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NATURAL INFECTION PEANUT WITH *Peanut mottle virus* (PEMV) IN EGYPT.

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ABSTRACT

A virus causing mottling, yellowing, necrosis, malformation and stunting on *Arachis hypogaea L.* plants was identified as *Peanut mottle virus* (PeMV) on the basis of host range, symptomatology, serology and RT-PCR. The purified virus formed a single zone in the density gradient columns. The absorption spectrum of the purified virus had $\mathbf{A_{max}}$ at 260 nm and $\mathbf{A_{min}}$ at 245 nm. The $A_{260/280}$ and $\mathbf{A_{max/min}}$ ratios were 2.6 and 1.3, respectively. The estimated yield of the purified virus was 0.7 mg / 100g of peanut tissue. The virus particles had about 720-750 nm long and 13 nm width. Antiserum produced against PeMV had a titer of 1/1024 using indirect ELISA. Reverse transcription-polymerase chain reaction (RT-PCR) based method was developed for testing peanut (*Arachis hypogaea L.*) plants for infection by *Peanut mottle virus* (PeMV). The PCR product (340 bp) of PeMV/CP was successfully amplified by using one set of specific primers designed from conserved sequences within the capsid region of the virus. A good correlation was obtained between virus detection by RT-PCR method and virus detection from the same plants by enzyme-linked immunosorbent assay (ELISA).

Keywords: PeMV, electron microscopy, ELISA, TBIA, DBIA, and RT-PCR.

INTRODUCTION

Peanut (*Arachis hypogaea L.*) is widely cultivated in Egypt for its value as food and forage. In Egypt, peanut is mostly cultivated in certain Governorates, which include Giza, Isamilia, Behaira and El-Sharkia. Several viruses have been reported to infect peanut naturally such as *peanut chlorotic streak virus*, *peanut clump virus*, *peanut green mosaic virus*, *peanut stripe virus*, *peanut stunt virus*, *peanut yellow spot virus* and *peanut mottle virus* (Brunt *et al.* 1996). *Peanut mottle virus* (PeMV) is the type member of plant infecting *Potyvirus* in the family *Potyviridae*.

Peanut mottle virus (PeMV) was reported from peanut plants in Georgia in 1961, and the virus was found to be seed transmitted at a rate of 2% and reduced yield by about 25% in experimental plots (Kuhn, 1965). In Egypt, PeMV has been reported and isolated for the first time from peanut (*Arachis hypogaea L.*) by Abdel Salam *et al.* (1986). PeMV was isolated from peanut in Korea by Koo *et al.* (2001) and from Bambara groundnut seed in Zimbabwe by Sibiya *et al.* (2002).

Paguio and Kuhn 1973; Demski *et al.* 1975 pointed out that the predominant strain of PeMV in the United States is a mild strain which caused a faint mottling of the youngest leaves and little or no reduction in vegetative plant growth (Kuhn, 1965). Field surveys indicate the presence of several other strains causing more severe symptoms, including the necrosis (N), necrosis/chlorosis (NC), chlorotic stunt (CS), and chlorosis (C)

strains (Sun and Hebert 1972; Paguio and Kuhn 1973; Kuhn *et al.* 1984; Mitchell *et al.* 1992). PeMV caused systemic necrosis of leaves, petioles, stems and pods of *Phaseolus vulgaris* in west texas, USA during 1987.

Virus detection is currently done by enzyme linked immunosorbent assay (ELISA) using seed or vegetative tissues (Demski and Warwick 1986; Pinnow *et al.* 1990; Sherwood *et al.* 1992).

The seedborne nature of this and other viruses in peanut is of considerable interest in the international exchange of germplasm because of the potential economic damage that could result from the introduction of new strains of the viruses into peanut-growing areas. Currently, there is an effort to detect all seedborne viruses in newly introduced peanuts and to eliminate the viruses from the germplasm before it is grown in fields or distributed.

In this study, we aimed to prove the incidence, insect transmissibility of PeMV and prepare a specific polyclonal antiserum, which can be used for serological diagnosis such as indirect ELISA, TBIA and DBIA. To accommodate screening of potentially virus-infected plants in a timely fashion, a high capacity, sensitive method RT-PCR was used.

MATERIALS AND METHODS

1- Source of virus:

Samples of peanut plants showing typical symptoms of peanut mottle virus (PeMV) were collected from open fields in El-Sharkia Governorae (El-Kasasein and Kafer Saker), the observed symptoms included mottling, mosaic, yellowing, necrosis, malfomation and stunting. PeMV was detected in the collected samples by ELISA (Clark & Adams, 1977) using specific polyclonal antiserum (Sanofi, Sant Animal, Paris, France).

