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Induction of Resistance in *Arachis hypogaea* L. against *Peanut Mottle Virus* by Nitric Oxide and Salicylic Acid

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Peanut mottle virus (PeMV) causing mottling, yellowing, necrosis, malformation and stunting was isolated from naturally infected peanut plants grown in El-Sharkiya Governorate. The virus was isolated mechanically and identified by indirect ELISA using both specific and induced polyclonal antiserum. Effect of virus infection on cell organelles, using electron microscopy, showed different degrees of degenerative changes in chloroplasts, mitochondria, nucleus, revealed amorphous and cylindrical cytoplasmic inclusions in infected leaf cells as pinwheel and laminated bundles. Pinwheel inclusion bodies are characteristic of *Potyviruses* which include PeMV.

Two field experiments were conducted in 2006 and 2007 to study the effect of spraying sodium nitroprusside (SNP) and salicylic acid (SA) at 50, 100, 200µmol / l to induce resistance against PeMV infection in peanut plants (cv. Giza 5). All treatments induced resistance against PeMV-infection, when plants were sprayed before inoculation with the virus. Also, all tested treatments gave a significant increase in photosynthetic pigments and activity of peroxidase (POD), ascorbate peroxidase (AS-POD), catalase (CAT), superoxide dismutase (SOD) and phenylalanine ammonia – lyase (PAL) compared with infected plants. Moreover, all treatments recorded increments in seed protein and oil contents at harvest time. Also, the total unsaturated and saturated fatty acids content increased in the treated peanut seeds compared with the untreated ones.

Keywords: Antioxidant enzymes, *Arachis hypogaea* L., nitric oxide, peanut, *Peanut mottle virus*, PeMV, salicylic acid, sodium nitroprusside, virus resistance.

Peanut or groundnut (*Arachis hypogaea* L.) is considered as an important oil, food and forage crop, cultivated mainly in tropical and subtropical regions of nearly 100 countries around the world. Peanut is highly nutritious food (25 to 34% protein) and the fifth most important oil seed crop (44% to 56% oil). High quality seeds are used for industries, while low quality seeds and shoots of harvested plants are used for animal feeding (Grosso *et al.*, 1997).

Peanut mottling is caused by *Peanut mottle virus* (PeMV) is classified as the type member of the *Potyvirus* genus in the family Potyviridae. All members of the Potyviridae form cylindrical inclusion bodies in infected cells (Matthews, 1991 and Allam *et al.*, 2000). PeMV produces a range of symptoms on peanut varied from mild mottle to mosaic patterns, necrosis, chlorosis and stunting. (Kuhn *et al.*, 1984; Ghanem,1986; Puttaraju *et al.*, 2001 and Khattab *et al.*, 2007). PeMV is mechanically transmissible. It is also transmitted, under field conditions, in a non persistent manner by a number of aphids. The natural host-range of virus includes several Legume crops such as soybean, cowpea and bean (Morales *et al.*, 1991 and Sibiya *et al.*, 2002).

The virus is also seed transmissible; the percentage of seed transmission was varied from 1-7% (Puttaraju *et al.*, 2001 and Khattab *et al.*, 2007). There is evidence that seed transmission in peanuts can be a source of primary and secondary spread of PeMV (Demski *et al.*, 1975). PeMV is considered to be economically important on a global scale. Also, in Georgia losses due to PeMV were estimated as 5-6% and in India susceptible cultivars crop losses may reach 40%, whereas it reduced yield by about 25% in experimental plots (Kuhn, 1965).

Plants have a natural way of defence against pathogens attack by an array of biochemical response (Faheed and Mahmoud, 2006). Nitric oxide (NO) was suggested to act as a signal molecule mediating responses to biotic and abiotic stress in plants (Durner and Klessig, 1999 and Rashad and Abou-Elalla, 2009). Furthermore, NO was suggested to be involved in the responses to disease resistance (Shi *et al.*, 2005). NO is also considered as a potent antioxidant in plants and direct scavenger reactive oxygen species (ROS) (Beligni and Lamattina, 2002) and NO is indispensable to salicylic acid functioning as a systemic acquired resistance (SAR) inducer (Qiao and Fan, 2008). Additionally, salicylic acid and nitric oxide treatments induced accumulation of defence related genes in plants (Shi *et al.*, 2005). On the other hand, the plant hormones e.g. salicylic acid is involved in the regulation of basal resistance against different pathogens (Ton *et al.*, 2001).

Also, salicylic acid is an important component in the signal transduction pathway and involved in local and systemic resistance to pathogens (Delaney *et al.*, 1995). In addition concentration of endogenous salicylic acid increases at the site of hypersensitive response and acts as a transducer signal for activation of defence response (Delaney *et al.*, 1994). Thus, the exogenous application of salicylic acid is required for the expression of resistance as well as for the enhancing the defensive capacity of tissues with acquired resistance (Szepesi *et al.*, 2005).

Therefore, the aim of this work was to study the cytological effects of virus infected plants using electron microscopy. Also, the effect of different concentrations of sodium nitroprusside (SNP), as a nitric oxide (NO) donor and salicylic acid (SA) in control of PeMV-infection in peanut plants under field conditions was studied. The effect of these treatments on photosynthetic pigments content and antioxidant enzymes activities in peanut leaves was also studied. Moreover, changes in the chemical composition of seed's oil were also estimated.

