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Original Paper

Enhance potato resistance to Potato virus Y^{NTN} using curcumin nanoparticles

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Nanoparticles (NPs) are considered the best way to manage plant viruses. Therefore, the aim of the study was to compare curcumin (Cur) and curcumin nanoparticles (CurNPs) as protective materials against the Potato virus Y^{NTN} (PVY^{NTN}). Besides, changes in gene expression and defensive enzymes in the potato plants treated and untreated were determined. The average CurNPs size at optimum conditions was about 45 nm and the zeta potential was negative (-18.1) determined by Zeta seizer. Transmission electron microscope (TEM) imaging showed a smooth, spherical shape, and an almost homogenous nanoparticle structure. The fourier transform infrared (FTIR) spectrum of CurNPs was recorded. In the FTIR spectrum of CurNPs, peaks were observed at 1,631; 1,464; 1,157; and 1,073 cm^{-1}. The potato plants treated with 10 $mg.ml^{-1}$ CurNPs + V recorded the highest significant reduction in percentage of disease severity (98%). Besides, treatment with CurNPs increased the rates of systemic acquired resistance (SAR) in the potatoes. On the other hand, SDS-PAGE showed clear variations in the content of the protein among potato plants treated with CurNPs or Cur and inoculated with PVY^{NTN}, compared with the control. In addition, the plants sprayed with CurNPs or Cur pre-inoculation virus induced an increase or decrease in peroxidase (POX) activities. However, there were no substantial differences in activities of polyphenol oxidase (PPO) isozymes were recorded. Therefore, CurNPs could be used in potato breeding programs to control PVY.

Keywords: FTIR, enzyme activities, systemic acquired resistance, SDS-PAGE, TEM

1 Introduction

PVY is one of the main factors limiting the productivity of potato yields in Egypt and worldwide (Jha et al., 2011). It causes losses in potato yield ranging from 10 to 80% (De Bokx and Huttinga, 1981). PVY belongs to the genus Potyvirus, the family Potyviridae. It is transmitted by aphids in a non-persistent manner. Many researchers discovered that medicinal plant extracts could be used to control plant viruses, by inducing resistance in the plant host to the virus (Shabana et al., 2016). Furthermore, the association of curcumin with nanoparticles (CurNPs) increases its bioavailability, solubility, and antiviral effect. Therefore, nanoparticles are considered the best way to manage plant viruses, either by using nanotechnology as a diagnostic tool for viruses or by providing controlled delivery of functional particles (Sharon et al., 2010). *Curcuma longa*, or turmeric is a perennial herbaceous, rhizomatous plant of the ginger family, Zingiberaceae. It is commonly used in Siddha Ayurveda, and traditional Chinese medicine for its medicinal purposes, e.g., antimicrobial, analgesic, anti-inflammatory activity, antiviral, and antiproliferative (Padmanaban and Rangarajan, 2016; Karimi et al., 2019). Indian turmeric is considered one of the best spices in the world market, due to its high content of curcumin (Cur) (Prasad and Aggarwal 2011). The medicinal properties of turmeric are ascribed to three major compounds known as curcuminoids: curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Stanic and Girousi 2012). Curcumin, or diferuloylmethane is the most bioactive compound in turmeric. It exhibits various pharmacological

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activities, involving anti-bacterial, antioxidant, anti-inflammatory, immunomodulatory activity, and antiviral (Padmanaban and Rangarajan, 2016; Karimi et al., 2019). Cur has been shown to have antiviral activity against both non-enveloped and enveloped viruses, including herpes viruses, papillomavirus, chikungunya, HIV, influenza, hepatitis, respiratory syncytial viruses, noroviruses, Zika, dengue, and arboviruses (Celum et al., 2010; Flores et al., 2016; Mounce et al., 2017). The mechanism of Cur action includes a virucidal substance, virus penetration inhibition into the host cell, and interference with viral replication machinery (Mathew and Hsu, 2018; Jennings and Parks, 2020).

Plant immune activators can prevent crop infection with several plant viruses by activating intrinsic immune mechanisms in plants and are widely used in agricultural production. In previous reports, it was observed that curcumin analogs (pentadienone and quinazoline) displayed efficient biological activity against many plant viruses, especially protective activity. Bioassay results found that curcumin analogs (compound A13) had inhibitory activity for *Nicotiana tabacum* plants against Tobacco mosaic virus (TMV), compared with untreated plants. The inhibitory activity is due to defense-related genes, defense enzymes, and photosynthesis. This was confirmed by the up-regulated expression of proteins that mediate stress responses and oxidative phosphorylation (Yin et al., 2018).

The aim of the study was to compare Cur and CurNPs as protective materials against the Potato virus Y^{NTN}. Besides, changes in gene expression and defensive enzymes in the potato plants treated and untreated were determined.

2 Materials and methods

2.1 Plant material

Virus-free potato tubers cv. Spunta are supported by the Potato Brown Rot Project, Ministry of Agriculture and Land Reclamation, Dokki, Giza, Egypt.

2.2 Source of the virus

Potato virus Y^{NTN} strain (accession no. EF 502038) was obtained from the Virology lab., Faculty of Agriculture, Ain Shams University (El-Dougdoug et al., 2007; Mahfouze et al., 2012). PVY^{NTN} strain was propagated on *Datura metel* L. Five healthy plants of the same species and age were left without inoculation as a control. The plants were kept in insect-proof greenhouse conditions. 21 days post-inoculation, the external symptoms were recorded.

2.3 Detection of PVY^{NTN} *by Immuno lateral flow*

0.1 g of *D. metel* L. leaf inoculated with PVY^{NTN} and the healthy control were put into extraction bags, and 3 ml of AgriStrip extraction buffer A was added. The tissues were homogenized for 2–3 sec. Two drops of extract and two drops of extraction buffer were transferred into cuvettes. The end of the stripe was inserted into the extracts, and the formation of color was recorded. The PVY AgriStrips are manufactured by BIOREBA AG, Reinach, Switzerland.

