Original Research Article

Viricidal activity of silicon dioxide nanoparticles against cowpea aphid borne mosaic virus

UNDER PEER REVIEW

ABSTRACT

The management of viruses in cowpea is a challenging task and the use of nanoparticles (NPs) presents a promising opportunity for novel antiviral strategies. This study evaluates the viricidal activity of silicon dioxide nanoparticles (SiO_2NPs) against cowpea aphid-borne mosaic virus (CABMV) under controlled conditions.Silicon dioxide nanopowder formulation was used in this study. The virus, molecularly characterized as CABMV, was maintained through mechanical transmission.Pre- and post-inoculation of virus followed by NP foliar sprayat different concentrations were done in local lesion host, *Chenopodium amaranticolor,* and propagative host, cowpea.The studies using various concentrations of SiO_2NPs on *C. amaranticolor* and cowpearevealed that pre- or post-inoculation of the virus combined with a foliar spray of SiO_2NPs at 1000 ppm is an effective treatment, exhibitingno local lesions compared to 34 local lesions in the viral control. In cowpea, among the various concentrations tested,foliar application of SiO_2NPs at 1000 ppm before CABMV inoculation reduced the vulnerability index (VI) to zero, followed by SiO_2NPs at 500 ppm, which achieved a VI of 2.77compared to viral control (VI – 94). The efficacy of NP treatment was further validated by assessing the viral titre using DAS-ELISA, with treated plants showing a sevenfold reduction in viral titre compared to the control.A significant reduction in the number of local lesions in *C. amaranticolor* and the vulnerability index in cowpea demonstrated the pronounced impact of SiO_2NPs on viral particles.Simultaneous inoculation of the virus and NPs showed reduced, diffused local lesions on leaves treated with viral sap-containing NPs, compared to a higher number of intact lesions in the control. This reduction is likely due to the disintegration of viral particles by NP treatment, leading to decreased disease severity.These findings further emphasize the potential of SiO_2 NPs in mitigating the severity of cowpea aphid-borne mosaic disease.

Keywords: CABMV, Diffused local lesions, Vulnerability index, DAS-ELISA, RT-PCR

1. INTRODUCTION

Cowpea, an annual tropical grain legume, plays a vital role in human nutrition, particularly in developing countries across the tropics and subtropics. Known as the "poor man's meat," cowpea is a cost-effective protein source, containing approximately 25% protein and 64% carbohydrates (Bressani, 1993). Viral diseases are significant biotic stressors severely impacting cowpea production at all growth stages of the crop. Cowpea viral diseases result in poor pod formation and quality and are a major constraint in most parts of the world. To date, 20 different viruses have been reported to infect cowpea

(Hampton *et al.,* 1997; Thottappilly and Rossel, 1997). Among them, the most damaging viruses contributing to yield losses is the cowpea aphid-borne mosaic virus (CABMV, family *Potyviridae*, genus *Potyvirus*). Most of the worldwide commercial varieties and landraces of cowpea are susceptible to viruses. Taiwo *et al.,* 1982 observed that the CABMV caused the most aggressive and severe symptoms compared to the cowpea mottle virus. CABMV is a significant seed-borne pathogen that inflicts substantial damage on cowpea. Once established in seedlings as seed-borne inoculum, these viruses are further disseminated within fields by insect vectors. The emergence of new viral strains through evolution, coupled with an expanding host range that undermines host resistance, presents a considerable challenge to effective management. Consequently, controlling viral diseases remains a major hurdle in cowpea cultivation. It is essential to devise effective management strategies to combat the losses caused by viruses in economically important crops. The emergence of Nanophytovirology in managing plant viral diseases by defense induction and biostimulation is quite promising. The potential of nanoparticles (NPs) in plant disease management is increasingly recognized. Their advantages lie in their extremely small size, large surface area, and strong reactivity (Jeevanandam*et al.,* 2018). The unique physicochemical properties of NPs enhance interactions with viruses, often leading to effective virus suppression. Numerous studies have highlighted the efficacy of NPs against various phytoviruses, showing promising results. Silver nanoparticles have been reported as effective antiviral agents (Jain and Kothari, 2014). El-Sawy *et al.,* 2017 demonstrated reduced disease severity and delayed symptoms in tomatoes infected with the tomato leaf curl virus when treated with silicon nanoparticles. In the present investigation, the antiviral activity of silicon dioxide nanoparticles was evaluated against CABMV in cowpea.

2. MATERIAL AND METHODS

2.1 Symptomatology and maintenance of virus

Virus-infected plants displayed symptoms such as vein-banding, vein clearing, mosaic patterns, stunted growth, leaf distortion, reduced leaf area, severe chlorosis, and leaf deformation. Purposive sampling was done and infected leaf samples were collected from Thrissur and Palakkad districts of Kerala. A disease score chart (scale of 0–5) was prepared to assess disease severity based on the vulnerability

index (Fig.1).