2- Isolation:

The positive samples reacted with PeMV antiserum was used as a source for virus biologically purified from single local lesion produced on top crop bean plants. The resulting local lesion was back inoculated onto peanut plants cv. Giza 5 for virus propagation.

3. Identification of the virus isolate

3.1 Host rang and symptomatology.

The infectious crude extracts of the peanut plants, were used in mechanical inoculation experiment. Thirty plant species and varieties belonging to three different families were mechanically inoculated with PeMV infectious sap. Inoculated plants were maintained under greenhouse conditions (30 °C) for symptom development. Four weeks later, the expressed symptoms were recorded weekly and the symptomless plants were checked for virus infection by indirect – ELISA.

3.2 Cultivars susceptibility

Reactions of different peanut cvs. to infection with (PeMV) under greenhouse conditions were determind by visual inspection and/or indirect - ELISA.

Twenty peanut seeds from each peanut cvs Giza 5, Giza 6, Ismailia 1 and C.623 were sown in 25 cm diameter pots under greenhouse conditions. The emerging seedlings were mechanically inoculated with PeMV. Seedlings, 4 weeks later were examined for symptoms expression on first trifoliate leaves and then the percentage of infection were recorded.

4. Modes of transmission

4.1 Mechanical transmission

Mechanical inoculation was carried out by grinding the infected peanut plants 1:5 (wt /vol) in 0.01 M Tris, pH 7.8, containing 0.01 M Na_2SO_3. All inoculation were made by rubbing Carborundum-dusted leaves with inoculum-soaked cotton wool applicator sticks as described by **Hoffmann *et al.* (1998).** Inoculated and healthy peanut plants were kept in insect-proof green house and examined for external symptoms.

4.2 Insect transmission

Myzus persicae Sulz, *Aphis craccivor* koch, and *Aphis gosspii* Glover were used as experimental vectors of PeMV in this study. Colonies of non-viruliferous aphids of each species were maintained on Chinese cabbage seedlings in insect proof cages. Aphids (starved for one hour) were divided into two groups; the first were fed on peanut plants infected with the studied virus isolate. The second was permitted to feed on healthy peanut plants. They were allowed to feed for 15 min (acquisition period). Viruliferous aphids were then transferred to healthy peanut seedlings for 60 min inoculation access period (IAP) using ten insect per plant and then sprayed with Malathion (1.5ml/L). The plants were maintained in an insect proof greenhouse (30 ^oC) for 30 days and were inspected daily for symptoms development and the percentages of transmission were recorded.

4.3 Seed transmission

Four commercial peanut cvs. namely Giza 5, Giza 6, Ismailia 1 and C.623 were tested for virus transmission. The Peanut seeds were kindly provided by the Oil Crop Research Institute, Agricultural Research Center, Ministry of Agric. Virus-free seeds of each peanut cvs. Tested (checked by ELISA) were cultivated in 20 cm clay, kept in an insect-proof greenhouse. Two weeks later, resulting seedlings were inoculated with the virus isolate. One hundred seeds from each peanut cvs. Were harvested from virus infected plants and treated with 2% w/v sodium hypochlorite for 5 min, then washed with distilled water for several times and planted in plastic pots. Seeds were sown (2 per pot) in 15 cm diameter pots and kept under greenhouse conditions. Upon germination, seedlings were tested serologically with indirect ELISA using the locally produced antiserum for peanut mottle virus (PeMV) as shown latter.

5. Serological detection:

Indirect ELISA technique was performed for virus detection as described by Converse and Martin (1990) using specific antiserum supplied by (Sanofi, Sant Animal, Paris, France). Microtitre plates were coated with

100 µl/well of leaf extract diluted 1/10 in coating buffer pH, 9.6. PeMV antiserum was added to each well at dilution of 1/500. Anti-rabbit alkaline phosphatase conjugate (Sigma, A-8025) was used at a dilution of 1/7000. Absorbance readings were recorded at 405nm.

6. Virus purification

PeMV was purified from mechanically inoculated peanut plants cv. Giza 5 according to the mehod described by Alconero *et al*. (1986) with some modifications mentioned by Kheder *et al*. (2002).

7. Electron microscopic examination

Purified virus preparation was negatively stained with 2% Uranyl acetate, pH 7.0 as described by (Noordam, 1973). The air-dried grids were then examined under JOEL-100 CX electron microscope, at the Electron microscopy Unit, Zagazig Univ., Egypt.

