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Inhibitory Effects of Salicylic Acid and Silver Nanoparticles on *Potato Virus Y*-Infected Potato Plants in Egypt

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ABSTRACT

Potato virus Y (PVY) is one of the most important plant viruses that cause economic loses to potato plants. For this importance, we aimed to identify and molecularly characterize the isolated PVY infecting potato plants in Egypt, study the efficiency of salicylic acid (SA) at 200 µmol/L, silver nanoparticles (AgNPs) at 0.1µg/µL and mixture of them with the same concentrations to produce resistance against infection with PVY in potato plants and study the effects of these treatments on yield reduction and quality parameters of potato tubers cv. Spunta. To achieve our aim, the virus has been isolated from naturally infected potato plants grown in the experimental Farm (Faculty of Agriculture, Cairo University) and biologically purified on both *Chenopodium amaranticolor* and *Chenopodium quinoa* as indicator host plants which showed large and irregular chlorotic local lesions. Then, it has been transmitted mechanically and propagated in healthy potato seedlings cv. Spunta. The virus identification has been done by direct ELISA technique using specific antiserum and confirmed by reverse transcription polymerase chain reaction (RT-PCR) using two different pairs of primers designed for amplification of two different regions of PVY coat protein gene. An approximately 810 and 1000 bp fragment was amplified with the two specific primers. Transmission electron microscopy (TEM) revealed the presence of flexuous rod-shaped virus particles (849 nm). Spherical AgNPs with average size 12nm were successfully synthesized from 1 mM $AgNO_3$ via wet chemical reduction method and characterized using UV- Visible absorption spectroscopy, X-ray Diffraction (XRD) and TEM. All the used compounds could induce resistance to virus infection when applied to the plants as a spray treatment. The most effective treatments were that applied 24h. after inoculation with the virus and treatment with AgNPs at 0.1µg/µL and the mixture of SA and AgNPs at the same concentration applied before 3 and 7 days of virus infection which led to an important decrease in virus concentration (0.280,0.308 and 0.140, respectively) and infection percentage (10, 30 and 10%, respectively). However, weak reduction in virus concentration and percentage of infection was observed when SA was sprayed at the pre-and after viral infection (90,80 and 60%, respectively). Also, all tested treatments gave a significant increase in total number of harvested tubers /plant, total tuber yield, average of plant height (cm), tuber diameter, tuber length, starch and total soluble sugars content compared to healthy plants.

Key words: *Potato virus Y*, Salicylic acid, Silver nanoparticles, Yield loss, Potato (*Solanum tuberosum* L.), ELISA, PCR, Transmission electron microscopy

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in terms of quantities produced and consumed worldwide (FAO, 2005). It is the fourth largest food crop cultivated in more than 100 countries throughout the world and has gained a status of globally traded commodity (He *et al*., 2012). Potato is affected by many pests, numerous fungal, bacterial and viral diseases. Among these diseases, viruses cause in the field and during post-harvest varies from mild symptoms to heavy crop losses (Yardımci *et al*., 2014 and Dupuis, 2017). The most common RNA

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viruses found in potatoes are *Potato Virus Y* (PVY), *Potato Virus X* (PVX), *Potato leaf roll virus* (PLRV) *Potato Virus A*(PVA), *Potato viruses' S and M* (PVS and PVM) (Burrows and Zitter, 2005). Among the several viruses reported to infect potatoes, PVY (family, *Potyviridae*, genus, *Potyvirus*) is one of the most important plant viruses affecting potato production worldwide (Dupuis, 2017) and causes a mosaic leaf pattern, vein clearing and leaf necrosis, although the type and severity of symptoms will differ among potato cultivars and it reduces both tuber yield and quality, ranged from 10 to 80% (De Bokx and Huttinga, 1981, Whitworth *et al.,* 2006; Tsedaley, 2015). PVY has a wide host range naturally infecting plants in more than nine families, including 14 genera of the *Solanaceae* such as pepper, tomato, eggplant and tobacco (El- Dougdoug *et al.,* 2014). Also, the virus has a flexuous particle 680–900 nm long and 11– 13 nm wide (Moury and Verdin, 2012) and a genome of single-stranded, positive-sense RNA~10kb long (Hari *et al*., 1979; Hari, 1981 and Ha *et al*., 2008).

Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) tests are the only certain means of ascertaining the infection status of potato tubers, especially symptomless carriers and ELISA is used in seed certification programmers to process large amounts of samples rapidly and ensures that infection levels in seed potatoes are low (Robert *et al*.,2000).

Salicylic acid(SA) is considered one of the key endogenous signals involved in the activation of numerous plant defense responses and is an important signal molecule in plants that is required for the induction of systemic acquired resistance (SAR) against a wide variety of pathogens including fungi, bacteria and viruses (Palukaitis and Carr 2008).In addition , SAR is broad spectrum in nature and can even be induced in plants independently of a resistance gene-mediated hypersensitive response (HR) if they are treated with SA or one of its derivatives of functional analogs (Baebler *et al.,* 2014).

Silver nanoparticles (AgNPs) attracted the attention of many research groups working on different fields thanks to their unique features and large range of applications such as food technology, medicine, agriculture and environmental technology (Elbeshehy, *et al*. 2015).Also studies on metal nanoparticles, especially on those produced from silver or gold revealed that nanoparticles exhibit a veridical activity against a broad spectrum of viruses and surely reduce viral infectivity of cultured (Galdiero *et al.,* 2011).The antimicrobial activity of AgNPs is attributed to cell death as a result of sequestration and inactivation of vital sub- cellular organelles, for which the silver ions have high affinity. It has also been suggested that AgNPs inhibit viral nucleic acid replication while, their antiviral activity depends on the particle size, as well as on the distribution of interacting ligand, receptor molecules (Papp *et al*., 2010 and Xia *et al*.,2016).