Materials and Methods

Plant and pathogen:

Peanut seeds of cv. Giza-5 highly susceptible to PeMV (Khattab *et al.*, 2007) used in this investigation were obtained from the Oil Crops Research Division, Agricultural Research Centre (ARC), Giza, Egypt while the PeMV used in this investigation was obtained from naturally infected peanut young leaves collected from open fields in El-Sharkiya Governorate (El-Kasasein and Kafr Sakr). Plants show symptoms of mottling, mosaic, yellowing, necrosis, malformation and stunting. Samples were detected by indirect ELISA (Converse and Martin, 1990) using specific polyclonal antiserum (Sanofi, Saint Animal, Paris, France) and induced antiserum (Khattab *et al.*, 2007).

Isolation and Propagation of PeMV:

The positive samples reacting with PeMV antiserum were used as a source of virus and were propagated by serial inoculations in peanut plants cv. Giza 5. Primary leaves of peanut plants were dusted with carborandum (600 mesh) and after inoculation with PeMV the leaves were washed with water and plants were placed in the greenhouse. Two weeks later, leaves with systemic mosaic and necrosis were ground in a mortar with 0.01 M tris, pH 7.8, containing 0.01M Na_2SO_3 at a ratio of 1:1 (w:v). The homogenate was then filtered and used as a virus inoculum in field experiments. On the other hand, leaves inoculated 20 days before with PeMV and also healthy ones were collected and used to study the cytological changes using electron microscopy.

Field experiments:

Under field conditions in seasons, 2006 and 2007, the effect of sodium nitroprusside (SNP) and salicylic acid (SA) at three concentrations (50, 100 and 200 µmol/l) on peanut plants for resistance to PeMV was studied. This experiment was carried out at the experimental station of the Faculty of Agriculture, Cairo University, Giza, Egypt. Plots, each consisting of five rows, ten plants /row were used as experimental unit. Seedlings of peanut plants cv. Giza-5 at the 2-3 leaf stage were divided into four groups ; the first was sprayed with water (negative control), the second was infected with PeMV (positive control), the third group was sprayed with (SNP) at 50, 100 and 200µmol /l and the fourth group was sprayed with (SA) at 50, 100 and 200µmol / l. Tested plants were kept for three days then groups 2, 3 and 4 were infected with the virus, which was previously prepared using mechanical inoculation. Five days after inoculation, 25-50 samples from inoculated peanut plants were taken at random. Two other samples were taken from each positive and negative control. Collected samples were examined with indirect ELISA to calculate the inhibition percentage of virus infectivity, determination of photosynthetic pigments and antioxidant enzymes activities. On the other hand, at harvest time, peanut seeds were harvested to determine protein, oil content and fatty acids profile.

Effect of virus infection on cell organelles:

Ultra structures of peanut leaves were investigated to detect the effect of virus infection using the following procedure. *Arachis hypogaea* L., leaves inoculated 20 days before with *Peanut mottle virus* (PeMV), and healthy ones were collected, the cytological changes were investigated with transmitted electron microscopy

based on the method described by Spurr (1969) and Weintraub and Ragetli (1966) with some modifications adopted by Allam *et al.* (2000). The samples were cut into small pieces (2x2 mm) and fixed in cold 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 for 2-3 hrs. Samples were rinsed in distilled water then washed three times, for ten min each, with 0.1M sodium phosphate buffer, pH 7.2. The specimens were post fixed in 1% osmium tetra-oxide for 60-90 min. and then dehydrated in ascending dilutions of ethanol 30, 40, 50 and 60% twice, 30 min each. The blocks were sectioned using ultramicrotome and the gold /or silver sections which were stained with uranylacetate and acetone 1:1(v/v) for 10 min, followed by staining with reynolds lead citrate for 20 min and washed with double distilled water several times and dried on a filter paper. The ultrathin sections were examined by transmitted electron microscopy using JOEL-100 CX electron microscopy, Faculty of Science, Zagazig University, Zagazig, Egypt.

Source of chemicals:

Sodium nitroprusside a nitric oxide (NO) donor and salicylic acid (analytical grade) were obtained from Sigma Company and used with three concentrations 50, 100 and 200µmol/l.

A- Chemical analysis of leaves:

1- Photosynthetic pigments:

Chlorophyll a, b and carotenoids were determined after extraction in 80% acetone according to the method reported by Holden (1965).

2- Soluble protein content:

Soluble protein was estimated by using the Coomassie brilliant blue G-250 reagent according to the method of Bradford (1976) with bovine serum albumin as standard.

3- Enzyme extraction:

The leaf tissue 3:1 (buffer volume : fresh weight) was homogenized in a pestle and mortar with 100 mM phosphate buffer pH 7.5 containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrrolidone. The homogenate was centrifuged at 10,000 g for 30 min and the supernatant was stored in separated aliquots at – 40 °C prior to determinate of peroxidase (POD), ascorbate peroxidase (AS-POD), catalase (CAT) and superoxide dismutase (SOD) activities as adopted by Angela *et al.* (2001).

3-1 Peroxidase activity:

Peroxidase activity was assayed in leaves extracts by the photochemical method as described Amako *et al.* (1994).

3-2 Ascorbate peroxidase activity:

Ascorbate peroxidase was assayed as the decrease in absorbance at 290nm (an absorbance coefficient of 2.8/mM per cm) as ascorbate was oxidized according to Fielding (1978).

