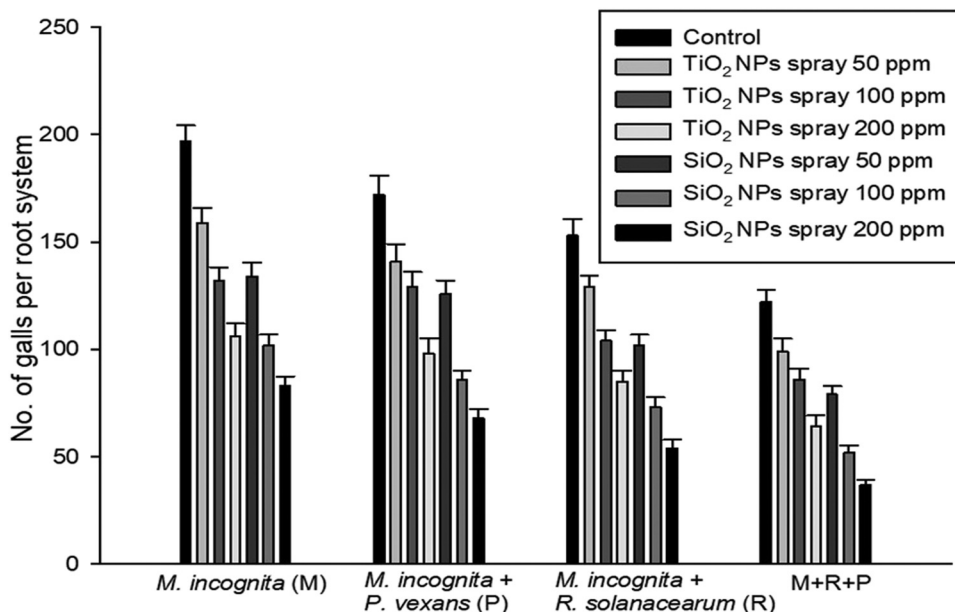


Figure 4: Effect of SiO₂-NPs and TiO₂-NPs on root galling of *M. incognita* (M) inoculated alone (M) and in combination with *P. vexans* (P) and *R. solanacearum* (R). Error charts reflect the standard deviation.

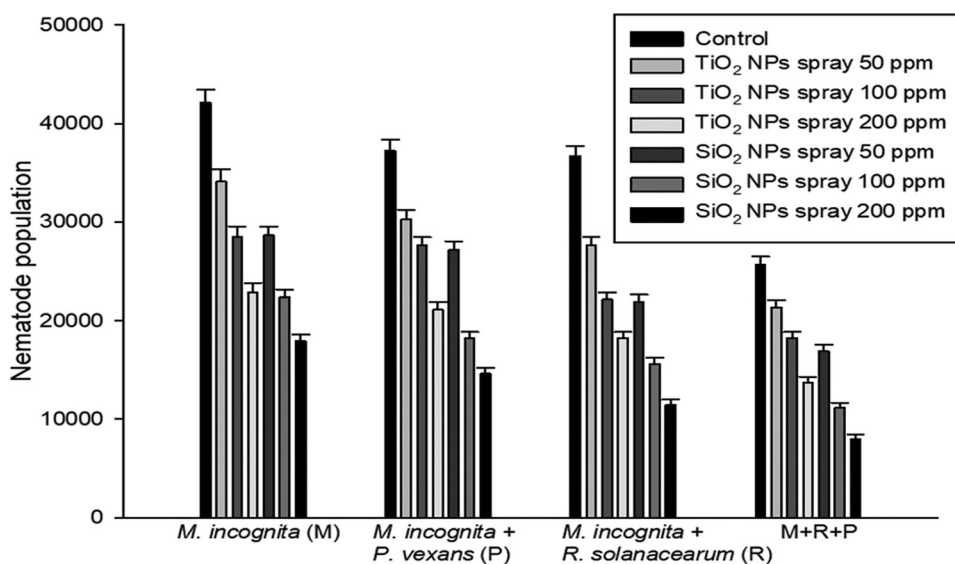


Figure 5: Influence of SiO₂-NPs and TiO₂-NPs on the multiplication of *M. incognita* (M) inoculated alone (M) and in combination with *P. vexans* (P) and *R. solanacearum* (R). Error bars reflect the standard deviation.

