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Research article

Antiviral properties of titanium nanoparticles and features of their influence on the morphology of virions

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Abstract

The search for new antiviral substances and the development of modern antiviral preparations and disinfectants is an important area of modern science. Nanoparticles (NPs) and nanomaterials (NMs) have received increasing attention from scientists as promising antiviral substances recently. Our study aimed to investigate the antiviral activity of citrate-stabilized titanium nanoparticles (Ti NPs) against Teschovirus A (TV-A) and Potato virus Y (PVY). The biological activity of PVY has been tested using cultivated tobacco plants (*Nicotiana tabacum* L.) and bell pepper plants (*Capsicum annum* L.). It has been found that injection of plants with the virus has led to the appearance of PVY infection symptoms with further death of the plants. The threshold limit value (TLV) for Ti NPs in embryonic pig kidney cell line culture is 12.5 μg/cm^3. According to the prophylactic and treatment plots, Ti NPs have slight antiviral activity against TV-A strain Dniprovskyi 34, decreasing its titer according to both plots by 0.5 lg TCD_{50}/cm^3. However, according to the virucidal plot at TLV concentration, Ti NPs show high antiviral activity against TV-A strain Dniprovskyi 34, significantly decreasing its titer in embryonic pig kidney cell line culture by 4.46 lg TCD_{50}/cm^3. Ti NPs do not have antiviral activity against PVY, according to the prophylactic and treatment plots. Nevertheless, according to the virucidal plot Ti NPs showed high antiviral activity against PVY, inactivating the virus in the reaction mixture *in vitro*. According to the results of TEM, Ti NPs bind to virus particles. Virus particles with NPs attached to them, deformed and partially destroyed virus particles have been found in samples of virus-containing suspension, which were incubated with Ti NPs. We suggest that Ti NPs are useful as disinfectants to control viral infections, particularly TV-A and PVY.

Keywords: Antiviral, Titanium nanoparticles, Cytotoxicity, Virions, Potato virus

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Introduction

The search for new antiviral compounds and the development of innovative antiviral drugs and disinfectants is a crucial research area in various scientific fields. Agriculture is one of the essential components of the economy, without which humanity's existence is impossible. Thus, there is a need to search for new drugs and compounds that can improve the efficiency of agricultural production. In particular, the development of new methods for treating and preventing viral infections in animals and plants is highly important (Huang et al., 2023). Several viruses can cause significant damage to agricultural production and are challenging to manage. One such virus is Teschovirus A (TV-A), which causes enzootic encephalomyelitis in pigs, also known as Teschen disease (Malik et al., 2020). This virus can also cause reproductive system disorders, diarrhea, pneumonia, pericarditis, and myocarditis. Many European countries do not develop vaccines against TV-A because of the high frequency of sporadic cases that are relatively mild. However, due to a large number

of serotypes, high recombination frequency, and frequent occurrence of new poly antigenic strains, it is necessary to develop a multivalent vaccine, which is a challenging task (Malik et al., 2020). However, infections caused by TV-A remain a topical problem. The possibility of an atypical infection course with damage to various systems of organs and high lethality makes TV-A a dangerous pathogen and causes economic losses. There is also a large number of plant pathogens, which cause economic losses among plant viruses, and they are difficult to control due to various factors.

Potato virus Y (PVY) is among these viruses. This virus infects potato plants and can cause yield losses of up to 80% (van Regenmortel and Mahy, 2009). Necrotic serotype PVY^N can cause necrosis of leaves and tubers, leading to yield losses of up to 100% (Warren et al., 2005). Potato viruses often occur as multi-infections. Thus, simultaneous infection by an ordinary strain of Potato virus Y – PVY and Potato virus X can cause yield losses of up to 63.7% (Kaniewski et al., 1990). PVY can cause major economic losses. Thus, direct and indirect economic losses due to PVY in Idaho, USA, comprise $34 million annually (McIntosh and O'Connell, 2014). Control of PVY is a difficult task. There are no antiviral drugs against it yet, and control methods are limited to the control of seed potato quality, selection of resistant cultivars, quarantine measures, and control of transmission vectors.

In view of the above, the search for new antiviral substances to control viruses in farm animals and crops is a highly important task. Organic compounds that are often used as antiviral substances have certain limitations, as they can be relatively expensive and toxic to humans, animals, and plants. Recently, nanoparticles and other nanomaterials have attracted the increasing attention of researchers as potential antiviral agents. NPs of many simple substances and chemical compounds have high antiviral activity and can have low toxicity. Scientists have shown that various titanium dioxide NMs (TiO_2 NMs) have significant antiviral activity against some viruses in farm animals and crops. Newcastle disease virus (Avian orthoavulavirus 1) (Akhtar et al., 2019), Porcine epidemic diarrhea virus, Transmissible gastroenteritis virus (*Alphacoronavirus* 1) (Kim et al., 2006), and Broad bean strain virus (Elsharkawy and Derbalah, 2019) are suitable for the treatment of viral infections in animals after a detailed study of the mechanisms of their action (Akhtar et al., 2019).

It has been found that various titanium nanomaterials (Ti NMs) have antiviral activity against human viruses and bacteriophages, in particular against influenza A virus (Nakano et al., 2012; Amirkhanov et al., 2015) and bacteriophage MS2 (Liga et al., 2011; Syngouna and Chrysikopoulos, 2017). In addition to the fact that Ti NMs have antiviral activity *in vitro*, scientists report the possibility of their use for the treatment of viral infections (Akhtar et al., 2019). Moreover, Sankar et al. (2014) and other scientists report the effectiveness of TiO_2 NPs for wound treatment in rats (Sankar et al., 2014; Sivaranjani and Philominathan, 2016; Ismail, 2019).

Thus, Ti NMs not only have antiviral activity against various viruses but may also be low in toxicity, which makes them promising candidates in the search for antiviral substances against viruses of farm animals and crops. Therefore, we aimed to investigate the antiviral activity of citrate-stabilized titanium nanoparticles (Ti NPs) against TV-A and PVY viruses, as well as possible mechanisms of their antiviral activity.

Materials and methods

Plants, viruses, and cell lines

The research was conducted from 2021 to 2023 and involved the use of the following experimental plants: culture potato (*Solanum tuberosum*), variety Souvenir Chernihivsky, obtained from Private Joint Stock Company "Scientific and Production Association "Chernihivelitkartoplya"; vegetable pepper (*Capsicum annum L.*), for which the seeds were obtained from National Botanical Garden named after M.M. Gryshko of the National Academy of Sciences of Ukraine; and real tobacco (*Nicotiana tabacum*), obtained from the Transcarpathian Institute of Agricultural Production of the National Academy of Agrarian Sciences of Ukraine (UA).

