JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 803-812

Effect of Biosynthesized Silver Nanoparticles on Physiological Parameters of *Vicia faba* Infected by Bean Yellow Mosaic Virus

Essam K.F. Elbeshehy[1a, 2], Omar A. Almaghrabi[1a], Wael M.A. Mahmoud[1b, 3] and Ahmed M. Elazzazy[1a,3]*

[1a]Biological Sciences Department, Faculty of Science; [1b]Department of Medical Genetics, Faculty of Medicine (North Jeddah), King Abdul aziz University, Saudi Arabia.
[2] Botany Department, Faculty of Agriculture, Suez Canal University, Egypt.
[3]National Research Centre, Cairo, Egypt.

(Received: 21 June 2014; accepted: 03 September 2014)

In this study, physiological and morphological modifications of infected *Vicia faba* leaves by bean yellow mosaic virus (BYMV) in response to biosynthesized silver nanoparticles treatments were examined. Infected leaves showed symptoms including deformations, mottling, size reduction, crinkling and severe mosaic. Three weeks after virus inoculation, the percentage of BYMV infection, disease severity and virus concentration were significantly reduced in response to virus infection. BYMV-infected cells showed lower chloroplast number in comparison to the control. Two kinds of inclusions were detected in BYMV- infected leaves: slightly curved or straight bands sometimes looped at the end, and electron opaque crystals with varied shapes. Spraying of biosynthesized silver nanoparticles on *Vicia faba* leaves helped to reduce or prevent the harmful effects produced after virus infection. Application of silver nanoparticles NPs (9-11nm) treatments after 24 hours of inoculation restored the metabolism of infected leaves to the levels of healthy controls. Silver nanoparticles treatments improved plant health by increasing the photosynthesis rates, pigment contents and levels of other parameters studied similar to control values. Moreover, silver nanoparticles treatments increased plant resistance against BYMV. This was observed through induction of chloroplast number, reduction in percentage of infected plants, decrease in disease severity and virus concentration of plants treated with silver nanoparticles treatments prior to BYMV inoculation. The present results provide an overview of the positive effects of silver nanoparticles treatments in induction of resistance against BYMV infection in faba bean leaves from physiological perspectives. To the best of our knowledge, this is the first report on the effect of biosynthesized AgNPs on physiological parameters of infected *Vicia faba* by Bean yellow mosaic virus.

Key words: Silver nanoparticles, Antiviral, Potyviruses, BYMV, and Faba bean.

Faba bean, *Vicia faba* L. is one of the major pulse crops grown in Arabic region. It is a multi-purpose crop that plays an important role in the socio-economic life of farming communities (Agegnehu and Fessehaie, 2006).

Faba beans can get better soil fertility and decrease the occurrence of diseases and pests when full-grown in rotation with other crops , under certain environmental conditions (Mwanamwenge *et al*. 1998 and Torres *et al*., 2006). In general, the production of faba bean has been constrained by several biotic and abiotic factors as riverward by El-Bramawy and Abdul Wahid (2005); Agegnehu, and Fessehaie, (2006). Diseases are among the most important biotic constraints limiting the production of faba bean.

There are different viruses in the genus *Potyvirus* of the family *Potyviridae* , such as BYMV. A viral disease caused by Bean yellow mosaic virus (BYMV) is one of the economically

* To whom all correspondence should be addressed.
E-mail: ahmedazazy8@hotmail.com

important diseases that damages the foliage, limits photosynthetic activity (depending on the infection stage), and reduces faba bean production globally (Cheng *et al*., 2002 and Miteva *et al*., 2005). In the Arabic region loss due to BYMV disease can be reached up to 30% on susceptible cultivars of faba bean (Khalil and Erskine, 2001). In Ismailia, a specific Egyptian Governorates, the BYMV infection could be reduce the faba bean production around 55.6 % and 81.00 % due to different environmental conditions and the wide distribution range of insects in the Ismailia district (El-Beshehy, 1999).

Symptoms of BYMV infection include severe symptoms as mosaic, mottling, crinkling, size reduction and deformation (Radwan *et al*., 2008).

Nanoparticles are appraised to be one of the most important molecules of recent scientific research and attracted the attention of scientists from different fields. Several methods are used for nanoparticles production such as physical, chemical and hybrid methods (Tsuji *et al*., 2003, Chol *et al*., 2005, Li *et al*., 2005 and Mazumdar H. and G.U.Ahmed, 2012**)**, which despite being rapidly production methods they possess the use of unsafe chemicals, undesirable side products, high energy, high temperature and wasteful purifications especially when being contacted by plant or human (Jae and Beom, 2009). Therefore, recently the scientists have made efforts to make the use of microorganisms as far as possible effective, harmless, eco-friendly nano-factories for the synthesis of nanoparticles (Speshock *et al*., 2010).

