Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 08 | Issue :01 |Jan.-Mar.| 2018
Pages: 100-111

Evaluation of Silver Nanoparticles as Antiviral Agent Against ToMV and PVY in Tomato Plants

Noha K. El-Dougdoug [1], A.M. Bondok [2] and K.A. El-Dougdoug [3]

[1]*Botany Dept., Fac. of Sci., Benha Univ., Egypt.*
[2]*Plant Pathology Dept., Fac. of Agric., Ain Shams Univ., Egypt.*
[3]*Microbiology Dept., Fac. of Agric., Ain Shams Univ., Egypt.*

Received: 06 Nov. 2017 / Accepted: 07 Dec. 2017 / Publication date: 10 Feb. 2018

ABSTRACT

The current study was carried out during the season of 2016 under greenhouse conditions to evaluate the efficiency of silver nanoparticles (AgNPs) as antiviral reagent to induce a systemic acquired resistance (SAR) against *Tomato mosaic virus* (ToMV) and *Potato virus Y* (PVY) and suppress their infection on tomato plants. Silver nanoparticles (AgNPs) were sprayed 7 days before inoculation with both viruses at 50, 60 and 70 ppm. The results showed that disease severity as well as relative concentration of both viruses were decreased dramatically by the treatment of AgNPs at 50 ppm compared to the other treatments. Examination the clarified sap of both viruses with TEM showed morphological evidenced that AgNPs bind to coat protein virus particles. The induction of SAR against ToMV and PVY was confirmed chemically by several parameters as indicated by increasing photosynthetic pigments, total soluble protein and the activities of peroxidase (POD) and polyphenol oxidase (PPO) compared with infected control

Key words: Tomato, ToMV, PVY, Silver nanoparticles, total soluble Protein, antioxidant enzymes, TEM.

Introduction

Plant viruses are worldwide in nature and cause severe diseases in different plants. They caused serious losses in crop quality and yield. Out of 15% of the global food production is lost due to plant diseases, virus diseases account for 47% of this loss (Anderson *et al*., 2004; Popp and Hantos 2011; Boualem *et al*., 2016). These effects might be attributed to the changes which occurred at the physiological, molecular and biochemical levels (Bondok and Ibrahim., 2014; Ibrahim *et al*., 2015).

Tomato virus diseases consider one of the most important problems facing tomato production in many countries (Daniela *et al*., 2009; Torsten *et al*., 2009 and Pradeep and Masato, 2010). Among these viruses TMV and PVY were known to be distributed worldwide under warm conditions. causing severe damage to tomato plants and significant losses in their yield (Shukla *et al* 1994; Sutic *et al*., 1999; Mohamed, 2010; Binyam, 2015).

Recently nanoparticles (NPs) show improved properties compare to bulk material and have applications in different fields including DNA sequencing, surface-enhanced Raman spectroscopy and pharmaceuticals. Moreover, it is well documented that NPs have an important role in disease management since they could be used as antimicrobials in addition to helping in the bimolecular detection, diagnostics, DNA sequencing and surely reduce viral infectivity of cultured cells (Panáček *et al* 2006; Murphy 2008; Rai *et al* 2009).

One of the most promising uses for this technology is depending on silver nanoparticles (AgNPs) which had been proved to have several properties in this respect ranged from the antimicrobial potential against bacteria, pathogenic viruses in human (Galdiero *et al*., 2011) and plants (Radwan *et al*., 2008).

The current study was carried out to evaluate the efficiency of silver nanoparticles at different concentrations suppression of the virus infection and to induce the systemic acquired resistance (SAR) against *Tomato mosaic virus* (ToMV) and *Potato virus Y* (PVY) in tomato plants under protective greenhouse condition.

.**Corresponding Author:** K.A. El-Dougdoug, Microbiology Dept., Fac. of Agric., Ain Shams Univ., Egypt
E-mail: drdougdoug@yahoo.com

Materials and Methods

Plant materials:

Pot experiment were conducted on the 25th of March 2016 under greenhouse conditions to investigate the effect of silver nanoparticles (AgNPs) to induce systemic resistance on ToMV and PVY in tomato plants. Healthy tomato seeds (*Lycopersecon esculantum)* cv. Stren B sown under greenhouse conditions (25±5 ◦C), in trays with 50 individual cells (4X4X6) containing a sterilized sandy-loamy soil (2:1 w/w). After 15 days, seedlings uniform in size were selected for transplanting in pots 25 cm in diameter filled by 8 kg soil in insect proof cages under greenhouse conditions; Plant Pathol. Dept., Fac. of Agric., Ain Shams Univ. Cairo, Egypt.

The Foliar Treatments of AgNPs:

Silver nanoparticles were purchased from Sigma in a liquid form. Tomato seedlings at the four-leaf stage were uniformly foliar-applied with AgNPs at concentration of 50, 60, 70 and 80 ppm. The foliar applications were sprayed on the plants until runoff on both plants at 7 days before virus inoculation with ToMV and PVY.

Source of viruses:

ToMV and PVY kindly provided by Plant Virus Lab., Plant Pathol. Dept., Fac. of Agric., Ain Shams University and confirmed on differential hosts (Table,1). ToMV was propagated on *Nicotiana tabacum* cv. white burly and PVY was propagated on *Datura metel*.