The experiments used the strain Dniprovskyi 34 of species TV-A, serotype 1 (TV-A1), and the necrotic strain ML 1 of species PVY from the virus collection of the Institute of Agricultural Microbiology and Agroindustrial Production (IAMAP) of the National Academy of Agrarian Sciences of Ukraine (NAAS). The colloidal system

of citrate-stabilized Ti NPs was obtained from a doctor of technical sciences, Kaplunenko V.H., LLC "Nanomaterials, and nanotechnologies". The NPs were obtained by the method of erosion-explosive dispersion (Kosinov and Kaplunenko, 2007). The experiments used a transferable culture of the embryonic pig kidney cell line (EPKT, IAMAP, Kyiv, Ukraine) obtained from the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" of NAAS and tobacco plants (*Nicotiana tabacum* L.). For cell culture growth, M-199 medium (BioTestLab LTD., Ukraine), Eagle's minimal essential medium, 0.5% solution of lactalbumin hydrolysate (LAG), 5% hemoglobin solution, cattle serum (JSC "Konotopmyaso", Konotop, Ukraine), and fetal bovine serum (Sigma-Aldrich, USA) were used.

Refreshment, accumulation, concentration, and purification of viruses

Refreshment of the TV-A strain Dniprovskyi 34 has been performed in EPKT transferable cell culture for three passages. The virus was accumulated in the same cell culture, which was grown in culture vials and flat flasks in an amount sufficient for the research. Cell culture cultivation was performed in flat flasks and test tubes at 37°C. Cells were washed out from the glass using a 0.02% Versene solution heated to 37 °C. The initial concentration of cells in flat flasks was 60,000–100,000 cells/mL in test tubes to 100,000–150,000 000 cells/mL. The pH value of cell suspension was adjusted to 7.2–7.4, and antibiotics were added at the concentrations of 100 IU of benzylpenicillin sodium salt and 100 μg of streptomycin sulfate per 1 cm^3 of the medium.

Tenfold dilutions of the virus-containing suspension in saline solution were prepared in order to evaluate the biological activity of the TV-A strain Dniprovskyi 34. The virus was incubated at a temperature of 37°C. The results of the cytopathic effect (CPE) were evaluated after 3–7 days. The titer of the virus was calculated by the method of Reed and Muench (Reed and Muench, 1938). The degree of the CPE was evaluated by the "++++" system, where each + corresponds to the destruction of 25% of the cell monolayer. Refreshment and accumulation of the PVY strain ML 1 were performed using potato plants (*Solanum tuberosum* L.). Infected potato plants were grown in rooms for vegetation at a temperature of 20–25°C and a photoperiod of 16 h. The apical leaves were sampled in the flowering phase in order to obtain inoculum. The inoculum was further used for indicator plants' inoculation.

Visual observation, an agglutination test with antiserum, and transmission electron microscopy (TEM) of samples were used to identify the presence of PVY in plants. Antiserums used in agglutination tests were produced in the laboratory of virology of IAMAP NAAS. To evaluate the biological activity of the PVY strain ML 1, indicator plants were used: cultivated tobacco plants (*Nicotiana tabacum* L.) and bell peppers (*Capsicum annum* L.). The plants were inoculated at the stage of 3–4 true leaves (4–8 weeks of age) by mechanical inoculation with a preliminary dusting of carborundum (grain size 500–600 mesh). The inoculum was prepared from the leaves of infected potato plants with the addition of a buffer solution at 1:1–1:3 ratios. 0.01 M phosphate buffer saline with pH values 7.2–7.5 was used. After the injection, the surface of the indicator plant leaves was washed with distilled water. Distillation water was used as a control. The plants were grown in rooms for vegetation at a temperature of 20–25°C and a photoperiod of 16 h.

Evaluation of cytotoxicity of NPs in cell culture and their acute toxicity in animals

The cytotoxicity of NPs was studied in EPKT cell culture. A nutrient medium containing the tested NPs at different concentrations was added to the fully formed monolayer of cells. 2-fold dilutions of colloidal systems of NPs were used. Four test tubes with cell culture were used for each concentration. The refreshment of the nutrient medium was also performed in the controls, but the NPs were not added.

The results were evaluated daily within seven days period. The monolayer of cells was examined for the presence of cytotoxic effects of the NPs using an optical microscope and the cytotoxic effects were evaluated by disruption of the integrity of the monolayer. The degree of toxicity was measured by a 4-cross system, where each "+" corresponds to the degeneration of 25% of the area of the cell monolayer.

The acute toxicity of NPs in vivo was studied in white mice. Acute toxicity was assessed according to Test No. 425, which is named Acute Oral Toxicity: Up-and-Down Procedure (OECD Guidelines for the Testing of Chemicals, Section

4). This method allows us to determine the main parameters of toxicity of substances, namely the average lethal dose and its standard error (LD_{50}±SE) for substances with unknown toxicity or to observe the presence or absence of clinical toxicity signs if the LD_{50} is above 2,000mg/kg.

Investigation of the antiviral activity of NPs against viruses TV-A and PVY

Before use in studies, the colloidal system of NPs was diluted to concentrations of 125 μg/cm^3 and 25.0 μg/cm^3, and the pH value was adjusted to neutral. Next, the dilutions were sterilized by autoclaving at a pressure of 0.6 atmosphere for 20 min. The prepared suspensions of NPs were added to nutrient media and buffer solutions at a concentration of 12.5μg/cm^3 before being finalized and added to the cell culture. The antiviral activity of Ti NPs against the studied viruses was investigated using prophylactic, treatment, and virucidal plots. According to the prophylactic plot, either the cell culture or indicator plants were treated with a suspension of Ti NPs at a concentration of 12.5 μg/cm^3. After 24 h, either cell culture or indicator plants were inoculated with viruses. According to the treatment plot, the EPKT cell culture or healthy indicator plants were infected with viruses, after which they were treated with Ti NPs at a concentration of 12.5μg/cm^3.

According to the virucidal plot, the virus-containing suspensions and Ti NPs were mixed at a ratio of 1:1 and kept for 24 h at a temperature of +4°C, after which the EPKT cell culture or indicator plants were inoculated with this mixture. The NPs were used at the threshold limit value (TLV), which is 12.5μg/cm^3 for EPKT cell culture. The difference between titers determined the antiviral activity of Ti NPs. If the titer of the virus decreased in the presence of NPs by 2.0 lg of 50% tissue cytopathic doses (TCD_{50}/cm^3) or more, the TI was calculated. In order to calculate TI, the minimum effective concentration (MEC), i.e., the concentration that reduces the infectious titer of the virus by 1.25–1.5 lg TCD_{50} compared to the titer of the virus in control, was determined. The TI was calculated as the ratio of TLV to MEC. A *t*-test for independent samples was used to compare the infectious activity of virus strains, and the significance of the difference between the two samples was evaluated. The calculated t-values were compared with the table of critical values of t (Cressie and Whitford, 1986), and the significance was determined. The calculations were performed in Microsoft Office Excel software.