In this article we hypothesis that the discovery of new silver reducing strains and the use of charge-free or charge- credit production rout, may lead toa greater possibility for economical, eco-friendly silver nanoparticles production method and investigate the effects of BYMV infection and silver nanoparticles treatments on several physiological and growth parameters, especially total photosynthesis pigments contents, total soluble proteins and total phenols. The leaf ultrastructure of chloroplasts and cell organelles in relation to symptom development was also analysed. Finally, this work aimed to examine whether the silver nanoparticles treatments can induce signs of resistance against BYMV in infected faba bean leaves.

MATERIALS AND METHODS

The bacterial isolates obtained in our previous study were used for the synthesis of AgNPs. Out of the eleven bacterial isolates only (D15) strain was used, where D15 was found to have the ability to form AgNPs and this isolate was confirmed as strain coming under *Bacillus* sp. by by 16S rDNA sequencing analysis.

Isolation and characterization of Bacteria and Silver nanoparticles

The selected bacterial strain was inoculated in nutrient broth and incubated for 24 h in a rotating shaker at room temperature uner agitation conditions. The biomass was harvested after 24 h. of growth and centrifuged at 5000 rpm for 10 min. The supernatant of bacterial culture was collected and separately added to the reaction vessels containing silver nitrate at concentrations of 1 mM. The reaction between these supernatant and silver ions was carried out in dark conditions for 24 h. Medium without silver nitrate was also running with the experiment flasks as a control. The stability detection of silver nanoparticles was carried out by visual observation of the culture filtrate at different time intervals at 12, 24, 48 and 72 hrs. These samples were later subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer and scanning the spectra between 200-1000 nm at the resolution of 1 nm, according to the method described by Kirubha and Alagumuthu (2013). Further characterization involved the use of scrutinize transmission electronmicroscope (TEM) to comprehend the morphology, size and the distribution of Ag+NP, (Nagati *et al*., 2012).

Isolation and identification of BYMV

BYMV source

Faba bean leave samples were tested serologically against BYMV, using specific polyclonal antibodies by DAS-ELISA test, according to the methods described by Clark and Adams (1977). Leaf samples which gave positive reaction with specific BYMV antibodies, were extracted and diluted to be used for inoculation faba bean cv. Giza 3 (Radwan *et al*., 2008).

Molecular characterization

RNA purification

Total RNA was extracted by SV total RNA isolation system by spin protocol as recommend

by the manufacturer instructions of Promega, fifty mg of BYMV -infected leaves of broad bean leaves were ground in liquid nitrogen. The purified RNA was stored at -20°C.

RT-PCR for virus identification

After total RNA extraction, detection was carried out using QIAGEN One Step reverse transcription- polymers chain reaction (RT-PCR) Kit (Qiagene, Germany). To amplify the BYMV genome, primers were designed from the CPregion and evaluated by RT-PCR. Using of the forward primer 5'CAGTTTATTATGCAGCGG3' and reverse primer 5'GTTATCATCAATCTTCCTGC3', which will yield a 700-bp product (Hammond and Hammond, 1989 and Hiroyuki Uga, 2005), and amplify only BYMV. Same region was utilized by most of workers for designing specific BYMV primers (Castro *et al.*, 1993; Rosner *et al.*, 1994). The procedure for one step RT-PCR consists of a 50 °C for 30min, incubation at 95 °C (15 min for the first cycle), 94 °C for 1 min, 53 °C for 1.30 min and 72 °C for 1 min (35th cycle for annealing temperature) 72 °C for 10 min and 40°C for hold. These conditions were used to run the cycles for all the primers. The PCR products were analyzed by gel electrophoresis on 1% agarose gel and stained by ethidium bromide and examined using UV transilluminator and the PCR fragments of BYMV will confirm as 700 bp. (Sambrook *et al*., 1989).

Plant resources and Ag+NPs applications

Cultivar of *Vicia faba*, cv. Giza 3 was planted in sterilized soil (pH 6.7) in natural and favorable condition suitable for broad bean growth. BYMV used in these experiments were prepared from fresh severely infected leaves of faba bean cv. Giza 3. After 21 days of growth. Plants with similar size were selected and divided into3 groups. Each group consists of 4 treatments. The names of the treatments were as follows, T1: (Healthy), T2: (Healthy & NPs), T3: (Infected), T4: (Infected & NPs). Each treatment consist of 3 replicates (a replicate is one pot containing3healthy seedling). The names of the groups was as follows, group 1: Inoculation by virus with NPs,group2: spray by NPs before 72 hours from virus inoculation,group3: spray by NPs after 24 hours from virus.