pathogens together caused a significant reduction in shoot dry weight (Table 5). The application of different concentrations of SiO₂-NPs and TiO₂-NPs resulted in a significant ($p < 0.05$) increase in shoot dry weight of pathogen-inoculated plants. Foliar spray of 0.20 mg/mL TiO₂-NPs to plants with *P. vexans* plus *M. incognita* caused a 51.49% increase in shoot dry weight while *M. incognita* plus *R. solanacearum*, *P. vexans* plus *R. solanacearum*, and all the three pathogens together caused 63.62, 58.03, and 66.98%

increases over their respective controls. Similarly, the use of 0.05 mg/mL SiO₂-NPs to plants with *P. vexans* plus *M. incognita* caused a 49.34% increase in shoot dry weight while *M. incognita* plus *R. solanacearum*, *P. vexans* plus *R. solanacearum*, and all the three pathogens together caused 55.76, 54.29, and 67.46% increases over their respective controls. Foliar spray of 0.20 mg/mL SiO₂-NPs to plants with *P. vexans* plus *M. incognita* caused a 78.64% increase in shoot dry weight while *M. incognita*

Table 4: Effect of test pathogens inoculated in combination on the plant growth, chlorophyll, and carotenoid contents of eggplant

Treatment	Plant-length (cm)	Plant-fresh weight (g)	Shoot-dry weight (g)	Root-dry weight (g)	Chlorophyll in fresh leaves (mg/g)	Carotenoid in fresh leaves (mg/g)	Wilt/blight index
Control	71.74a	55.67a	11.05a	2.12a	1.764a	0.0595a	—
M + P	51.60c	37.88c	8.75c	1.62c	1.410c	0.0418b	3.14
M + R	47.38d	37.67c	8.32d	1.46d	1.328d	0.0407c	3.14
R + P	53.41b	40.69b	9.52b	1.71b	1.532b	0.0436c	3.14
M + R + P	40.56e	28.73d	6.96e	1.36e	1.090e	0.0386d	3.85
Least mean square	52.93	40.12	8.92	1.65	1.424	0.0440	3.31
LSDP = 0.05	0.892	0.604	0.263	0.035	0.022	0.001	
Control	47.98f	35.74g	6.36g	0.94g	0.933g	0.0322g	5
TiO ₂ -0.05 mg/mL	49.97e	37.15f	8.13f	1.25f	1.118f	0.0349f	4.25
TiO ₂ -0.10 mg/mL	51.74d	38.90e	8.78e	1.54e	1.352e	0.0443e	3.25
TiO ₂ -0.20 mg/mL	53.55c	40.43d	9.35c	1.82c	1.481c	0.0479c	3.25
SiO ₂ -0.05 mg/mL	52.42d	39.68c	9.07d	1.69d	1.399d	0.0456d	3.25
SiO ₂ -0.10 mg/mL	55.76b	42.82b	9.94b	2.02b	1.692b	0.0506b	2.25
SiO ₂ -0.20 mg/mL	59.14a	46.17a	10.80a	2.32a	1.999a	0.0527a	2
Least mean square	52.93	40.12	8.92	1.65	1.424	0.0440	3.32
LSDP = 0.05	1.05	0.715	0.311	0.042	0.026	0.001	

Data analysis was done with Duncan's multiple range test at $p \leq 0.05$. Within a column, the same letters are not significantly different.