The antiviral activity of Ti NPs against PVY was studied in a vegetation experiment. Indicator plants were infected in the phase of 3–4 true leaves by means of mechanical inoculation with preliminary dusting with carborundum (grain size 500–600 mesh). The inoculum was prepared from the leaves of infected potato plants by adding a buffer solution at 1:1–1:3 ratios. 0.01 M phosphate buffer saline with pH values 7.2–7.5 was used. After the inoculation, the surface of the indicator plants' leaves was washed with distilled water. Distillation water was used as a control. The plants were grown in rooms for vegetation at a temperature of 20–25°C and a photoperiod of 16 h.

The severity of the PVY disease in plants was assessed by observation of visible symptoms, TEM, and an agglutination test. The antiserums were produced in the laboratory of virology of IAMAP NAAS. TEM studied the mechanisms of antiviral activity of Ti NPs. The TEM was performed on a transmission electron microscope JEOL JEM–1400 at the Danylo Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The microscopy was performed on copper grids with a carbon-formvar coating at an accelerating voltage of 100kV and a magnification of 12,000–150,000. Samples of the colloidal system of Ti NPs were applied to the grids, dried in air and viewed without further contrasting. Samples of virus-containing suspensions with TV-A or PVY were contrasted with 1% uranyl acetate solution for 30 s before microscopy.

To study the interaction between Ti NPs and virus particles, virus-containing suspensions and the colloidal system of Ti NPs were mixed and incubated. In order to do this, virus-containing suspensions were applied to the grids with the formvar film and air-dried. Next, the colloidal system of Ti NPs was added, and the mixture was kept for one hour, after which the excess of the colloidal system was washed with sterile distilled water. Thus, only the NPs that have bound to the virus particles remained on the grid. The dimensions of the NPs, their aggregates, and virus particles were measured using a scale label in ImageJ 1.53c software.

Statistical processing of results

The primary calculations and data preparation

for statistical analysis were carried out using Microsoft Office Excel. The STATISTICA 8.0 (StatSoft.Inc) program was used for statistical analysis of the data, using either parametric or non-parametric methods depending on the data distribution. The normality of the data distribution was checked using the Shapiro-Wilk test and the Lilliefors test, while homoscedasticity was checked with Levene, Brown-Forsyth, Bartlett's, and Cochran's G-tests. The statistical significance of the data was determined using one-factor analysis of variance (Fisher's Least Significant Difference test and Duncan's New Multiple Range test), t-test for independent samples (Two-sample Student's test), Mann-Whitney U-test, and other relevant methods of statistical analysis.

Results

Refreshment, accumulation, concentration, and purification of viruses

The refreshment, accumulation, concentration, and purification of TV-A strain Dniprovskyi 34 have been performed. The titer of the virus was 6.46 lg TCD_{50}/cm^3. CPE manifested after 24–72 h as degenerative changes in cell culture, the appearance of single spherical cells, with a further increase in their number to the destruction of the monolayer. There were no distinct types of CPE (Figure 1). It has been found that according to the "++++" system, the degree of CPE ranged from "+++" to "++++". The refreshment and accumulation of the PVY strain ML 1 have been performed in potato plants. The observation of visible symptoms, agglutination tests, and TEM of sap samples have confirmed the presence of the virus. The biological activity of the PVY strain ML 1 has been tested using indicator plants. It has been found that the plants developed symptoms of necrotic PVY infection after inoculation (Figure 2). Such symptoms as lightening of the veins and an indistinct mosaic on the apical leaves appeared within 5–7 days after injection. Chlorosis, necrosis of leaf veins, deformation of leaves, deformation of veins, reduction of leaf size, and growth retardation were observed after 10–14 days (Figure 2). The death of plants occurred on the 28th day. Control plants remained asymptomatic throughout the whole observation period. The presence of the virus in the infected plants has been confirmed by the observation of visible symptoms, agglutination test, and TEM of sap samples.

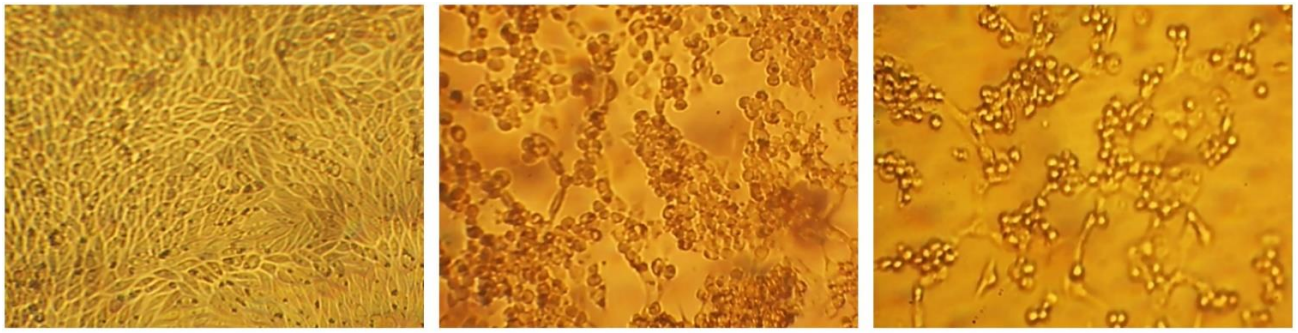


Figure 1: Cytopathic effect of Teschovirus A (TV-A) strain Dniprovskyi 34 in embryonic pig kidney cell line : (a) control of cell culture without virus; (b) Cytopathic effect of 50–75% (+++) after 24–48 h; (c) the Cytopathic effect of >75% (++++) after 48–72 h.

The cytotoxicity of NPs in cell culture and their acute toxicity in animals

It has been found that the TLV of Ti NPs is 12.5μg/cm^3. The Ti NPs were used at TLV for further studies of antiviral activity. Evaluation of the antiviral activity of NPs is possible only at TLV or lower concentrations. Degenerative changes in the EPKT cell culture were observed with the use of higher concentrations of Ti NPs: disruption of the integrity of the monolayer, rounding, flatness, and groove of cells, vacuolization, and granularity of cells, changes in the nutrient medium color (Figure 3).

Evaluation of acute toxicity was performed according to Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure (OECD Guidelines for the Testing of Chemicals, Section 4), which is described above. It has been found that Ti NPs are not toxic to white mice at a concentration of 2,000mg/kg.

Figure 2: Biological activity of the Potato virus Y (PVY) strain ML 1 in cultivated tobacco plants: Control plants without PVY (a and b); plants showing the symptoms of PVY infection in infected plants (c and d).