8. Antiserum production

A white New Zealand rabbit was immunized through six intramuscular injections (1mg/ml, each). Purified virus preparations emulsified with an equal volume of freund's incomplete adjuvant, were injected at weekly intervals. The rabbit was bled from the ear marginal vein, 11 days after the last injection.

9. Titration of antiserum

Titration of antiserum was determined using indirect ELISA. Infected and healthy leaves of peanut were extracted in 0.05 M coating buffer, pH 9.6 diluted 1: 10 (w/v) and then centrifuged for 10 min at 10.000 xg. The antiserum was diluted from a concentration of 1/1 up to 1/2048 with serum buffer. The antigen was detected with goat anti-rabbit antibody conjugated with alkaline phosphatase (diluted 1/7000 in conjugate buffer), followed by the addition pNpp substract solution. The reaction was stopped by adding 5 µl 0.5 N NaOH to each well. Absorbance readings were recorded at 405 nm using Dynatech-MR7000 ELISA reader.

10. Serological tests using antiserum produced against PeMV

10.1 Serologic relationship between (PeMV) and other potyviruses by using indirect ELISA:

Five Antisera specific to different viruses belonging to *Potyvirus* group i.e. *Potato virus y* (PVY), *Cowpea aphid born virus* (CABV), *Bean common mosaic virus* (BCMV) and *Bean yellow mosaic virus* (BYMV) were used to study the serlolgical relationship with (PeMV). The indirect enzyme linked immunosorbent assay technique was used as described by Converse and Martin (1990).

10.2 Dot-Blot immunobinding assay (DBIA):

DBIA technique was carried out for PeMV detection according to the method described by **Abdel-Salam (1999).**

10.3 Tissue blot immunoassay (TBIA):

The technique of TBIA described by **Lin *et al.* (1990)** was carried out for PeMV detection with some modifications in which the NCM is blocked for 1 min in polyvinyl alchohol (1mg/ml) then incubated with PeMV antiserum

diluted in TBS (1/500) for one hour at room temperature. Finally, the NCM was stained with (NBT / BCIB) chromogenic substrate for 10 min. After the appropriate color has developed, the membranes were rinsed quickly with H_2O and then air-dried.

11. Total RNA extraction

Total RNA was extracted from healthy and virus infected peanut leaf tissues for the development of the RT-PCR procedures: 0.5 g of tissue was ground in 500 µl RNA extraction buffer containing guanidine isothiocynanate, mixed with phenol:chloroform: isoamyl alcohol (24:24:1) (*AB Gene, Company*). The mixture was mixed gently and centrifuged at 10,000 xg to separate the phases. The upper aqueous phase was transferred to a clean eppendorf tube and 1/10 vol of 7.5 M ammonium acetate and 0.7 vol of isopropanol was added. The tubes were then centrifuged at 14,000 xg for 25 min and the RNA pellets were washed with 70 % Ethanol and air-dried.

12. RT-PCR

One step RT-PCR reaction containing 10 µl of total RNA in a total volume of 50 µl of RT-PCR mix (4 µl of 5× first-strand reverse transcription buffer (Roche), 2 µl of 0.1 M DTT, 2.6 µl of 10 mM dNTP, 0.1 µl of RNasin RNase inhibitor (Roche), 0.5 µl of AMV Reverse Transcriptase (Roche Molecular Biochemicals), 3 µl of 10× PCR buffer (Alliance Bio, Inc), 4.2 µl of 25 mM $MgCl_2$, 1 µl of reverse primer (primers at 100 pm/µl) 0.5 µl of forward primer, 0.5 µl of *Taq* polymerase (Alliance Bio, Inc.), and 21.6 µl of nuclease free water) was carried out in UNOII Biometra thermal cycler. The tubes were then treated as follows: 37°C for 1 h, 94°C for 2 min, (35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s), and 72°C for 10 min. The forward PeMV primer sequence (5' to 3') was (IKP_3) AAT GCA AAG CCA ACA TTC and the reverse PeMV primer was (IKM_4) CTA ATA CGA ACA CCA AGC AT (Metabion, GmbH) with an expected product size of 340 bp based on a PeMV capsid sequence reported by **(Demski, and Lovell, 1985)**. Ten-microliter aliquots of the amplification reactions were analyzed by 1.5 % agarose gel electrophoresis at 60 V in TAE (40 mM Tris/acetate, 1 mM EDTA, pH 8.0) and stained with ethidium bromide.