Therefore, the objectives of this work were to: (I) identify and molecularly characterize PVY isolate infecting potato plants in Egypt (II) determine the inhibitory property of AgNPs at 0.1 µg/µl, SA at 200 µmol/l and mixture from them with the same concentrations at pre-and after infection with PVY on potato plants cv. Spunta under greenhouse conditions. The effects of these treatments on yield, yield components, starch and total sugars contents of the potato tubers were also determined to evaluate their efficiency for potato virus control.

Materials and Methods

Source of potato cultivar:

One hundred and seventy tubers of potato (*Solanum tuberosum* L.) cultivar Spunta were obtained from Daltex company- Kafr El-Zayat, ELGharbia governorate and detected before cultivation and inoculation for the presence of the viruses (PLRV, PVX and PVY) serologically, directly from sprouting tubers (Gugerli and Gehriger, 1980) by using double antibody sandwich ELISA (DAS-ELISA) technique using specific polyclonal antibodies (Clark and Adams, 1977). ELISA Kits were supplied by LOEWE Biochemical, GmbH,DSMZ, Germany .

Virus isolation and propagation:

Naturally diseased potato plants showing symptoms including necrosis and veinal necrosis suspected due to viral infection were obtained from the experimental Farm (Faculty of Agriculture, Cairo University) .Obtained samples were tested serologically by DAS – ELISA as described by Clark and Adams, (1977) using 3 specific antisera against some potato viruses , PLRV, PVX and

PVY .The positive samples reacted with PVY antibodies were used as a source for the virus under study and biologically purified from the single local lesion produced on both *Chenopodium amaranticolor* and *Chenopodium quinoa* plants as indicator host by local lesion technique according to Kuhn (1964) and also inoculated onto *N. tabacum* cv. White Burley. Five plants for each were inoculated. An equal number of healthy seedling were left without inoculation to serve as control. Inoculated plants were kept under observation in the greenhouse. The purified isolate was propagated on potato (*Solanum tuberosum* L.) cv. Spunta and used as a source of PVY for the further experiments. The virus was identified as PVY on the basis of indicator host, transmission experiment, electron microscopy and molecular biological studies.

Mechanical transmission:

Twenty tubers from potato cv. Spunta which gave negative results with DAS – ELISA test for PLRV, PVX and PVY were planted in plastic pots of 30 cm containing sterilized soil (one tuber per pot) in virology greenhouse, Virus and Phytoplasma Research. Department. PVY isolate was mechanically inoculated on ten plants (2 weeks old) as well as ten plants without inoculation left as negative control. 0.1M phosphate buffer pH 7.2 (1:2 w/v) was used in mechanical inoculation. Inoculated plants were maintained in the greenhouse. Three weeks later, leaf samples from each pot were collected and examined for virus presence by DAS-ELISA using the specific antiserum.

Transmission electron microscopy (TEM):

To study the morphology of virus particles and confirm the virus infection, dip preparation technique described by Noordam (1973) was used. Carbon coated cupper grid (400mesh) were dipped in sap expressed from PVY infected leaves, then negatively stained by 2% Phosphotungestic Acid (PTA) for 2min, then air dried and examined using transmission electron microscope JEOL (JEM-1400TEM, Japan) at the Electron Microscope Unit, Faculty of Agriculture, Cairo University, Research Park (FARP). Images were captured using camera at 80.000×

Total RNA extraction of Potato Virus Y:

Total RNA of PVY was extracted from fresh leaves of potato samples (*i.e.,* mechanically inoculated with PVY, potato plants treated with both SA, AgNPs and in mixture of them with the same concentrations to confirm the positive results in ELISA test according to Zhang *et al*. (2015).

Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR):

The cDNA synthesis was performed with cDNA synthesis kit (Invitrogen, USA) using RNA as template. The semi-quantitative RT-PCR was accomplished using two different pairs of primers designed for amplification of two different regions of PVY code protein gene. The sequence of first pair of primer as suggested by Shalaby *et al*. (2002) for amplicon size of 810 bp is CPf 5'TCAAGGATCCGCAAATGACACAATTGATGCAGG3', and CPr 5' AGAGAGAATTTCATC ACATGTTCTT GACTCC3'. The second pair of primer is suggested by Fakhrabad *et al*. (2012) for amplicon size of 1000 bp, the sequence of the forward prime (PVY-P1): 5'CAA CT CCA G A T GGAACAATTG3' and the reverse prime is (PVY-P2): 5'CCATTCATCACAGTTGGC3'. The PCR reaction was performed using GoTaq® Green Master Mix (Promega, USA) by applying manufacturer protocol. The thermal cycler programme was adjusted as follow: 94°C for 30 min, 35 cycles of 94°C 1 min, 55°C for 1 min and 72°C for 1 min, and the final extension was 72°C for 7 min. The reaction was stopped by pausing the tubes containing the mixture at 4°C for at least 30 min. The product of the PCR test was visualized on 1% agarose gel stained by Ethedium bromide on UV table, and photographed by Gel Doc XR system (Biorad, USA).

Preparation of AgNPs:

AgNPs were prepared using chemical reduction method according to Li *et al.* (2012) by adding 0.5g of PVP (Polyvinylpyrrolidone, 30K) to 45 mL of 1mM silver nitrate (AgNO3) solution with

stirring. 112.5 ml cold solution of $NaBH_4$ was added to the $AgNO_3$ solution drop-wisely. The color of the $AgNO_3$ solution was changed to greenish yellow that means the formation of silver nanoparticles.