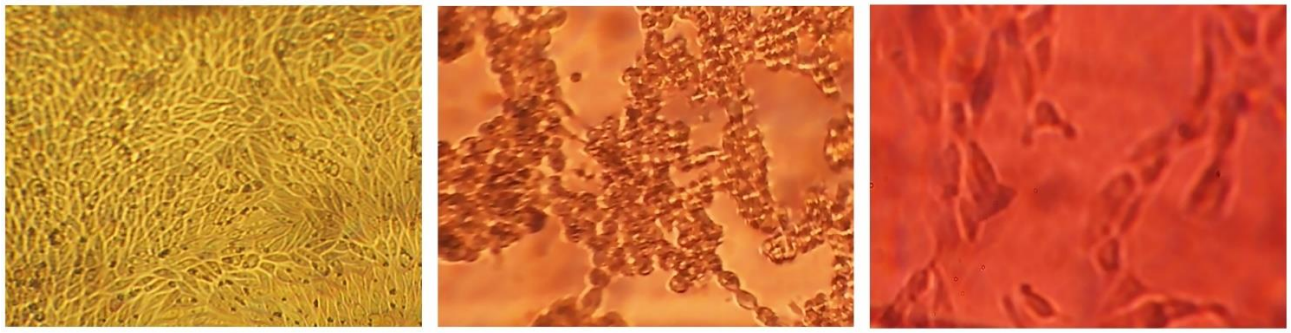


Figure 3: Degenerative changes in the transferable culture of embryonic pig kidney cell line under the influence of high concentrations of Ti nanoparticles: (a) the cell culture in the absence of nanoparticles; (b, c) degenerative changes of cells under the influence of Ti nanoparticles.

Antiviral activity of Ti NPs against TV-A strain Dniprovskyi 34

Studies according to prophylactic and treatment plots were performed in the EPKT cell culture in order to determine the antiviral activity of Ti NPs. It has been found that Ti NPs have insignificant. antiviral activity, reducing the virus titer according to both plots by only 0.50 lg TCD_{50}/cm^3. The virucidal activity of Ti NPs has been investigated. It has been found that after the exposition within 24 h at the TLV, Ti NPs show high virucidal activity against the TV-A1 strain Dniprovskyi 34, significantly reducing its infectious titer in EPKT cell culture by 4.46 lg TCD_{50}/cm^3. The dependence of the virucidal activity of Ti NPs at the TLV against the TV-A1 strain Dniprovskyi 34 on the exposure time has been studied. It has been found that the virucidal activity of Ti NPs manifests from the first hour and increases over time (Table 1).

At the next stage, MEC and TI for Ti NPs were determined. The results are presented in Table 2. It has been found that Ti NPs at the TLV reduced the virus titer by 4.46 lg TCD_{50}/cm^3 within 24 h.

Table 1: Virucidal activity of titanium nanoparticles (Ti NPs) at the threshold limit value (TLV) against the Teschovirus A (TV-A1) strain Dniprovskyi 34 in the embryonic pig kidney cell line, depending on the exposition time.

Exposition time in an hour	Treatment	Virus titer, lg TCD_{50}/cm^3	Differences in the titers, lg TCD_{50}/cm^3
1	Ti NPs	3.67±0.11	2.56*
	Control	6.23±0.12	–
3	Ti NPs	3.23±0.10	3.00*
	Control	6.23±0.12	–
12	Ti NPs	2.77±0.10	3.46*
	Control	6.23±0.12	–
24	Ti NPs	1.77±0.12	4.46*
	Control	6.23±0.12	–

*According to the results of the t-test for independent samples, the difference between virus titers in the control and treatment with Ti NPs is statistically significant. * refers to a significance level of $p<0.05$.

Table 2: Virucidal activity of titanium nanoparticles (Ti NPs) against the Teschovirus A (TV-A1) strain Dniprovskyi 34 in the embryonic pig kidney cell line cell culture at different concentrations (exposition time 24 h).

Treatment	Concentration of nanoparticles, µg/cm^3	Virus titer, lg TCD_{50}/cm^3	Differences in the titers, lg TCD_{50}/cm^3	Therapeutic index
Ti NPs	12.5	1.77±0.12	4.46*	10
	1.25	2.23±0.14	4.00*	
	0.5	2.77±0.12	3.46*	
	0.25	3.50±0.18	2.73*	
	0.125	5.00±0.14	1.23*	
	0.062	6.50±0.12	0.73*	
Control	–	6.23±0.12	–	–

*According to the results of the t-test for independent samples, the difference between virus titers in the control and treatment with Ti NPs is statistically significant. * refers to a significance level of $p<0.05$.

At a concentration of 0.125µg/cm^3, the virus titer decreased to 1.23 lg TCD_{50}/cm^3. We consider this concentration to be MEC. Thus, the TI for Ti NPs is 10.

Therefore, Ti NPs have high antiviral activity against TV-A and can be recommended for the development of antiviral drugs, virucidal substances, and disinfectants.

Antiviral activity of Ti NPs against PVY

The antiviral activity of Ti NPs against the PVY strain ML 1 has been investigated in cultivated tobacco plants according to the prophylactic, treatment, and virucidal plots. It has been found that Ti NPs do not show antiviral activity against PVY according to both prophylactic and treatment plots. It has been found that Ti NPs have high antiviral activity according to the virucidal plot (Figure 4).

For 28 days of observation, experimental plants did not show symptoms of viral infection and remained healthy (Figure 4a), as did plants in negative control (Figure 4b). According to the results of agglutination tests and TEM, there was no PVY in these plants, and no visible symptoms of PVY infection have been observed. Inoculation of control plants with PVY strain ML 1 caused the appearance of necrotic infection symptoms (Figure 4c and d). First, lightening of the veins and an indistinct mosaic on the apical leaves were observed (Figure 4c). Further, the development of infection was manifested as necrosis and deformation of leaves and necrosis of leaf veins, which is typical for necrotic PVY strains (Figure 4d). Both the agglutination test and TEM confirmed the presence of PVY in the plants. Thus, Ti NPs inactivate PVY in the reaction mixture *in vitro*, according to the study's virucidal plot.

According to the TEM results, the samples of the colloidal system Ti NPs contain spherical NPs with sizes from 2 to 22nm, which are either discrete or form aggregates with sizes from 128 to 888nm (Figure 5a and b).

Morphology of the TV-A strain Dniprovskyi 34 virus particles

According to the TEM results, the samples of

virus-containing suspension with TV-A strain Dniprovskyi 34 contain spherical virus particles with a size of 25–30nm, which morphologically corresponds to the typical virus particles of TV-A (Figure 5c).

Interaction of Ti NPs with virus particles of TV-A strain Dniprovsky 34

It has been found that Ti NPs interact with TV-A virus particles; in particular, they adsorb on the surface of virus particles and affect their morphology (Figure 6). Intact TV-A virus particles with sizes of 25–27nm surrounded by Ti NPs (Figure 6a), TV-A virus particles (size 29nm) with Ti NPs (size 12nm) adsorbed on their surface (Figure 6b), Ti NPs with sizes 3–10nm (Figure 6 c) and particles similar morphologically to TV-A virus particles, but with smaller sizes (Figure 6d) have been found in the sample of virus-containing suspension with TV-A, which was incubated with Ti NPs.

Morphology of PVY virus particles

According to the TEM results, the samples of virus-containing suspension with PVY contain non-enveloped filamentous virus particles with a length of 603–770nm, which corresponds to the morphological features of the typical PVY virus particles (Figure 7).