Determination of photosynthetic pigments

The photosynthetic pigments were described by Holden (1965) in the third leaves of various broad bean cultivars after 21 days from inoculation by (BYMV). Leaves were collected and ground in a mortar with 10 ml of 85% acetone, approximately 2-3 gm purified sand, and 2gm $CaCo_3$ were added to neutralize the acidity of the sap and to prevent transformation of chlorophyll into pheophytuin . Acetone extract was filtered through a center glass funnel of fine porosity (G4) and the residues were washed with small volumes of acetone 85% until being free from pigments .Each filtrate was made up to 50 ml volume with 85% acetone and optical density was measured at wave length of 662, 644 and 440.5 nm for chlorophylla, chlorophyll b and total chlorophyll a+b , respectively , using Beck man DK-2 spectrophotometer . Concentrations of chlorophyll a , b and total chlorophyll a+b werecalculated as follows:

Chlorophyll a = (9.784 × E662) - (0.99 × E644) = mg /g (F.wt.)

Chlorophyll b = (21.426 × E644) - (4.56 × E662) = mg /g (F.wt.)

Chlorophyll a + b = Chlorophyll a + Chlorophyll b

Proteins content

The leaves of broad bean cultivar were collectedto determine total soluble protein contents of infected and NPs treated leaves compared with control. Total proteins contents were extracted from 50 gm broad bean leaves in SDS reducing buffer. The sample was diluted at least 1:4 with sample buffer and the extract was centrifuged at 10.000 rpm for 20 minutes. The total soluble protein method was described by Bradford. (1976) by using bovine serum albumin as a standard protein content and was adjusted to 2 mg / ml per sample. (Tucci *et al* ., 1991) . Then 10 µl of total soluble protein was taken for electrophoresis or stored at -20 according to (Juo and Stotzky , 1970) .

Total phenolics content

A modified folin-ciocalteu method (William, *et al*.1965) was used as shown below. For determination of total phenols in various leaf samples, 1ml of ethanolic extracts of each leaf and tuber sample was added to 1ml of 2N folin-ciocalteu. Test tubes were heated to about 70 cæ% in water bath, and then left for slow cooling at room temperature. An optical density of these samples was measured on 650 nm. Using a Beckman DK-2 spectrophotometer. Concentration of total phenols in the extracts was calculated as mg/gm (D.Wt.) of the extracted tissues using a pyrogallol

standard curve.

Total mineral constituents (NPK).

Broad bean leave samples were taken to determine total nitrogen,phosphorous and potassium contents. Total nitrogen was determined as mg /g dry matter using micro- kjeldahl method using Sodium hydroxide, Sulphoric acid and BGG +MR (Jakson,1958).

Phosphorous was determined Colourimetrically as mg / g dry matter using Ammonium molybdate, Hydroquinon and Sodium sulfite solution as reagents (Snell and Snell , 1967). Standard curve were carried out using known concentration ranging from zero to 100 ppm.

The potassium concentration was determined in the wet ash as ppm using Beck man flame photometer according to the method described by Braun and Lilleland (1946) .

Arithmetical analyses

ANOVA type one-way were used to calculate the noteworthy diversity in the averages of the experimental treatments. A probability at level of 0.05 or less was measured considerable.

RESULTS AND DISUSSION

Among the many bacterial isolates used for the study, culture supernatant of Bacillus sp. (D15) was found to have the potential to form AgNPs (Fig. 1). This was indicated by the colour change of the supernatant when incubated with 1 mM $AgNO_3$ (data not shown). This colour change, which is due to the excitation of surface plasmon resonance of AgNPs, is a well-known proof of AgNPs formation (Kumar and Mamidyala, 2012).

The production of AgNPs was further monitoredby UV-Vis spectral analysis, as part of its preliminarycharacterization (Kirubha and Alagumuthu 2013**)**.Silver nanoparticles present an absorption peak at 425 nm after 12 h of reaction (Fig. 2), and that is the attribute surface plasmon resonance (SPR) band of AgNPsprobably owing to the stimulation of longitudinal plasmonsensations in Ag+NPs in the mixture (Mulvaney, 1996; Mock *et al*., 2002; Kumar and Mamidyala, 2012). The absorbance intensity increases steadily with increasing reaction time to 72 h, this denoted continuous reduction of silver nanoparticles subsequently increase in the concentration of AgNPs (Kirubha and Alagumuthu

Table 1. Effect of different silver nanoparticles antiviral on estimate of virus concentration , percentage of infection and diseases severity of faba bean, cv. Giza3 in the presence Bean yellow mosaic virus under greenhouse condition.

Treatments	Virus concentration by DAS- ELIZA			Percentage of infection (%) Virus infectivity (I)						Percentage of disease severity (DS %) *(Disease grade)		
				Group1		Group2		Group3		Group1	Group2	Group3
	Group1	Group2	Group3	I	%	I	%	I	%			
H (Healthy)	0.037(-)	0.037(-)	0.036(-)	0/9	00.00	0/9	00.00	0/9	00.00	00.0%*(0)	00.0%*(0)	00.0%*(0)
H+NPs	0.033(-)	0.035(-)	0.032(-)	0/9	00.00	0/9	00.00	0/9	00.00	00.0%*(0)	00.0%*(0)	00.0%*(0)
I (Infected)	1.744(+)	1.431(+)	1.150(+)	9/9	100.00	9/9	100.00	9/9	100.00	100%*(4)	100%*(4)	100%*(4)
I+NPs	0.400(-)	1.412(+)	0.091(-)	0/9	00.00	9/9	100.00	0/9	00.00	00.00%*(0)	50%*(2)	00.00%*(0)

The values are means (M) of three replicates. In Elisa test for virus concentration, the positive and negative control are 1.492 and 0.113 respectively. Positive control means infected leaves showed symptoms typically and negative control means infected leaves showed no symptoms.