Differential host:

ToMV and PVY were confirmed by observing the viral infection symptoms that developed on differential host plants. were observed on ToMV inoculated leaves of *Ch. amranticolor; D. stramonium ; D.metle ; N. glatinosa* ; *Ch.quinoa* and *N.tabacum cv* .white burly.

Experimental design:

All treatment application was conduct under two different conditions, first without inoculation with TMV or PVY as control and the second inoculated with TMV or PVY. Twenty-one days old tomato plants (150 plants) were divided to 15 treatments, each one contains 10 pots with ten plants. were maintained for each treatment. All the pots were arranged in a completely randomized block design (22 - 28 ºC) and watered as needed. Tomato healthy plants were mechanically inoculated with ToMV and PVY using phosphate buffer solution (Garcia-cano *et al*., 2006) as follow in Table (1). Symptoms were recorded 20 days after inoculation.

Table 1: Foliar application treatments under greenhouse conditions.

Treatment	Concentration
Negative Control	Distilled water
Positive control ToMV	-
Positive control PVY	-
AgNPs	50 ppm
AgNPs	60 ppm
AgNPs	70 ppm
AgNPs	80 ppm
AgNPs 7 dpi ToMV	50 ppm
AgNPs 7 dpi PVY	50 ppm
AgNPs 7 dpi ToMV	60 ppm
AgNPs 7 dpi PVY	60 ppm
AgNPs 7 dpi ToMV	70 ppm
AgNPs 7 dpi PVY	70 ppm
AgNPs 7 dpi ToMV	80 ppm
AgNPs 7 dpi PVY	80 ppm

Evidences of SAR induction

Virus infectivity:

Twenty days after TMV and PVY inoculation; The percentage of virus infection was determined and calculated relatively to the infected control. For determination of disease severity (DS), all plants in each treatment were examined weekly for virus symptoms development. The symptoms were recorded using the following rating scale: 0 = no symptoms, 2 = vein clearing, 4 = 50% of leaves showing mosaic symptoms, 6 = 100% of leaves showing mosaic symptoms, 8 = 50% of leaves showing severe mosaic and malformation and 10 = 100% of leaves showing severe mosaic and malformation. Disease severity values were calculated using the following formula according to Yang *et al.* (1996).

$$(DS) = \frac{10\Sigma \text{ (disease grade} \times \text{number of plants in each grade)}}{\text{total number of plants} \times \text{highest disease grade}} \times 100$$

Relative concentration of TMV and PVY:

Enzyme-linked immunosorbent assay (Clark and Adams, 1977) was used to determine ToMV and PVY levels in sap of young leaves of inoculated tomato plants30 days after inoculation. Absorbance values were determined using ELISA reader (BIE & BERNTSEN A.S) at 405 nm [1 hr. after addition of the substrate].

Determination the effect of silver nanoparticles of ToMV on Nicotiana glutinosa:

Three concentrations of silver nanoparticles were examined separately on ToMV crud sap 0 distilled water (+ control), 50, 60 and 70 ppm respectively on average number of local lesions of ToMV on *Nicotiana glutinosa* inoculated leaves by add 1 ml of each concentrate on 1 ml of ToMV crude sap then mixed well followed by incubation for 10 min. then inoculated on ten replicate *N. glutinosa* plants for each concentrate.

Effect of Silver nanoparticles on ToMV or PVY in partial purified preparation:

ToMV and PVY partial purified preparation obtained from infected *Nicotiana tabacum* cv. Samson leaves treated with Silver nanoparticles at concentration 50 ppm were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. The film on the grid was allowed to dry by putting it under a mercury lamp for 5 min. The grids were examined by Transmission electron microscopic (Joel JEM-1400 TEM machine), Al Azher University.

Total soluble protein (TSP): was estimated according to the method of Bradford (1976) by using bovine serum albumin as a standard protein. Protein concentration was adjusted to 2 mg / ml per sample.

Peroxidase activity (POD) was estimated according to the method of Kochba *et al.* (1977). Peroxidase activity was estimated by measuring the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 at 425 nm.

Polyphenoloxidase activity (PPO) was assayed in a reaction mixture composed of phosphate buffer (0.2 M, pH 7.0); catechol (100 mM) and crude extract (0.5 mL) and incubated at 50°C for 3 mins. according to Maria *et al* (1981)and estimated at 410 nm O.D. by spectrophotometer (spectronic 601) as the change in O.D.g $^{-1}$fresh weight mins^{-1} (Mayer 1987).

Photosynthetic pigments:

Total chlorophyll a, b and carotenoids concentration in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001).

Proline determination:

Proline colorimetric determination proceeded according to Bates *et al.* (1973), Marín *et al*., (2009) based on proline's reaction with ninhydrin.

Total soluble Phenol compounds:

One gm of fresh tomato leaves were macerated in 50 ml 80% ethanol for at least 24 hr at 0°C, the alcohol was clarified the remained residue was extracted with 10 ml 80% ethanol 3times. At the end, the clarified extract was completed to 50 ml using 80% ethanol. Extraction and determination of phenolic compounds according to method described by Daniel and George (1972) using UV-spectrophotometer (UNICO 2000) at wave length 725 nm.