Characterization of AgNPs:

UV–Visible (UV–Vis) spectra were measured with a Perkin Elmer Lambda 40 UV–visible spectrophotometer using 1-cm path length Hellma quartz cuvettes. Transmission electron microscopy (TEM) images were obtained with a Joel JEM-1230 electron microscope operated at 120 kV equipped with Gatan UltraScan 4000SP 4K 9 4K CCD camera. A drop from a diluted sample dispersion was deposited onto an amorphous carbon film on 400 mesh copper grids and left to evaporate at room temperature. X-ray diffraction (XRD) measurements were performed using a Philips PW1710 X-ray diffractometer using Cu Ka radiation (k = 1.54186 A °). The XRD patterns were recorded from 20°to 70°2H with a step size of 0.020°2H and collecting 10 s per step.

Greenhouse experiment:

Greenhouse experiments were conducted during September 2016 in the greenhouse of vegetable crops department, Faculty of Agriculture, Cairo University, Giza, Egypt to study the effect of treatments with SA at 200 µmol/L according to kobeasy *et al*. (2011), AgNPs at 0.1µg/µL according to Elbeshehy *et al*. (2015) and mixture of SA and AgNPs with the same concentrations on potato plants to produce resistance to PVY infection. On the other hand, study the effect of these treatments on total number and total weight of tubers per pot after 90 days from planting, average plant height (cm), tuber length (mm), tuber dry weight (g), tuber diameter (mm), starch and total sugars content of tubers.

Experimental design:

In greenhouse experiment, one hundred and fifty tubers of potato cultivar Spunta which gave negative results DAS – ELISA test, the most susceptible cultivar to PVY infection of eleven potato cultivars tested by DAS-ELISA according to El-Dougdoug *et al.* (2014) were planted in steam-sterilized potting compost (mix of soil, sand and peat) of 30 cm plastic pots (one tuber per pot) and grown from eye cores under usual and propitious conditions for potato plants. After 21 days of growth, plants of similar size were chosen and separated into three groups (i.e., I, II, III), the identity of the groups is as follows: Group [1], plants treated (sprayed) after 24h. of virus inoculation, group [2] plants sprayed 3 days before virus inoculation and group [3] plants sprayed 7 days before virus inoculation. Each group consisting of five subgroups and containing10 plants / subgroups. Subgroups [1, 2] in all groups included healthy potato seedlings (negative control) and potato seedlings inoculated with PVY (positive control), respectively. Subgroup [3] included potato seedlings sprayed with SA at 200 µmol/L, Subgroup [4] included potato seedlings sprayed with AgNPs at 0.1µg/µL, and Subgroup [5] included potato seedlings sprayed with mixture of SA and AgNPs with the same concentrations. Symptom manifestations were examined weekly. Four weeks later, leaf samples from each pot were collected and examined by DAS-ELISA to calculate the inhibition percentage of virus infectivity using PVY specific polyclonal antiserum and by RT-PCR using two different pairs of primers designed for amplification of two different regions of PVY code protein gene. Different measurements have been recorded at harvest time (after 90 days of planted) such as, average plant height (cm), tuber length (mm), tuber diameter (mm). Also, total number and total weight of tubers per pot, tuber dry weight (g), starch and total sugars content of tubers were also determined.

Chemical analysis of potato tuber:

Starch content:

Starch content was determined according to the method of Cleeg (1956)

Total soluble sugars

The ethanol extract of potato tubers at harvest were used to determine total soluble sugars according to Dubois *et al. (*1956)

Statistical analysis

The obtained data were statistically analyzed according to the method described by Snedecor and Cochran (1982).

Results and Discussion

Virus detection in potato tubers:

Virus detection was relied on the extracted sap from sprouting potato tubers cultivar Spunta obtained from Daltex company- Kafr El-Zayat by using DAS-ELISA technique. Results of DAS-ELISA test found to be free of PLRV, PVX and PVY or mixed infection.

Virus source and symptomatology:

Natural symptoms induced by PVY infection which isolated from the experimental Farm (Faculty of Agriculture, Cairo University) were characterized by necrosis, veinal necrosis, stunting and slight inverted cupping of leaflets (Fig.1-A) and confirmed by DAS-ELISA against specific polyclonal antibodies for PVY. Such collected symptoms have previously been described for PVY infection on potato (Ahmed *et al*, 2004; El-Araby, *et al.* 2009; El-Dougdoug, *et al*. 2014 and Tsedaley, 2015).

Virus isolation and propagation:

Several samples of naturally infected potato *(Solanum tuberosum* L.) that suspected to be due to single infection with PVY as detected by DAS-ELISA were used and biologically purified on both *Chenopodium amaranticolor* and *Chenopodium quinoa* as indicator host plants which showed large and irregular chlorotic local lesion (Fig.2). This result agrees with similar results obtained by El-Dougdoug *et al*. (2014) and EL- Banna *et al.* (2015). The isolated virus was successfully transmitted using mechanical transmission by the single local lesion and propagated in healthy potato seedlings cv. Spunta which used as a source of PVY. Veinal necrosis was produced on *N. tabacum* cv. White Burley, 5-7 days after virus inoculation. The successful transmission was confirmed utilizing back inoculation and /or by DAS-ELISA. All healthy potato plants inoculated with PVY showed symptoms of necrosis, veinal necrosis, stunting and slight inverted cupping of leaflets after 16 days of inoculation (Fig.1-B, C, D) compared with healthy plants (Fig.1-E).