RESULTS AND DISCUTION

In this study, PeMV was isolated from naturally infected peanut plants, showing mottling, yellowing, necrosis, malformation and stunting collected from commercial fields in different localities in El-Sharkia Governorate. The virus was identified as PeMV based on symptomatology, host range, modes of transmission, serology and RT-PCR.

1. Isolation and identification of the virus isolate:

1.1 Host range and symptomatology:

Results in **(Table 1)** indicated that there were eighteen out of thirty plant species including cvs. found susceptible to PeMV. Infection was confirmed by ELISA technique. Symptoms that appeared on host plants divided into three groups as follows:

Group I: Plants reacted only with systemic symptoms. These are *Arachis hypogaea L.* cvs. Giza 4, Giza 5, Giza 6, and Ismailia 1; *Pisum sativum* cv. Sugar sweet and Master B, *Glycine max* cv. Giza 11 and Giza 21; *Vicia Faba* cv Sakha 1, Sakha 3, Egypt 1 and Giza 843, *Phaseolus Vulgaris* cv. Bountiful and Pronco; *Trifolium*, *incarnatum, Lathyrus odoratus* and *Vigna unguiculate.*
Group II: Plants reacted with local lesions followed by systemic symptoms which include *Phaseolus vulgaris* cv. Top crop which reacted first with necrotic local lesions, 3-5 days post inoculation followed by systemic yellow mosaic, 10-12 days post inoculation.
Group III: Plant species and cultivars that have no reaction upon mechanical inoculation with PeMV are the following: *Chenopodium quinoa*, *C. album, C. murale, C. amaranticolor, Datura stramonium, D. metel, Capsicum annum, Nicotiana rustica, N. tabacum, N. clevelandii, Petunia hybrida and Gomphrena globosa.* These results agreed with that obtained by Bock and Kuhn (1975), Edwardson, (1987), Silbernagel and Mills (1991), Morales *et al*. (1991) and Sibiya *et al.* (2002).

Table (1) Host range of *Peanut mottle virus* tested by mechanical inoculation.

Families	Host species	Cultivars	Symptoms	Days after inoculation	ELISA readings at A_{405}	
Fabaceae	*Arachis hypogaea L.*	Giza5	M.Y.CK	17	0.701	+
		Giza 6	M.Y	17	0.67	+
		Giza 4	N.	20	0.557	+
		$Ismailia_1$	D.M.N	14	0.592	+
	Pisum sativum L.	Sugar sweet	M	18	0.598	+
		Master B	VC, Mm	20	0.606	+
	Glycine max L.	$Giza_{11}$	M.ST	14	0.643	+
		$Giza_{21}$	M.ST	14	0.6612	+
	Vicia faba L.	$Sakha_1$	Mm	18	0.619	+
		$Sakha_3$	M	14	0.575	+
		Egypt 1	Mo	16	0.621	+
		Giza 843	M	18	0.648	+
	Phaseolus vulgaris L	Top crop	N.LL,M	3	0.664	+
		Bountiful	Y,Vn	7	0.59	+
		Pronco	M	14	0.652	+
	Trifolium incarnatum		Mm, VB	14	0.633	+
	Lathyrus odoratus		M.Y	18	0.627	+
	Vigna unguiculata L		Mo	18	0.557	+

VC: vein clearing, D: deformed leaves, NLL: necrotic local lesions, VB: vein banding, M: mosaic, Mm: mild mosaic, Mo: mottling, Y: yellowing, CK: crinkle, ST: stunting, Vn: vein necrosis, N: necrosis. Optical density values of the uninoculated control ranged between 0.130 and 0.135

1.2 Cultivar susceptibilities:

Results in **Fig. (1)** indicated that all four different commercial peanut cvs. (Ismailia 1, Giza 5 and Giza 6) were found to be susceptible to PeMV when tested by ELISA using the locally produced antiserum. The percentages of infection in different peanut cvs ranged from 48 - 90% as shown in **Fig. (2).**