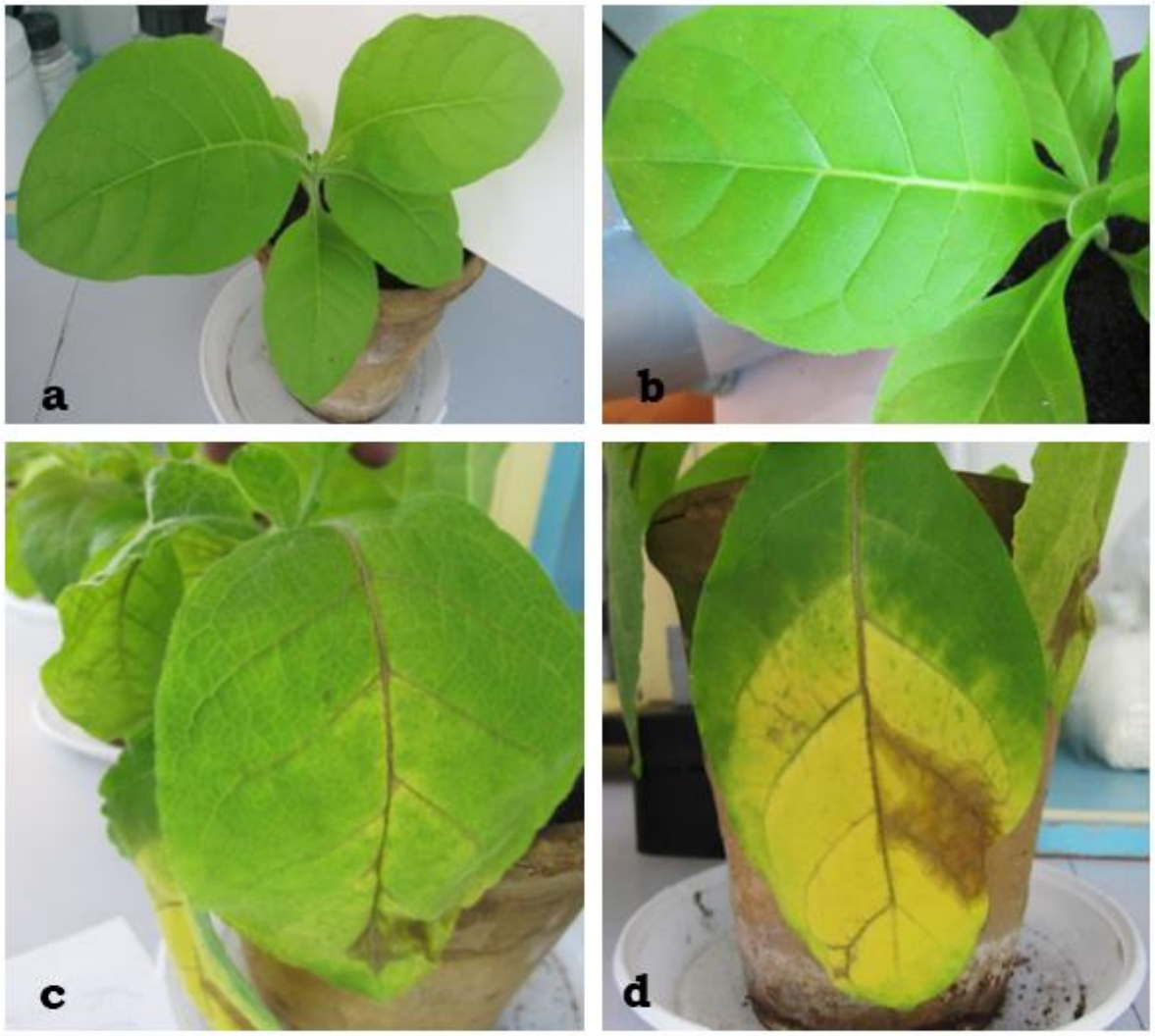


Figure 4: Study of antiviral activity of Ti nanoparticles against Potato virus Y in tobacco plants: (a) experimental plants without symptoms; (b) negative control; (c, d) symptoms of Potato virus Y infection (positive control).

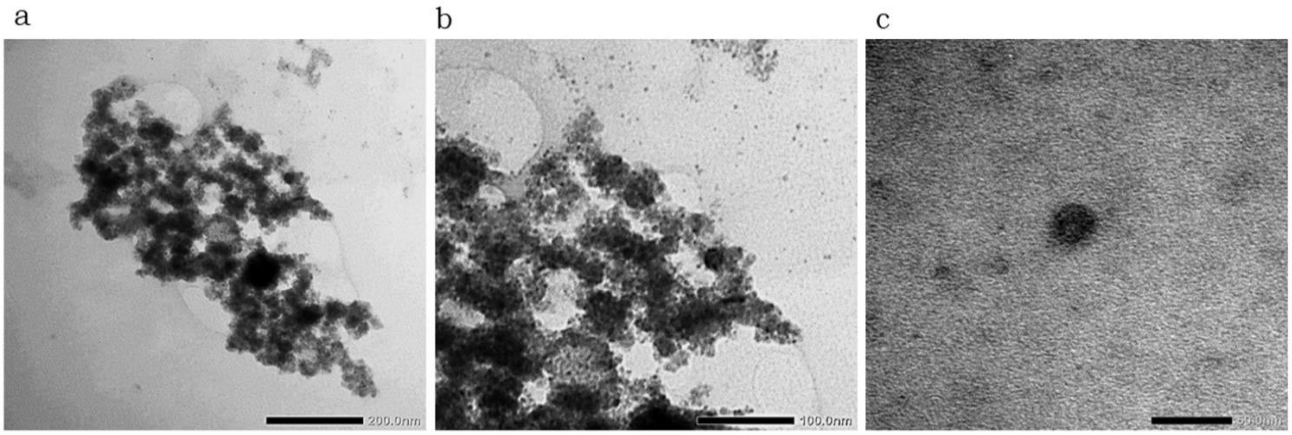


Figure 5: Transmission electronic microphotographs of a sample of a colloidal system of Ti nanoparticles (a, b) and sample of virus-containing suspension with Teschovirus A (c).