3-3 Catalase activity:

Catalase activity was assayed according to the method of Chance and Maehly (1995) by measuring the decrease in the absorbance due to the disappearance of H_2O_2 at 240nm.

3-4 Superoxide dismutase activity:

Superoxide dismutase activity was assayed by the photochemical method described by Giannopolitis and Ries (1977).

3-5 Extraction and determination of phenylalanine ammonia-lyase (PAL) activity:

Extraction and assay of phenylalanine ammonia-lyase (PAL) were done according to Edwards and Kessmann (1992) as follows:-

One gram of fresh tissue sample was ground in 2ml of extraction buffer containing 50 mM Tris-HCl (pH 8.5), 14 mM 2-mercaptoethanol and 5% (w/v) polyvinyl pyrrolidone. The homogenate was immediately centrifuged at 10,000 g for 10 min at 4 °C and the supernatant was immediately taken for enzyme assay.

For the assay of PAL activity, 100 µl of the supernatant was incubated at 40 °C with 0.9 ml of 50 mM Tris-Hcl (pH 8.5) containing 12.1 mM L-phenylalanine with parallel incubation of 100 µl of the supernatant with 0.9 ml of 50 mM Tris-HCl (pH 8.5) containing 12.1 mM D-phenylalanine check as a control. The formation of cinnamic acid was monitored using spectrophotometer by reading the absorbance at 30 min intervals and up to 2 hr at 290 nm. PAL activity was calculated on the basis of soluble proteins (n kat /g protein) according to the following equation:

$$\text{PAL activity (n kat/g protein)} = \frac{.27780 \text{ X } (\Delta A_{290} \text{ L-Phe/60min} - \Delta A_{290} \text{ D-Phe/60min}}{\mu\text{g protein per incubation}}$$

B- Seed chemical analysis:

1- Crude protein:

The total nitrogen was determined by micro Kjeldahl method according to A.O.A.C. (2000). The nitrogen content was multiplied by the factor 5.46 to obtain the protein content.

2- Oil content:

The oil content of the seeds was determined according to the procedure reported in A.O.A.C (2000).

Fatty acid composition:

The seed oil content was extracted according to A.O.A.C (2000) and separated to fatty acids and unsaponifibles matter according to Ahmed *et al.* (1986) and the standard and the sample fatty acids were converted to methyl esters using ethereal solution of diazomethane according to Vogel (1975). The fatty acid methyl esters were determined by GC-MS using Trace GC Model 2000 series produced by Thermo equipped with Selective Detector Mass Spectroscopy Model SSQ 7000 produced by Finnegan (USA). This equipment was interfaced via HP chemstation version A 02.12 software (Hewlett-Packard. Avondale, PA). The gas chromatography was equipped with DB-5 (5%-phenyl methylpolysiloxane 25µ capillary column, 50mx0.25mm i.d, 1.5m thickness. The operating conditions for gas chromatography were as follows: injector temperature 250°C, carrier gas helium at 30 cm/sec, measured at 150°C, oven temperature 50°C for 4 min, 150°C for 4 min and held at 250°C until the chromatogram was completed. The temperature was 280°C, mass spectroscopy operating parameters were electron ionization at 70 ev,

accelerating voltage 10 kV and scan M/Z range from 50 to 500. Identification of fatty acid constituents way was carried out by comparing retention times with those of authentic reference compounds, or peak-matching library (NIST) Standard Mass Library, Version 2.0 and available literature.

C- Statistical analysis:

Statistical analysis of the data was performed using statistical programme. Duncan's Multiple Range Test was used to determine significant differences of the means at a 5% level. Different letters (a-g) indicated significant differences at $P \leq 0.05$ levels among treatments according to Duncan's multiple range tests.

Results and Discussion

Symptomatology:

Symptoms induced by PeMV isolated from El-Sharkiya governorate included systemic mottling, mosaic, yellowing, malformation and stunting. Primary reaction of the experimentally inoculated peanut plants with PeMV included systemic yellow mosaic on leaves and necrosis and malformation on both leaves and seeds (Fig. 1 A, B, C). These results are similar to those recorded by Puttaraju *et al.* (2001) and Sibiya *et al.* (2002).

Indirect ELISA:

Indirect ELISA was used at the beginning and the end of experiments as a diagnostic test. In the early, indirect ELISA confirmed the presence of PeMV in some infected peanut samples, while in the later test demonstrated that inhibition percent of infection with PeMV as shown in Table (1).

Effect of PeMV infection on cell organelles:

In this study, ultrathin sections of peanut leaves showed different changes in the structure after 20 days from inoculation with PeMV. Virus infection affected the fine structure of cell organelles. This effect depended on virus concentration, infected host and period after infection (Walkey, 1985 and Matthews, 1991). Examination of ultrathin sections of healthy peanut, showed healthy mesophyll cells which were nearly round chollenchyma with uniformly thin cell walls and contained nucleus, chloroplasts and mitochondria (Fig. 2). Different degrees of degenerative changes in chloroplast appeared as aggregated, partially destructed and filled with vacuoles (Fig. 3) Also, the chloroplasts were reduced in size and number , the cell walls showed extrusion (Fig. 4). Finally, chloroplasts and mitochondria were completely destroyed and degenerated in the cytoplasm. These results are similar to those obtained by Morghal and Francki (1981) and Omar *et al.* (1995). PeMV caused hypertrophy of the nucleus as nuclear membrane was irregular and its chromatin stained dark. Also, amorphous inclusions which were aggregated adjacent to the cell wall. These results were similar to those found by Weintraub and Ragetli (1966) and Omar *et al.* (1995) in *Vicia faba* cells infected with PYMV. In particular, cylindrical cytoplasmic inclusions were also found .These inclusions appeared as pinwheels and bundles (Fig. 5). These results were similar to those reported on *Datura* sp. infected with Potyvirus by Matthews (1991).