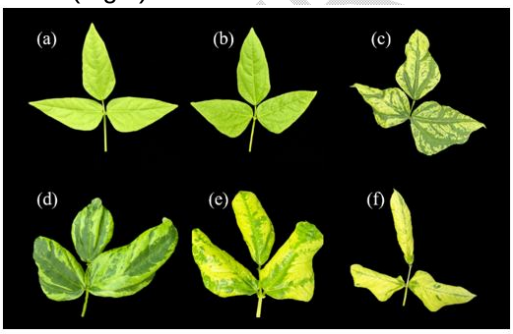


Fig 1.Disease score chart (a) **Score 0** (No symptoms) (b) **Score 1** (Slight vein-clearing & mosaic symptoms) (c) **Score 2** (Vein banding symptoms) (d) **Score 3** (Severe vein banding and cupping of leaves) (e) **Score 4** (Severe mosaic, vein-banding & reduction in leaf area) (f) **Score 5** (Chlorosis and severe mosaic).

UNDER PEER REVIEW

The virus was maintained through sap transmission to susceptible cowpea (Var. Anaswara) and *Chenopodium amaranticolor*, which is the local lesion host of the virus, under insect-proof glasshouse conditions. The symptom development was monitored and documented on both hosts. The viral inoculum was maintained in susceptible cowpea plants and inoculated using potassium phosphate buffer (0.01 M, pH 7.2). Severe mosaic symptoms were selected, and leaf samples were homogenized in a 1:2.5 sample buffer ratio, using a pre-cooled mortar and pestle. The inoculum was then applied to cowpea leaves at the cotyledonary stage and *C. amaranticolor* leaves at the 8-leaf stage, with celite dusted on the leaves to facilitate abrasion. Viral sap was gently applied along the veins, and the leaves were rinsed with distilled water after inoculation.Evening hours were identified as optimal for inoculation, and regular observations were conducted to record symptom development and the time taken for symptoms to appear.

2.2 Molecular detection of virus

The virus was identified at the molecular level using reverse transcription polymerase chain reaction (RT-PCR). RNA was extracted from 100 mg of infected cowpea leaf tissue (Var. Anaswara), and ground with liquid nitrogen and polyvinylpyrrolidone (PVP), followed by the addition of 1 mL of Trizol reagent and 10 µl of β-mercaptoethanol. The homogenate was incubated at room temperature, mixed with chloroform, and centrifuged at 10,000 rpm. The RNA was precipitated with ice-cold isopropanol, rinsed with 75% ethanol, air-dried, and resuspended in RNase-free water. RNA quality and concentration were assessed spectrophotometrically (OD 260/280) and electrophoretically on a 2% agarose gel stained with ethidium bromide. The total RNA was converted into complementary DNA (cDNA) using the G-Biosciences cDNA synthesis kit, incubating 1 µg of RNA at 42°C for 20 min. and then inactivating at 85°C for 5 min. The quality of the synthesized cDNA was also assessed spectrophotometrically. RT-PCR was conducted using Takara Emerald AmpR GT PCR Master Mix (2X-Premix) with cDNA as the template and specific primers for cowpea aphid-borne mosaic virus. A 20 µl reaction mixture was prepared, and the cycling conditions were optimized with an annealing temperature of 54^{c}C for the coat-protein gene-specific primers CABMV – F (5'–GGA TGC GGA GAA TCT GTG–3') and CABMV – R (5'–GAT TGA CGT CCC TTG CAG–3'), targeting an expected amplicon size of 812 bp (Bhadramurthy and Bhat, 2009).

2.3 Serological detection of virus by DAS-ELISA

The serological detection was carried out using a specific antiserum for cowpea aphid-borne mosaic virus (DSMZ -PV RT-0417) following the standard procedure (Clark and Adams,1977). Antibody for CABMV was diluted in coating buffer in the ratio of 1:1000 and 200 µl was added to each well of a microtiter plate. The plate was covered and incubated at 37°C for 4 hours, then washed three times with phosphate buffer saline tween (PBS-T), including a soaking step, and blotted dry. Samples were extracted at 1:20 (w/v) in extraction buffer (PBS-T), and 200 µl aliquots were added to duplicate wells, followed by overnight incubation at 4°C. After washing, 200 µl of enzyme-conjugated antibody, diluted in conjugate buffer, was added, and plates were incubated at 37°C for 4 hours. Following another wash, 200 µl of freshly prepared substrate (1 mg/mL para-nitrophenyl-phosphate in substrate

UNDER PEER REVIEW

buffer) was added to each well and incubated at 37°C for 60 min. Results were assessed by measuring the absorbance at 405 nm in an ELISA- reader (BIO-RAD iMarkTM microplate reader).

2.4 Characterization and preparation of nanoparticle suspension for treatments

SiO_2 NPs purchased from Sisco Research Laboratories PVT. LTD. was used in the present study. The silicon nanoparticle had an average primary particle size of 15 nm and purity percentage, of 99.5%.The NPs were characterized using FE-SEM (Fig 3). To prepare a homogeneous nanoparticle suspension, the nanopowder was suspended in double-distilled water, the suspension was sonicated for 30 min. using a probe sonicator.

2.5 Assay for antiviral activity of silicon dioxide nanoparticles

One mL of nanoparticle formulation of each concentration was added to one mL of CABMV crude sap, and the mixture was thoroughly mixed to ensure uniformity. 50,100, and 200 ppm of SiO_2NP suspension were prepared and mixed with viral sap. This mixed suspension was then incubated for 10 min. to allow for optimal interaction between the nanoparticles and the viral particles. Following the incubation period, the mixture was used for inoculating *C. amaranticolor* plants, facilitating the assessment of the effect of nanoparticles on viral infection in the host plants. This was done to evaluate the potential antiviral activity of the nanoparticles against CABMV.