Table 5: Effect of SiO₂-NPs and TiO₂-NPs on simultaneous inoculation of *P. vexans*, *M. incognita*, and *R. solanacearum*, as well as plant development, chlorophyll, and carotenoid content of eggplant

Treatment	Pathogens	Plant-length (cm)	Plant-fresh wt (g)	Shoot-dry wt (g)	Root-dry wt (g)	Chlorophyll in fresh leaves (mg/g)	Carotenoid in fresh leaves (mg/g)	Wilt/ Blight index
Control	C ^A	67.72d	52.17e	9.71ghi	1.32no	1.192k	0.0508de	—
	M + P	46.84klm	34.11qr	6.04rs	0.82q	0.893n	0.0316pq	5
	M + R	42.66no	33.23r	5.47s	0.76q	0.802o	0.0302qr	5
	P + R	49.13jk	36.51nop	6.41qr	1.09p	1.013m	0.0331p	5
	M + R + P	33.58q	22.72u	4.21t	0.73q	0.769o	0.0290r	5
TiO ₂ - 0.05 mg/mL	C ^B	69.76cd	53.22de	10.13efg	1.61jk	1.427i	0.0527d	—
	M + P	48.91jk	35.92op	7.83no	1.28o	1.023lm	0.0401mn	4
	M + R	44.89mn	35.22pq	7.65op	1.05p	1.018m	0.0394n	4
	P + R	50.91ij	37.63lmno	8.87jklm	1.33no	1.211k	0.0359o	4
	M + R + P	35.42q	23.77u	6.18rs	1.02p	0.912n	0.0326pq	5
TiO ₂ - 0.10 mg/mL	C ^B	71.55bc	54.94c	11.04bcd	1.92fgh	1.698f	0.0576bc	—
	M + P	50.75ij	36.96no	8.47klmn	1.54kl	1.339j	0.0421jklm	3
	M + R	46.14lm	37.11no	8.26lmno	1.39mn	1.238k	0.0422jklm	3
	P + R	52.15hi	39.18jkl	9.44ghij	1.54kl	1.475hi	0.0409klmn	3
	M + R + P	38.11p	26.32t	6.71qr	1.35no	1.014m	0.0388n	4
TiO ₂ - 0.20 mg/mL	C ^B	72.12bc	55.89c	11.49bc	2.28cd	1.869d	0.0598b	—
	M + P	52.62hi	38.29jklmn	9.15hijk	1.83h	1.503h	0.0471fg	3
	M + R	48.42jkl	39.13jklm	8.95ijkl	1.55kl	1.343j	0.0453gh	3
	P + R	54.41gh	40.93hi	10.13efg	1.87gh	1.611g	0.0451ghi	3
	M + R + P	40.22op	27.94s	7.03pq	1.57kl	1.079l	0.0426ijklm	4
SiO ₂ - 0.05 mg/mL	C ^C	70.94bc	54.77cd	10.89cd	2.21d	1.708f	0.0559c	—
	M + P	50.94ij	37.55lmno	9.02ijkl	1.67ij	1.436i	0.0459g	3
	M + R	47.04klm	37.32mno	8.52klmn	1.47lm	1.321j	0.0432hijk	3
	P + R	52.81hi	40.03ij	9.89fgh	1.71i	1.513h	0.0428hijkl	3
	M + R + P	40.37op	28.73s	7.05pq	1.39mn	1.017m	0.0405lmn	4
SiO ₂ - 0.10 mg/mL	C ^C	73.25b	57.76b	11.76ab	2.59b	2.067b	0.0691a	—
	M + P	53.73gh	39.76ijk	9.95fg	1.98f	1.691f	0.0487ef	2
	M + R	50.42ij	39.12jklm	9.33ghij	1.95fg	1.663fg	0.0467fg	2
	P + R	55.55fg	43.78g	10.55def	2.08e	1.806e	0.0461g	2
	M + R + P	45.87lm	33.72qr	8.14mno	1.51kl	1.235k	0.0426ijklm	3
SiO ₂ - 0.20 mg/mL	C ^C	76.87a	60.94a	12.34a	2.91a	2.391a	0.0707a	—
	M + P	57.41ef	42.82g	10.79cde	2.23d	1.987c	0.0503de	2
	M + R	52.12hi	42.35gh	10.08efg	2.11e	1.915d	0.0488ef	2
	P + R	58.93e	46.78f	11.38bc	2.37c	2.098b	0.0491ef	2
	M + R + P	50.38ij	37.97klmn	9.43ghij	1.98f	1.606g	0.0447ghij	2
L.S.D. $p = 0.05$ NPs × pathogens		1.59	0.695	0.093	0.057	0.002	0.001	