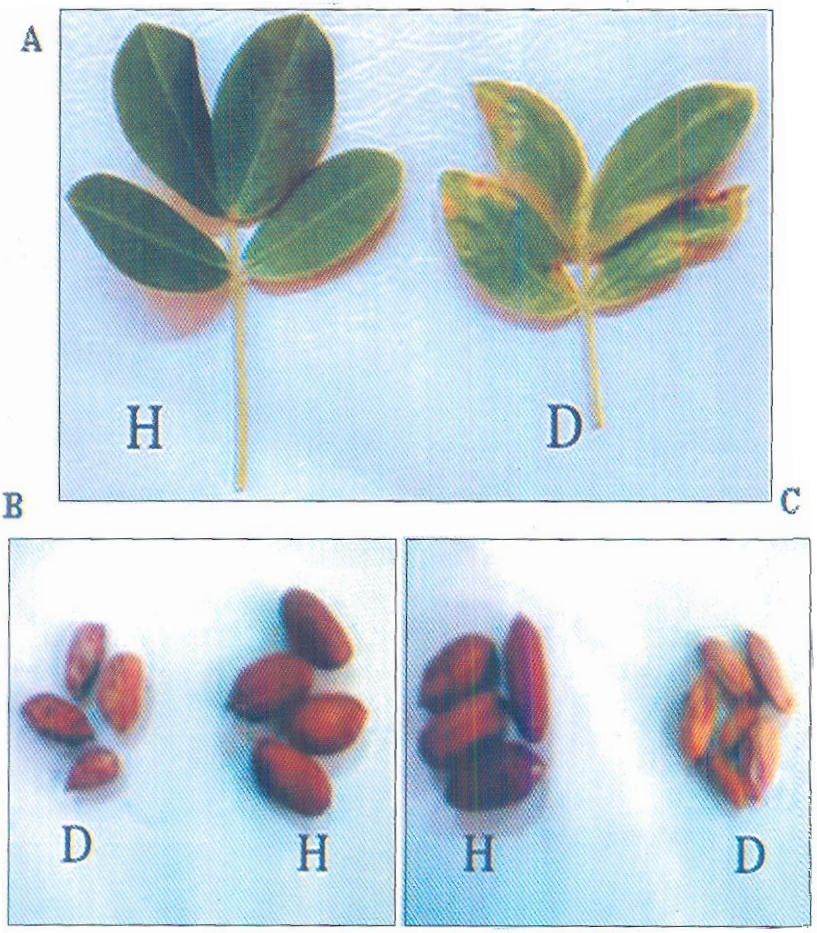

Fig. 1. PeMV-developed symptoms after mechanical inoculation of peanut plants and seeds (cv. Giza 5) showing systemic yellow mosaic and necrosis on leaves (A), necrotic local lesion (B) and malformation (C) on diseased (D) peanut seeds, comparing with the healthy control (H).

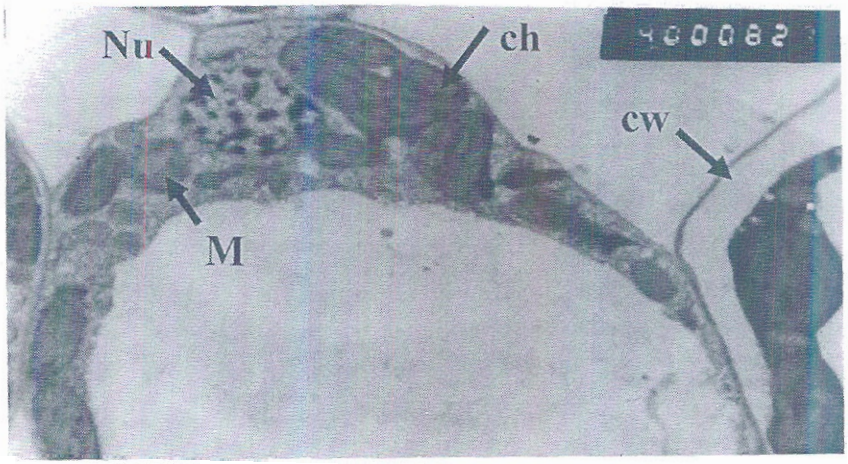

Fig. 2. Electron micrograph of ultrathin section of mesophyll cell of healthy peanut leaf showing the cell walls (cw), nucleus (Nu), chloroplast (ch) and rounded mitochondria (M). (X-8000).