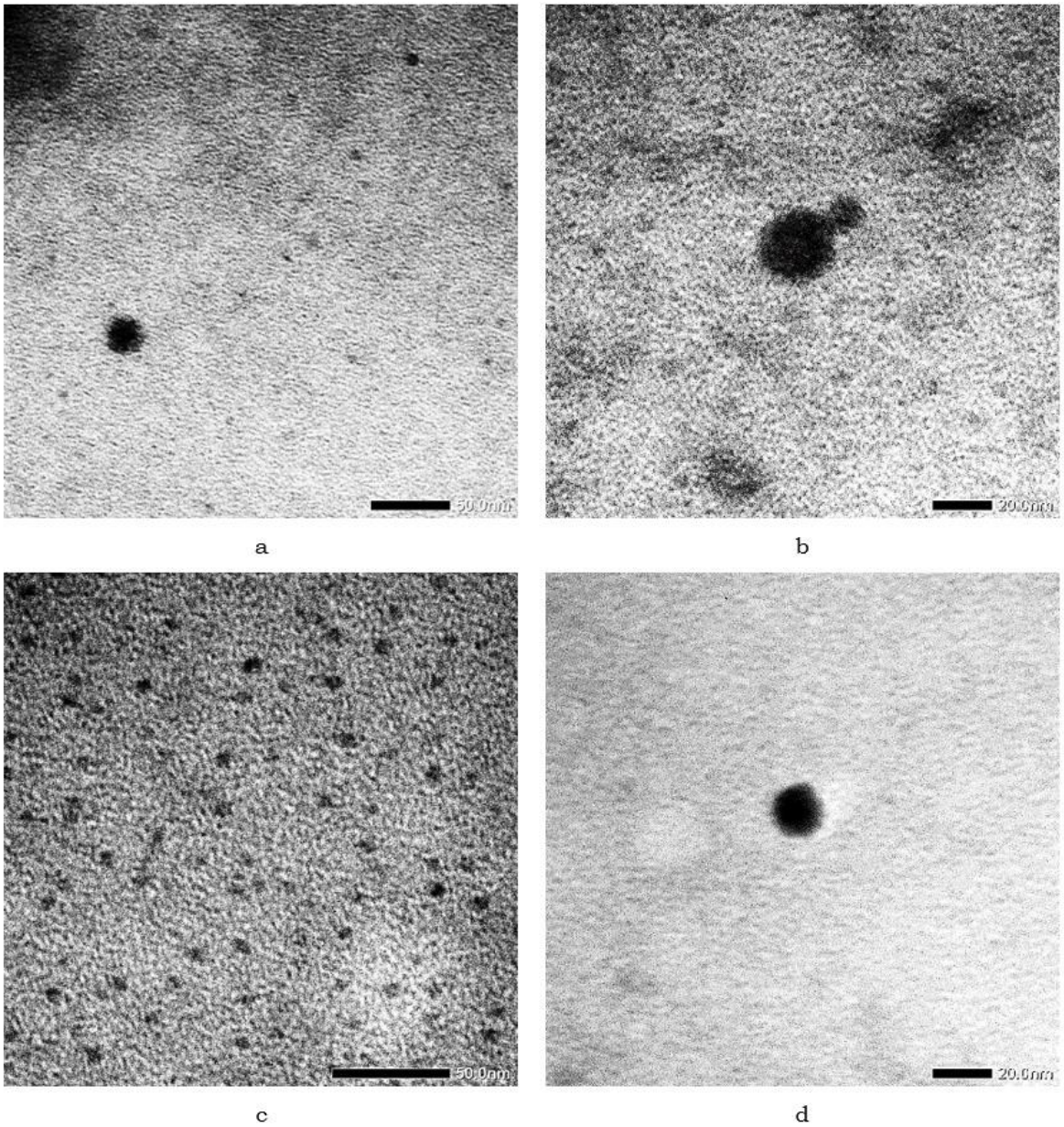
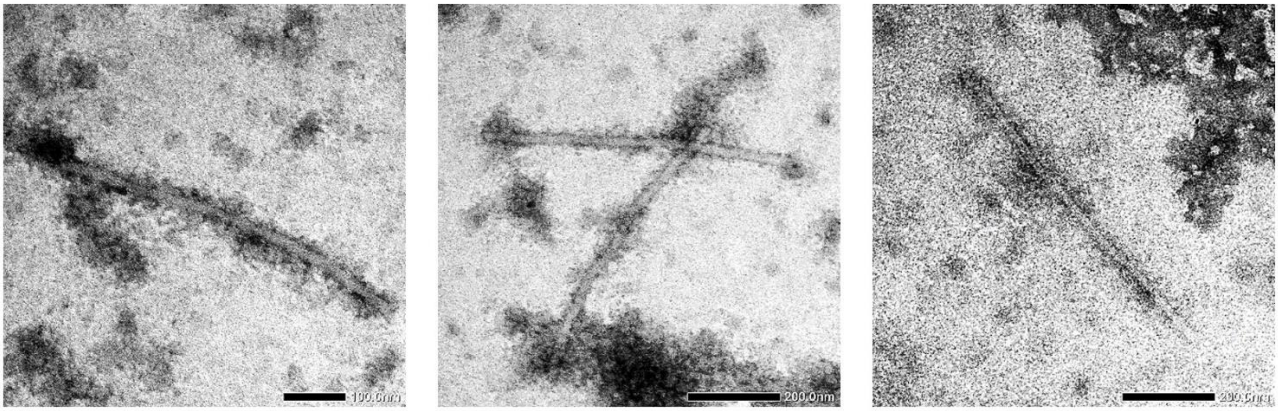


Figure 6: Transmission electron micrographs of a sample of virus-containing suspension with Teschovirus A (TV A), which was incubated with Ti nanoparticles. (a) virus particle of normal size (26.5nm) surrounded by Ti nanoparticles; (b) TV-A virus particle with Ti nanoparticle adsorbed on its surface; (c) titanium nanoparticles present in the sample; (d) virus particle of reduced size.

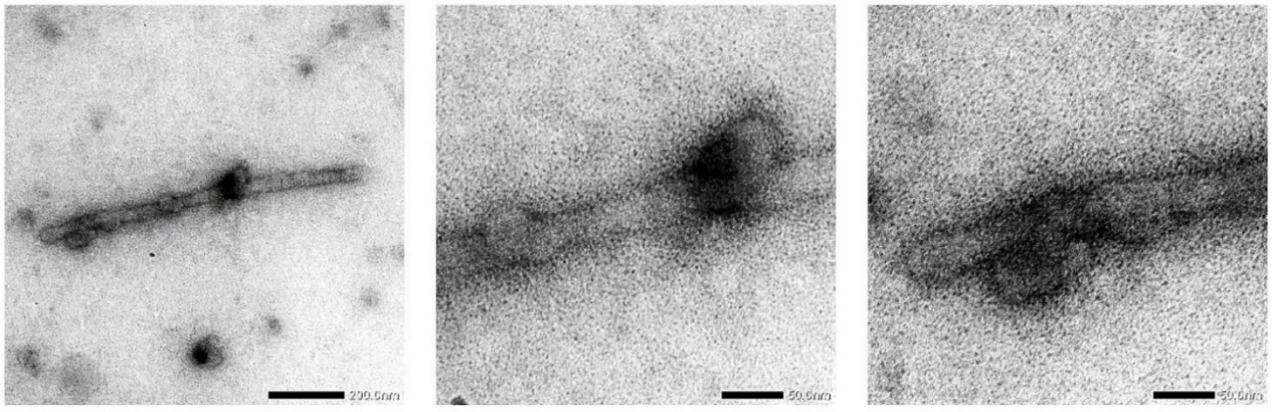


a b c

Figure 7: Transmission electron micrographs of a sample of virus-containing suspension with Potato virus Y.