2.6 Pre-and post-inoculation studies in *Chenopodium* and cowpea

Leaves of *C. amaranticolor* at the 8-leaf stage and Cowpea (Var. Anaswara) at the cotyledonary leaf stage were treated with nanoparticles both before and after virus inoculation. For the pre-inoculation studies, the plants were first challenge-inoculated with a freshly prepared virus inoculum, followed by a foliar spray of nanoparticles 24 hours later. For post-inoculation, nanoparticle formulation was sprayed at various concentrations 24 hours before challenge inoculation with CABMV. 50, 100, 200, 500, and 1000 ppm of SiO_2 NP suspension were prepared and used. Based on the scoring of plants subjected to various treatments, the vulnerability index (VI) was calculated by using the formula given by Bos, 1982.

$$VI = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5)}{n_t(n_c - 1)} \times 100$$

VI – Vulnerability Index
n_0, n_1..... n_5 – Number of plants in disease category of 0, 1, 2, 3, 4 and 5
n_t – Total number of plants
n_c – Number of categories

2.7 Statistical analysis

Data generated from the experiment were subjected to ANOVA. Analyses were conducted using the statistical software KAU GRAPES 1.1.0 (Gopinath *et al.,* 2021) and evaluated the statistical significance of the analysis results at a probability value (p-value) ≤ 0.05. The result's significance was indicated in letters in descending order where (a > b > c) and the same letters indicated equal significance. All results were represented as means of triplicate with the corresponding standard deviations (SD).

3. RESULTS AND DISCUSSION

Cowpea plants showing varied diseased symptoms were observed and the symptoms were documented. The cowpea infected with CABMV had severe vein banding

UNDER PEER REVIEW

symptoms, leaf distortion, and mosaic symptoms. The virus from the infected leaves could be successfully transferred to the propagation host, cowpea (*Vigna unguiculata* var. Anaswara) as well as the local lesion host *Chenopodium amaranticolor* by mechanical inoculation with 0.01M phosphate buffer. There are reports of *C. amaranticolor*as a good host for potyvirusproducing chlorotic local lesions (Hollings, 1956; Bock, 1973). Here in this study, chlorotic local lesions were observed 7 days after mechanical inoculation and later turned necrotic in *C. amaranticolor*. In cowpea, most inoculated plants showed systemic symptoms, and the symptoms were observed in emerging trifoliate with slight vein-clearing symptoms initially, followed by severe vein banding and distortion of leaf size. The mechanical transmission rates were 97.45% in *C. amaranticolor* and 98.13% in cowpea (Table 1).

The virus cultured in cowpea and *C. amaranticolor* was molecularly confirmed as CABMV (Fig 2). The virus was identified as a strain of Bean common mosaic virus showing more than 95% similarity to CABMV. The sequence was deposited in NCBI with accession number PQ511232. Further studies were carried out using this virus culture maintained in cowpea.

PEER REVIEW

Table 1. Mechanical transmission of CABMV in *C. amaranticolor* and cowpea

Sl No.	Crop / Variety	Days taken for symptom development	Mechanical transmission (%)	Nature and Type of symptom
1	***Chenopodium amaranticolor***	9-10	97.45	Definite chlorotic spots with a yellow halo later necrotize at the center
2	Cowpea (**Anaswara**)	7-8	98.13	Vein banding, vein clearing, and slight mosaic symptoms

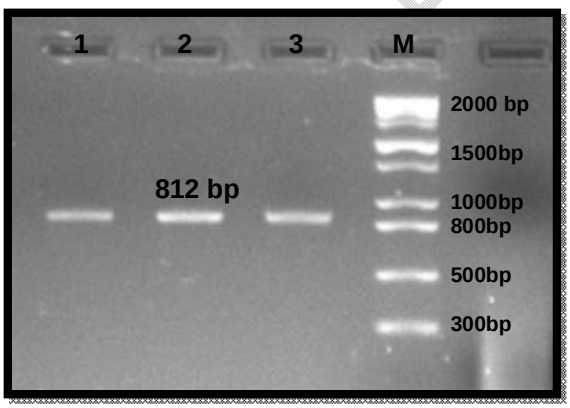


Fig 2. 2% Agarose gel profile of PCR products with CABMV-specific primer Lanes 1, 2 & 3: PCR products Lane M: 1kb ladder