Infected tubers produced from PVY-inoculated potato plants were harvested 3 months' post inoculation. The tubers collected showed necrotic ringspot, small sized and malformation (Fig.1-F). These results are similar to those recorded by Lung'aho *et al*. (2007); Dupuis *et al*. (2013); Lindner, *et al*. (2015) and Dupuis (2017).

Serologic detection of PVY:

Direct ELISA technique was used at the beginning and the end of experiments as a diagnostic test. In the beginning, direct ELISA confirmed the presence of PVY in both naturally infected and mechanically inoculated potato plants using specific antiserum of PVY only, while in the later test, demonstrated the inhibition percent of infection with PVY. Result showed that, all samples were positive for PVY after 30 min of adding substrate. The absorbance value greater than threefold of a negative control value and with a visually detectable yellow color was rated as a positive reaction as shown in Table (1). Such results are confirmed by several authors applying ELISA tests for PVY identification (Hane and Hamm, 1999; El-Dougdoug *et al*., 2014; Khassanov *et al*., 2016 and Dupuis, 2017). Nevertheless, polyclonal antibodies do not discriminate between PVY strains so, monoclonal antibodies specific to O and N strains or polyclonal specific to N strain have been used to characterize selected PVY isolates (Gamal El-Din *et al*.,1997 and Gugerli and Fries ,1983).

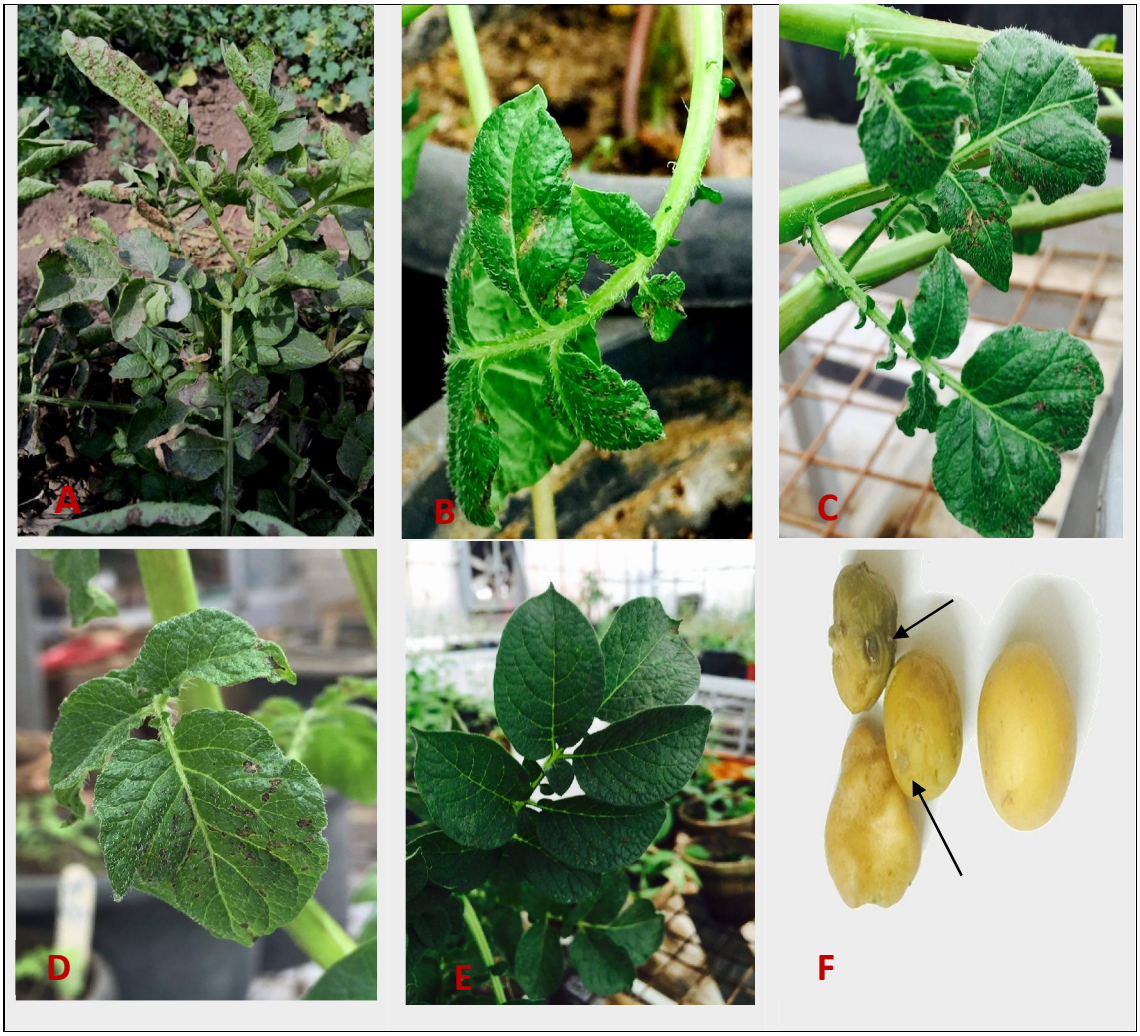

Fig. 1: Natural infection as well as mechanical transmission of PVY on potato plants A: Symptoms of naturally infected potato plants with PVY in the field showing severe necrosis, veinal necrosis, stunting and slight inverted cupping of leaflets. B, C and D: mechanically inoculated potato plants showing necrosis, veinal necrosis and infected leaflets may eventually roll downward giving the plants a dropping appearance. E: healthy plant and F: PVY symptoms on potato tubers cv. Spunta showing tuber necrotic ringspot, small sized and malformation compared with healthy tuber (right).