2.4 Synthesis of CurNPs

Curcumin (Cur) powder was stored under dry conditions at -20 °C when not in use and was allowed to warm the space to room temperature (≈25 °C) before the synthesis of nanoparticles and antibacterial tests. Colloids of curcumin nanoparticles (CurNPs) with an average diameter of 20–40 nm were prepared in according to the method described previously (Basniwal et al., 2011; Adahoun et al., 2017).

2.5 Characterization of the prepared Curcumin

2.5.1 Transmission electron microscopy analysis (TEM)

Transmittance electron microscopic (TEM) analysis was done using the Joel JEM-1400 TEM machine in the Faculty of Agriculture, Cairo University, Giza, Egypt. The samples were prepared on a carbon-coated copper grid by just dropping a very small amount of the sample on the grid, an extra solution was removed using a blotting paper, and then the film on the TEM grid was allowed to dry for 5 min.

2.5.2 Particle size and Zeta potential analysis

A Malvern Instrument, MAL 1071664, USA at the Nanotechnology Lab in the Petroleum Research Institute, Cairo, Egypt, was used to determine the standard size of particles, and zeta potential of nanoparticles. Disposable zeta cell measurement was carried out with ultra-pure water at 25 °C. The mean zeta potential was determined using the phase analysis light scattering technique.

2.5.3 Infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy was used to ascertain the structure of CurNPs. FTIR-4100 types A, B154461016 Model of FTIR spectrophotometer within the range of 4,000–400 cm^{-1} in the Faculty of Science; Cairo University, Giza, Egypt. Approximately 5 mg was mixed with KBr (100 mg) and condensed into a pellet using a hydraulic press.

2.6 Application of CurNPs as an antiviral agent

The potato tubers were sown in clay pots (25 cm) filled with clay soil : vermiculite : sand (3 : 1 : 1 w) mixture and transferred to the greenhouse. Plants at the 4-leaf stage were used for experimental work. The experiment carried out 14 treatments, as follows: The first group: Potato

plants were used as healthy controls (H). The second group: Potato plants were inoculated with the virus only (V). The third group: Potato plants sprayed with three different concentrations of 1, 5, and 10 mg.ml^{-1} of curcumin nanoparticles with a volume of 100 ml. plant^{-1} (CurNPs) (Nabila et al., 2020). The fourth group: Potato plants were sprayed with three concentrations of curcumin (100 ml.plant^{-1}) (Cur). The fifth group: potato plants were sprayed with three concentrations of 1, 5, and 10 mg.ml^{-1} of curcumin nanoparticles and inoculated with the virus after a week (100 ml.plant^{-1}) (CurNPs + V).The sixth group: Potato plants were sprayed with three concentrations of 1, 5, and 10 mg.ml^{-1} of curcumin and inoculated with the virus after a week (100 ml.plant^{-1}) (Cur + V). The experiment was conducted with a completely randomized design.

2.7 Assessment of disease severity

After 14 days of PVY inoculation, all potato plants in each treatment were examined weekly for the appearance of external virus symptoms. The symptoms were recorded using the following rating scale: 0 – no symptoms, 1 – blackening and banding of veins, 2 – 50% of leaves showed mosaic symptoms, 3 – 100% mosaic symptoms appeared on all leaves, 4 – 50% of infected leaves showed venial necrosis and severe mosaic, and 5 – 100% leaves showed severe mosaic and leaf crinkling. Disease severity values were calculated using the following formula, according to (Mughal and Khan, 2001):

$$disease\ severity\,(A) = \frac{\sum(B \cdot C)}{(D \cdot E)}$$

where: B – disease grade; C – number of plants in each grade; D – a total number of plants; E – the highest disease grade

2.8 Detection of the virus isolate by DAS-ELISA

Virus detection was carried out to determine its value by the Double Antibody Sandwich-Enzyme Linked Immune Sorbent Assay (DAS-ELISA). One gram of sample leaves from each treatment was used to detect serologically using DAS-ELISA according to Clark and Adams (1977).

2.9 Electrophoretic analysis of proteins by SDS-PAGE

SDS-PAGE was done according to Laemmli (1970) as modified by (Studier, 1973). After, the electrophoresis gel was stained with Coomassie Brilliant Blue dye, and then destained to visualize the protein bands.

2.10 Polyphenol oxidase (PPO) and peroxidase (POX) isoforms

For the assay of antioxidant enzymes, POX and PPO were extracted based on the method described in Stagemann et al. (1985). PPO and POX isozymes were separated by Native-Polyacrylamide Gel Electrophoresis (Native-PAGE). The activities of POX and PPO were determined according to Baaziz et al. (1994). Relative mobility (*Rf*) values were calculated for each band based on the migration of the band relative to the front or tracking dye (*Rf*). The gels were scored as the presence (+) or absence (-) of isozyme bands.

2.11 Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) among treatments. Means were compared by least significant differences (LSD) at $p \leq 0.05$.

3 Results and discussion

3.1 PVY^{NTN} strain

The PVY^{NTN} strain was propagated on *D. metel* L. plants. After 21 days of inoculation, vein clearing, crinkle, and severe mosaic symptoms appeared on plants (Figure 1). These results were confirmed by lateral flow immunoassay, which gave a red-colored band in the test line. In contrast, the healthy control has not scored any color in the test line (Figure 2).