Interaction of Ti NPs with PVY virus particles

According to the TEM results of the samples of virus-containing suspension with PVY incubated with the colloidal system of Ti NPs, Ti NPs are able to adsorb on the surface of PVY virus particles and influence their morphology (Figure 8-10). Thus, Figure 8 shows a PVY virus particle (length 870 nm) surrounded by small electron-dense particles with sizes of 2–6 nm, which are apparently Ti NPs. Figure 9 shows a virus particle with a length of 750nm and altered contrasting surrounded by small spherical electron-dense particles with sizes 2–8nm. The small particles are scattered around the virus particle, but some are located directly on its surface. This indicates that Ti NPs can adsorb on the surface of PVY virus particles.



a b c

Figure 8: Transmission electron micrographs of the Potato virus Y particle, which titanium nanoparticles surround. (a) general view of the particle at low magnification; (b, c) fragments of the virus particle at high magnification, surrounded by small electron-dense particles.

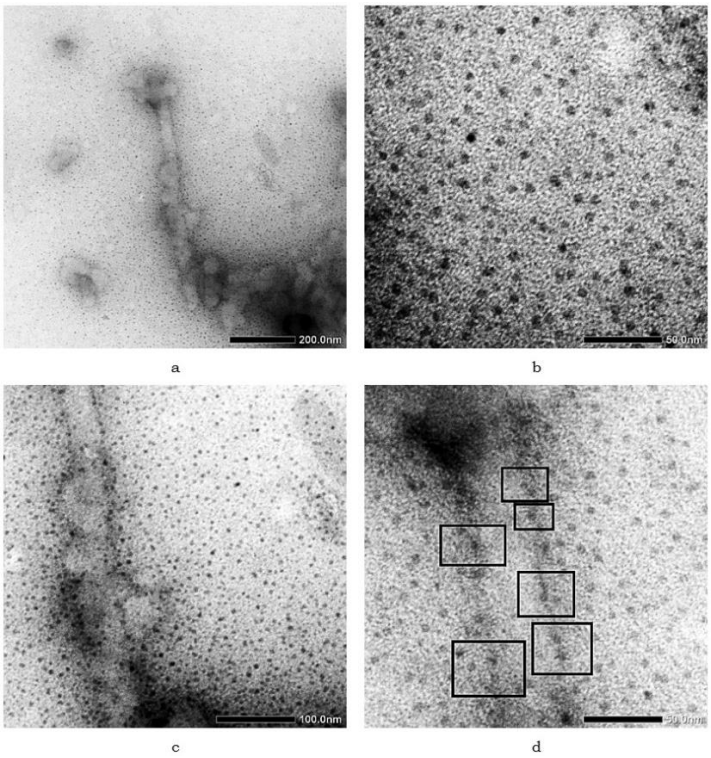


Figure 9: Transmission electron micrographs of the Potato virus Y particle, which titanium nanoparticles surround. (a) general view of the particle at low magnification; (b) NPs surrounding the virus particle; (c, d) enlarged areas of the virus particle (NPs located on the surface of the virus particle are highlighted with rectangles).

It should be noted that the virus particle has an altered contrast. Thus, the contours of the virus particle are quite contrasted. However, in general, the virus particle looks much less contrasted than the virus particles in control without NPs (Figure 10). The central part of the particle looks especially weakly contrasted (Figure 10a), which can indicate the absence of the virus genome in the central canal.

Fragments of PVY virus particles, some of which were deformed, have also been found in samples of virus-containing suspension incubated with the colloidal system of Ti NPs (Figure 10). Thus, figure 10a shows two fragments of PVY virus particles with a length of 250 (fragment on the left) and 370 (fragment on the right) nm. Figure 10b shows a structure that is apparently a cluster of virus particle fragments 130–210nm long around a deformed virus particle 530nm long. Figure 10c shows a virus particle with a length of 900 nm and a fragment of a virus particle with a length of 230nm.

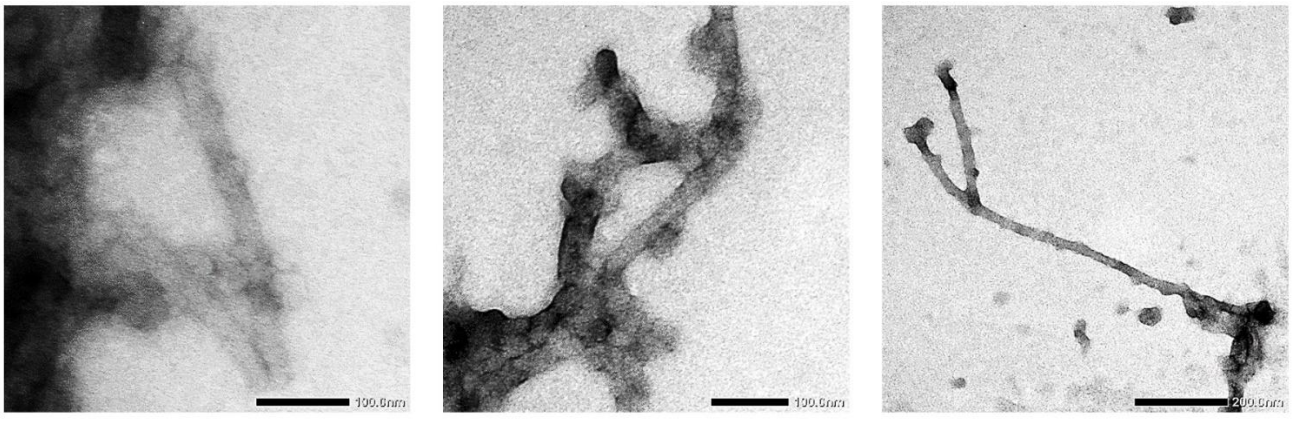


Figure 10: Transmission electron micrographs of deformed Potato virus Y particles treated with titanium nanoparticles.

Discussion

It has been discovered that Ti NPs have the ability to reduce or eliminate the virulence of TV-A and PVY viruses. This is achieved by mixing the virus-containing suspension with the colloidal system of Ti NPs and allowing them to incubate together for 24 hours. The antiviral activity of Ti NPs is due to their adsorption on the surface of virus particles, which can cause deformation and fragmentation of the virus particles. This interaction between Ti NPs and virus particles is believed to be the mechanism behind the high virucidal activity of Ti NPs when they come into direct contact with the virus-containing suspension. TEM microphotographs have revealed four phenomena associated with the interaction of NPs and virus particles: adsorption of NPs on the surface of virus particles, the appearance of virus-like particles that are smaller in size, fragmentation of virus particles, and deformation of virus particles. Observations have shown that NPs are adsorbed on the surface of both TV-A and PVY virus particles. Single Ti NPs are adsorbed on the surface of the TV-A virus particles, while a large number of NPs are concentrated around the PVY virus particles, forming a denser layer directly near the surface of the virus particles. Some NPs are located directly on the surface of the capsids, which is evidence of adsorption.