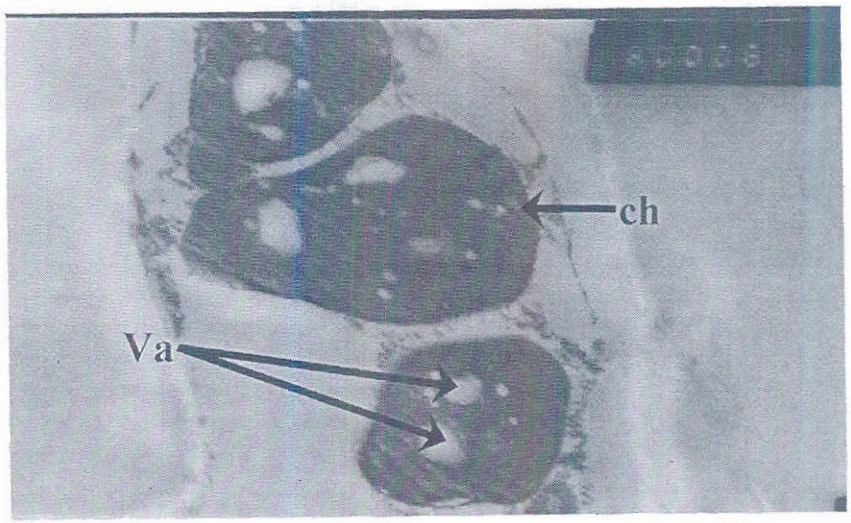

Fig. 3. Ultrastructure of ultrathin section of infected peanut leaf infected with PeMV, the chloroplast (ch) was destroyed, irregular and swollen, and filled with vacuoles (Va). (X-10000).

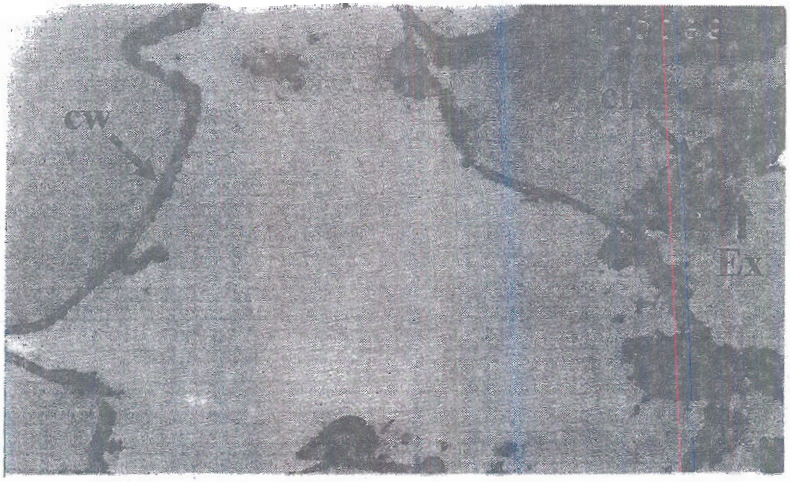

Fig. 4. Ultrathin section of infected peanut leaf with PeMV, the chloroplast (ch) was slightly affected and reduced in size and number, extrusion (Ex) from cell walls (cw) also appeared. (X-5000).

Fig. 5. Cytoplasmic cylindrical inclusions appeared as pinwheel (PW) and laminated bundles (Lb) (X-28000).

1- Effect of treatment with sodium nitroprusside and salicylic acid on reducing infection with PeMV in peanut plants:

Sodium nitroprusside (SNP) NO generator and salicylic acid (SA) at three concentrations (50, 100 and 200 µmol /l) were tested for their ability to inhibit PeMV multiplication and spread of virus infection in systemically infected peanut plants using indirect ELISA test as shown in Table (1).

Table 1. Effect of sodium nitroprusside (SNP) and salicylic acid (SA) on reducing infection with PeMV in peanut plants cv. Giza 5

Treatments (µmol /l)	Reduction (%)	Infection (%)
SNP 50	44	66
SNP 100	56	44
SNP 200	40	60
SA 50	28.8	72.2
SA 100	36	64
SA 200	42	58
Infected (positive control)	0.00	100

Data demonstrated in Table (1) reveal that all the used compounds could induce resistance to virus infection when applied to the plants as a spray treatment. SNP was more effective at 50 and 100 µmol /l than salicylic acid at all the used concentrations as the percentage of PeMV infection was decreased by 44, 56 and 40% in case of SNP 50, 100 and 200 µmol /l, respectively, compared with SA where the percentage were 28.8, 36 and 42% of 50, 100 and 200 µmol /l, respectively. Reduction in infection by using SNP may be due to nitric oxide which has been identified as an essential molecule that mediates hypersensitive cell death (HR) after infection and the free radical gas NO acts as a signalling molecule in plants, mediating responses to a variety of developmental and environmental stimulus including plant-pathogen interactions (Floryszak-Wieczorek *et al.*, 2007) and nitric oxide (NO) may directly or indirectly interact with other signalling molecules such as H_2O_2, salicylic acid and cystolic Ca^{2+} (Wendehenne *et al.*, 2006).

At the same time, reduction in infection with different concentrations of salicylic acid delayed systemic symptoms development by PeMV and suppressed virus multiplication and decreased the accumulation of virus. Also, treatments have affected the entry of the virus into vasculature and affected the long distance movement (Gilliland *et al.*, 2003). Also, SA inhibited indirectly, replication and the cell to cell movement of TMV in tobacco inoculated leaf tissue as shown by Murphy and Carr (2002).

Also, these results are in agreement with Van Loon and Antoniw (1982) who suggested that salicylic acid at high concentrations may induce the full set of systemic acquired resistance (SAR) genes.