3.2 Size, Zeta Potential, and Morphology of CurNPs

The CurNPs prepared in the experiment exhibit a yellow solution. The Zeta-sizer analysis was used to determine the mean size and size distribution of each batch of CurNPs suspension. The size distribution profile is shown in Figure (3), which represents a typical batch of CurNPs with a mean diameter of 45 nm. It was observed that the surfaces of CurNPs have a negative charge of about -18.1 mV as shown in Figure (4). CurNPs were observed by the transmission electron microscope. The particle

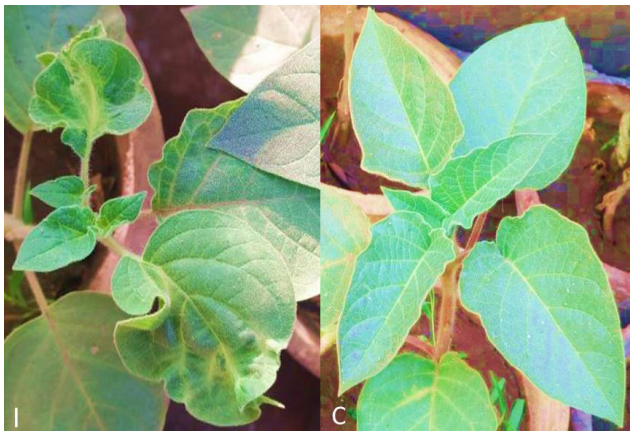

Figure 1 Photograph of *D. metel* L. plants mechanically inoculated with PVY^{NTN} strain showing vein clearing, crinkle, and severe mosaic symptoms (I), compared with healthy control (C)

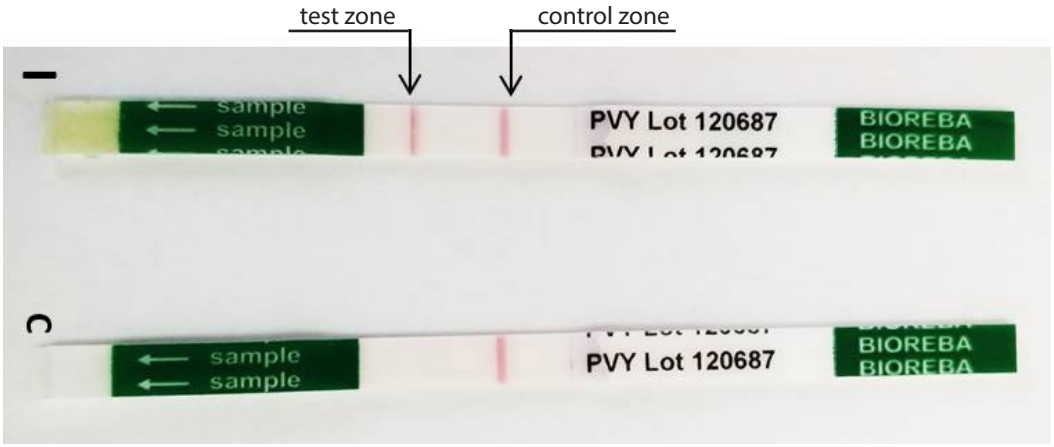

Figure 2 Detection of PVY^{NTN} strain in *D. metel* L. plant inoculated with virus (I) by lateral flow immunoassay, compared with the control (C)

shape was less than 20 nm in diameter with a smooth and spherical shape. The particle size was decreased due to its aggregation and high specific surface energy (Figure 5).

3.3 Infrared Spectroscopy

The FTIR spectrum of CurNPs was recorded. In the FTIR spectrum of CurNPs, peaks were observed at 1,631; 1,464; 1,157; and 1,073 cm^{-1} (Figure 6). In FTIR analysis,

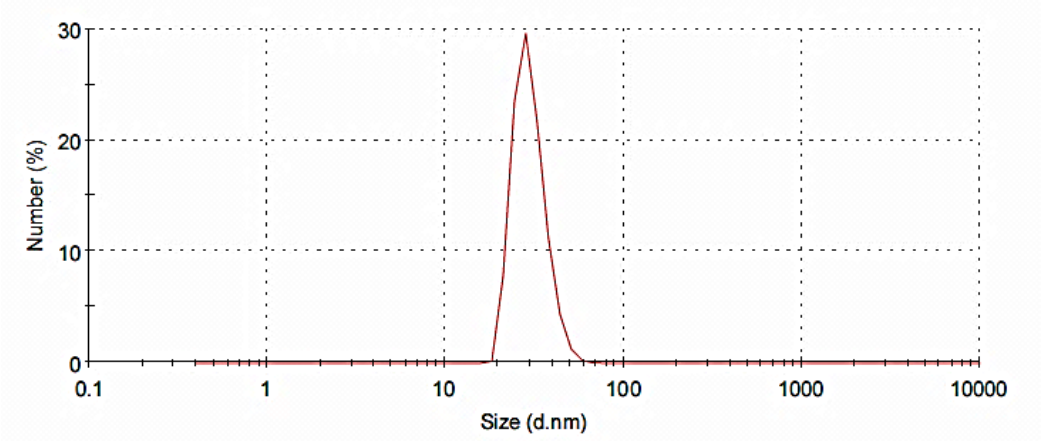

Figure 3 Size distribution of curcumin nanoparticles (CurNPs) with a mean diameter of 45 nm.as measured by zeta-sizer analysis

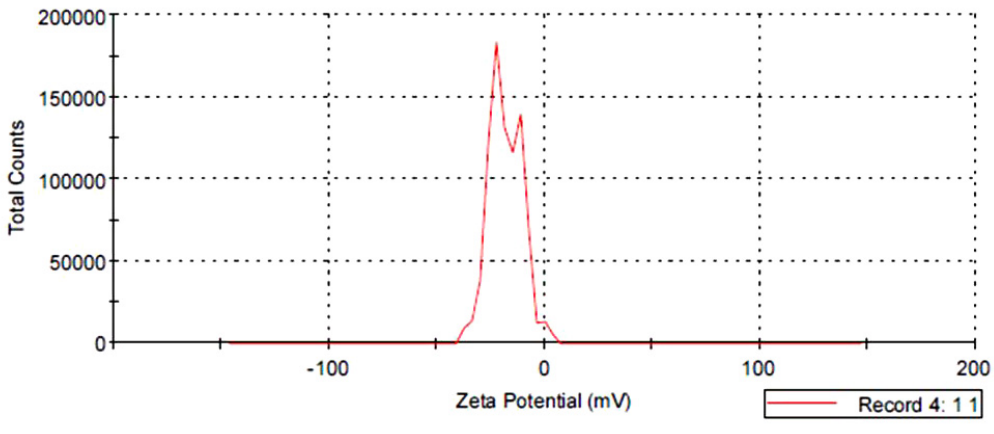

Figure 4 Zeta potential distribution of curcumin nanoparticles (CurNPs). CurNPs exhibit a zeta potential charge with -18.1 mV