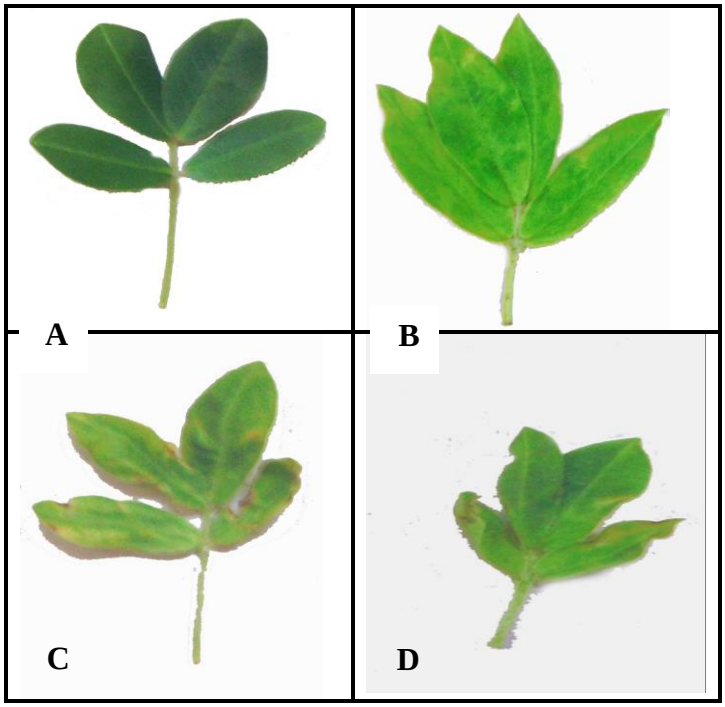

Fig (1): Symptoms expression on peanut plants upon mechanical inoculation with PeMV. A: Healthy leaf. B: Systemic mosaic on *Arachis hypogaea* cv. Giza 6. C: Systemic yellow mosaic and necrosis on leaves of cv. Giza 5. D: Mosaic and deformed peanut leaves on Ismailia 1 cv.

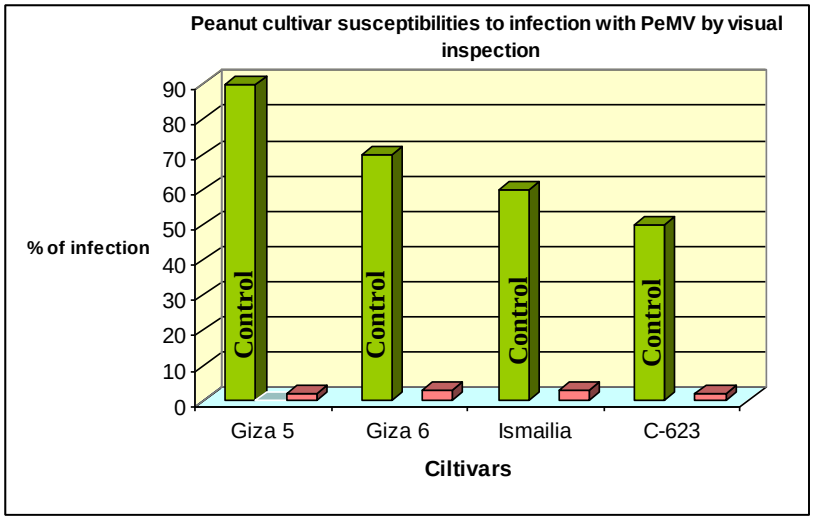

Fig. (2). Percentage of infection of different peanut cultivar upon mechanical inoculation by visual inspection. Giza 5, showed about 90 % of infection, Giza 6 showed about 65 % of infection, Ismailia 1, showing 58% of infection, C623,

2. Modes of transmission

2.1 Mechanical transmission:

The virus under study was readily transmitted by mechanical inoculation of sap extracted from infected peanut leaves to all tested seedlings. External symptoms were examined by visual diagnosis which confirmed by ELISA. In most cultivars, the final level of disease incidence was reached 4 weeks after inoculation, with 80–100% of the inoculated plants exhibiting visible symptoms of PeMV infection and detectable virus concentrations (Table 1). These results are similar to those obtained by Anjos *et al.* (1998).

2.2 Insect transmission: *Aphis craccivora, Aphis gossypii* and *Myzus presicae* were used successfully to transmit the virus isolate. The percentage of transmission was 80%, 60% and 40% for the three aphid species (Table 2).The obtained results were in agreement with the data recorded by Morales *et al.* (1991) and Pietersen & Garnett (1992).

Table (2): Percentage of Transmission of PeMV by three different Aphid species

Insect	No. of inf. Plants/ Total no of plants	% infection
A. Cracivora	8/10	80
A. gossypi	6/10	60
M. persica	5/10	50

2.3 Seed transmission:

Data tabulated in **(Table 3)** indicated that PeMV was transmitted through seeds of all the peanut cultivars tested. Percentage of seed transmission was 3 %, 1 %, 7 % and 6 % for Ismilia, Giza 5, Giza 6 and C 623 cvs., respectively. These results were similar to that obtained by Demski *et al.* (1983) and Puttaraju *et al.* (2001) indicating the high epedimic potential for this virus.