Table 2. Changes of photosynthetic pigments in control and bean yellow mosaic virus-infected broad bean leaves under effect of different silver nanoparticles antiviral

	Photosynthetic pigments in control (mg g-1 fresh weight)								
	Group1			Group2			Group3		
	Chl a (mg g_1 fresh weight)	Chl b (mg g_1 fresh weight)	Total Chl (a + b)	Chl a (mg g_1 fresh weight)	Chl b (mg g_1 freshweight)	Total Chl (a + b)	Chl a (mg g_1 fresh weight)	Chl b (mg g_1 freshweight)	Total Chl (a + b)
H (Healthy)	0.643 ± 0.001528	0.341 ± 0.009644	0.984 ± 0.009815	0.543 ± 0.006028	0.358 ± 0.016653	0.901± 0.015716	0.678 ± 0.037723	0.403 ± 0.006807	1.081± 0.458784
H+NPs	0.640 ± 0.006083	0.339 ± 0.033151	0.979 ± 0.03923	0.608 ± 0.017039	0.322 ± 0.020502	0.931 ± 0.037541	0.654 ± 0.015044	0.364 ± 0.01044	1.018 ± 0.006351
I (Infected)	0.305 ± 0.032787	0.087 ± 0.011015	0.392 ± 0.043432	0.274 ± 0.024705	0.117 ± 0.037859	0.390 ± 0.020502	0.310 ± 0.010017	0.157 ± 0.064671	0.467 ± 0.056471
I+NPs	0.651 ± 0.018502	0.340 ± 0.007638	0.992 ± 0.063553	0.493 ± 0.005508	0.314 ± 0.014295	0.808± 0.018583	0.648 ± 0.00611	0.362 ± 0.011533	1.009 ± 0.015948

Mean of three replication and ± is standard deviation

2013**).**The concentration of the AgNPs band amplifiedshortestfraction to the incubation period of nanoparticle synthesis, but there were no increase in the intensity after 48 h in AgNPs, indicating the completion of the reaction (Kumar and Mamidyala, 2012) and good stability of biosynthesized AgNPs (Mittal *et al*., 2014).

The TEM images and XRD analysis confirmed the production of silver particles at nano-scale (Fig. 3). TheNPs were mono-dispersed and sphere-shaped(Nagati *et al*., 2012). The silver nanoparticle formed was in the size range of (9-11nm). This also may reveal that the biosynthesized silver nanoparticles present a perfect dispersion inside the bio-reduced aqueous solution (Kathiresan *et al*., 2010).

Fig. 1. Phylogenetic tree based on the nucleotide sequences of 16S rRNA genes

Fig. 2. UV-Vis absorption spectrum of obtained silvernanoparticles

Naturally infected samples of faba bean plants exhibiting venial yellowing, followed by obvious yellow mosaic, vein clearing with yellowish line patterns were collected from open field (Fig.4). These samples were tested serologically against BYMV, using specific polyclonal antibodies by direct ELISA test, leaf samples which gave positive reaction with specific BYMV antibodies, were collected separately and used for virus inoculation on faba bean cv. Giza 3.and symptoms appeared severe mosaic, crinkling and size reduction, These findings were in harmony with results reported by Arneodo *et al*., (2005) ; Radwan *et al*.,(2008) ; El-Bramawy and El-Beshehy (2011) and El-Bramawy and El-Beshehy (2012).

The RT-PCR was used for detection of BYMV coat protein (cp) gene in six samples from faba bean leaves cv. Giza3. PCR fragment of correct size 700bp was amplified with the specific primers for BYMV-cp. gene. These results were in agreement with Hammond and Hammond, (1989) and Hiroyuki Uga (2005).

Systemic yellow mosaic, crinkling, and leave malformation were appeared on infected broad bean leaves cv.Giza3 by BYMV in comparison to healthy leaves. In opposition, silver nanoparticles submission abridged the appearance of destructive symptoms caused by virus progress, especially when plants spread by silver nanoparticles after 24 hours from inoculation