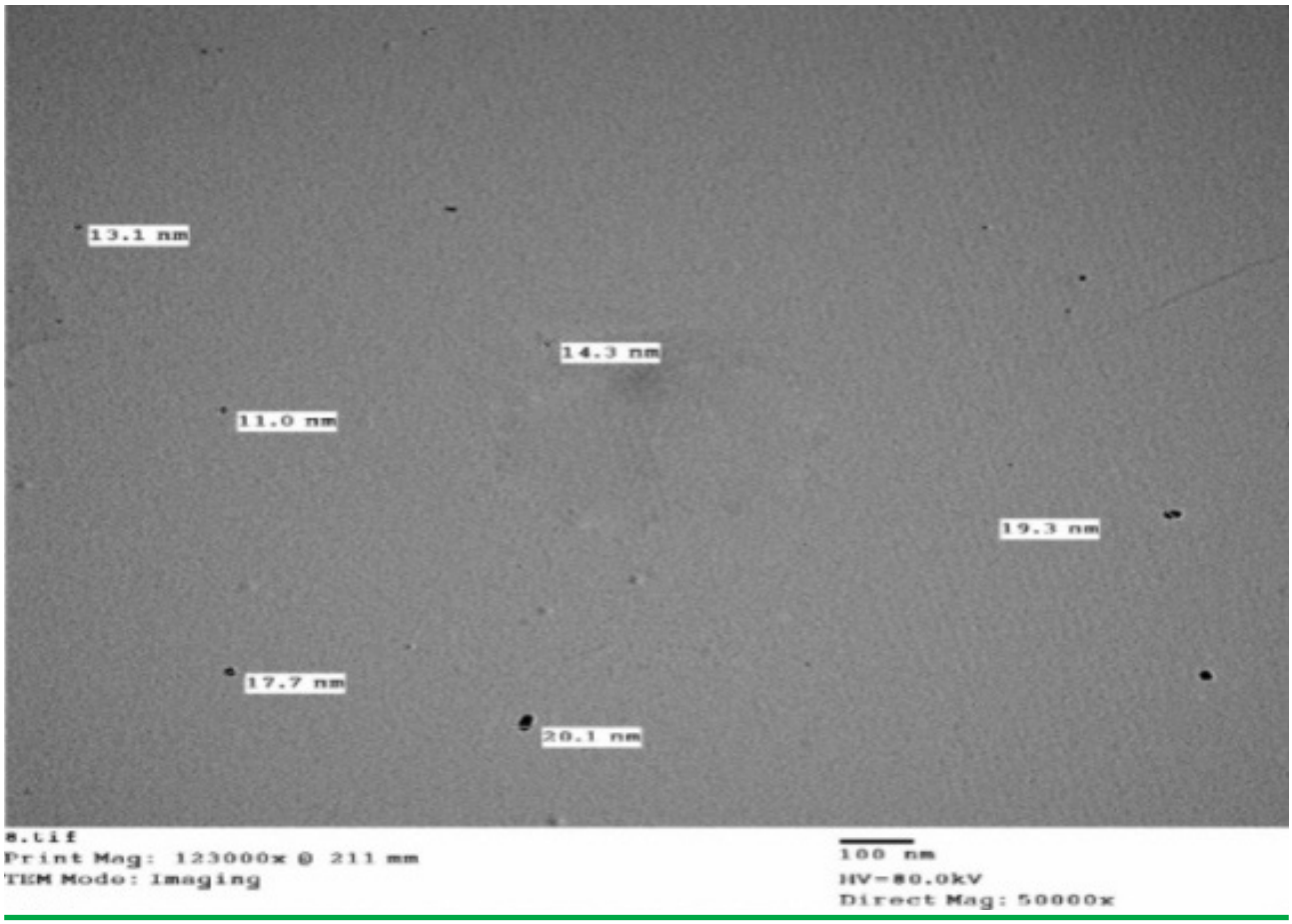

Figure 5 Photograph showing Transmission electron micrograph of curcumin nanoparticles (CurNPs)

peaks correspond to different functional groups. Among these, the absorption peak at 1,631 cm^{-1} can be assigned to C=C stretching, 1,464 cm^{-1} corresponds to C=H, and the absorption at 1,157 cm^{-1} is due to C–H stretching. The absorption peak at 1,073 cm^{-1} might be due to C–N stretch. The absorption spectra of control might be attributed to functional groups such as benzene ring, the C–O–C bond, and aromatic C–H stretching.