At $p \leq 0.05$, data analysis was done with Duncan's multiple range test. The same letters do not differ significantly within a column. M = *M. incognita*; P = *P. vexans*; R = *R. solanacearum*; AC = control without pathogen without SiO₂-NPs and TiO₂-NPs; BC = control without pathogen with TiO₂-NPs; C^C = control without pathogen with SiO₂-NPs.

plus *R. solanacearum*, *P. vexans* plus *R. solanacearum*, and all the three pathogens together caused 84.28, 77.54, and 123.99% increases over their respective controls (Table 5).

3.3.2 Effect on chlorophyll and carotenoid contents

Inoculation of test pathogens in combination resulted in a noteworthy reduction in contents of chlorophyll over

the uninoculated control (Table 4). Inoculation of *M. incognita* plus *P. vexans* caused a 25.08% reduction in chlorophyll contents while a 32.72% reduction was caused by *M. incognita* plus *R. solanacearum* (% data not shown). Inoculation of *P. vexans* plus *R. solanacearum* and all the three pathogens together caused 15.02 and 35.49% reductions in chlorophyll contents, respectively. Foliar spray of 0.20 mg/mL TiO₂-NPs to plants with *M. incognita* plus *P. vexans* caused a 68.31% increase in chlorophyll contents

while *M. incognita* plus *R. solanacearum*, *P. vexans* plus *R. solanacearum*, and all the three pathogens together caused 67.46, 59.03, and 40.31% increases over their respective controls. Foliar spray of 0.20 mg/mL SiO₂-NPs to plants with *M. incognita* plus *P. vexans* caused a 122.50% increase in chlorophyll contents while *M. incognita* plus *R. solanacearum*, *P. vexans* plus *R. solanacearum*, and all the three pathogens together caused 138.78, 107.11, and 108.84% increases over their respective controls (Table 5).

Inoculation of all the pathogens together caused a high reduction in carotenoid contents (Table 4). Inoculation of *M. incognita* plus *P. vexans* caused a 37.80% reduction in carotenoid contents while a 40.55% reduction was caused by *M. incognita* plus *R. solanacearum* (% data not shown). Inoculation of *P. vexans* plus *R. solanacearum* and all the three pathogens together caused 34.84 and 42.91% reductions in carotenoid, respectively. Spraying three pathogens together caused 50.0, 36.25, and 46.90% increases over their respective controls. Similarly, the use of 0.05 mg/mL SiO₂-NPs to plants with *M. incognita* plus *P. vexans* caused a 54.11% increase in the carotenoid content while *M. incognita* plus *R. solanacearum*, *P. vexans* plus *R. solanacearum*, and all the three pathogens together caused 54.63, 39.27, and 46.90% increases over their respective controls. Spraying with 0.20 mg/mL SiO₂-NPs to plants with *M. incognita* plus *P. vexans* caused a 59.18% increase in the carotenoid content while *M. incognita* plus *R. solanacearum*, *P. vexans* plus *R. solanacearum*, and all the three pathogens together caused 61.59, 48.34 and 54.14% increases over their respective controls (Table 5).

3.3.3 Blight and wilt indices

Blight and wilt indices were 5 when *P. vexans*, *R. solanacearum*, and *M. incognita* were inoculated in combinations (Table 5). Indices were recorded as 2 when plants inoculated with two or three pathogens were sprayed with 0.10 (mg/mL)/0.20 (mg/mL) SiO₂-NPs except for plants inoculated with three pathogens sprayed with 0.10 mg/mL SiO₂-NPs (Table 5).