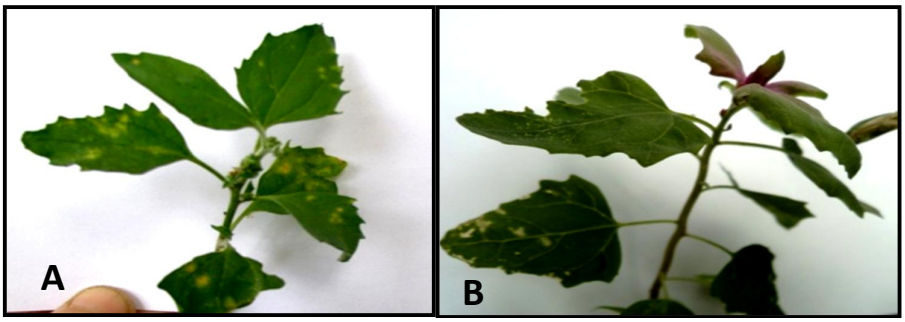

Fig. 2: Symptoms of PVY as a result of artificially inoculated *Chenopodium quinoa* (A) and *Chenopodium amaranticolor* (B) as indicator host plants showing a large and irregular chlorotic local lesion.

Transmission electron microscopy (TEM):

Electron microscopy of the virus dip preparation, negatively stained with 2% phosphotungestic acid obtained from PVY – infected potato leaves revealed as shown in Fig (3) the presence of flexuous rod-shaped particles (849 nm), as reported by Mcdonald and Kristjansson (1993); Moury and Verdin (2012) and EL- Banna *et al*. (2015).

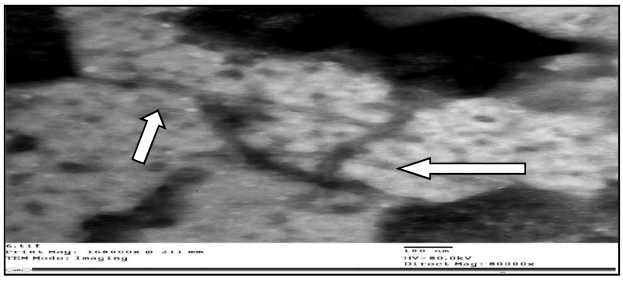

Fig. 3: Electron micrograph of PVY particles negatively stained with 2% phosphotungestic acid (Magnification 80,000 x).

Molecular characterization of the PVY – CP:

In order to consolidate the obtained results through ELISA, RT-PCR method was carried out. The RNA extracted from mechanically inoculated potato plants by PVY (positive control), potato plants treated with both SA, AgNPs and mixture of SA and Ag- NPs with the same concentrations were used for the RT-PCR amplification of the *PVY*-CP gene. To molecularly characterize the *PVY*-CP, two different pairs of primers designed for amplification of two different regions of *PVY* code protein gene were used. Electrophoresis analysis of RT-PCR product showed a single band at 810 bp representing the expected size for the amplified fragment of the PVY-CP with primer suggested by Shalaby *et al.* (2002) and 1000 bp using primer suggested by Fakhrabad *et al*. (2012) for either the positive control or treated samples (Fig. 5). Similar results were reported in different studies (Shalaby *et al*., 2002, Zhiming *et al*., 2005, Fakhrabad *et al*., 2012 and Yardımci *et al*.,2014)

Preparation and characterization of AgNPs

Figure (4) shows the characterization of the prepared AgNPs that have spherical shape with average size 12±1.3nm and maximum absorption band at 406nm. Intense XRD peaks of the formed Ag NPs were observed corresponding to the (111), (200), (220), (311) planes at 2θ angles of 38.29°, 44.38°, 64.56°, and 77.64°, respectively

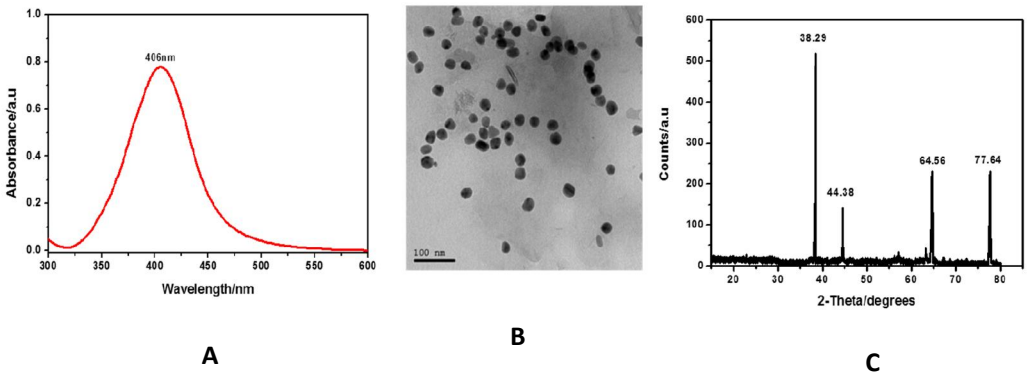

Fig. 4: Absorption spectrum of the prepared AgNPs (A), TEM image of AgNPs (B) and XRD pattern of the formed AgNPs in C.

Effect of SA and AgNPs treatments on PVY - infected potato plants:

SA at 200 µmol/L, AgNPs at 0.1µg/µL and mixture of them at the same concentrations were tested for their ability to inhibit PVY multiplication and spread of virus when potato plants sprayed after 24h. and before 3 or 7 days of virus inoculation using direct ELISA test and RT-PCR as shown in Table (1) and Fig. (5).