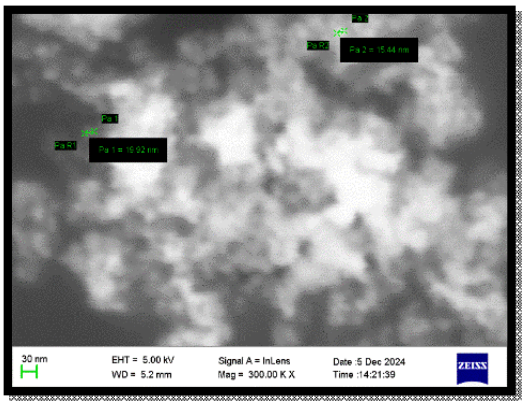


Fig 3.Field Emission Scanning Electron Microscopic image of SiO_2 Nanoparticles

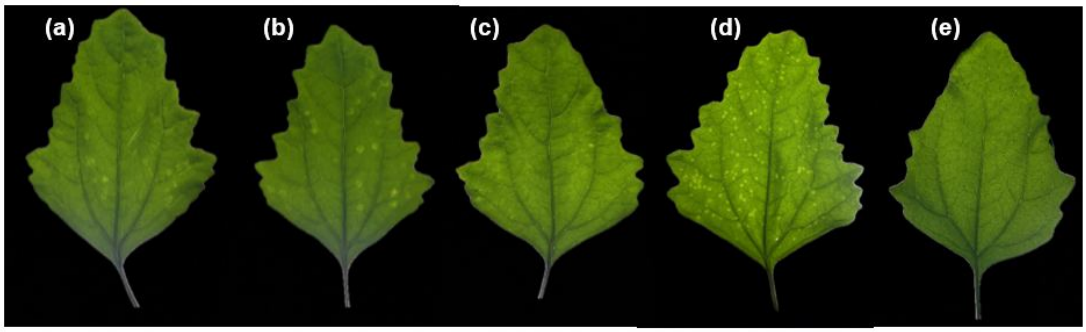


Fig 4.Efficacy of NPs against virus on simultaneous inoculation of NP and virus to *C.amaranticolor*.(a),(b) & (c) diffused local lesions in 50,100 and 200 ppm of SiO_2 NPs (d) intact chloroticlocal lesions in viral control (e)Absolute Control.

The virucidal effects of nanoparticles (NPs) on viral particles represent an area of considerable scientific interest. A direct inoculation study was conducted to investigate the direct action of NPs, where viral sap mixed with NPs was inoculated onto *C. amaranticolor*. The evaluation of silicon dioxide nanoparticles (SiO_2NPs) in this co-inoculation study revealed notable results. Apart from a reduction in the number of local lesions, diffused spots were observed on the leaves, in contrast to the intact chlorotic spots seen in viral control plants (Fig. 4). Among the tested concentrations of SiO_2NPs—50 ppm, 100 ppm, and 200 ppm—200 ppm proved most effective, resulting in the least number of local lesions (4), compared to 36 in the viral control (Table 2). SiO_2NPs at 50 ppm and 100 ppm also reduced lesion counts to 6 and 10, respectively. The interaction between the virus and nanoparticles likely reduced the infectivity of viral particles by causing disintegration or impairing their movement and stability. Nanoscale particles possess unique physicochemical properties that enable them to bind to and potentially destroy viral particles effectively. Such nanoparticles have demonstrated virucidal activity against various viruses, significantly lowering their infectivity. Cai *et al.,* (2019) reported similar findings, demonstrating the antiviral activity of ZnONPs and SiO_2NPs against TMV in vitro. Their study revealed that the direct interaction of metal nanoparticles with TMV resulted in capsid protein injury, aggregation, and disintegration of the virus, further highlighting the potential of nanoparticles in antiviral applications. Similar studies conducted by El-Dougdoug*et al.,* (2018) demonstrated that AgNPs bind to the coat protein of Potato virus Y and tomato mosaic virus, thereby inhibiting viral replication. The interaction between the virus and SiO_2NPs likely contributed to the reduction in viral titre and the milder symptom expression observed in this study.

Table 2. Symptom development on simultaneous inoculation of NP and virus to *Chenopodium amaranticolor*

Sno.	Treatments	Local Lesions*	Percent disease inhibition over viral control
1	SiO_2 50 ppm	$\mathbf{06.33 \pm 1.155}^{c}$	**81.73**
2	SiO_2 100 ppm	$\mathbf{10.33 \pm 3.215}^{c}$	**70.19**
3	SiO_2 200 ppm	$\mathbf{04.00 \pm 1.732}^{b}$	**88.45**
4	Viral control	$\mathbf{34.66 \pm 2.309}^{a}$	-

5	Absolute control	**00.00±0.000^{d}**	**-**
CD –0.94		**P Value – 0.00**	

**** Mean of three replications. In each column figure followed by same superscript do not significantly differ according to DMRT***

The efficacy of nanoparticles SiO_2NPs in curative and protective strategies was assessed through pre- and post-inoculation studies using a local lesion host and further confirmed in the propagative host, cowpea. Pre-inoculation of the virus via mechanical inoculation, followed by foliar application of SiO_2NPs at varying concentrations, resulted in a significant reduction in the average number of local lesions compared to the viral control (34 lesions) in *C. amaranticolor*. All tested concentrations of SiO_2NPs demonstrated a notable decrease in lesion numbers (Fig. 5). A decreasing trend in lesion count was observed with increasing concentrations of SiO_2NPs, with 100 ppm showing 9 lesions, 200 ppm showing 8 lesions, and no local lesions observed at 1000 ppm in pre-inoculation studies (Table 3). In post-inoculation studies, where a foliar spray of SiO_2NPs was applied before virus inoculation, the lesion count was significantly lower compared to the viral control. This prophylactic approach proved to be more effective than the curative method, where the virus was inoculated first, followed by NP application.SiO_2NPs at 1000 ppm completely suppressed local lesion formation, with no lesions observed, while foliar sprays at 50 ppm and 200 ppm resulted in an average of 3 local lesions each (Table 3). Additionally, symptom expression in SiO_2 nanoparticle-treated plants was delayed by 3-4 days compared to the viral control, where symptoms appeared within 7 days post-inoculation. Silver nanoparticles (AgNPs) with a size of 12.6 nm were evaluated for their impact on the tomato spotted wilt virus (TSWV) in *Solanum tuberosum*. The findings revealed a notable suppression of local lesions and a decrease in TSWV infection(Shafie *et al.,* 2018). El-Dougdoug*et al.,* 2018 reported that the tomato plants treated with AgNPs at a concentration of 50 ppm exhibited a greater reduction in disease severity, disease incidence, and relative viral concentration compared to other tested concentrations. Additionally, AgNPs at 50 ppm significantly decreased the average number of local lesions caused by tomato mosaic virus in *N. glutinosa.*