Lozovski et al. (2012) have reported that NPs can form stable systems with virus particles, known as “virus-nanoparticle” systems. We have also observed the formation of such systems on micrographs. According to Lozovski and colleagues, these systems are stable and are formed due to the action of local fields of NPs (Lozovski et al. 2012). However, the geometry of the "virus-nanoparticle" system is different from the intact virus particle, which reduces its ability to penetrate sensitive cells. This may prevent the attachment and penetration of the virus particle to susceptible cells since the correct geometry of the virus particle is crucial for this purpose.

It is possible that the activity of NPs can be attributed to a physical mechanism where the spatial structure of proteins and glycoproteins that interact with host cell receptors is altered. NPs can create local fields that affect the spatial conformation of these molecules or destroy them, which can lead to a reduction or loss in the ability of virus particles to penetrate susceptible cells. The deformation and fragmentation of virus particles and their size reduction, as observed by TEM, can also be caused by NPs. These effects may be due to the action of local fields of NPs on the spatial conformation or destruction of proteins and glycoproteins. Overall, these findings suggest that changes in the spatial conformation or destruction of proteins and glycoproteins can explain the deformation, fragmentation, and size reduction of virus particles. (Lozovski et al., 2012).

According to a study by Elechiguerra et al. (2005), silver nanoparticles (Ag NPs) bind to the gp120 glycoprotein spikes of HIV-1 virus particles. However, the study did not explain the physical mechanisms of this interaction. The researchers suggested that the most likely binding sites for NPs are the disulfide bonds of the gp120 glycoprotein, which has nine such bonds. Additionally, Elechiguerra et al. (2005) noted that Ag NPs probably interact most strongly with cysteine residues containing thiol groups when binding to bovine serum albumin used as a coating agent. TV-A surface proteins include 1B (VP2), 1C (VP3), and 1D (VP1). According to the Protein database, VP2 protein contains eight cysteine residues and two methionine residues, VP3 protein contains three cysteine residues and nine methionine residues, and VP1 protein – 5 cysteine residues and eight methionine (Doherty et al., 1999). Depending on the spatial conformation, cysteine residues can form disulfide bonds, which are probable sites of NPs binding. In addition, according to Elechiguerra et al. (2005) thiol groups of cysteine residues by themselves are able to interact with NPs strongly. Since TV-A surface proteins contain cysteine residues, they are apparently able to interact with NPs.

It is likely that Ti NPs, like Ag NPs, interact with disulfide bonds and thiol groups of cysteine residues. The interaction may be due to the local fields of NPs. The TV-A surface proteins contain cysteine and methionine residues that have a sulfur atom, making it possible for NPs to interact with these proteins strongly. The PVY capsid is made up of coat (capsid) protein (CP). As in the case of TV-A surface proteins, the CP protein of PVY contains cysteine and methionine residues, which may explain the binding of Ti NPs to PVY virus particles (Elechiguerra et al., 2005).

Reactive oxygen species (ROS) generation can be another possible mechanism of viruses’ inactivation by Ti NPs, according to the virucidal

plot of studies. According to Ma et al. (2012), nanoparticles of titanium dioxide (TiO_2 NPs) generate ROS under the influence of sunlight in aqueous suspension. UV-A (315–400nm) has been found to be the most important band of the spectrum, which is crucial for ROS generation. There is no significant reduction in the generation of ROS by TiO_2 NPs when sunlight passes through the glass of Petri dishes, optical microscope filters, or silicate window glass since these types of glass significantly remove UV-B band (280–315 nm), but remove only a small fraction of UV-A band. UV-A is contained in sunlight in quantities sufficient for ROS generation by TiO_2 NPs (Ma et al., 2012). The radiation of incandescent lamps also contains a certain percentage of UV-A. Window glass, glass of Petri dishes, and glass vials in which the colloidal systems of TiO2 NPs can be stored do not absorb the UV-A waves, which makes ROS generation possible in the presence of artificial light.

According to Murray et al. (2008), ozone-induced ROS inactivate both enveloped (Human alpha herpesvirus 1, Indiana vesiculovirus, and Vaccinia virus) and non-enveloped viruses (Human adenovirus type 2). Ozone is ROS itself. In addition, it produces other ROS, such as H_2O_2. According to Murray et al. (2008) ROS inactivates enveloped viruses due to lipid peroxidation, which destroys the envelope of the virus particle and makes binding of the virus to a susceptible cell impossible. Inactivation of non-enveloped viruses occurs due to the peroxidation of proteins, which also disrupts the normal binding of the virus to a susceptible cell. Virus anti-receptors and lipid envelopes can be the main targets of ROS. Non-enveloped viruses show slightly higher resistance to ROS but are also inactivated by ROS (Murray et al., 2008).

It is likely that the inactivation of TV-A and PVY by Ti NPs involves multiple mechanisms, including the following: i) The action of local fields of NPs creates stable "virus-nanoparticle" systems. These systems have a different geometry from intact virus particles, which reduces or completely disrupts the virus's ability to infect susceptible cells. ii) The spatial conformation of virus molecules can be altered, or the action of local fields of NPs can destroy the molecules. This changes the geometry of the virus particle and can destroy the virus's anti-receptors, which are essential for binding to susceptible cells. iii) NPs attach to capsid proteins by strongly binding to disulfide bonds and thiol groups of cysteine residues. This can affect the spatial structure of the virus particle and block anti-receptors, preventing them from binding to susceptible cells. iv) NPs generate ROS, which can inactivate viruses. Sunlight and artificial light are sufficient to generate ROS by NPs.

Conclusions

Ti NPs have been found to have antiviral activity against TV-A and PVY. This activity is only observed in the virucidal plot of the study, while the prophylactic and treatment plots do not show any significant antiviral activity. After incubation of a mixture of a colloidal system of Ti NPs and virus-containing suspension with TV-A, the infectious titer of the virus in the EPKT cell culture was significantly reduced by 4.46 lg TCD_{50}/cm^3. Similarly, plants inoculated with a mixture of the colloidal system of Ti NPs and virus-containing suspension with PVY did not show any symptoms of virus infection throughout the observation period, and the absence of the virus in plants was confirmed by agglutination test and TEM.

The TEM analysis showed that Ti NPs can bind to TV-A and PVY virus particles. Deformed virus particles and virus particles of reduced size have also been found in the samples of virus-containing suspensions incubated with a colloidal system of Ti NPs. This indicates that the direct interaction of NPs with virus particles is one of the mechanisms of antiviral activity of Ti NPs. The literature suggests that the binding of NPs to virus particles can lead to several consequences that can inactivate the virus, such as changes in the geometry of the virus particle, spatial conformation of proteins, destruction of proteins, and blocking of anti-receptors. Other possible mechanisms of antiviral activity of Ti NPs include the generation of ROS, which can damage capsid proteins.

Ti NPs are considered to have a high potential as an antiviral agent, and their low toxicity makes them a more favorable option than commonly used disinfectants. They are even used to facilitate wound healing. Therefore, they are considered promising for the development of novel methods to control TV-A and PVY viruses.

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