2- Effect of sodium nitroprusside and salicylic acid on photosynthetic pigments of peanut leaves:

The effect of SNP and SA with different concentrations on the photosynthetic pigments of peanut leaves after foliar application and inoculation with virus are

Shown in Table (2). The obtained proved that the healthy plants (negative control) recorded the highest content of chlorophyll a, b and carotenoids while the lowest values were found in plants infected with the virus (positive control). A significant increase was noticed in chlorophylls and carotenoids content in different treatments of SNP and SA. These data also show that chlorophylls and carotenoids have gradually increased according to increasing of antiviral compound concentration. These changes in chlorophylls and carotenoids content may be due to virus infection frequently involves yellow mosaic mottling or generalized yellowing of the leaves (Naidu *et al.*, 1986). Also virus infection inhibits chlorophyll biosynthesis of dark grown barely seedlings (Harsanyi *et al.*, 2006) and different treatments with SNP and SA delayed systemic symptoms development by PeMV and suppressed virus multiplication thus increase the chlorophylls and carotenoids contents . Also, nitric oxide has been shown to prevent chlorophyll loss in some situations where the main stresses seem to come from the generation of reactive oxygen species (ROS) (Neill *et al.*, 2008).

Table 2. Chlorophyll and carotenoids content (mg/g) in leaves of peanut after treatment with antiviral compounds

Treatment (µmol/l)	Chl (a)	Chl (b)	Total chlorophyll	Carotenoids
SNP 50	4.43 ± 0.06^{c}	2.46 ± 0.04^{c}	6.89 ± 0.10^{c}	1.95 ± 0.01^{ef}
SNP 100	5.06 ± 0.08^{b}	2.82 ± 0.07^{b}	7.88 ± 0.16b	2.09 ± 0.02^{c}
SNP 200	4.01 ± 0.09^{d}	2.22 ± 0.05^{d}	6.23 ± 0.12^{d}	2.17 ± 0.03^{b}
SA 50	3.22 ± 0.08^{f}	1.84 ± 0.04^{f}	5.06 ± 0.13^{f}	1.90 ± 0.02^{f}
SA 100	3.66 ± 0.11^{e}	2.09 ± 0.07^{e}	5.75 ± 0.10^{e}	1.99 ± 0.04^{de}
SA 200	3.90 ± 0.04^{d}	2.17 ± 0.05^{de}	6.07 ± 0.14^{d}	2.06 ± 0.04^{cd}
Healthy	7.67 ± 0.19^{a}	4.21 ± 0.09^{a}	11.88 ± 0.17^{a}	2.68 ± 0.08^{a}
Infected	2.75 ± 0.06^{g}	1.55 ± 0.05^{g}	4.30 ± 0.09^{g}	1.81 ± 0.03^{g}
LSD 5%	0.164	0.106	0.208	0.074

[a,b,c] Means within the same column followed by different letters are significantly different at $P<0.05$. Values are means of three replicates (±SE).

3- Resistance and antioxidant enzymatic activities:

To counteract the toxicity of ROS in response to the variety of stress antioxidant defence system will be activated in plants thus the antioxidant and resistance enzymes peroxidase (POD), ascorbate peroxidase (AS-POD), catalase (CAT), superoxide dismutase (SOD) and phenylalanine ammonia lyase (PAL) were extracted from peanut leaves assayed as specific activities and the data (Table 3) indicate that infection with PeMV increase activities of these enzymes by three folds compared with healthy plants while treatments with SNP and SA increased the activities of these enzymes more than the infected plants.

Table 3. Effect of treatment with SNP and SA on resistance enzymes specific activity

Treatment (µmol/l)	POD units $min^{-1} mg^{-1}$ protein	AS-POD units min^{-1} mg^{-1} protein	CAT µmol min^{-1} mg^{-1} protein	SOD units $min^{-1} mg^{-1}$ protein	PAL n Kat/g protein
SNP 50	68.10 ± 1.4^{c}	9.12 ± 0.19^{e}	55.70 ± 1.16^{c}	52.80 ± 2.10^{c}	31.26 ± 0.55^{d}
SNP 100	76.10 ± 1.9^{a}	11.4 ± 0.18^{a}	67.12 ± 2.09^{a}	65.3 ± 2.00^{a}	43.66 ± 0.57^{a}
SNP 200	60.33 ± 1.6^{d}	8.80 ± 0.18^{f}	50.66 ± 1.67^{d}	40.6 ± 1.50^{e}	29.00 ± 0.39^{f}
SA 50	58.88 ± 1.4^{de}	9.70 ± 0.15^{d}	49.36 ± 1.95^{d}	36.17 ± 1.43^{f}	30.15 ± 0.27^{e}
SA 100	65.99 ± 2.0^{c}	10.1 ± 0.13^{c}	54.56 ± 1.50^{c}	46.20 ± 1.83^{d}	33.24 ± 0.38^{c}
SA 200	72.54 ± 2.03^{b}	10.89 ± 0.19^{b}	63.3 ± 1.30^{b}	58.22 ± 1.92^{b}	39.12 ± 0.30^{b}
Healthy	17.80 ± 0.89^{f}	3.13 ± 0.11^{h}	19.50 ± 0.67^{f}	20.40 ± 0.80^{h}	8.33 ± 0.19^{g}
Infected	57.10 ± 1.0^{e}	7.70 ± 0.12^{g}	43.31 ± 1.72^{e}	32.30 ± 1.36^{g}	30.52 ± 0.27^{e}
LSD 5%	2.671	0.283	2.703	2.835	0.665

[a,b,c,...] Means within same column followed by different letters are significantly different at $P \leq 0.05$. Values are means of three replicates (±SE).