Statistical analysis:

The experiment layout was a completely randomized design (CRD). All percentages were transformed to arcsine to be analyzed. Data were subjected to convenient statistical analysis methods for calculations of means using MSTATC software. Mean separations was estimated by calculating LSD values at 5% probability level according to Snedecor and Cochran (1980).

Results and discussion

Differential host:

ToMV and PVY infectious sap was confirmed by observing the viral infection symptoms that developed on differential host plants. Necrotic local lesions were observed on ToMV inoculated leaves of *Ch .amranticolor; D. stramonium ; D.metle ; N. glatinosa* and little chlorotic local lesions *on Ch.quinoa* at 5-7 days after inoculation systemic symptoms severe mosaic on *N. tabacum cv .white burly*. Whereas Systemic symptoms; Severe mottling on *D. metel*; Mild mosaic on *N. glutinosa*; Top necrotic on *N. tabacum cv* .white burly ; Little chlorotic local lesions on *Ch. quinoa* and no Symptoms on *Ch .amranticolor* and *D. stramonium* were observed on PVY inoculated leaves. Generally, systemic symptoms appeared 20 days after inoculation (Table,2).

Table 2: Differential hosts and symptoms of ToMV and PVY infectious sap.

Differential hosts	Symptoms	
	ToMV	PVY
Ch. amranticolor	L.L	no Symptoms
Ch. quinoa	L.ch.L	L.ch.L
D. stramonium	L.L	no Symptoms
D. metle	L.L	Severe mottling
N. glutinosa	L.L	Mild mosaic
N. tabacum cv .white burly	Severe mosaic	Top necrotic

L.L.= Local lesion; L.ch.L = Little local chlorotic lesion.

Evidences of SAR induction:

Disease severity and Reduction of virus infectivity:

Tomato plants were foliar-applied with AgNPs reacted with different systemic symptoms ranged latent at (50 ppm**)** to mild at **(**60 and 70 ppm**)** on the other hand 80 ppm concentration showed phytotoxicity at 7 days after virus inoculation with ToMV and PVY. While infected tomato plants non foliar-applied with Ag NPs were reacted with different systemic symptoms ranged between mild and severe mosaic, associated with blisters, distortions and leaf deformity, alone or in combinations. Generally, systemic symptoms appeared 20 days after inoculation.

The results in Table (3) show that all treatments have different percentages of disease severity (DS), virus infection and reduction of virus infection (RI). The AgNPs at concentration 50 ppm was more effective (89.30%), (17.50%) against ToMV and (85.25%), (20.25%) against PVY of both virus reduction and disease diversity respectively than 60 and 70 ppm to induce the SAR in tomato plants against ToMV and PVY (table,3).

Table 3: Effect of the foliar applications of AgNPs at 20 days after virus inoculation on TMV and PVY infectivity in tomato plants.

Treatments	ToMV			PVY		
	% of infection	% of RI*	% of DS**	% of infection	% of RI*	% of DS**
Infected control	100.00	0.00	84.00	100.00	0.00	91.5
50 ppm AgNPs	10.70	89.30	17.50	15.75	85.25	20.25
60 ppm AgNPs	21.50	75.50	39.00	26.76	75.28	35.12
70 ppm AgNPs	32.30	62.70	46.00	28.15	67.52	41.25
LSD ≤ 0.05	5.64	9.47	10.47	4.54	8.75	10.85

**RI: Reduction of virus infection* ***DS: Disease severity*

The current study was carried out to evaluate the efficiency of silver nanoparticles to suppression plant virus infection and induce systemic acquired resistance (SAR) against ToMV and PVY in tomato. ToMV and PVY infectious sap was confirmed by observing the distinct viral symptoms that developed on differential host plants. Generally, systemic symptoms appeared 20 days after inoculation. As a result, foliar application of 50 ppm of silver nanoparticles then inoculated with ToMV or PVY on tomato plants showed reduction of disease severity and reduction of virus infection. This finding suggested that, silver nanoparticles are effective antiviral agent. Furthermore, another results in this study confirmed that silver nanoparticles bind to the virus particles and inactivates the virus by inhibiting virus replication in host plants (Fig.2). The silver nanoparticles were bind to coat protein virus particles using transmission electron microscopy. Those results were confirmed by Jain and Kothari (2014) they reported that, spray application of 50 ppm aqueous solution silver nanoparticles on cluster bean leaves inoculated with sunhemp rosette virus (SHRV) showed complete suppression of the disease, suggesting that silver nanoparticles are effective antiviral agent. Furthermore, it in harmony with Elbeshehy *et al*. (2015) they found that treatment with NPs led to decrease percentage of infection and disease severity of BYMV.

Relative concentration of ToMV and PVY:

Foliar-application of Silver nanoparticles after 30 days of virus inoculation with ToMV and PVY on tomato plants lead to caused reduction of virus relative concentration using DAS ELISA (Table, 4). The Optical density DAS ELISA (405 nm) was 0.127 (- ve result), generally treatment with AgNPs at 50 ppm on relative concentration on ToMV and PVY were the lowest relative concentration, followed by 60 and 70 ppm were reduced the relative concentration less than positive control. These results are in harmony with (Elbeshehy *et al.,* 2015) they found that AgNPs led to decrease in virus concentration against BYMV infection. Also (Lu *et al*., 2009; Papp *et al*., 2010) they suggested that AgNPs inhibit viral nucleic acid replication and their antiviral activity due to several factors like: the particle size, the distribution of interacting ligand/receptor molecules.