Table (3): Seed transmissibility of PeMV using different peanut cultivars as checked by the locally produced antiserum.

Peanut cultivars	*Ratio	% seed transmission
Giza 6	7/100	7
C.6 23	6/100	6
Ismailia 1	3/100	3
Giza 5	1/100	1

*** No of infected seedlings/No of tested ones**

3. Virus purification:

PeMV was successfully purified with a high degree of purity. After sucrose density gradient centrifugation purified PeMV migrated as a single zone, 3 cm below the meniscus of the tube. This zone was found infectious when tested on the host plants and gave a typical ultraviolet absorption spectrum of nucleoprotein with A maximum at 260 nm and A minimum at 245 nm, the $A_{260/280}$ and $A_{max/min}$ ratios were 2.64 and 1.3 respectively **(Fig. 3).**

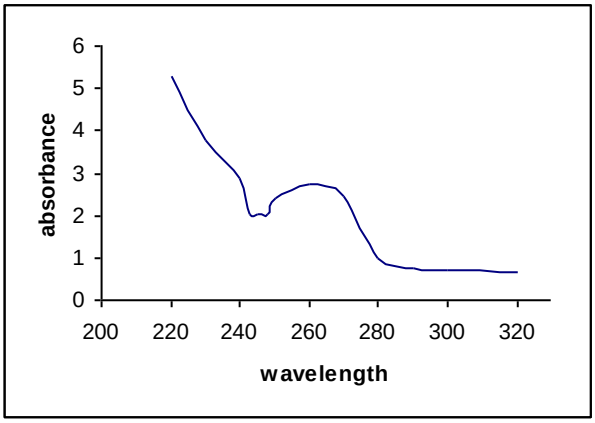

Fig (3): Ultra-violet absorption spectrum of purified PeMV

The yield of viral zone, using the extinction coefficient of 2.4 was 7mg/kg of infected peanut leaves**.** These results suggested that, the method of purification described in this study was quite successful in purifying PeMV. These results were in agreement with Abdel-Salam *et al.* 1986; Abdel-Salam & El Kady, 1991; and Pietersen and Garnett, 1992 who used two cycles of differential centrifugation instead of using polyethyleneglcol (PEG 6000) for precipitation of PeMV due to its harmful effect on the virus particles precipitated at the bottom of the tube.

4. Electron microscopy

Electron microscopic examination of purified preparation of PeMV demonstrated the presence of long, flexuous virus particles 720-750 nm in length **(Fig. 4)**. These results were identical to those mentioned by Abdel-Salam *et al.* (1986), Pietersen & Garnett (1992) and Sibiya *et al.* (2002).

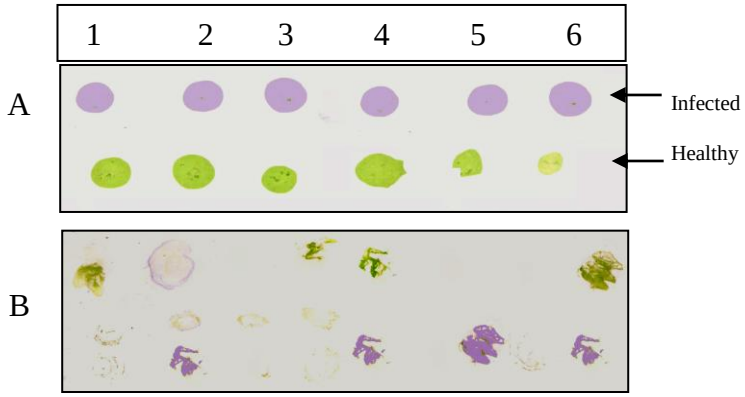

Fig. (4): Electron micrograph of purified PeMV negatively stained with 2% Uranyl acetate. Bar represents 200 nm.

5. Serological detection:

The virus isolate was detected by indirect ELISA using specific PeMV antiserum. Positive reaction was obtained with PeMV and PeMV specific antiserum.

5.1 Production of PeMV/ antiserum

The polyclonal antibodies developed against PeMV after rabbit immunization were obtained from bleeding taken 11 days after the last injection. The titer of antiserum as determined by indirect ELISA were 1/1024 **(Table 4).** Healthy control sap gave no reaction with the obtained antiserum.

The production of polyclonal antibodies specific to PeMV is one of the main objectives of this work to cover the continuous increasing needs for accurate and fast virus detection, especially if the virus is seed transmissible.