These changes in enzymes activity due to virus infection promotes (ROS) formation significantly and exerts oxidative stress to the plant (Shi *et al.*, 2005). Also, infection by the pathogen increased lipid peroxidation, CAT and SOD activities. APX, GPX and GR activities were also increased in infected roots and seeds Amari *et al.* (2007) and NO can protect oxidative stresses by decreasing the carbonyl group and H_2O_2 contents and the decrease in H_2O_2 concentration was probably due to a direct NO-H_2O_2 interaction (Qiao and Fan, 2008). Also, Mackernessa *et al.* (2001) mentioned that it is highly possible that the protective effect of NO may be increased level of expression genes encoding active oxygen scavenging enzyme under virus infection.

Also, pre-treatment of plants with SA enhanced antioxidant enzyme activities in concentration dependant manner and increased the stress tolerance of seedlings (Faheed and Mahmoud, 2006). SA has been reported to potentate the expression of phenylalanine ammonialyase (PAL) and other defence-related genes allowing higher levels of expression in response to virus infection (Karabal *et al.*, 2003).

These results are in agreement with those of Chen *et al.* (1995)who reported that intracellular H_2O_2 accumulation activities, H_2O_2-scavenging enzymes such as catalase and peroxidase in plants, in which salicylic acid is required for induction of these antioxidant enzymes.

4- Effect of SNP and SA on protein, oil percentage and fatty acids composition of peanut seeds:

4-1- Protein content:

Data in Table (4) indicate that infection with PeMV gave a significant decrease in protein content of the seeds (26.13%) compared with healthy plants (30.22%) while all treatments with antiviral compounds caused increase in protein content and SNP (100 µmol/l)) and SA (200 µmol/l) gave the highest increase (28.69 and 29.13),

Table 4. Effect of SNP and SA on protein and oil contents of peanut seeds

Treatment (µmol /l)	Protein (%)	Oil (%)
SNP 50	26.88	39.54
SNP 100	28.69	43.56
SNP 200	27.11	37.67
SA 50	26.69	38.13
SA 100	27.83	41.52
SA 200	29.13	42.41
Healthy	30.22	45.34
Infected	26.13	36.79

respectively. These changes due to infection with virus affect on host protein synthesis, this effect is one of the commonest effects of viruses that cause mosaic and yellowing diseases. Also at a late stage of infection photosynthetic activity was lower than in controls and there was a substantial diversion of the products of photosynthetic carbon fixation away from sugars and into organic acids and amino acids (Badbrook and Mattews, 1973). In addition, treatments with SNP and SA inducing PR-proteins and resistance to virus infection as describe before.

4-2 Oil content:

It is clear from the data in Table (4) that all treatments with SNP and SA gave a significant increase in oil content of the seeds. SNP at 100 µmol/l and SA at 200 µmol/l recorded the highest increase (43.56, 42.41%) while other concentrations gave a moderate increase in oil content compared with the positive control (36.79%). These changes may be due to that virus infection caused expression of lipid transfer proteins which are implicated in defence responses to virus infection (Harsanyi *et al.*, 2006).

4-3 Fatty acids composition:

Fatty acid profiles of oil from healthy, infected and treated peanut seeds contained marked variation in the composition of saturated and unsaturated fatty acids (Table 5). The unique feature of peanut oil is the accumulation of large amounts of oleic and linolenic acid. The amount of total unsaturated fatty acids ranged from 66-80% while the amount of total saturated fatty acids ranged from 19-33%.

These changes may be due to the virus infection reduced total lipids content and amount of unsaturated fatty acids because membrane fluidity plays an important role in the replication of virus which depends on the presence of unsaturated fatty acids (Lee *et al.*, 2001). These results are in agreement with (Harsanyi *et al.*, 2006) they showed that BSMV infection reduced fluidity of the chloroplast membranes and reduced the amount of highly unsaturated linolenic acid in dark grown barely seedlings and treatments with SNP and SA induced plant resistance to viral infection.

Therefore, it could be concluded that treatments with 100 µmol/l SNP and 200 µmol/l SA prevent peanut plants from infection with PeMV under field conditions, at the same time these treatments didn't effect on the quality of yielded seeds.