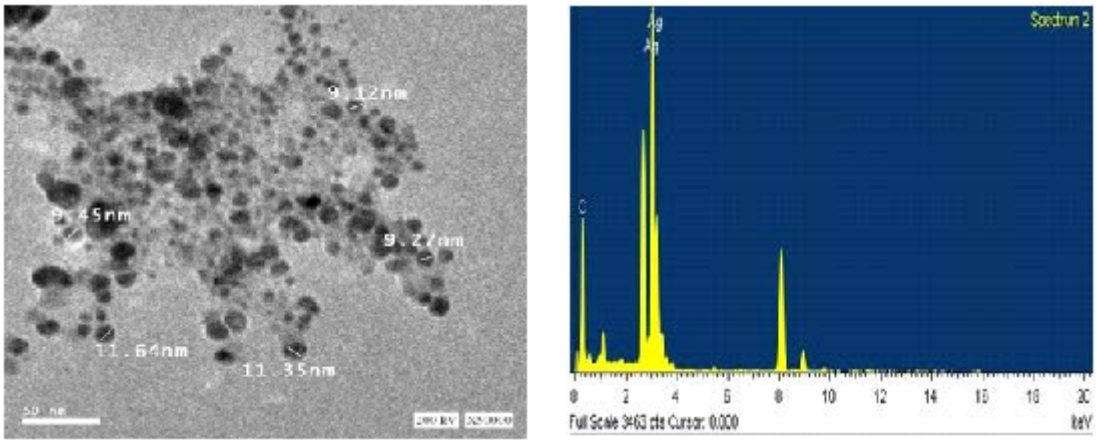

Fig. 3. TEM image and EDX analysis of silver nanoparticles synthesized by *Bacillus* sp. D15of biosynthesized silver nanoparticles

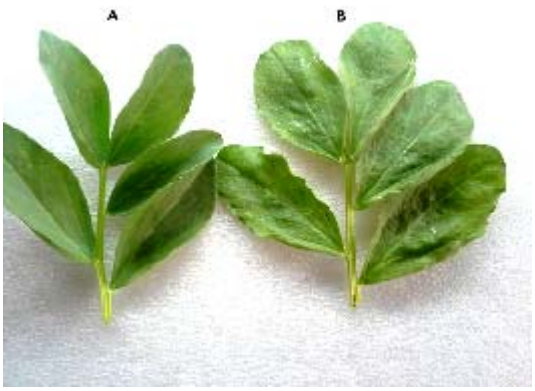

Fig. 4. Symptoms caused by natural infection of BYMV on broad bean leaves viewing severe mosaic, crinkling and size reduction. (A) Healthy broad bean leave, (B) Infected broad bean leave

compared with infected and healthy controls ,while, Mild BYMV symptoms were obtained when plants treated by silver nanoparticles after zero time from inoculation (with viral inoculums sap). On the other hand, plants treated by silver nanoparticles before 72 hours from inoculation had not given any effect on viral infection compared with other silver nanoparticles treatments.

Silver nanoparticles treatment in group1 were recorded a middling velocity of decreased compared with control. On the other hand, we observed negative effect of silver nanoparticles treated before 72 hours from inoculation on BYMV infection. Ended that silver nanoparticles synthesized from bacteria D15 in group3 caused an almost complete reduction of the impact of the virus and viral infection on infected broad bean

Table 5. Changes of chemical components (N, P and K) in control and infected bean yellow mosaic virus-infected broad bean leaves under effect of different silver nanoparticles antiviral

Treatments	Chemical components (N, P and K)								
	Group1			Group2			Group3		
	N	P	K	N	P	K	N	P	K
H (Healthy)	1.937± 0.051316	31.167± 1.997807	130.827± 0.430039	1.927± 0.049329	31.580± 0.629603	131.680± 0.961613	1.903± 0.032146	32.410± 1.090825	130.790± 0.451331
H+NPs	1.843± 0.005774	30.997± 1.351493	131.647± 0.241937	1.863± 0.020817	31.837± 1.380012	130.707 ± 0.090738	1.827± 0.011547	32.437± 0.623244	130.780± 0.508626
I (Infected)	2.365± 0.021213	52.547± 1.048872	209.217± 3.527695	2.367± 0.011547	41.307± 2.200508	204.830± 5.111996	2.427 ± 0.035119	51.430± 1.213919	213.177± 3.114488
I+NPs	2.147± 0.047258	38.763± 0.336056	236.297± 58.02677	2.530± 0.036056	51.213± 0.851959	210.943± 0.292803	2.030± 0.060828	36.970± 0.25865	200.8533± 0.876603

Mean of three replication and ± is standard deviation
(N) Total nitrogen content (mg/g dry matter) (P) Phosphorous content (mg/g dry matter) (K) Potassium content (PPm)

leaves, which led to a reduction in the concentration of the virus and symptoms of infection does not appear on the infected leaves by BYMV.

The BYMV concentration, percentage of infection, and disease severity were summarized in Table 1. Low incidence of symptoms occurred with silver nanoparticles NPs sprayed after 24hr of infection and significant decreases in BYMV concentration, percentage of infection and disease severity (0.091), (0.00%) and (0.00%) respectively, compared with another treatments. Moderate reductions in all symptoms were exhibited when the silver nanoparticles sprayed at the same time of infection in group1. This suggests that the mode of action of viral neutralization by silver nanoparticles occurs during the early phases of viral replication. In contrast, weak to rare reduction occurred in virus concentration (1.41), percentage of infection (100%) and disease severity (50%), when silver NPs1, NPs2 and NPs3 sprayed pre-viral infection. This may be due to the inability of the silver nanoparticles to activate the induced systemic resistance of the plant against BYMV infection.