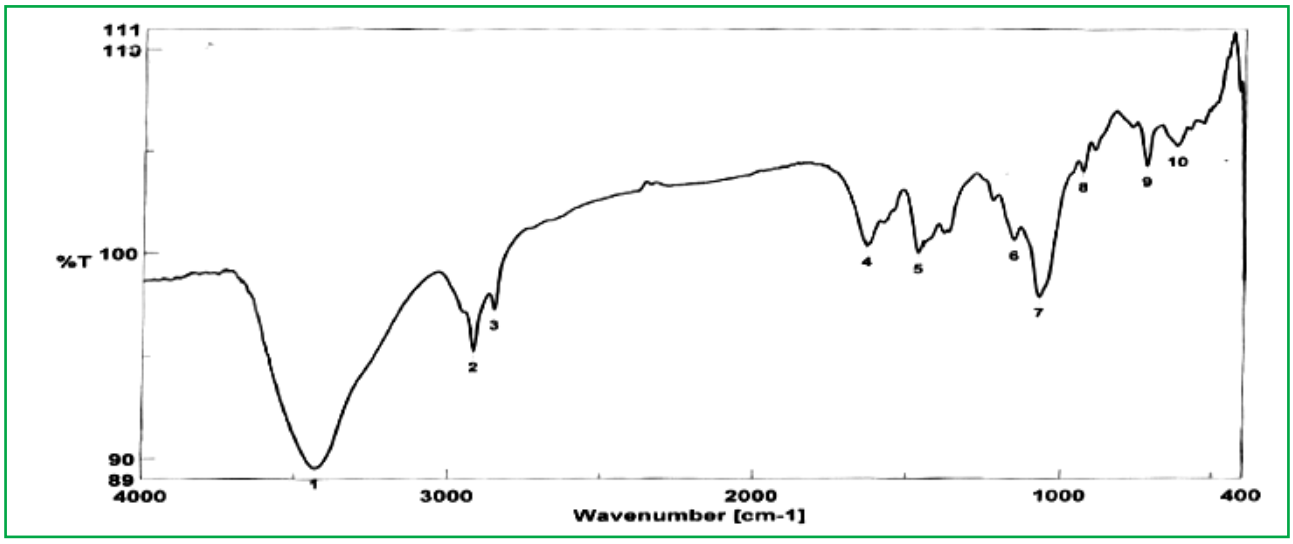

Figure 6 Histograms show FT-IR spectra of curcumin nanoparticles

3.4 Application of CurNPs or Cur as antiviral agents

Data in Table 1 indicated that all concentrations of CurNPs or Cur was able to reduce disease severity after 25 days of inoculation in potato plants treated with different concentrations of CurNPs or Cur pre-inoculation. Reduction percentages were observed with all different concentrations compared with the infected control. Data in Table 1 and Figures (7, 8, and 9) showed that the potato plants treated with 10 mg.ml^{-1} CurNPs + V recorded the highest significant reduction in disease severity, followed by 1 and 5 mg.ml^{-1} CurNPs + V,10 mg.ml^{-1} Cur + V, 1 mg.ml^{-1} Cur + V, and 5 mg.ml^{-1} Cur + V, compared with the infected control.

3.5 Assessment of virus concentration

The virus concentration was measured after 21 days of virus inoculation using ELISA. The results of ELISA presented in Table 2 demonstrated that all treatments reduced the virus concentration, compared to the control (potato plants inoculated with PVY^{NTN} only). After 21 days of inoculation, a concentration of 10 mg.ml^{-1} of CurNPs + V showed the lowest significant concentration of the virus, followed by concentrations of 1 and 5 mg.ml^{-1}. Besides, a concentration of 10 mg.ml^{-1} of Cur + V showed the lowest significant concentration of the virus, followed by concentrations of 1 and 5 mg.ml^{-1}.

3.6 Effect of treatment with CurNPs or Cur on gene expression by SDS-PAGE

The SDS-PAGE revealed clear variations in the content of the protein among potato plants treated with three different concentrations of CurNPs or Cur, and then inoculated with the PVY^{NTN}, compared with the control (Figure 10). The SDS-PAGE was estimated depending on the molecular weights (MWs) (kDa) and the number of bands. A total of 24 polypeptides were detected, ranging in size from 5 to 255 kDa; eight of these were polymorphic (33.33%), while 16 were monomorphic (66.67%). The highest number of protein subunits (24 subunits) was scored in the healthy control (C) (24 bands) and potato plants treated with 1 mg.ml^{-1} (CurNPs + V); followed by plants treated with 10 mg.ml^{-1} (CurNPs) (23 bands).

Table 1 Disease severity and virus concentration of PVY^{NTN} infected potato plants treated with Curcumin (Cur) and curcumin nanoparticles (CurNPs) under greenhouse conditions

Parameters			Infected plants (n =10)	Symptoms index					Virus infectivity	
				mM *(2)	sM *(4)	R *(6)	Y *(8)	N (10)	% Inf	% disease severity
Infected plant (control)			9	2	0	3	0	4	90	62a
Post PVY infection	NanoCurcumin (CurNPs)	1 mg.ml^{-1}	2	2	0	0	0	0	20	4 bc
		5 mg.ml^{-1}	2	2	0	0	0	0	20	4 bc
		10 mg.ml^{-1}	1	1	0	0	0	0	10	2 c
	Curcumin (Cur)	1 mg.ml^{-1}	4	1	1	2	0	0	40	18 bc
		5 mg.ml^{-1}	4	1	0	3	0	0	40	20 b
		10 mg.ml^{-1}	3	0	1	2	0	0	30	16 bc

values in the same letters are not significantly different (P <0.05); total inoculated plants – 70 plants, % Inf – % infection; * degree of symptoms index (2) mM – mild mosaic, (4) sM – sever mosaic, (6) R – rugosity, (8) Y – yellow, (10) N – necrosis

Table 2 Effect of curcumin nanoparticles (CurNPs) and curcumin (Cur) concentrations on the PVY^{NTN} concentration in potato plants after 21 days of inoculation

ELISA result**	Treatment
0.468	infected plant control
0.125	healthy control
0.185	plants treated with 1 mg.ml^{-1} nano curcumin
0.168	plants treated with 5 mg.ml^{-1} nano curcumin
0.159	plants treated with 10 mg.ml^{-1} nano curcumin
0.195	plants treated with 1 mg.ml^{-1} curcumin
0.175	plants treated with 5 mg.ml^{-1} curcumin
0.165	plants treated with 10 mg.ml^{-1} curcumin