Table 1: Effect of SA, AgNPs and mixture of SA and AgNPs on PVY infection in experimental potato plants cv. Spunta according to direct ELISA test.

Treatments (Subgroups)	No. of tested plants	Virus concentration by DAS –ELISA Groups			Infection (%) Groups		
		I	II	III	I	II	III
SA (200 µmol /L)	10	1.200	1.053	0.935	90	80	60
AgNPs (0.1 µg/µL)	10	0.280	0.632	0.750	10	60	80
SA + AgNPs	10	0.551	0. 308	0.140	60	30	10
Positive control	10	1.220	1.100	1.020	100	100	100
Negative control	10	0.061	0.065	0.045	0	0	0

Group I: plants treated 24h. after virus inoculation
Group II: plants treated 3 days before virus inoculation
Group III: plants treated 7 days before virus inoculation

Data demonstrated in Table (1) and Fig. (5) revealed that all compounds used could induce resistance to virus infection when applied to the plants as spray treatments. The most effective treatment of infected plants was that applied 24h. after inoculation with AgNPs at 0.1µg/µL and the mixture of SA and AgNPs with the same concentrations applied before 3 and 7 days of virus inoculation which led to an important decrease in virus concentration (0.280,0.308 and 0.140, respectively) and percentage of infection (10,30 and 10%, respectively). However, weak reduction in virus concentration (1.053, 0.935 and1.200) and percentage of infection (80, 60% and90%, respectively) was observed when SA was sprayed at pre-and after viral inoculation. Reduction in infection by SA treatments delayed systemic symptoms developed by PVY, suppressed virus multiplication, decreased the accumulation of the virus, moreover treatments have affected the entry of the virus into the vascular system. Cytological and biochemical characterization have revealed that SA is a crucial factor for inhibition of the spread of PVY in parenchymal tissue, while a lack of SA results in delayed early transcriptional events, which can lead to inefficient defense responses. In addition, these results are in agreement with Van Loon and Antoniw (1982) who suggested that SA at high concentrations may induce the full set of systemic acquired resistance (SAR) genes. At the same time treatments with exogenous SA induces protein resistance (PR) genes. Also resistance induce by SA may be due to SA act as signaling compounds during senescence and defense against pathogens (Zhou *et a*l 2014). However, the treatments of inoculated plants with AgNPs induced different plant responses, depending on treatment time (pre- or post-viral inoculation). In particular the pre-inoculation treatment with AgNPs (*i.e*, 3 and 7 days) had weak effect on virus concentrations (0.632 and 0.750, respectively) and percentage of infection (60% and 80%, respectively).On the contrary, remarkable results were observed in post-inoculation treatment *i.e*, 24 h. after inoculation in which of AgNPs prevented all destructive symptoms caused by the virus, while weak PVY symptoms were observed when plants treated by AgNPs after inoculation. These changes may be due to the treatments with AgNPs lead to an important decrease in virus concentration 0.280 and percentage of infection 10% correspondingly , it has been reported that the AgNPs are effective against a prototype arena virus when administered early after initial virus exposure (Speshock *et al* 2010).These finding may suggest that the AgNPs activity is more dramatic at the early phases of viral replication .However, moderate reduction in virus concentration and percentage of infection were observed when AgNPs was sprayed at the pre-viral inoculation stage, indicating inability of the AgNPs to activate the inducible systemic resistance of the plant against PVY infection (Elbeshehy *et al*. 2015). Also AgNPs may enter the cell and fulfill their antiviral activity through interactions with the viral nucleic acids (Galdiero *et al.,* 2011). At the same time nanosilver has shown to have antibacterial, antifungal and antiviral effects .Studies have demonstrated that silver ions interact with sulfhydryl groups of proteins

as well as with the bases of DNA leading either to inhibit respiratory processes or DNA unwinding lead to inhibit of cell division (Davod *et al.*,2011).These results are agree with Jain and Kothari (2014) who suggested that AgNPs bind to the virus particles and inactivates the virus by inhibiting virus replication in host plant and it has ability as effective antiviral agent

Antiviral activity of silver ions has been recorded, interaction with sulfhydryl groups has been implicated in the mode of action. Contact time and temperature can have impact on both the rate and extent of antimicrobial activity (Dibrov *et al*., 2002 and Xia *et al*., 2016).