Table 5. Fatty acid composition of healthy, infected and treated peanut seeds

Fatty acid	Healthy	Infected	SNP 50 µmol/l	SNP 100 µmol/l	SNP 200 µmol/l	SA 50 µmol/l	SA 100 µmol/l	SA 200 µmol/l	Mean
Myristic acid (14:0)	2.35	1.32	2.05	2.14	1.70	1.88	2.06	2.09	1.95 ± 0.32
Palmitic acid (16:0)	13.65	8.89	10.36	12.48	9.92	9.21	11.09	11.75	10.92 ± 1.65
Stearic acid (18:0)	9.56	3.34	4.04	7.09	3.93	3.58	5.35	5.63	5.32 ± 2.13
Oleic acid (18:1)	49.74	37.22	42.57	48.89	39.30	40.24	47.40	48.19	44.19 ± 4.93
Linoleic acid (18:2)	28.28	25.7	26.12	27.04	25.99	24.16	27.88	28.11	26.66 ± 1.43
Arachidic acid (20:0)	3.86	3.41	3.22	3.54	3.19	3.04	3.40	3.71	3.42 ± 0.28
Gadoleic acid (20:1)	3.32	3.09	2.65	3.24	2.36	3.03	2.99	3.28	2.98 ± 0.45
Behinic acid (22:0)	2.12	1.55	1.76	2.02	1.44	1.53	2.25	2.26	1.87 ± 0.34
Lignoceric acid (24:0)	1.63	1.38	1.07	1.44	1.22	1.15	1.53	1.72	1.39 ± 0.23
TS[1]	33.17	19.89	22.50	28.71	21.40	20.39	25.68	27.16	24.84 ± 4.66
TUS[2]	80.70	66.01	71.34	79.17	67.65	67.43	78.27	79.58	73.74 ± 6.30
US/S[3]	2.43	3.32	3.17	2.75	3.16	3.30	3.05	2.93	3.03 ± 0.32

[1] Total saturated fatty acids, [2] Total unsaturated fatty acids, [3] Ratio of total unsaturated fatty acids to total saturated fatty acids. Mean ± SD of healthy, infected and treated peanut seeds.

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استحثاث مقاومة الفول السودانى للإصابة بفيروس تبرقش الأوراق بواسطة أكسيد النيتريك وحمض السلسليك

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يُعد محصول الفول السوداني من محاصيل البقوليات الهامة في العالم حيث أنه يُعتبر مصدر جيد للبروتينات والدهون اللازمة لتغذية الإنسان ويصاب بالعديد من الفيروسات أهمها فيروس تبرقش الأوراق والذي تم عزله من نباتات الفول السوداني المصابة طبيعياً والمنزرعة في محافظة الشرقية. وأعراض الإصابة عبارة عن تبرقش واصفرار وبقع ميتة وتشوه وتقزم للنباتات، وقد تم عزل وتعريف الفيروس ميكانيكياً باستخدام طريقة الاليزا – غير المباشرة بواسطة الأنتيسيرم المُنتج والمعروف والخاص بالفيروس. وقد أظهرت الدراسة تأثير الإصابة الفيروسية على التركيب المتناهي الصغر لمكونات خلايا أوراق الفول السوداني المصابة باستخدام الميكروسكوب الالكترونى النافذ تغيرات في شكل وحجم كلا من الكلوروبلاست والميتوكوندريا والنواة كما شوهدت أجسام محتواة ذات شكل صفائحي اسطواني وأخرى بريمية الشكل وهي المميزة للمجموعة الفيروسية التي يتبعها الفيروس Potyvirus وهذا الفيروس لما له من أضرار بالغة للنباتات تتمثل في قلة المحصول وقلة القيمة الغذائية للبذور الناتجة لذلك كان لا بد من إيجاد وسيلة لتقليل الأضرار الناتجة عن الإصابة بهذا الفيروس ومن أهم هذه الوسائل المعاملة بالمواد الكيمائية الاقتصادية والمضادة لتضاعف وانتشار الفيروس وقد تم اختبار نتروبروسيد الصوديوم وحمض السلسليك وتم إجراء المعاملات رشاً لكل منهما بثلاث تركيزات (٥٠ ، ١٠٠ ، ٢٠٠ ميكرومول/لتر) للنباتات عند عمر ثلاث ورقات وبعد المعاملة بثلاث أيام تم إجراء العدوى الفيروسية ميكانيكيا وتم التأكد من نجاح عملية النقل الميكانيكي باستخدام طريقة الاليزا غير المباشرة وتم دراسة اثر العدوى بالفيروس والمقاومة الكيميائية على بعض مكونات أوراق وبذور الفول السوداني ونشاط بعض الإنزيمات المضادة للأكسدة والمقاومة.

ووجد أن العدوى بالفيروس أدت إلى انخفاض محتوى الكلوروفيل والكاروتين وصاحب ذلك زيادة نشاط بعض الإنزيمات المضادة للأكسدة مثل إنزيم البيروكسيديز والاسكوربات بيروكسيديز والسوبراكسيد ديسميوتيز والكتاليز والفينايل الانين امونيالييز المسئول عن تخليق المركبات الفينولية.

وجد أن المعاملة بالتركيزات المختلفة من كل نتروبروسيد الصوديوم وحمض السلسليك أدت تقليل شدة الإصابة ويرجع ذلك إلى حدوث مقاومة داخلية نتيجة تنشيط بعض جينات المقاومة والتي ظهرت بزيادة نشاط الإنزيمات المضادة للأكسدة وانزيمات المقاومة وأيضا تم تحليل البذور الناتجة من المعاملات المختلفة ووجد اختلاف واضح بين البذور الناتجة من الإصابة والمعاملات المختلفة في كل من نسب البروتين والزيت ومحتوى الأحماض الدهنية المشبعة وغير المشبعة.

وبذلك يمكن التوصية باستخدام نيتروبروسيد الصوديوم (١٠٠ميكرومول/لتر) أوحمض السلسليك (٢٠٠ميكرومول/لتر) رشاً على نباتات الفول السوداني عند عمر الثلاث ورقات تحت الظروف الحقلية لتجنبها الإصابة بفيروس تبرقش الأوراق والأضرار المصاحبة لها لما فى هذه المعاملات من فائدة إقتصادية عالية.