** virus concentration was determined at the means of three replicates by DAS ELISA (optical density at 405 nm)

Figure 7 Development of PVY^{NTN} symptoms on the potato plants cv. spunta

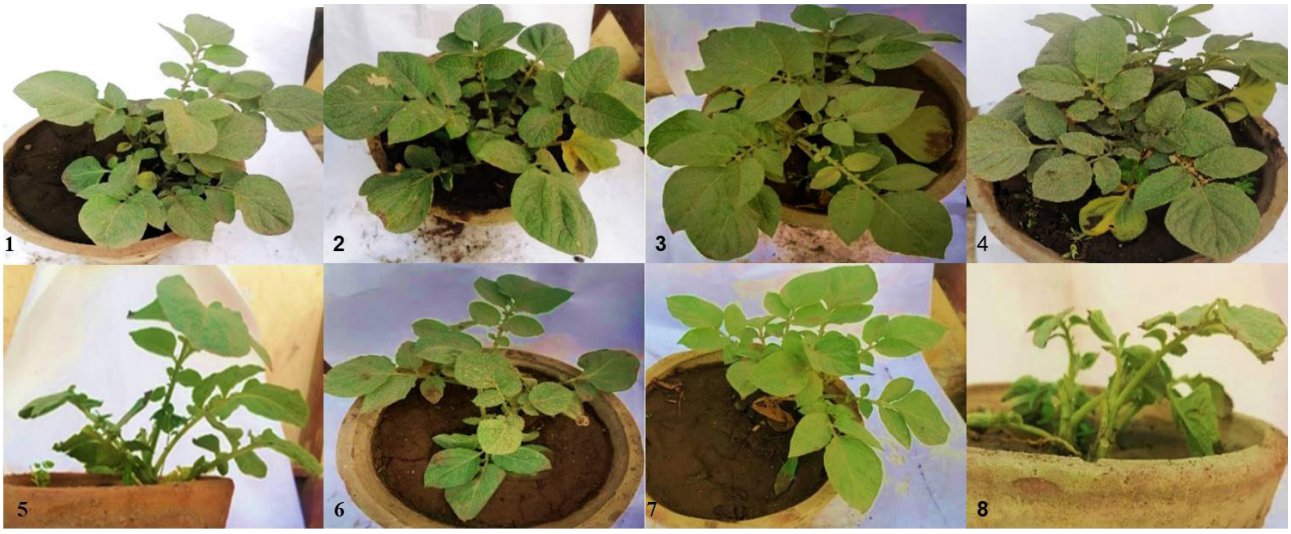

Figure 8 Assaying of the disease severity on potato plant inoculated with PVY^{NTN} after 21 day and treated with curcumin nanoparticles (CurNPs)
1 – 1 mg.ml^{-1} CurNPs, 2 – 5 mg.ml^{-1} CurNPs, 3 – 10 mg.ml^{-1} CurNPs, 4 – healthy control, 5 – 1 mg.ml^{-1} CurNPs + virus, 6 –5 mg.ml^{-1} CurNPs + virus, 7 – 10 mg.ml^{-1} CurNPs, 8 – virus control

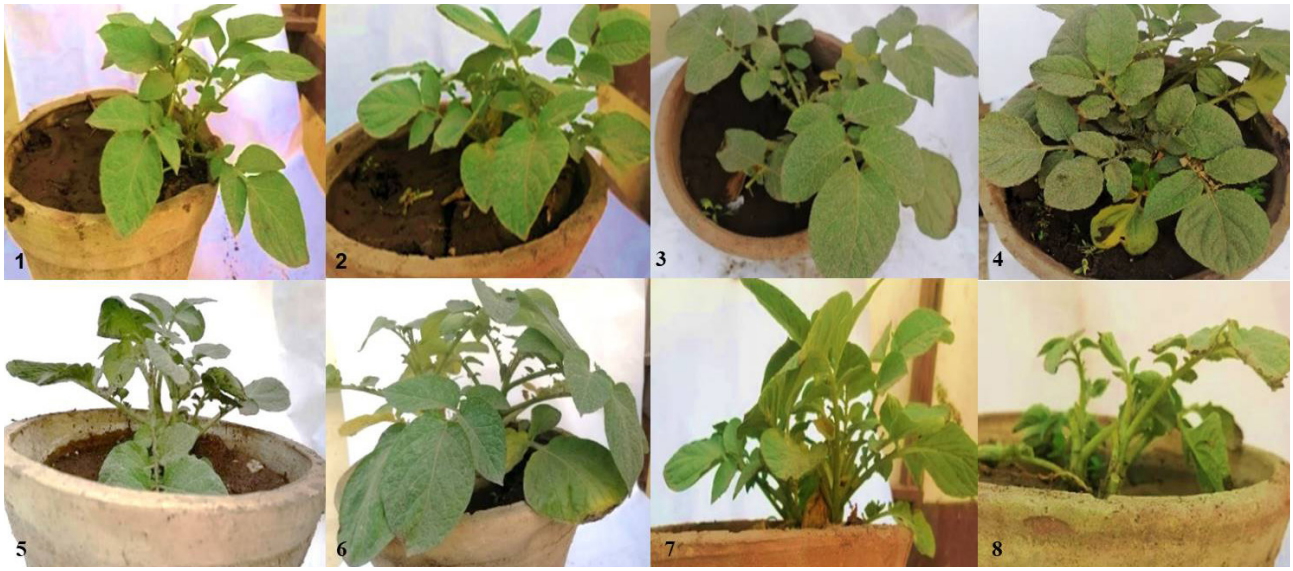

Figure 9 Assaying of the disease severityon potato plant inoculated with PVY^{NTN} after 21 day and treated with curcumin (Cur)
1 – 1 mg.ml^{-1} cur, 2 – 5 mg.ml^{-1} cur, 3 – 10 mg.ml^{-1} cur, 4 – Healthy control, 5 – 1 mg.ml^{-1} cur + virus, 6 – 5 mg.ml^{-1} cur + virus, 7 – 10 mg.ml^{-1} cur + virus, 8 – virus control

In contrast, the lowest number of polypeptides was found in plants treated with 1 and 5 mg.ml^{-1} CurNPs only (16 polypeptides). On the other hand, the highest number of bands disappearance was scored in 1 and 5 mg.ml^{-1} CurNPs (7 subunits), followed by, the plants treated with 1 and 5 mg.ml^{-1} Cur (5 polypeptides), 5 and 10 mg.ml^{-1} CurNPs + V (4 bands), and 5 mg.ml^{-1} Cur + V (3 subunits). In contrast, the least number of bands disappearance was recorded at 10 mg.ml^{-1} of CurNPs or Cur + V, and 1 mg.ml^{-1} of CurNPs + V (1 subunit).

3.7 Effect of treatment with CurNPs or Cur on gene expression by enzyme activities

3.7.1 POX

Changes in the POX activities in potato plants treated with three concentrations of CurNPs or Cur and infected with PVY^{NTN} were determined by Native-PAGE, compared with the control (Figure 10). POX enzymes produced nine different isoforms, with *Rf* values ranging from 0.280 to 0.945. Three isozyme bands were monomorphic, and six were polymorphic. The results showed clear variations between the treatments.