Table (4): Determination of PeMV antiserum titer

Antiserum dilution	ELISA readings at A_{405}		
	Inf.		H
1/1	1.299	+	0.495
1/2	1.189	+	0.399
1/4	1.095	+	0.301
1/8	0.997	+	0.289
1/16	0.899	+	0.250
1/32	0.785	+	0.204
1/64	0.694	+	0.199
1/128	0.589	+	0.179
1/256	0.469	+	0.145
1/512	0.301	+	0.121
1/1024	0.242	+	0.101
1/2048	0.101	-	0.096

O.D. readings after 30 min incubation with pNpp substrate. Readings greater than twice the A_{405} value of healthy controls was considered positive. Inf. = infected plants, H = Healthy plants.

6. Serological tests using antiserum produced against PeMV

6.1 Serological relationship between PeMV and other potyviruses by using Indirect ELISA

Serological studies indicated positive serological relationships between PeMV, Potato (PVY), and *cowpea aphid-borne mosaic virus* (CabMV). The PVY/AS showed more specific serological reactivity to PeMV than that of CabMV/AS.

On the other hand, PeMV did not react with either *Bean common mosaic virus* (BCMV) or *Bean yellow mosaic virus*, (BYMV) antisera **(Table** 5). The obtained results therefore, confirmed that PeMV under study is a Potyvirus member and this result is similar to that obtained by (Rajeshwari *et al.* 1983; and Meyer 1983) who reported strong positive cross reactivity between PeMV, PVY and CabMV and poor reactivity with BYMV, BCMV antisera.

7. Detection of PeMV using the locally produced PeMV/AS by DBIA & TBIA

DBIA and TBIA was successfully applied for detection of PeMV, either in the green house or in naturally infected peanut leaves in the field using the induced PeMV/AS (Fig 5 A &B). These results are confirmed by the results obtained by Lin *et al.* 1990; and Abdel-Salam, 1999) who used TBIA and DBIA for detection of tomato yellow leaf curl virus (TYLCV).

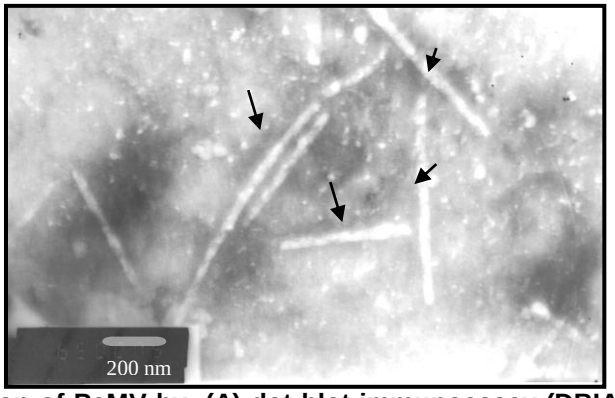

Fig (5): Detection of PeMV by: (A) dot blot immunoassay (DBIA) and (B) tissue blot immunoassay (TBIA) using locally produced PeMV/antiserum. 5 µl of infected and healthy extracts from peanut leaves collected from green house and/or from the open field were spotted onto nitrocellulose membrane. A purplish blue color indicates positive reactions, whereas extracts from healthy plants remained green indicated negative reactions.

8. Detection of PeMV using RT-PCR

Results in Fig. (6) showed that the capsid specific primers designed for the amplification of PeMV (IKP_3 and IKM_4) were able to amplify products of 340 bp of the coat protein gene of PeMV from *Arachis hypogaea L.* cvs. Giza 5, Giza 6, and Ismailia 1, *Vicia faba L* cv. C-623*, Phaseolus vulgaris* cv. Top crop, and *Pisum sativum L.* cv. sugar sweet. These results were in accordance with that obtained by Gillaspie *et al.* (2000) who reported that the IC-RT-PCR method is more sensitive than ELISA, and is useful for testing large numbers of seed lots of peanut germplasm, and could be adapted to test other plants and detect other viruses.

The RT-PCR procedure was used to detect *Bean Yellow mosaic virus* in *Vicia faba* seeds in an epidemiological study (El-Said *et al.* 2002) and to detect *Banana bract mosaic virus, Beet necrotic ringspot virus,* and *plum pox virus* in other plant tissues (Abou El-Nasr *et al*. 2004; Osman *et al.* 2005 and Abdel-Salam *et al*. 2006). The technique offers improved sensitivity over that of ELISA. Many other viruses probably can be detected by this method. All that is required is a set of primers selected to a highly conserved region, such as the primers reported here, so that all isolates of PeMV virus could be detected.