PREPRINT

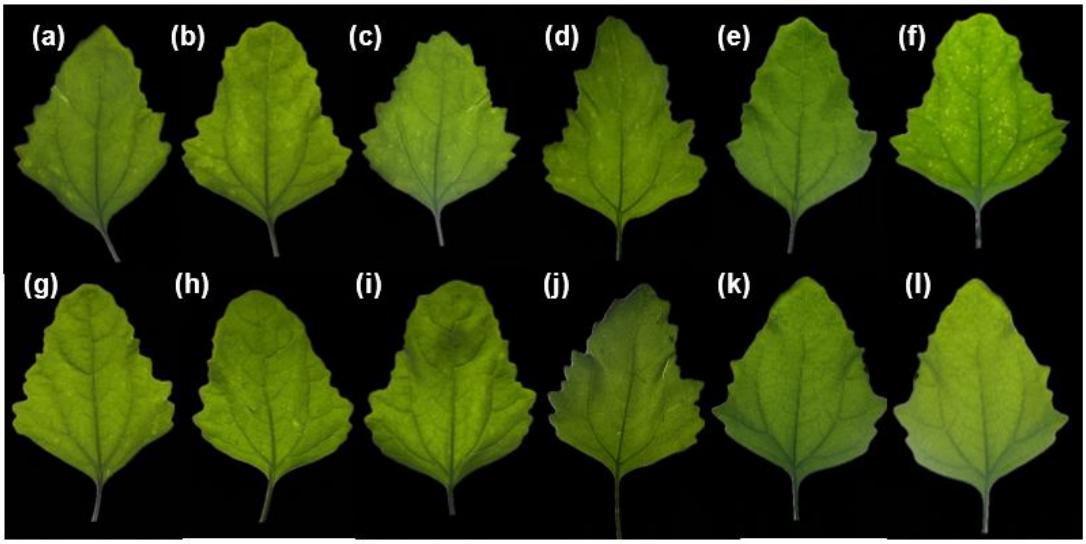


Fig. 5.Efficacy of different concentrations of NPs on pre- and post-inoculation of virus in *C.amaranticolor*based on local lesions, Pre inoculation(a)SiO_2 NP 50ppm (b)SiO_2 NP 100ppm (c)SiO_2 NP 200ppm (d)SiO_2 NP 500ppm (e)SiO_2 NP 1000ppm (f)Viral control, **Post inoculation** (g)SiO_2 NP 50ppm (h)SiO_2NP 100ppm (i)SiO_2 NP 200ppm (j)SiO_2 NP 500ppm (k)SiO_2NP 1000ppm (l)Absolute control

The pre-and post-inoculation studies were conducted on cowpea plants and observed for two weeks to verify the reduction in disease severity. Disease severity in cowpea was evaluated using the vulnerability index (VI), which was derived from disease scores. A VI of less than 20 indicates a higher tolerance to virus inoculation. In cowpea, foliar application of SiO_2 NPs at 1000 ppm before virus inoculation demonstrated the best control, with a VI of zero, as no symptoms were observed for up to two weeks in treated plants. Across all treatments in cowpea, the VI was significantly lower compared to the viral control, which exhibited the highest VI of 94 (Fig. 6). SiO_2 NPs at 1000 ppm were followed by 500 ppm, with a VI ranging from 5 to 9 in pre-inoculation studies and 2 to 6 in post-inoculation studies (Table 3). In studies involving pre-inoculation of the virus, followed by foliar application of SiO_2 at concentrations of 1000 ppm, 500 ppm, and 100 ppm, the treatments demonstrated good efficacywith 100%, 94%, and 85% reduction in VI, respectively. In a prophylactic (post-inoculation) approach, foliar application of SiO_2NPs at 1000 ppm, 500 ppm, and 200 ppm was found to be highly effective, resulting in 100%, 97%, and 87% reduction in VI compared to the untreated viral control. A threshold level of NP dosage is needed to induce resistance and inhibit viral particles at the same time. In the present investigation, SiO_2NPs 1000 ppm on both prophylactic and curative approacheswere observed with lower VI and reduced viral titre indicating 1000 ppm as the optimum dosage for reducing disease severity. El-Shazly et al., 2017 found that potato plants treated with AgNPs at a concentration of 0.1 µg/µL exhibited a decrease in both virus concentration and disease incidence 24 hours post inoculation with Potato virus Y.The use of chitosan NPs at a concentration of 100 µg/mL significantly reduced alfalfa mosaic virus (AMV) accumulation levels, decreased disease severity, and induced systemic resistance compared to the control(Abdelkhaleke*t al.,* 2021).