It was observed that the potato plants infected with virus only (V) recorded the highest decrease in POX activities, compared with the control. In addition, the potato plants treated with 10 mg.ml^{-1} CurNPs, 5 ml Cur, and (1 and 10 mg.ml^{-1} of CurNPs + V) induced an increase in POX activities (nine loci). In contrast, there was a decrease in POX activities in potato plants treated with 1 and 10 mg.ml^{-1} of Cur (eight isozyme bands), and 5 mg.ml^{-1} (Cur + V) (seven loci). However, the remaining treatments have not caused any changes in POX activities (Figure 10). On the other hand, it was observed that disappearance of one band *Rf* 0.364 in both potato plants treated with 5 mg.ml^{-1} (CurNPs + V) or (Cur + V), while it appeared in all treatments. Also, one locus of *Rf* 0.375 was absent from potato plants treated with 1 and 5 mg.ml^{-1} (CurNPs), 1 and 10 mg.ml^{-1} (Cur), and 1, 5, and 10 mg.ml^{-1} (Cur + V). Besides, one band with *Rf* 0.604 was revealed in all treatments except the potato plants infected with virus (V), the plants treated with 1 and 10 mg.ml^{-1} (Cur). Furthermore, one locus of *Rf* 0.669 was found in all treatments and disappeared in V and 1 mg.ml^{-1} of Cur (Figure 10).

3.7.2 PPO

Changes in the PPO isozymes in potato plants treated with three concentrations of CurNPs or Cur and inoculated with PVY^{NTN} were determined, compared with the control (Figure 10). POX enzymes produced ten different isoforms with *Rf* values ranging in size from 0.434 to 0.965. Eight bands out of ten were monomorphic, two were polymorphic. It was observed that one locus of *Rf* 0.434 disappeared in all treatments and appeared in (1 mg.ml^{-1} CurNPs), (1 and 5 mg.ml^{-1} CurNPs + V), and (10 mg.ml^{-1} Cur + V). Besides, one locus with *Rf* 0.965 was shown in the potato plants treated with (5 and 10 mg.ml^{-1} CurNPs + V) and (1 mg.ml^{-1} Cur + V). On the other hand, one isozyme marker with *Rf* 0.847 was absent only at 1 mg.ml^{-1} Cur and appeared in all other treatments (Figure 10).

In recent years, potatoes have received a lot of attention, because of PVY is the most important disease problem in the production of seed potatoes in many areas of the world. The virus causes a decrease in the quantity and quality of potato yields (Jha et al., 2011).

In this work, the PVY^{NTN} strain was propagated on *D. metel* L. plants, which gave systemic symptoms including vein clearing, crinkle, and severe mosaic (Mahfouze et al., 2012). These results were confirmed by lateral flow immunoassay (LFIA). These results were agreed with Bahadir & Sezginturk (2016) who mentioned that LFIA is a simple, easy, and sensitive technique for the detection of potato viruses. Furthermore, it takes about a few minutes (10–15 min).

In the current study, the mean size and size distribution of each batch of CurNPs suspension were analyzed by the Zeta-sizer analysis. The mean diameter of CurNPs was 45 nm. It was observed that the surfaces of CurNPs have negative charges of about -18.1 mV. CurNPs were examined by the transmission electron microscope, and they were less than 20 nm in diameter with smooth and spherical shapes. The particle size was decreased due to its aggregation and high specific surface energy. These results were confirmed by Sudyajai (2006) who found that the uniformity of the CurNPs could be estimated by measuring the polydispersity index (PI). PI values close to 0 indicate a homogeneous dispersion. However, PI values >0.3 are high heterogeneity. Apt et al. (2015) estimated the zeta potential of CurNPS by a zeta potential analyzer. The zeta potentials of CurNPs (A1, A2, B1, and B2) were (-) 2.57, (+) 27.55, (+) 19.94, and (+) 21.19, respectively.

In this finding, TEM analysis was carried out to examine the size and shape of CurNPs. It was found that the particle showed a spherical shape and the diameter size was less than 20 nm. Besides, the FTIR spectrum of CurNPs was recorded, and peaks were observed at 1,631; 1,464; 1,157; and 1,073 cm^{-1}. These findings are supported by many researchers (Yadav et al., 2009; Yen et al., 2010; Sav et al., 2012). According to the phenomenon of systemic acquired resistance, the present work was carried out to study potato resistance against PVY^{NTN} infection. CurNPs and Cur were sprayed in three different concentrations to enhance the potato plants to reduce PVY^{NTN} infection and increase potato crop productivity.