Silver nanoparticles may affect the RNA copying during viral multiplication and it is obvious that silver nanoparticles significantly affect the inhibition of viral nucleic acid replication when silver nanoparticles particles become less than the size of the particles (Toshikazu, 1999; Lee *et al*., 2003; Galdiero *et al*. 2011 and Narasimha *et al*., 2012).

From the data exposed in Table 2, the concentrations of photosynthetic pigments (chlorophyll a, b and total chlorophyll a + b) increased in all plants treated with silver nanoparticles, while in infected plants the pigments concentration decreased. Sameh (2005) reported that the pigment contents (chlorophylls a, b and carotenoids), water-soluble carbohydrates, total soluble proteins, and total free amino acids were estimated in leaves of two host plants (Vicia faba) inoculated with BYMV. In Vicia faba the virus isolate induced a highly gradual decline in photosynthetic pigments and an increase in soluble proteins with age.

High values of total soluble protein contents were recorded in infected plants treated with NPs (35.63, 50.71, and 33.02) in comparison with infected plants (54.01, 40.79, and 43.16) and

Table 3. Changes of soluble proteins (mg g^{-1} fresh weight) in control and bean yellow mosaic virus-infected broad bean leaves under effect of different silver nanoparticles antiviral

Treatments	Soluble proteins (mg g-1 fresh weight)		
	Group 1	Group 2	Group 3
H (Healthy)	30.567±0.145717	31.863±0.346747	32.137±0.638931
H+NPs	29.957±0.126623	30.457±0.095044	31.137±0.694718
I (Infected)	54.010±0.426732	51.797±0.622682	53.160±0.806164
I+NPs	36.637±0.050332	47.710±0.60531	35.020±0.348281

Mean of three replication and ± is standard deviation

Table 4. Changes of total phenols (mg g^{-1} dry weight) in control and bean yellow mosaic virus-infected broad bean leaves under effect of different silver nanoparticles antiviral

Treatments	Total phenols (mg g-1 fresh weight)		
	Group 1	Group 2	Group 3
H (Healthy)	0.500± 0.01	0.513 ± 0.015275	0.500± 0.01
H+NPs	0.543 ± 0.005774	0.513± 0.01	0.553 ± 0.005774
I (Infected)	0.357± 0.011547	0.363 ± 0.005774	0.353 ± 0.005774
I+NPs	0.477± 0.015275	0.383 ± 0.005774	0.487± 0.005774

Mean of three replication and ± is standard deviation

healthy plants (30.56, 31.83, and 32.13) (Table 3). Therefore, all treatments of silver nanoparticles showed lower accumulated total soluble protein content compared with infected leaves, Sameh (2005).

The total phenolics content (mg /g^{-1} dry weight) reached (0.35, 0.36, and 0.35) in susceptible cultivar Giza3 when inoculated by BYMV compared with healthy plants (0.5, 0.51, and 0.5). Broad bean leave treated with silver nanoparticles showed highly significant increase of phenolic content at all levels of treatment (Table 4). Balogun *et al*., (2004) and Rai *et al*., (2010) mentioned that the studies on the changes in total phenol among resistant and susceptible genotypes during disease reaction indicated that there are a wide variation in the enzyme activity among different condition. Under stress condition, including viral infection, stimulation and increased activity of phenolics play role in defense mechanism.

All plants treated by silver nanoparticles showed higher accumulation of phenolic contents compared with infected leaves. Duarte *et al*., (2005) suggested that a significant decrease in the contents of phenols and alkaloids were observed in the leaves of *Datura stramonium* inoculated with Potato virus X (PVX (X-I).

Total nitrogen, phosphorus, and potassium contents of healthy and infected broad bean leaves were determined. Results in table (5) indicated that the infected leaves contained high amounts of total mineral constituents (NPK) than the healthy ones.

Generally, (NPK) content increased in all silver nanoparticles applications compared with healthy control. Awadhesh (1973) mentioned that the virus infection generally results in the drastic bio-chemical and physiological changes in the host plants. These changes might be used in setting up the characteristic of the disease as a supplement to the symptomatology. Duarte *et al.*, (2005) have been studied various physiological changes associated to viral infection such as decrease in photosynthetic activity, increase in respiration rate, accumulation of nitrogen compounds, and increase in polyphenol oxidase activity and alterations in hormonal and secondary metabolisms.