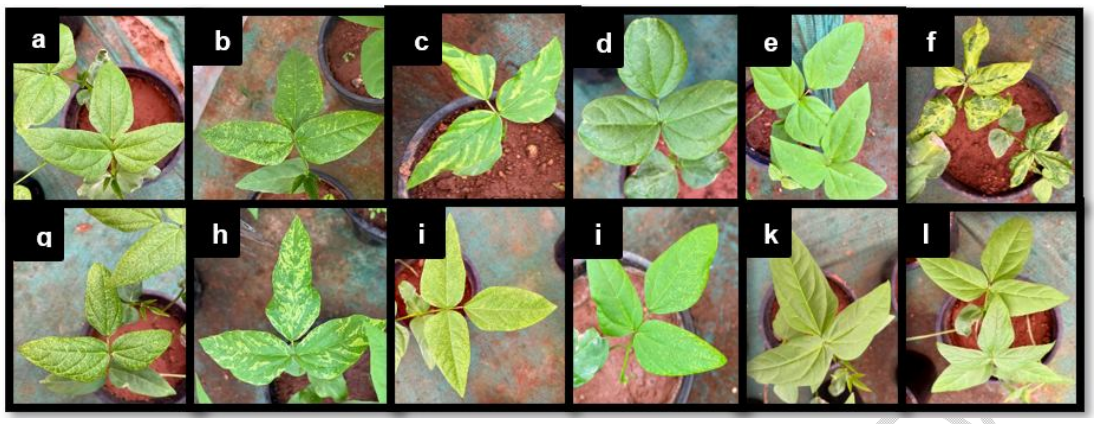
Table 3. Efficacy of different concentrations of NPs on pre- and post-inoculation of virus onto*C. amaranticolor* and cowpea

Pre/post	Treatments	Average local lesions*	Percent inhibition over viral control	Vulnerability index*	Percent inhibition over viral control
Pre-inoculation silicon	SiO_2 50 ppm	**8.66**±2.30^{b}	**74.7**	**16.66**± 08.33^{d}	**82.36**
	SiO_2 100 ppm	**9.33**±9.29^{b}	**72.6**	**13.88**± 12.72^{de}	**85.30**
	SiO_2 200 ppm	**8.66**±0.57^{b}	**74.7**	**33.33**± 08.33^{b}	**64.71**
	SiO_2 500 ppm	**5.33**±2.08^{bc}	**84.4**	**05.55**± 04.80^{ef}	**94.12**
	SiO_2 1000 ppm	**0.00**±0.00^{c}	**100**	**00.00**± 00.00^{f}	**100**
Post inoculation silicon	SiO_2 50 ppm	**3.00**±1.73^{c}	**91.1**	**22.22**± 04.81^{cd}	**76.47**
	SiO_2 100 ppm	**1.66**±1.52^{c}	**95.2**	**30.55**± 09.61^{bc}	**67.65**
	SiO_2 200 ppm	**0.66**±.57.0^{c}	**98.2**	**13.88**± 04.80^{de}	**85.30**
	SiO_2 500 ppm	**2.66**±4.61^{c}	**92.3**	**02.77**± 04.80^{f}	**97.07**
	SiO_2 1000 ppm	**0.00**±0.00^{c}	**100**	**00.00**± 00.00^{f}	**100**
Control	Viral control	**34.66**±2.30^{a}	-	**94.44**± 04.81^{a}	-
	Absolute control	**0.00** ± 0.00^{c}	**-**	**00.00**± 00.00^{f}	-
Local lesions (CD – 1.54, P Value – 0.0)			**Vulnerability index (CD – 3.07, P Value-0.0)**		

**** Mean of three replications. In each column figure followed by same superscript do not significantly differ according to DMRT***

Fig 6.Efficacy of different concentrations of SiO_2NPs on pre- and post-inoculation studies in cowpea based on VI
Pre-inoculation (a)50ppm (16.6) (b)100ppm (13.8) (c)200ppm (33.3) (d)500ppm (5.5) (e)1000ppm (0) (f)Viral control (94.4) **Post-inoculation** (g)50ppm (22.2) (h)100ppm (30.5) (i)200ppm (13.8) (j)500ppm (2.7) (k)1000ppm (0) (l)Absolute control (0)

UNDER PEER REVIEW



UNDER PEER REVIEW

Viral symptom expression is not an absolute confirmation of reduction in viral titre in plants, hence the reduction in viral titre was further confirmed by DAS ELISA in selected treatments. ELISA results showed a lower titre in treatments with a reduced fold increase in absorbance for samples taken from SiO_2NP-treated plants. A two-fold increase in absorbance at 405 nm compared to the healthy negative control was considered positive for the virus, and the increase in absorbance was used to estimate viral concentration. Viral control plants showed a 14.5-fold increase in absorbance at 405 nm relative to the healthy control, indicating a high viral titre in virus-inoculated plants. In plants treated with SiO_2NPs at 1000 ppm before virus inoculation, the viral concentration was significantly reduced, as indicated by only a two-fold increase in absorbance compared to the viral control. Although these plants appeared symptomless and healthy upon visual inspection, DAS-ELISA confirmed the presence of the virus, albeit with a much lower titre than in the viral control. The less viral titre at 1000ppm on post-inoculation studies indicates the curative effect of SiO_2NPs against CABMV. In contrast, plants subjected to pre-inoculation of the virus followed by SiO_2NPs 100 ppm foliar spray showed a 10-fold increase in absorbance, reflecting a relatively high viral concentration. However, the viral titre in these plants was still lower than in the viral control.Post-inoculation SiO_2NPs treatments at 200 ppm and 1000 ppm showed significantly lower viral titre, with only 1.9-fold and 2.5-fold increases, respectively (Table 4). Similar studies were reported by Abdelkhalek et al. (2021), where the ELISA test revealed that alfalfa mosaic virus-inoculated control plants exhibited the highest viral concentration, with an ELISA value of 0.991, compared to 0.0585 in mock-inoculated control plants. Among the chitosan NPs treatments, the inactivating treatment was the most effective, showing an ELISA value of 0.083, followed by protective and curative treatments with values of 0.380 and 0.293, respectively.The antiviral activity of Si NPs has been reported against phytoviruses (Elsharkawy and Mousa, 2015; El-Sawy*et al.*, 2017, Sangwan *et al.,* 2023). Foliar application of SiNPs (100nm) before challenge inoculation with the virus along with ginger and horsemint extract delayed