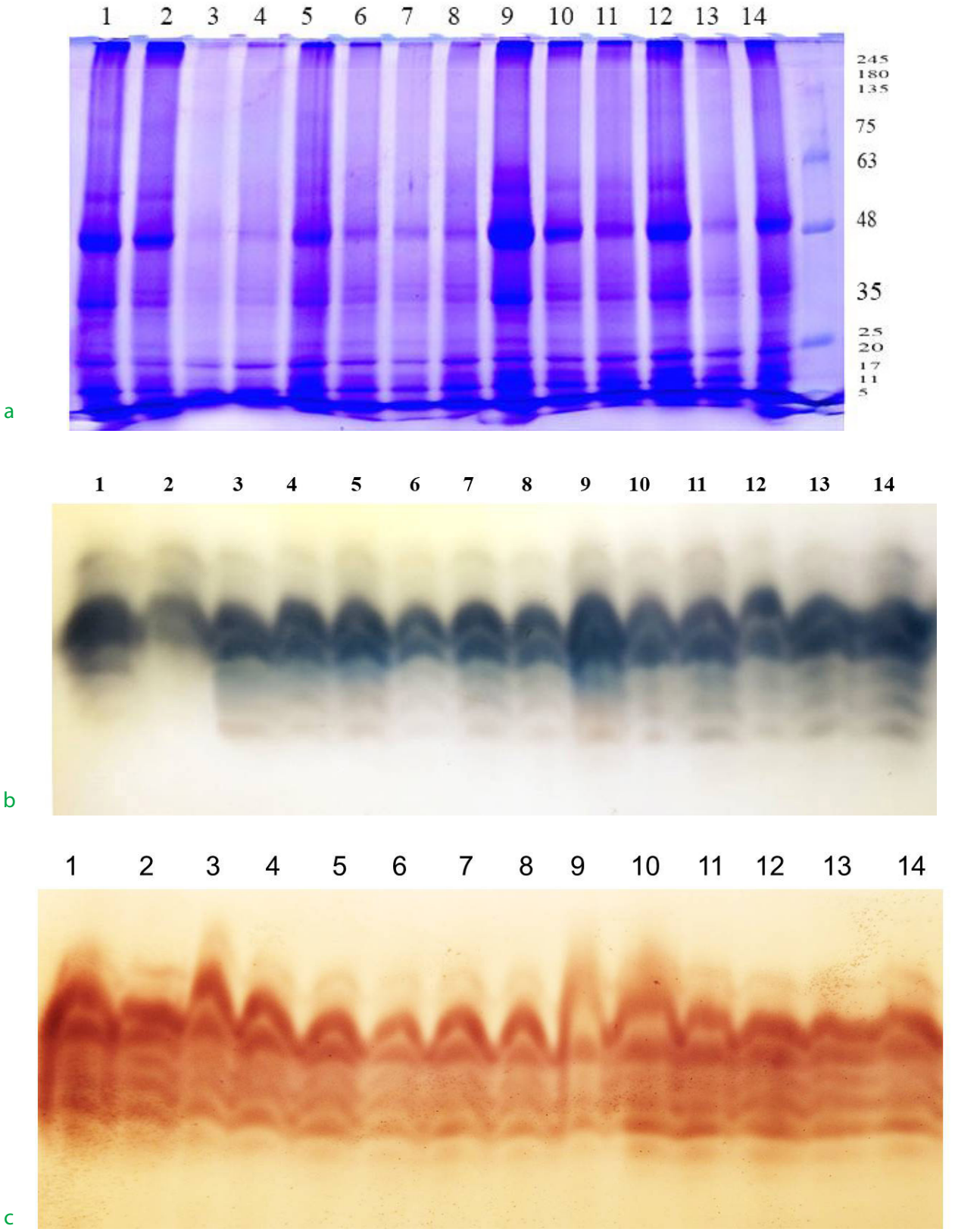

Figure 10 a – SDS-PAGE analysis; b – POX isozymes; c – PPO isozyme profiles of potato plants inoculated with PVY^{NTN} and treated with CurNPAs or Cur

Lane M – protein marker; lane 1 – healthy control, lane 2 – potato plants infected with PVY^{NTN}, lane 3 – 1 mg.ml^{-1} CurNPAs, lane 4 – 5 mg.ml^{-1} CurNPs, lane 5 – 10 mg.ml^{-1} CurNPs, lane 6 – 1 mg.ml^{-1} Cur, lane 7 – 5 mg.ml^{-1} Cur, lane 8 – 10 mg.ml^{-1} Cur, lane 9 – 1 mg.ml^{-1} CurNPAs + V, lane 10 – 5 mg.ml^{-1} CurNPAs + V, lane 11 – 10 mg.ml^{-1} CurNPAs + V, lane 12 – 1 mg.ml^{-1} Cur + V, lane 13 – 5 mg.ml^{-1} Cur + V, lane 14 – 5 mg.ml^{-1} Cur + V

In this study, it was observed that the potato plants treated with a concentration of 10 mg.ml^{-1} CurNPs + V displayed the highest reduction in percentage of disease severity (98%), followed by 1 and 5 mg.ml^{-1} CurNPs + V (96%), 10 mg.ml^{-1} Cur + V (84%), 1 mg.ml^{-1} Cur + V (82%), and 5 mg.ml^{-1} Cur + V (80%), compared with the infected control (38%). These results were confirmed by Taha et al. (2019) who used curcumin-milk protein nanoparticles as an antiviral agent against PVY. Praditya et al. (2019); Jennings and Parks (2020) mentioned that Cur was used as an antiviral agent against enveloped and non-enveloped DNA and RNA viruses. The mode of action of Cur includes the inhibition of penetration of the virus into the plant host and interference with the mechanism of virus replication. Furthermore, Cur works as an antiviral agent, acting on the viral proteins or envelope.

In our study, SDS-PAGE showed clear variations in the content of the protein among potato plants treated with CurNPs or Cur, and inoculated with PVY^{NTN}, as compared with the control. The results showed that CurNPs or Cur pre-inoculation of the virus caused the disappearance of some high molecular weight polypeptides, which is attributed to post-translational changes and differential expression of particular proteins. These proteins are expected to detect a fundamental cellular mechanism underlying the action of CurNPs or Cur (Azad et al., 2013)

In the current investigation, it was observed that the potato plants infected with virus only (V) recorded the highest a decrease in POX activities, compared with the control. Furthermore, the plants treated with CurNPs or Cur pre-inoculation of the virus-induced an increase or decrease in POX activities. However, there were no substantial differences recorded between the effects of either CurNPs or Cur before virus inoculation on PPO isozymes. These results were reported by Taha et al. (2019) who mentioned that there were no significant differences between the effects of both Cur and chitosan on the POX and PPO isozymes. Besides, antioxidant enzymes were significantly increased by increasing the concentration of the native form of the CurNPs as compared with Cur, chitosan, and the native milk proteins.

4 Conclusions

This work shows that CurNPs have inhibitory activity against PVY^{NTN} at a concentration of 10 mg.ml^{-1}. This protective agent of CurNPs is due to its ability to induce systemic acquired resistance in potato plants by post-translational changes and differential expression of particular proteins and defense enzymes such as POX and PPO. We conclude that CuNPs have the potential to serve as a safe, inexpensive, highly efficient, and sustainable alternative for PVY control.

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