Table 4: Effect of AgNPs concentrations on ToMV and PVY relative concentrations in tomato plants using DAS ELISA.

Treatments	Optical density DAS ELISA (405 nm)	
	ToMV	PVY
Infected control	0.485	0.524
50 ppm nanosilver	0.175	0.235
60 ppm nanosilver	0.245	0.265
70 ppm nanosilver	0.283	0.285
+ve control	0.536	0.575
-ve control	0.127	0.127

Another finding suggested that, these results may be due to silver nanoparticles affect the RNA copying during viral multiplication and significantly affect the inhibition of viral nucleic acid replication when AgNPs become less than the size of the particles, also silver nanoparticles applications increased (NPK) content compared with healthy control. (Awadhesh 1973; Lee *et al*., 2003; Galdiero *et al*. 2011; Narasimha *et al*., 2012).

Effect of Silver nanoparticles on activity of ToMV on *Nicotiana glutinosa*:

Data in table (5) and Fig. (1) show that, after mix of 1 ml of Silver nanoparticles from different concentrations (0 + control,50, 60 and 70 ppm) on 1 ml of crude sap of TMV then inoculated on *N. glutinosa* showed different average number of local lesions on each concentration. Generally, concentration 50 ppm showed significantly ($P \leq 0.05$) lowest average number of local lesions followed by 60 and 70 ppm respectively compared with distilled water (+ control). This finding suggests that silver nanoparticles are effective antiviral agent that inactivates the virus by inhibiting virus replication in host plant. Further electron micrographic showed that silver nanoparticles bind to coat protein of the virus body (Fig. 2).

However positive control showed the highest number of local lesions as showed in table (5) and fig. (1). These results were in harmony with Jain and Kothari (2014) they reported that after 3-4 days from inoculation SHRV on guar plants untreated with silver nanoparticles showed 100:150 lesions on the other hand, the plants treated with silver nanoparticles had no virus lesions.

Table 5: Effect of foliar application of distilled water as +control, AgNPs at 50, 60 and 70 ppm on average number of local lesions of ToMV on *Nicotiana glutinosa* inoculated leaves.

Treatment	Mean number of local lesions
+ control	40.8
50 ppm AgNPs	8.8
60 ppm AgNPs	15.6
70 ppm AgNPs	21.1
LSD≤ 0.05	3.69

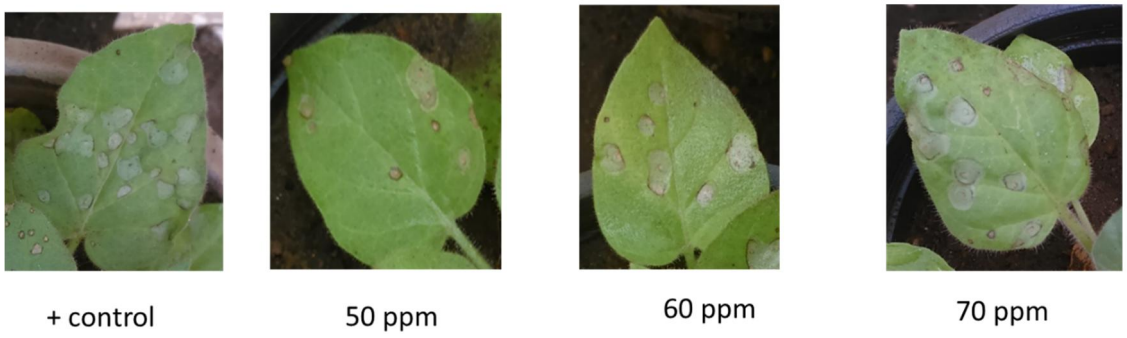

Fig. 1: Showed average number of local lesion on *N. glutinosa* after inoculation with crud sap of ToMV mixed with AgNPs concentrations +control, 50, 60 and 70 ppm.