tomato yellow leaf curl virus (TYLCV) symptoms and disease severity in tomato plants (El-Sawy*et al.,* 2017).

Table 4. Determination of viral titre by DAS-ELISA in different treatments in cowpea

**** Mean of three replications. In each column figure followed by same superscript does not significantly differ according to DMRT***

Sno.	Treatment	Absorbance at 405nm*	Fold increase over negative control	Reaction to virus
1	Negative Control	0.012 ± 0.016^{c}	-	-
2	Positive Control	0.174 ± 0.015^{a}	14.5	+ve
3	Pre- inoculation SiO_2 100 ppm	0.120 ± 0.102^{ab}	10.0	+ve
4	Post-inoculation SiO_2 200 ppm	0.023 ± 0.032^{c}	01.9	+ve
5	Post-inoculation SiO_2 1000 ppm	0.031 ± 0.017^{bc}	02.5	+ve
CD – 0.066		**P Value – 0.009**		

On perusal of the literature, no prior reports on the efficacy of SiO_2NPs against CABMV were reported. In the present study, based on the vulnerability index and the assessment of viral titre by ELISA, prophylactic application of SiO_2NPs 1000 ppm can reduce the disease severity of cowpea aphid-borne mosaic disease in cowpea. Nanoparticles can interact with the virus mainly in two methods; nanoparticles directly inactivate microbes by disrupting processes such as DNA replication, protein synthesis, cell wall formation, electron transport, viral coat protein synthesis, capsid degradation, and genome packaging, and indirectly by enhancing plant defense mechanisms by stimulating the production of enzymes and compounds like peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, superoxide dismutase, jasmonic acid, and ethylene (Vargas-Hernandez *et al.,* 2020, Rajani *et al.,* 2022, Dutta *et al.,* 2022, Warghane *et al.,* 2024). To balance plant defense responses and virus-NP interactions, low to medium concentrations of NPs are essential. This optimization is critical to effectively activate plant defense systems without causing phytotoxicity, ensuring sustainable antiviral activity. Here in this study, CABMV causes systemic infection in plants and it has a high replication rate as demonstrated by a high vulnerability index (94) and viral titre in (0.174) DAS-ELISA. The findings suggest that higher concentrations of SiO_2NPs are needed to disrupt viral particles or interfere with their replication effectively. Moreover, the prophylactic foliar application of 1000 ppm SiO_2NPsbefore virus inoculation significantly reduced disease severity compared to the curative approach.

4. CONCLUSION

Recent advancements in Nanophytovirology present promising opportunities for managing viral diseases, though understanding the precise mechanisms behind their effects remains a critical area for future research. The in vitro greenhouse assay conducted in this study highlights the antiviral potential of SiO_2 nanoparticles against cowpea aphid-borne mosaic virus. The pre- and post-inoculation studies exhibited a reduction in the number of local lesions in *C. amaranticolor* and a decrease in the vulnerability index in cowpea (var. Anaswara).These findings suggest that SiO_2NPs possess significant antiviral potential. The reduction in viral replication and viral titre was further validated through DAS-ELISA results.To confirm the field applicability of these findings, further studies involving field

UNDER PEER REVIEW

assays are essential. Additionally, deeper exploration into the mechanisms by which nanoparticles induce systemic resistance and their direct and indirect interactions with viruses is necessary to understand their role in disease management better. Integrating nanoparticle treatments with other antiviral management strategies may enhance the control of CABMV while minimizing the need for high nanoparticle doses. Such approaches could pave the way for more sustainable and effective viral disease management solutions.