Fig. (6). 1.5 % Agarose gel electrophoresis showing the RT-PCR products amplified from total RNA extracted from different plant tissues infected with PeMV. Lanes 1 to 7: RT-PCR products amplified from T-RNA extracted from different plant cultivars. Lane 1: *Arachis hypogaea* cv. Giza 6, Lane 2: *Arachis hypogaea* cv. Giza 5, Lane 3: *Arachis hypogaea* cv. Ismailia 1, Lane 4: *Vicia faba L,* Lane 5: *Arachis hypogaea* cv. C-623, Lane 6: *Phaseolus vulgaris* Top crop, Lane 7: *Pisum sativum L.* sugar sweet, Lane 8: Healthy control. The arrow indicates the PCR products amplified using the specific primers IKP3 and IKM4 expected size 340 bp. M: Molecular weight DNA Marker (Roche, XIV).

Finally, a relatively good concordance was found between ELISA and RT-PCR. Our results showed a high correlation between the two methods employed to assess the same samples. Results of ELISA and PCR based assays are compared to the pathogenicity assay for PeMV. Based on current data collected for all samples tested, the percentage of correct matches between ELISA and PCR was more than 90 %. The same samples were respectively found to be positive or negative by each of the two methods. The ELISA technique is dependent upon the virus being present and is reliable if using relatively fresh samples. However, it is not sensitive during the early stages of the disease and decreases in reliability when the specimen has autolyzed or decomposed. Although less sensitive, could be particularly useful for monitoring viral infection.

The advent of the PCR technique has increased our knowledge and this offers an understanding for the pathogenesis and diagnostics of viral as well as other pathogenic organisms. Experimentally, PCR has been shown to be highly sensitive and accurate. The studies cited above, with reference to PeMV, provide information which can be applicable for routine diagnostics.

Table (5). Serologic relationship between PeMV and other potyviruses.

Tested virus	Tested antiserum	In direct ELISA readings	
PeMV	Potato virus Y (PVY/AS)	0.789	+
	Cowpea aphid-born mosaic (CabMV/AS)	0.692	+
	Bean Common mosaic (BCMV/AS)	0.201	-
	Bean yellow mosaic virus (BYMV/AS)	0.192	-

Optical density values of the healthy control ranged between 0.131 and 0.152.

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اصابة الفول السودانى طبيعيا بفيروس تبرقش أوراق الفول السودانى فى مصر

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تسجل هذه الدراسة عزل و تعريف فيروس تبرقش أوراق الفول السودانى الذى يسبب اصفرار و تبرقش و بقع ميتة و تشوه و تقزم نباتات الفول السودانى و ذلك اعتمادا على المدى العوائلى و الأعراض و الأختبارات السيرولوجية و تفاعل البلمرة المتسلسل. و قد وجد الفيروس المنقى فى منطقة ترسيب مفردة فى أعمدة السكروز المتدرجة الكثافة وكان أقصى و أدنى امتصاص للفيروس المنقى عند طول موجى ٢٦٠ و ٢٤٥ نانوميتر على التوالى و كانت نسبة ٢٨٠/٢٦٠ هى 2.6 & 1.3 على التوالى. و قد بلغت كمية الفيروس المنقى 0.7 ملجم / ١٠٠ جم نسيج غض.

و قد بينت دراسة الميكروسكوب الألكترونى أن جزيئات الفيروس المنقى عصوية مرنة و ذات ابعاد تبلغ من ٧٢٠-٧٥٠ نانوميتر طولا و ١٣ نانوميتر عرضا.

و قد أوضحت الدراسات السيرولوجية أن تركيز المصل المنتج محليا ضد فيروس تبرقش اوراق الفول السودانى هو 1/1024 و ذلك باستخدام طريقة الأليزا الغير مباشرة.

وقد تم استخدام طريقة تفاعل النسخ العكسى و البلمرة المتسلسل (RT-PCR) للكشف عن الفيروس فى نباتات الفول السودانى المصابة و كانت نواتج تفاعل البلمرة المتسلسل قطعة طولها ٣٤٠ نيوكليوتيدة من جين الغلاف البروتينى للفيروس وذلك باستخدام زوج واحد من البادئات المتخصصة والتى صممت فى المنطقة الثابتة لجين الغلاف البروتينى للفيروس.

و قد اثبتت الدراسة وجود علاقة جيدة بين النتائج المتحصل عليها بالكشف عن الفيروس باستخدام تفاعل البلمرة المتسلسل و تكنيك الأليزا فى نفس النباتات المصابة.