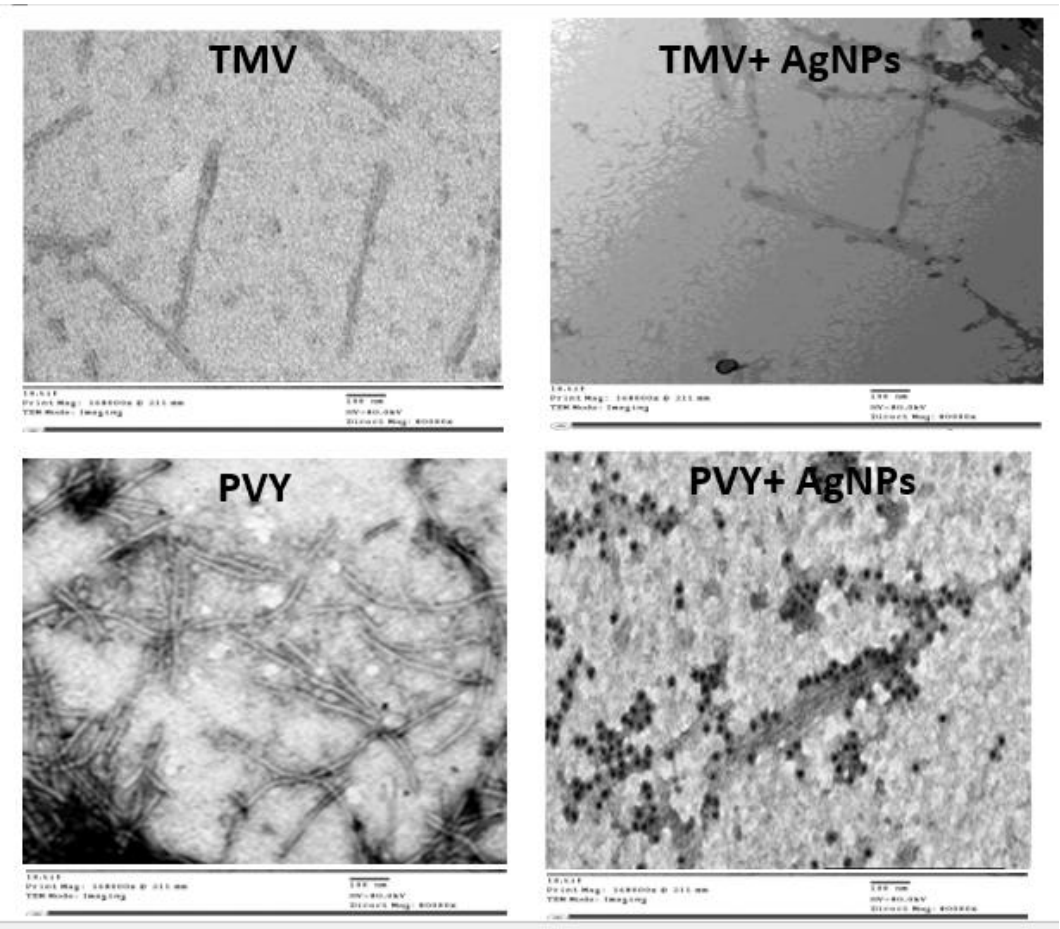

Fig. 2: TEM micrograph of: ToMV ; PVY treated and untreated with AgNPs. Arrows showed attached of AgNPs on coat protein of both virus particles in treated than untreated.

Effect of Silver nanoparticles on ToMV or PVY in partial purified preparation:

Total soluble protein (TSP), Peroxidase activity (POD) and Polyphenoloxidase activity (PPO):

Data presented in table (6) revealed that in general, the foliar applied plants with AgNPs inoculated with ToMV or PVY were showed significant increases in TSP and the activity of antioxidant enzymes (POD and PPO) compared to the both healthy and infected controls.

The considerable increases of protein in the infected plants compared to the healthy ones might be interpreted by two different suggested possibilities: firstly, the multiplication of virus particles inside plant cells lead to increasing the contents of proteins which are considered the main components of coat protein and replicase and movement proteins of virus infection. Secondly, these proteins may play important role in plants as pathogen related proteins which required for resistant viral infection. These results were confirmed in many previous studies (Wolf *et al*., 1989; Roth *et al*., 2004). They found that TMV encodes two replicase proteins, movement protein (MP) and coat protein (CP). In addition, *Tomato mosaic virus* (ToMV) and *Potato virus Y* (PVY), developed a counter-defensive strategy like viral proteins, referred as PTGS suppressors, block one or more steps of the PTGS pathway.

Regarding, the activities of POD and PPO; it was obvious that the activity of both antioxidant enzymes were increased significantly ($P \leq 0.05$) in the infected plants compared to the healthy ones. These findings could be attributed to the scavenging activities of both enzymes for reactive oxygen species (ROS) which are accumulated in the infected tissues as a response to the biotic stress.

As for the effect of AgNPs on TSP, POD and PPO; it was noticed that AgNPs caused significant increases ($P \leq 0.05$) for all previous traits compared to the untreated plants. The highest significant (P

≤ 0.05) results were achieved by the treatment of AgNPs at 50 ppm compared to the other treatments. It was well documented that the exposing of plants to AgNPs lead to altering TSP and the activities of antioxidant enzymes; for instance, Mehrian *et al*., (2015) found that the infected plants treated with AgNPs showed significant increases in TSP remarkable decrease in total soluble protein was observed with increase in concentration of AgNPs. Additionally, the application of AgNPs may cause an improving in releasing ROS in the plant tissues associated with an increasing in the activity antioxidant enzymes as an adaptive to the viral diseases (Jiang *et al*., 2014; Tripathi *et al*., 2017).

Table 6: Effect of foliar application AgNPs at 7 days after ToMV and PVY inoculation on total soluble protein (TSP); Peroxidase (POD) and Polyphenoloxidase (PPO) activities in tomato plants.