3.3.4 Nematode multiplication and galling

Galling and multiplication of nematode significantly reduced after the application of SiO₂-NPs followed by TiO₂-NPs (Figures 4 and 5). The spray of 0.20 mg/mL SiO₂-NPs resulted in the greatest reduction in galling and nematode population, whereas the spray of 0.05 TiO₂-NPs resulted in the least. Inoculation of *P. vexans* or *R. solanacearum* also

harmed galling and nematode multiplication. *R. solanacearum* showed a stronger detrimental effect on nematode multiplication and galling than *P. vexans*. Together had a greater adverse effect than alone (Figures 4 and 5).

3.4 Molecular docking analysis

R. solanacearum protein containing the X-ray crystallographic (PDB IDs: 1UQX and 3ZI8) was retrieved from the website of Protein Data Bank RCSB PDB. The bacterium *R. solanacearum*, which is found all over the world and causes deadly wilt in a wide range of crops, has been revealed to produce *R. solanacearum* lectin, a powerful L-fucose-binding lectin with a tandem repetition in its sequence of amino acids. The catastrophic bacterial wilt disease can be caused by a powerful L-fucose-binding lectin that can infect a wide spectrum of plants [21,22].

Docking was used to ascertain the best-docking pose of SiO₂-NPs' inactive pocket of amino acids of *R. solanacearum* of protein, as well as its binding affinity and confirmation within the binding sites. For the analysis and involvement of amino acids, the best-docking pose with a binding energy (global energy) of −31.61 and −43.00 kcal/mol was chosen for 1uqx.pdb and 3zi8.pdb, respectively. The important amino acids that participate in the non-binding interaction with SiO₂-NPs are LYS63, VAL5, GLN2, GLN3, ALA1, VAL76, LEU75, TYR101, ASP74, GLU94, SER73, PRO72, and LYS71 of the protein (1uqx.pdb) while amino acids, such as TYR37, THR38, GLU28, GLY39, ALA40, CYS30, TRP31, ASP32, LYS34, SER15, VAL13, TRP10, GLY11, TRP76, ASN79, GLY78, ILE59, LEU54, ALA58, SER57, and GLY56, for 3zi8.pdb found around the NPs stabilize the interaction between receptor and ligand (Figure 6).

4 Discussion

The growth of fungus *P. vexans* had been reduced when SiO₂-NPs and TiO₂-NPs were present in the PDA medium. In this study, SiO₂-NPs were found more effective against *P. vexans* compared to TiO₂-NPs. SEM photographs suggest that SiO₂-NPs showed disturbed mycelia and conidia of *P. vexans*. Moreover, confocal images showed penetration of SiO₂-NPs in mycelia and conidia. The antifungal property of silica NPs may be present due to the breakdown of the cell wall by forming hydrogen bonds between lipopolysaccharides present in the cell wall and hydroxyl groups present in silica NPs [23]. Silicon amendments have