REFERENCES

1. Agegnehu G.& Fessehaie R. Response of faba bean to phosphate fertilizer and weed control on nitisols of Ethiopian Highlands. *Ital. J. Agron. Riv. Agron*. 2006; **1**: 281-291.
2. Arneodo J.D., de Breuil S., LenardonS.L., Conci V.C. & ConciL.R. Detection of Bean yellow mosaic virus and Cucumber mosaic virus infecting gladiolus in Argentina. *Agriscientia*, 2005; **XXII** (2): 87-89.
3. Awadhesh B.S., 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.
4. Balogun O.S. & Teraoka T., Time-course analysis of the accumulation of phenols intomato seedlings infected with Potato virus X and Tobacco mosaic virus. Biokemistri, Llorin, v. 2004; **16**: 112-120.
5. Bradford M.M., A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*. 1976; **72**:248-254.
6. Braun J.D. &Lilleland O., Rapid determination of potassium and sodium in plant material and soil extracts by flame photometry.*Proc. Amor. Soc. Hort.Sci*. 1946; **48**: 341.
7. Castro S., Carazo G., Saiz M., Romero J.& Blas, C.De., Use of enzymatic cDNA amplification as a methodof detection of bean yellow mosaic virus. *Neth J. Plant Pathol*. 1993; **99**: 97-100.
8. Cheng Y., Jones R.A.C. &Thackrya D.J., Deploying strain specific hypersensitive resistance to diminish temporal virus spread. *Ann Appl Biol*. 2002; **140**: 69-79.
9. Chol S.H., Zhang Y.P., Gopalan A., Lee K.P. & Khang H.D., Preparations of catalytically efficient precious metallic colloids by γ-irradiations and characterizations colloids and surfaces A: Physiochemical and Engineering *Aspects*. 2005; **256**: 165-170.
10. Clark M.F. & Adama A.N., Characteristics of the microplate methods of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol*. 1977; **34**: 475-483.
11. Duarte L.M.L., Salatino M.L.F., Salatino A., Negri G. & Barradas M.M. Efeito do Potato virus X no conteúdo de fenóis totais e alcalóides em folhas de Datura stramonium. *Summa Phytopathologica*, 2008; **34**(1): 65-67
12. EL-Beshehy E.K.F., Pathological studies on some viruses infecting legume plants in Ismailia Governorate. Thesis M. Sc., Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, 1999; 122.
13. EL-Bramawy M.A.S. & El-Beshehy E.K.F., The Resistance of Bean Yellow Mosaic Virus (BYMV) in Faba bean (Vicia faba L.) with Diallel Analysis. *Journal of Biology and Life Science*. 2011; **2**(1): E1.
14. EL-Bramawy M.A.S. & El-Beshehy E.K.F., Inheritance of resistance to Bean yellow mosaic virus in Faba bean plants. *International journal of virology*. 2012; **8**(1): 98-105.
15. EL-BramawyM.A.S.& Abdul WahedO.A. Faba bean sources of resistance to chocolate and spot diseases, earliness and seed yield. The 7th ACSS Conference, 5-9 Sept., Uganda. 2005; **7**: 359-562.
16. Galdiero S., FalangaA., Vitiello M., Cantisani M., Marra V. & Galdiero, M. Silver Nanoparticles as Potential Antiviral Agents. Molecules 2011; **16**: 8894-8918.
17. Hammond J & Hammond R. W., Molecular cloning, sequencing and expression in *Escherichia coli* of the bean yellow mosaic virus coat protein gene. *J Gen Virol.* 1989; **70**(Pt. 8): 1961-1974.
18. Hiroyuki U., Use of crude sap for one-step RT-PCR based assay of Bean yellow mosaic virus and the utility of this protocol for various plant – virus combinations.*J.Gen Plant Pathol.*2005; **71**: 86-89.
19. Holden M., Chlorophyll in chemistry and biochemistry of plant pigments. Acadmic press Inc., New york, 1965.
20. Jae Y.S. & Beom S.K., Rapid Biological Synthesis of Silver Nanoparticles Using Plant Leaf Extracts. *Bioprocess Biosyst. Eng*., 2009; **32**: 79-84.
21. Jakson M.L. Soil chemical Analysis. Constabe and Co. LTD, London, 1958.
22. Juo P. & Stotzky G., Electrophoretic patterns of globulin and albumin in conifer seeds. *Gan. J. Bot*. 1970; **48**:1911-1912.
23. Kathiresan K.N.M., Alikunhi S., Pathmanaban A., Nabikhan S. &Kandasamy., Analysis of antimicrobial silver nanoparticles synthesized by coastal strains of *Escherichia coli* and *Aspergillus niger, Canad J. of Microbiol*. 2010; **56**: 1050.
24. Kirubha R. & Alagumuthu G., Morinda tinctoria fruit assisted biosynthesis of silver nanoparticles. *Asian J Pharm Clin Res.* 2013; **6**(4): 60-66.
25. Khalil S.A. &Erskine W., Combating disease problems of grain legumes in Egypt. *Grain Legumes* 2001; **32**(2): 24-26.