Treatment	TSP mg. g^{-1} f. wt		POD activity specific Unit $min^{-1}g^{-1}$ protein		PPO activity specific Unit $min^{-1}g^{-1}$ protein	
	ToMV	PVY	ToMV	PVY	ToMV	PVY
Healthy control	0.616	0.915	9.5	10.4	8.75	9.65
Infected control	1.002	1.075	11.42	12.35	9.75	10 21
50 ppm AgNPs	1.375	1.251	12.57	13.25	12.25	13.21
60 ppm AgNPs	1.202	1.242	12.24	12.92	12.21	12.10
70 ppm AgNPs	1.174	1.207	11.55	12.40	10.75	11.74
LSD ≤ 0.05	0.152	0.127	0.950	0.860	1.77	2.23

Photosynthetic pigments:

Data presented in table (7) show that there were significant (*P≤0.05*) decreases in all investigated photosynthetic pigments including chlorophyll a, b and carotenoids in the infected plants compared to the healthy ones. The reduction in the concentrations of up mentioned pigments in the infected plants may be attributed to increase ROS which cause a considerable damage in the chloroplast's membranes the main location for these pigments in leaf tissues. These findings are in agreement with Bondok and Ibrahim (2014).

Concerning, the influence of AgNPs, it was observed that there was a significant reducing in all plants treated with different concentrations of AgNPs compared to the heathy control. On the other hand, it can be noticed that the treatment of AgNPs at 50 ppm achieved an improving in all studied photosynthetic pigments compared to the infected control. These increases did not reach the level of significance (*P*≤0.05) in respect to carotenoids in the PVY infected plants. Conversely, the highest concentrations of AgNPs at 60 and 70 ppm revealed significant (*P*≤0.05) decreases compared to the other treatments this regard. The negative effect of AgNPs on the photosynthesis and its related pigments was proved previously in many investigations. This response could be attributed to the collapse the structure of chloroplasts and the inhibition of Ribulose- 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity and the photo-protective capacity of PSII (Da Costa and Sharma, 2016; Jiang *et al*., 2017).

Table 7: Photo pigment concentrations (mg. g^{-1} f.wt) of foliar application AgNPs at 7 days after ToMV and PVY inoculation in greenhouse condition.

Treatments	ToMV			PVY		
	Chlorophylls		Carotinoids	Chlorophylls		Carotinoids
	Ch a	Ch b		Ch a	Ch b	
Healthy control	4.75	1.45	1.59	6.50	4.62	1.96
Infected control	4.20	1.15	1.35	4.20	4.21	1.85
50 ppm AgNPs	4.25	1.21	1.50	6.45	4.54	1.91
60 ppm AgNPs	3.45	1.15	1.15	6.12	4.11	1.72
70 ppm AgNPs	3.25	1.05	1.20	3.85	3.10	0.92
LSD≤ 0.05	0.38	0.21	0.08	0.05	0.12	0.06

Total soluble phenols and free proline concentrations:

Results in table 8 show that in general, there was an increase in total soluble phenols in all examined treatments compared to the healthy control. Several studies reported that both viral infected plants or treated with nano-particles (NPs) leading to an enhancement production of phenolics, which might act as antioxidants to scavenge the ROS following by increasing the concentrations of the antioxidant molecule in plant tissues (Faisal *et al*., 2013; Comotto *et al*., 2014; Jiang *et al*., 2014; Ibrahim *et al*., 2015; Costa and Sharma, 2016; Vecerová *et al*., 2016)

As for free proline, it was obvious that the treatment of AgNPs at 50 ppm achieved the highest concentrations compared to the other treatments. This effect could be attributed to induce systemic acquired resistance (SAR) against both investigated viruses in this study. It had been confirmed that proline have a crucial protective role in plant cell under adverse conditions either environmental or biotic stresses. These effects may be explained by that the proline metabolic pathway was proposed to have a regulatory function in oxidation–reduction homeostasis and cell survival (Phang, 1985). Moreover, it can help plants in scavenging ROS, adjustment plant water status and stability of membranes structure and stabilization of enzymes /proteins (Hayat *et al*., 2012; Ibrahim *et al*., 2016). All of these effects could explain the positive effect of the treatment of AgNPs at 50 ppm under the circumstances of this study.

Table 8: Effect of foliar application AgNPs at 7 days after ToMV and PVY inoculation in tomato plants on total soluble phenols and proline concentrations.

Treatments	Total soluble phenols (mg. g^{-1} f.wt)		Proline (mg. g^{-1} f.wt)	
	ToMV	PVY	ToMV	PVY
Healthy control	1.34	1.12	0.72	0.75
Infected control	1.43	1.24	0.85	0.95
50 ppm AgNPs	1.81	1.45	1.00	1.25
60 ppm AgNPs	1.87	1.65	0.94	1.12
70 ppm AgNPs	1.93	1.85	0.91	1.05
LSD ≤ 0.05	0.45	0.48	0.08	0.06

Conclusion:

The results of this study indicated that the treatments of silver nanoparticles (AgNPs) to suppression of the virus infection and induce systemic acquired resistance (SAR) against *Tomato mosaic virus* (ToMV) and *Potato virus Y* (PVY). Generally, tomato plants sprayed with AgNPs at concentration 50 ppm showed reduction of disease severity, disease percentage and relative concentration than the other concentrations. As well as AgNPs at concentration 50 ppm reduced significantly average number of local lesions of ToMV inoculated on *N. glutinosa*. Examination the partial purified preparations of both viruses with TEM showed morphological evidenced that AgNPs bind to coat protein virus particles. Chemical parameters showed that, AgNPs at concentration 50 ppm increase the levels of total soluble proteins (TSP) and the activity of antioxidant enzymes (POD and PPO) compared to the both healthy and infected controls, significant decreases in all investigated photosynthetic pigments, increase in total soluble phenols and free proline. were detected in ToMV and PVY infected tomato plant respectively.