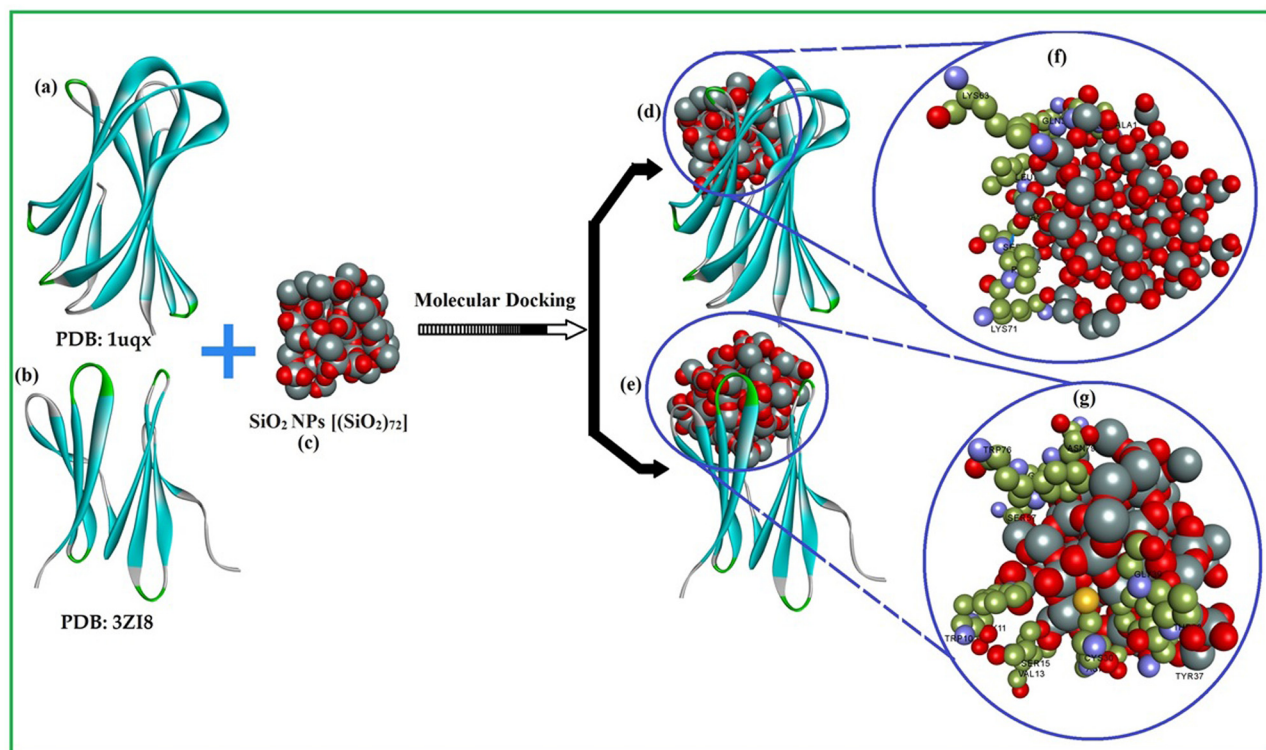


Figure 6: Molecular docking study of NPs (c) against two receptors (a) PDB: 1uqx and (b) PDB: 3zib of *R. solanacearum*, (d and e) demonstrating active sites around NPs and (f and g) different non-bonding amino acid interactions with NPs.

been shown to be effective at preventing fungal diseases [6,24]. SiO₂-NPs were found effective against bacterial pathogens, such as *Xanthomonas campestris* pv. *vesicatoria* and *R. solanacearum*, and a few other tested pathogens *in vitro* and under greenhouse conditions [25].

The application of SiO₂-NPs and TiO₂-NPs exhibited antibacterial activity against *R. solanacearum* in the current study. Deformed cells of *R. solanacearum* were observed under the SEM when the bacterium was grown in the medium with the disk of SiO₂-NPs. Foliar spray of SiO₂-NPs was demonstrated to be superior to the TiO₂-NPs foliar spray. Si mitigates disease intensity in crops [26]. Plants absorb Si in the mono-silicic acid form [27]. Si depositions in plant cell walls, leaves, and stems provide a barrier to host plants against pathogens. Lsi1 and Lsi2 are the transporters of Si present in the cell membrane of plants [28,29]. Polymerization of Si occurs in the extracellular spaces of epidermal cells and xylem vessels [30]. Ayana *et al.* [31] showed that the population of *R. solanacearum* was significantly reduced in Si fertilizer-treated tomato plants. Si could reduce bacterial diseases [32]. SEM micrograph showed deformed cells of *R. solanacearum* grown in the medium with the disk of TiO₂-NPs. TiO₂-NPs could inhibit the development of bacterial pathogens. TiO₂-NPs at 200 mg/L

inhibit the progress of fungus *Rhizoctonia solani*, bacterium *Pectobacterium betavascularum*, and hatching of root-knot nematode (*M. incognita*) [33]. Norman and Chen [34] found that TiO₂-NPs' treatment reduces 93% of lesions caused by *X. axonopodis* pv. *poinsetticola* in poinsettia plants treated with 75 mm. The TiO₂-NPs' treatment could modify the bacterial community structure [35]. Therefore, the current study confirmed that the application of foliar of SiO₂-NPs and TiO₂-NPs is effective in reducing the disease complex of eggplant.