REFERENCES

1. Bressani, R. (1993). Grain quality of common beans. Food Reviews International, 9(2), 237-297.
2. RO, Hampton., Thottappilly, G., & Rossel, H. W. (1997). Viral diseases of cowpea and their control by resistance-conferring genes. *Advances in cowpea research. Copublication of International Institute of Tropical Agriculture (UTA) and Japan International Research Center for Agricultural Sciences (JIRCAS). UTA, Ibadan, Nigeria. Prepared for publication by Sayce Publishing, Devon, UK. Printed by Colorcraft, Hong Kong*, 159.
3. Thottappilly, G., & Rossel, H. W. (1997). Identification and characterization of viruses infecting bambara groundnut (Vigna subterranea) in Nigeria. *International Journal of Pest Management*, *43*(3), 177-185.
4. Taiwo, M. A., Gonsalves, D., Provvidenti, R., & Thurston, H. D. (1982). Partial characterization and grouping of isolates of blackeye cowpea mosaic and cowpea aphid-borne mosaic viruses. *Phytopathology*, *72*(6), 590-596.
5. Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity, and regulations. *Beilstein journal of nanotechnology*, *9*(1), 1050-1074.
6. Jain, D., & Kothari, S. L. (2014). Green synthesis of silver nanoparticles and their application in plant virus inhibition. *J Mycol plant pathol*, *44*(1), 21-24.
7. El-Sawy, M. M., Elsharkawy, M. M., Abass, J. M., & Hagag, E. S. (2017). Inhibition of tomato yellow leaf curl virus by zingiber off cinaleandmentha longifolia. *International Journal of Antivirals &Antiretrovirology*, *1*(1), 1-6.
8. Bhadramurthy, V., & Bhat, A. I. (2009). Biological and molecular characterization of Bean common mosaic virus associated with vanilla in India.
9. Clark, M. F., & Adams, A. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, *34*(3), 475-483.
10. Bos, L.1982. Crop losses caused by viruses. *Crop Protection*. *1*(3):263-282.
11. Gopinath et al., (2021). grapesAgri1: Collection of Shiny Apps for Data Analysis in Agriculture. Journal of OpenSource Software, 6(63), 3437, https://doi.org/10.21105/joss.03437
12. Hollings, M. (1956). CHENOPODIUM AMARANTICOLOR AS A TEST PLANT FOR PLANT VIRUSES. *Plant Pathology*, *5*(2).
13. Bock, K. R. (1973). East African strains of cowpea aphid-borne mosaic virus. *Annals of Applied Biology*, *74*(1), 75-83.
14. Cai, L., Liu, C., Fan, G., Liu, C., & Sun, X. (2019). Preventing viral disease by ZnONPs through directly deactivating TMV and activating plant immunity in Nicotiana benthamiana. *Environmental Science: Nano*, *6*(12), 3653-3669.

UNDER PEER REVIEW

15. El-Dougdoug, N. K, K., Bondok, A. M., & El-Dougdoug, K. A. (2018). Evaluation of silver nanoparticles as an antiviral agent against ToMV and PVY in tomato plants. *Sciences*, *8*(01), 100-111.
16. Shafie, R. M., Salama, A. M., &Farroh, K. Y. (2018). Silver nanoparticles activity against Tomato spotted wilt virus. *Middle East J. Agric. Res*, *7*, 1251-1267.
17. El-Shazly, M. A., Attia, Y. A., Kabil, F. F., Anis, E., & Hazman, M. (2017). Inhibitory effects of salicylic acid and silver nanoparticles on potato virus Y-infected potato plants in Egypt. *Middle East J Agric Res*, *6*(3), 835-848.
18. Abdelkhalek, A., Qari, S. H., Abu-Saied, M. A. A. R., Khalil, A. M., Younes, H. A., Nehela, Y., &Behiry, S. I. (2021). Chitosan nanoparticles inactivate alfalfa mosaic virus replication and boost innate immunity in Nicotiana glutinosa plants. *Plants*, *10*(12), 2701.
19. Elsharkawy, M. M., & Mousa, K. M. (2015). Induction of systemic resistance against Papaya ring spot virus (PRSV) and its vector Myzuspersicae by Penicillium simplicissimum GP17-2 and silica (SiO2) nanopowder. *International journal of pest management*, *61*(4), 353-358.
20. El-Sawy, M. M., Elsharkawy, M. M., Abass, J. M., & Hagag, E. S. (2017). Inhibition of tomato yellow leaf curl virus by zingiber off cinaleandmentha longifolia. *International Journal of Antivirals &Antiretrovirology*, *1*(1), 1-6.
21. Sangwan, A., Gupta, D., Singh, O. W., Roy, A., Mukherjee, S. K., Mandal, B., & Singh, N. (2023). Size variations of mesoporous silica nanoparticle control uptake efficiency and delivery of AC2-derived dsRNA for protection against tomato leaf curl New Delhi virus. *Plant Cell Reports*, *42*(10), 1571-1587.
22. Rajani, Mishra, P., Kumari, S., Saini, P., & Meena, R. K. (2022). Role of nanotechnology in management of plant viral diseases. *Materials Today: Proceedings*, *69*, 1-10.
23. Vargas-Hernandez, M., Macias-Bobadilla, I., Guevara-Gonzalez, R. G., Rico-Garcia, E., Ocampo-Velazquez, R. V., Avila-Juarez, L., & Torres-Pacheco, I. (2020). Nanoparticles as potential antivirals in agriculture. *Agriculture*, *10*(10), 444.
24. Dutta, P., Kumari, A., Mahanta, M., Biswas, K. K., Dudkiewicz, A., Thakuria, D., ... & Mazumdar, N. (2022). Advances in nanotechnology as a potential alternative for plant viral disease management. *Frontiers in Microbiology*, *13*, 935193.
25. Warghane, A., Saini, R., Shri, M., Andankar, I., Ghosh, D. K., &Chopade, B. A. (2024). Application of nanoparticles for management of plant viral pathogen: current status and future prospects. *Virology*, 109998.

UNDER PEER REVIEW