26. Kumar C.G& Mamidyala S.K., Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa*. Colloids and Surfaces B: Biointerfaces 2012; **84**: 462-466.
27. Lee H.J., Yeo S.Y. & Jeong S.H., Antibacterial effect of nanosized silver colloidal solution on textile fabics. *Journal of Materials Science*. 2003; **38**: 2199-2204.
28. Li Z., Li Y., Qian X.F., Yin J.&Zhu Z.K., A simple method for selective immobilizations of silver nanoparticles. *Applied surface sciences.* 2005; **250**: 109-116.
29. Mazumdar H. & Ahmed G.U., Synthesis ofsilver nanoparticles and its adverse effect on seed germinations. *International Journal of Advanced Biotechnology and Research*. 2012; **2**(4): 404-413.
30. Mock J.J., Barbic M., Smith D.R., Schultz D.A. & Schultz S. Shape effects in plasmon resonance of individual colloidal silver nanoparticles. *Journal of Chemical Physics*, 2012; **116**(15): 6755-6759.
31. Mittal A.K., Bhaumik J., Kumar S.&Banerjee U.C., Biosynthesis of silver nanoparticles: Elucidation of prospective mechanism and therapeutic potential. *Journal of Colloid and Interface Science*. 2014; **415**: 39-47.
32. Miteva E., Hristova A.D., Nenova B.V. & Maneva S., Arsenic as a factor affecting virus infection in tomato plants: changes in plant growth, peroxidase activity and chloroplast pigments. *Sci Horticult*. 2005; **105**: 343-358.
33. Mulvaney P., Surface Plasmon Spectroscopy of nanosized metal particles; *Langmuir* 1996; **12**: 788–800.
34. Mwanamwenge J., Loss S.P., Siddique k.H.M. & Cocks P. 1998. Growth, seed yield and water use of faba bean (Vicia faba L.) in a short-season Mediterranean-type environment. Aust J Exp Agric., 38:171-80.
35. Nagati V. B., Rama K., Manisha R.D., Jahnavi A.& Karunakar R., Green Synthesis and characterization of Silver nanoparticles from Cajanus cajan leaf extract and its antibacterial activity. *International Journal of Nanomaterials and Biostructures*. 2012; **2**(3): 39-43.
36. Narasimha G., Khadri H.& Alzohairy M., Antiviral properties of silver nanoparticles synthesized by Aspergillus sps. *Der Pharmacia Lettre*, 2012; **4**(2):649-651.
37. Radwan D.E.M., Fayez K.A, Mahmoud S.Y. & Hamad A., Protective action of salicylic acid against bean yellow mosaic virus infection in Vicia faba leaves. *Journal of Plant Physiology.* 2008; **165**: 845-857.
38. Rai V.P., JaiswalN., KumarS., SinghS.P., Kumar R. & RaiA.B., Response of total phenols and peroxidase activity in chilli exposed to pepper leaf curl virus disease. *Veg.Sci*. 2010; **37**(1): 78-80.
39. Rosner A., Stein A., Levy S.& Lilien-Kipni, H., Evaluation of linked PCR-transcription amplification procedure for bean yellow mosaic virus detection in gladioli. *J. Virol. Methods*. 1994; **47**(1–2): 227-235.
40. Sambrook J.F.,Ritsch E.F. & Maniatis T., Molecular cloning: a laboratory Manual. vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y, 1989.
41. Sameh K.H., Effect of Bean Yellow Mosaic Virus on Physiological Parameters of Vicia faba and Phaseolus vulgaris. *International Journal of Agriculture & Biology*.2005; 1560–8530/07–2–154-57.
42. Snell F.D. & SnellC.T., Colloriemetric Method of Analysis. D. Van Nostard company, Inc., Torono, Newyork,1967.
43. Speshock J.L., Murdock R.C., Braydich-Stolle L.K., Schrand A.M.& Hussain S.M., Interaction of silver nanoparticles with Tacaribe virus. *J Nanobiotechnology*. 2010; **8**: 19.
44. Torres A.M., Roman B., Avila C.M., Satovic, D.Z., Rubiales J.C., Sillero, J. I., Cuber& Moreno M.T., Faba bean breeding for resistance against biotic stresses: Towards application of marker technology. Euphytica. 2006; **147**(1-2): 67-80.
45. Toshikazu T. Antimicrobial agent composed of silica-gel with silver complex, Inorganic *Materials.* 1999; **6**: 505-511.
46. Tsuji T., Watanabe N.& Tsuji M., Laser induced morphology change of silver colloids: Formations of nano-sized wires. *Applied surface sciences.* 2003; **211**: 189-193.
47. Tucci M., Capparelli R., Costa A. & Roa R. Molecular heterogenity and genetics of Vicia faba seeds storage proteins. *Theor. Appl. Genet.* 1991; **81**: 50-58.
48. William H., Chichilo P.A.C. & Reynolds H. Official methods of analysis of the association of agricultural chemists. Tenth edition, Published by ASS. of Agric. Chem. Washington D.C. 1965; 158.