References

Anderson, P. K., A.A. Cunningham, N.G. F.J. Patel, Morales, P.R. Epstein, P.D. aszak, 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers Trends Ecol. Evol., 19: 535-544

Awadhesh, B.S., 1973. The Effect of Infection with Papaya Leaf Reduction Virus on the Total Nitrogen and Carbohydrate Content of Papaya Leaves. Phyton (Austria) 1973; 15 Fasc. 1-2: 37-43.

Bate, L.S., R.P. Waklren and I.D. Teare, 1973. Rapid determination of free proline water stress studies. Plant Soil. 39: 205-207.

Binyam, T., 2015. A Review Paper on Potato Virus Y (PVY) Biology, Economic Importance and its Managements. Journal of Biology, Agriculture and Healthcare. Vol.5, No.9, 2015, 110-126.

Bondok, A.M. and M. F. M. Ibrahim, 2014. Citric and Ascorbic Acid Drive some Physiological, Biochemical and Molecular Aspects in Tomato Plants Inoculated with *Tomato spotted wilt virus* (TSWV). Middle East Journ al of Agriculture Research, 3(4): 1248-1261.

Boualem, A., C.Dogimont and A.Bendahmane, 2016. The battle for survival between viruses and their host plants. Curr. Opin. Virolol. 17: 32-38.

Bradford, M., 1976. A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

Clark, M. F. and A. N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34:475-483.

Comoto, M., A. A.Casazza, B.Aliakbarian, V.Caratto, M.Ferretti, and P.Perego, 2014. Influence of TiO2 nanoparticles on growth and phenolic compounds production in photosynthetic microorganisms. Sci. World J. 2014:9.

Costa, M.V. and P. K. Sharma, 2016. Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in Oryza sativa. Photosynthetica 54, 110–119.

Da Cosa, M. V. J. and P. K. Sharma, 2016. Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in Oryza sativa. Photosynthetica 54, 110–119.

Daniel, H.D. and C. M. George, 1972. Peach seed dormancy in relation to indogenous inhibitors and applied growth substances. J. Amer. Soc. Hort Sci. 97:651-654

Daniela, R., W. B.Jan, G.Rob and K.Richard, 2009. Tomato spotted wilt virus nucleocapsid protein interacts with both viral glycoproteins Gn and Gc in planta. Virology, 383, 1, 5, 121-130.

Elbeshehy, E. K. F., A. M. Elazzazy and G.Aggelis, 2015. Silver nanoparticles synthesis mediated by new isolates of *Bacillus* spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus and human pathogens. Front Microbiol. 2015; 6: 453.

Faisal, M., Q.Saquib, A. A.Alatar, A. A.Al-Khedhairy, A. K. Hegazy and J. Musarrat, 2013. Phytotoxic hazards of NiO-nanoparticles in tomato: a study on mechanism of cell death. J. Hazard. Mater. 250–251, 318–332.

Galdiero, S., A.Falanga, M.Vitiello, M.Cantisani, V.Marra and M.Galdiero, 2011. Silver nanoparticles as potential antiviral agents. Molecules 16, 8894–8918.

García-Cano, E., R. O.Resende, R.Fernández-Muñoz, and E.Moriones, 2006. Synergistic interaction between *Tomato chlorosis virus* and *Tomatospotted wilt virus* results in breakdown of resistance in tomato. Phytopathology 96:1263-1269.

Hayat, S., Q.Hayat, M.N.Alyemeni, A.S.Wani, J.Pichtel, and A. Ahmad, 2012. Role of proline under changing environments: a review. Plant Signal Behav 7:1456–1466

Ibrahim M.F.M., A.Faisal and S.A. Shehata, 2016. Calcium Chloride Alleviates Water Stress in Sunflower Plants through Modifying Some Physio-Biochemical Parameters. American-Eurasian J. Agric. & Environ. Sci., 16 (4): 677-693.

Ibrahim, M.F.M., H.G. Abd El-Gawad and A.M. Bondok, 2015. Physiological Impacts of Potassium Citrate and Folic Acid on Growth, Yield and some Viral Diseases of Potato Plants. Middle East Journal of Agriculture Research. 4 (3): 577-589.

Jain, D. and S.L. Kothari, 2014. Green Synthesis of Silver Nanoparticles and their Application in Plant Virus Inhibition. J Mycol Plant Pathol, Vol. 44, No.1, 2014.

Jiang, H. S., X. N.Qiu, G. B.Li, W.Li, and L. Y. Yin, 2014. Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant Spirodela polyrhiza. Environ. Toxicol. Chem. 33, 1398–1405.

Jiang, H. S., L. Y.Yin, N. N.Ren, S. T.Zhao, Z.Li and Y.Zhi, 2017. Silver nanoparticles induced reactive oxygen species via photosynthetic energy transport imbalance in an aquatic plant. Nanotoxicology 11, 157–167.