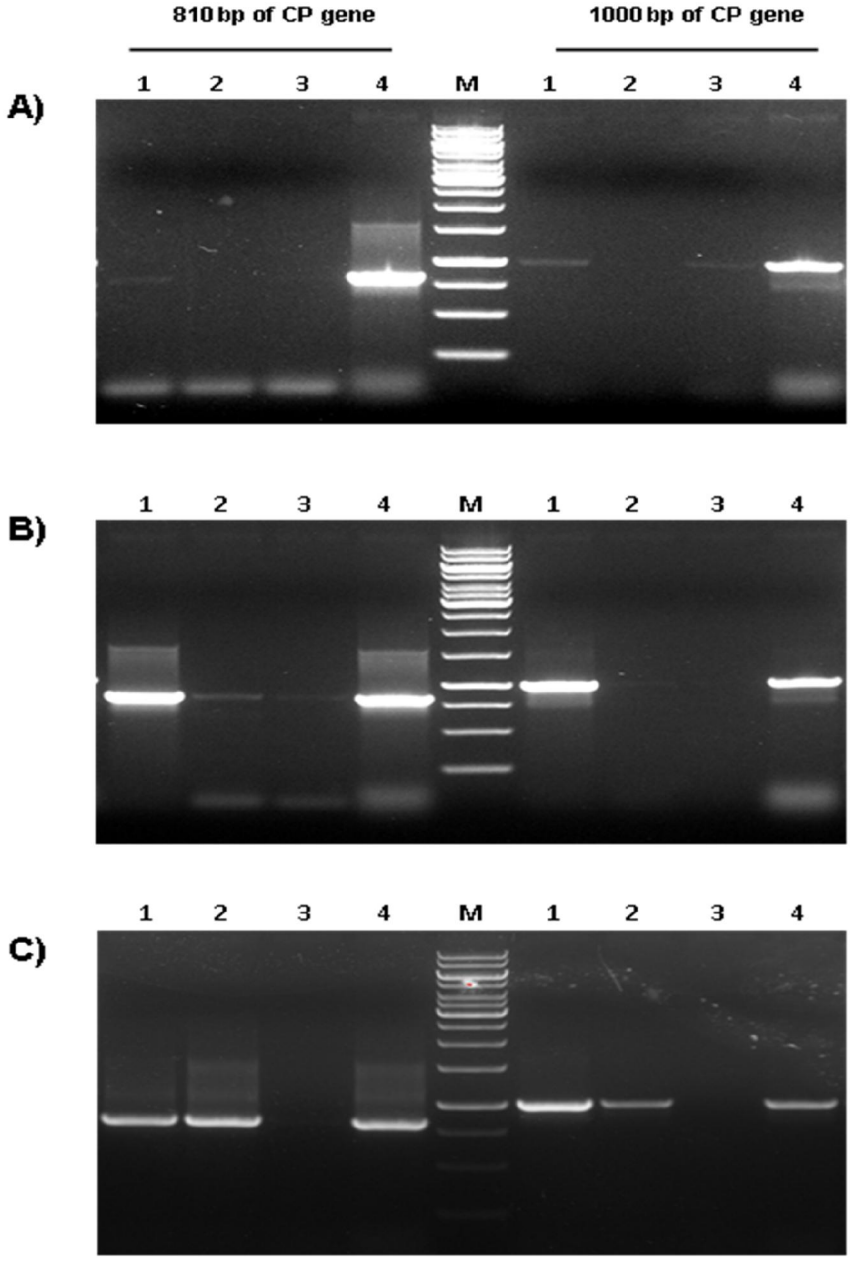

Fig. 5: Agarose gel electrophoresis showing the migration of two RT-PCR products of coat protein of PVY at molecular weight of 810 bp (left) and 1000 bp (right) of potato plants after 4 weeks of inoculation. **A**: potato plants treated 24h. after virus inoculation , **B**: potato plants treated 3 days before virus inoculation, **C**: potato plants treated 7 days before virus inoculation., **Lan1**:potato plants treated with 200µmol/L SA , **Lan 2**: potato plants treated with 0.1 µg/µL AgNPs, **Lan 3**: potato plants treated with mixture of SA and AgNPs at the same concentration, **Lan 4:** positive control, and **Lan** M: Gene-Ruler^{TM} Kb ladder.

Influence of SA and AgNPs treatments on starch and total sugars contents in PVY - infected tubers:

Dry matter and starch contents of potato tubers have important properties of potato food and non-food industries because the quality of final products and efficiency of processing tuber are directly linked to them (Haase, 2003). Also, sugars are yet another potato components affecting tuber suitability for cooking and processing, they include total sugars, reducing sugars and sucrose. Increased concentrations of these compounds resulted in edible tubers having a palpable sweeter taste and their flesh blackening more rapidly, which is a very undesirable process particularly in tubers destined for chips and crisps (Zarzecka *et al.,* 2015). Regarding to the data of SA and AgNPs treatments in Table (2) it could be noticed that starch percentage was decreased after infection with virus and reached to 10.14, 8.89, and 9.00% compared to negative controls 16.09, 15.66 and 15.22%, respectively.

Table 2: Influence of SA and AgNPs treatments on starch and total soluble sugars contents in PVY - infected tubers.

Treatments	Starch % Groups			Total soluble sugars% Groups		
	I	II	III	I	II	III
SA(200µmol/L)	11.15± 0.51c	10.24± 0.35d	11.48± 0.56b	0.89± 0.05b	0.98± 0.10a	0.79± 0.06c
AgNPs(0.1 µg/µL)	15.10± 1.21a	11.59± 0.71c	10.99± 0.44b	0.66± 0.07c	0.85± 0.03b	1.02± 0.05b
SA + AgNPs	13.22± 0.73b	13.97± 0.57b	15.03± 0.87a	0.81± 0.09b	0.76± 0.03b	0.69± 0.06d
Positive control	10.14± 0.47c	8.89± 0.50e	9.00± 0.92c	1.61± 0.07a	1.09± 0.09a	1.23± 0.04a
Negative control	16.09± 1.01a	15.66± 0.24a	15.22± 0.70a	0.61± 0.06c	0.56± 0.05c	0.69± 0.03d

Group I: plants treated 24h. after virus inoculation, Group II: plants treated 3 days before virus inoculation, Group III: plants treated 7 days before virus inoculation
The values are means ± SE. The mean values with different small letters within a column indicate significant differences ($p \leq 0.05$).

Values in Table (2) also indicated that the treatments with SA and AgNPs increased starch percentage from (8.89 to 15.10%) according to the treatment, compared with positive control. AgNPs 24h. after virus inoculation gave the highest increase (15.10%) compared with treatment with SA ,3 days before infection which gave the lowest value (10.24%). At the same time, different treatments decreased the percentage of total soluble sugars from 1.61 to 0. 66 % according to treatment. These changes may be due to virus infection in potato that reduced the percentage of dry matter, dry matter yield and reduced the marketable yield. Also, viral diseases show similar tendencies, generally, viruses reduce starch content but increase nitrogenous compounds. This is likely to be due to reduced rates of photosynthesis, insufficient water supply and disorders in assimilate movement within diseased potato plants (Abo-Elyousr *et al.,* 2016).