The adverse effect of SiO₂-NPs and TiO₂-NPs was also observed on eggs and J₂ was referred to the second-stage juveniles of *M. incognita*. Sudanophilic lipids and weakly acidic mucopolysaccharides are present in nematode cuticles. In addition, nematodes' hypodermis contains lipids, glycogen, and acidic muco-polysaccharides [36]. The SEM of second-stage juveniles of *M. incognita* confirmed that the application of SiO₂-NPs/TiO₂-NPs harmed nematodes cuticle by affecting glycogen, lipid, and mucopolysaccharides. These are also found to have the potential to reduce galling and multiplication of *M. incognita* on eggplant. Si application dramatically reduced root-knot nematodes in the roots of the hosts and Si-treated plants have high phenolic compounds and high callose deposition in roots after

nematode attack [37]. Similarly, *M. incognita* J₂ mortality was 4.3 and 2% was observed in 800 and 400 mg/mL of TiO₂-NPs [38].

Previous studies reported that 500 mg/L SiNPs and SiO₂-NPs' treatment increased plant growth and improved seed germination in maize crops [39,40]. Improvement in the seed germination of soybean was reported after treatment with nano-SiO₂ and nano-TiO₂ and they also improve the water- and nutrient-absorbing ability of seeds [8,41].

Plants sprayed with TiO₂-NPs have increased photosynthesis plant growth and chlorophyll contents [42]. Applications of TiO₂-NPs (<20 nm in size) increased shoot lengths, root length, chlorophyll content, and phosphorus uptake in wheat [43]. An increase in the growth of eggplants without pathogens by the application of SiO₂-NPs and TiO₂-NPs may be attributed to the above-mentioned reasons. Kang *et al.* [44] reported that silica NPs reduce the growth of *Fusarium* fungus and improve the growth of the Watermelon (*Citrullus lanatus*) plant. Ahamad and Siddiqui [45] found SiO₂-NPs to be most effective against *M. incognita* compared to ZnO and TiO₂-NPs. Spraying carrot plants with SiO₂-NPs had the greatest impact on plant growth parameters. The docking study of NPs showed better activity against *R. solanacearum* carried out to understand the involvement of various amino acids with hydrophobic and hydrophilic nature residues of the protein. Certain amino acids (Figure 6) in 1uqx.pdb, such as ALA1, GLN2, LYS63, ASP74, LEU75, VAL76, and ASP74, as well as some amino acids in 3zi8.pdb, such as TRP10, SER15, TYR37, SER57, ALA58, ASN79, and TYR37, form hydrogen bonds with oxygen atoms of SiO₂-NPs, and other nonbonding interactions can hinder microbe proliferation in the host plant. The nature of the contact, as well as active residues that are critical to the growth of *R. solanacearum* in various plants, is disclosed by molecular docking. To develop antimicrobial drugs in the future, a mechanistic method based on molecular docking could be interpreted by taking into account the amino acid residues of bacterial and fungal proteins.

5 Conclusion

Wilt and blight indices were reduced by the application of SiO₂/TiO₂-NPs. The reduction in disease indices by SiO₂/TiO₂-NPs also confirms the antibacterial and antifungal efficiencies of used NPs. The application of SiO₂-NPs will be eco-friendly and can be used as an alternative to chemical pesticides to manage the disease complex of eggplant. A molecular docking investigation was also conducted to

learn more about the interaction of SiO₂-NPs with the protein of *R. solanacearum* at the binding site. Certain amino acid residues of bacterium explained in the discussion part formed hydrogen bonds with SiO₂-NPs' surface.

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