Kochba, J., S.Lavee and P.Spiegel-Roy, 1977. Differences in peroxidase activity and isoenzymes in embryogenic and nonembryogenic ‘‘Shamouti’’ orange ovular callus lines. Plant Cell Physiol 18:463–467.

Laemi, U.K., 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature 224: 680-689.

Lee, C.H., D.S.An, H.F. Park and D.S. Lee, 2003. Wide-spectrum antimicrobial packaging materials incorporating nisin and chitosan in the coating. Packag Technol Sci. 2003;16:99–106.

Lichtenthaler, H.K. and C. Buschmann, 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy', in 'Current protocols in food analytical chemistry' pp. F4.3.1–F4.3.8. Wiley, New York.

Lu, J. M., X.Wang, C. M.Muller, H.Wang, P. H. Lin, and Y.Qizhi, 2009. Current advances in research and clinical applications of PLGA based Nanotechnology. Expert Rev. Mol. Diagn. 9, 325–341.

Maria, A.M., S. Galeazzi, S.Valdemo and M. Garbieri, 1981. SpirosIsolation, purification and physicochemical of polyphenol oxidase (PPO) from a dwarf variety of banana J Food Sci, 46 (1) pp. 150-155

Marin, J.A., P.Andreu, A.Carrasco and A. Arbeloa, 2009. Proline content in root tissues and root exudates as a response to salt stress of excised root cultures of *Prunus* fruit tree rootstocks.. ITEA 105:282–290.

Mayer A.M., 1987. Polyphenol oxidases in plants-recent progress. Phytochemistry 26: 11-20.

Mehrian, S.K., R.Heidari and F. Rahmani, 2015. Effect of silver nanoparticles on free amino acids content and antioxidant defense system of tomato plants. Ind J Plant Physiol. 20(3):257–263

Mohamed, E. F., 2010. Interaction Between Some Viruses Which Attack Tomato (*Lycopersicon esculentum* Mill.) Plants and Their Effect on Growth and Yield of Tomato Plants. Journal of American Science., 6 (8): 311-320

Murphy, C.J., 2008. Sustainability as an emerging design criterion in nanoparticle synthesis and applications. J Mater Chem 18: 2173-2176.

Narasimha, G., H.Khadri and M.Alzohairy, 2012. Antiviral properties of silver nanoparticles synthesized by *Aspergillus* spp. Der Pharmacia Lettre 4, 649–651.

Naser-El Din, M. A., 2007. Biological and molecular studies on potato virus Y. MSc. Banha. University, Egypt, pp: 205.

Panáček, A., L.Kvítek, R.Prucek, M.Kolár, R.Veeřová, N.Pizúrová, V. K.Sharma, T.Nevěčná, and R.Zbořil, 2006. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. Phys Chem B110: 16248.

Papp, I., C.Sieben, K.Ludwig, M.Roskamp, C. Böttcher and S.Schlecht, 2010. Inhibition of influenza virus infection by multivalent sialic-acidfunctionalized gold nanoparticles. Small 6, 2900–2906.

Phang, J.M., 1985. The regulatory functions of proline and pyrroline-5-carboxylic acid. Curr Top Cell Regul. ;25:91–132.

Popp J. and K.Hantos, 2011. The impact of crop protection on agricultural production. Stud. Agric. Econ. 113, 47–66.

Pradeep, S. and I.Masato, 2010. Tomato leaf curl Java virus V2 protein is a determinant of virulence, hypersensitive response and suppression of posttranscriptional gene silencing. Virology, 396,(1): 85-93.

Rai, M., A.Yadav and A.Gade, 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Advances 27: 6-83.

Roth, B.M., G.J. Pruss and V.B. Vance, 2004. Virus Res 102:97–108.

Shukla, D. D., C. W. Ward and A. A. Brunt, 1994. The Potyviridae. CAB International, Cambridge University Press, Cambridge.

Snedeor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Edition, Iowa State University Press, Ames.

Sutic D.D., R.E. Ford and M.T. Tosic, 1999. Handbook of plant virus diseases. CRC Press LLC., 139-141p.

Torsten, G., S.Beate and B. Sven-Erik, 2009. Replication of Tomato bushy stunt virus RNA in a plant in vitro system. Virology, 390, 2, 1, 250-260

Tripathi D. K., S.Singh, S.Singh, R.Pandey, V. P. Singh and N. C. Sharma, 2017. An overview on manufactured nanoparticles in plants: uptake, translocation, accumulation and phytotoxicity. *Plant Physiol. Biochem.* 110 2–12. 10.1016

Vecerová, K., Z.Vecera, B.Docekal, M.Oravec, A.Pompeiano, and J.Tríska, 2016. Changes of primary and secondary metabolites in barley plants exposed to CdO nanoparticles. Environ. Pollut. 218, 207–218.

Wolf , S., C.M. Deom, R.N. Beachy and W.J. Lucas, 1989. Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. Science 246:377–379.

Yang, X., K.Liangyi and P.Tien, 1996. Resistance of tomato infected with cucumber mosaic virus satellite RNA to potato spindle tuber viroid. Ann. Appl. Biol. 129: 543-555.