Effect of SA and AgNPs treatments on quality parameters of PVY - infected tubers:

Owing to the results in Table (3) it could be noticed that infection with virus decreased plant height from (51.4 to 26.3 cm), number of tubers/plant from (5 to 3 tubers/plant), tuber dry weight from (18.99 to12.14%), tuber length from (66.96 to 47.43mm), tuber diameter from (36.10 to 26.54mm), tuber weight from (64.98 to 27.64g) and total weight of tubers/ plant from (324.90 to 82.92g) compared with healthy plants. Also, treatment with AgNPs 24h. after virus inoculation was the best treatment in improvements of quality parameters of infected tubers compared with other treatment as shown in Table (3).At the same time, result in table (4 and 5) showed that treatments with SA and AgNPs improvement of yield and yield components and the best treatments were potato plants treated 3 and 7 days before virus inoculation with mixture of SA and AgNPs , these may be due to reduction in infection by induce resistance or the treatments have affected the entry of the virus

Table 3: Effect of treatments with SA and AgNPs on quality parameters of tubers from potato plants treated 24h. after virus inoculation

Treatments	Average of plant height (cm)	Number of tubers/ plant	Tuber dry weight (%)	Tuber length (mm)	Tuber diameter (mm)	Average of tuber weight(g)	Total weight of tubers/ plant(g)
SA (200µmol/L)	30.4±1.18e	5	13.89± 1.70b	58.98± 3.98b	24.20± 1.70b	35.93± 3.07b	179.65± 11.35d
AgNPs (0.1µg/µL)	48.4±2.00b	5	17.70± 1.10a	67.40± 4.60a	35.09± 2.90a	59.07± 5.05a	295.35± 15.65b
SA + AgNPs	38.9±2.59c	5	14.11± 0.19b	61.00± 5.05ab	30.03± 3.78b	41.33± 2.65b	206.65± 9.65c
Positive control	26.3±1.00e	3	12.14± 0.65b	47.43± 3.45c	26.54± 1.43b	27.64± 2.15c	82.92± 4.10e
Negative control	51.4±1.20a	5	18.99± 1.99a	66.96± 3.05a	36.10± 1.90a	64.98± 4.00a	324.90± 19.00a

The values are means ± SE. The mean values with different small letters within a column indicate significant differences ($p \leq 0.05$).

Table 4: Effect of SA and AgNPs treatments on quality parameters of tubers from potato plants treated 3 days before virus inoculation

Treatments	Average of plant height(cm)	Number of tubers/ plant	Tuber dry weight (%)	Tuber length (mm)	Tuber diameter (mm)	Average of tuber weight (g)	Total weight of tuber/ plant
SA (200µmol/L)	30.7± 1.40d	5	11.67± 0.55c	52.12± 2.88b	27.78± 1.89bc	33.90± 1.07d	169.50± 10.95d
AgNPs (0.1µg/µL)	37.5± 1.10c	5	14.39± 2.20b	59.88± 2.90a	29.66± 4.27ab	42.18± 2.18c	210.90± 10.90c
SA + AgNPs	47.2± 4.20b	5	15.47± 1.17ab	64.13± 2.15a	32.48± 3.04ab	55.20± 4.80b	270.00± 26.06b
Positive control	25.8± 0.50e	3	10.99± 1.80c	46.84± 4.05b	25.90± 2.10c	26.80± 2.79e	80.40± 5.40e
Negative control	52.1± 3.50a	5	17.96± 0.95a	61.27± 3.01a	35.30± 1.10a	65.12± 3.85a	325.60± 18.60a

The values are means ± SE. The mean values with different small letters within a column indicate significant differences ($p \leq 0.05$).

Table 5: Effect of SA and AgNPs treatments on quality parameters of tubers from potato plants treated 7 days before virus inoculation

Treatments	Average of plant height(cm)	Number of tubers/ plant	Tuber dry weight (%)	Tuber length (mm)	Tuber diameter (mm)	Average of tuber weight (g)	Total weight of tuber/ plant
SA (200µmol/L)	39.3± 2.80b	5	13.68± 1.48b	55.70± 2.30b	27.79± 2.20bc	44.13± 3.87b	220.65± 20.50b
AgNPs (0.1µg/µL)	32.0± 2.00c	5	12.99± 0.79bc	51.33± 4.30b	26.01± 2.00c	29.77± 0.75c	148.85± 9.15c
SA + AgNPs	47.7± 3.70a	5	16.50± 0.87a	62.61± 3.70a	31.97± 2.95ab	61.00± 5.00a	305.00± 8.00a
Positive control	27.1± 1.80c	3	11.10± 1.70c	44.01± 1.21c	24.24± 1.75c	25.89± 1.90c	77.67± 7.35d
Negative control	51.8± 3.20a	5	18.02± 0.88a	63.55± 4.02a	33.13± 2.87a	63.66± 6.35a	318.30± 25.90a

The values are means ± SE. The mean values with different small letters within a column indicate significant differences ($p \leq 0.05$).

into vascular system and indirectly inhibited replication of virus in case of treatment with SA while treatment with AgNPs may enter the cell and fulfill their antiviral activity through interactions with the viral nucleic acids (Jain and Kothari,2014 and Xia *et al.*,2016). The information is valuable for future registration and labeling of the AgNPs as antiviral agents for crop protection.

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