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A New Whitefly Transmitted *Geminivirus* Infecting Tomato Plants in Egypt

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Whitefly transmitted Geminiviruses cause severe damage to different crops in Egypt. *Geminiviruses* often occur in mixed infections and it is difficult to diagnose such mixtures by biological properties. Two types of yellow leaf curl symptoms were collected from tomato plants grown at different locations in Egypt. The collected plants were subjected to biological analysis, the viral causal agent vector of the two types of symptoms was found to be *Bemisia tabaci* (Gennadius.). Based on diagnostic host species and back inoculation assay two different types of symptoms were found, the first consists of identical *Tomato yellow leaf curl virus* (TYLCV) symptoms such as severe leaf chlorosis and distortion on tomato plants, named TYLCV-Giza isolate, and the other one showed yellow mosaic symptoms on tomatoes, named *Tomato yellow mosaic virus* (TYMV) -Qalubia isolate. The genetic diversity at the molecular level for the two isolates showed that the coat protein and the replicase genes of putative TYMV-Qalubia are not identical to TYLCV resembled genes, at least at the flanking regions of each gene. According to the results obtained from the PCR products and indicator host plants, it can be concluded that they are two different Geminiviruses. Based on host range and symptomatology, the TYMV-Qalubia appeared to cause infections only to some species of the family *Solanaceae*. In contrast to TYLCV, it was indicated that TYMV could be mechanically transmitted to several species of the family *Solanaceae* with the aid of a special buffer as well as by grafting and whitefly transmission. Electron microscopy studies of TYMV showed geminated virus particles with about 18- 20 x 30 diameters nm. The molecular weight of the viral capsids was estimated to be 28 KDa for TYMV. Serological analysis also proved that the putative TYMV belongs to whitefly- transmitted geminivirus by ELISA analysis using the whitefly transmitted- geminivirus (WTG) antiserum [immunogenic oligopeptides coat protein (Cp-3)]. Based on the previous results, the isolated virus TYMV is confirmed to be a whitefly transmitted *Geminiviruses* belonging to genus *Begomovirus* of the Geminiviridae family.

INTRODUCTION

Geminiviruses are DNA plant viruses and considered as worldwide important pathogens of either mono or dicotyledonous plants (Goodman, 1981). Some Geminiviruses are transmitted by whiteflies and others by leafhoppers in a persistent manner, but the viruses did not appear to replicate in their vector and are not seed transmitted (Bock, 1982). A few numbers of Geminiviruses can be transmitted experimentally by mechanical inoculation (Goodman, 1981; Bock, 1982). Most purified preparation of Geminiviruses contains geminate particles about 18x30 nm (Francki *et al.*, 1979). *Geminiviruses* had small single-stranded DNA (2.5 - 3.0 kb) in 1 or 2 parts, encapsidated by a shell consisting of two incomplete icosahedra built from a single species of polypeptide with M_r between 27-34X10^3 Da (Harrison *et al.*, 1977). According to the International Committee on the Taxonomy of Viruses (ICTV), Geminiviruses are subdivided into three subgroups based on the vector specificity, host range

and genome structure (Grimsley *et al.*, 1987). Recently it has been proposed, and accepted, by ICTV that the Geminiviridae family including four genus *Mastrevirus*, *Curtovirus*, *Begomovirus* and *Topocuvirus* (van Regenmortel *et al.*, 2000).

Tomato yellow leaf curl virus (TYLCV) belongs to genus *Begomovirus* of the Geminiviridae family infecting tomato in the Middle East, and is transmitted by the whitefly *Bemisia tabaci* (Genn.) (Murphy *et al.* 1995). In Egypt, the virus causes more than 80% loss in yield of tomato cultivation's planted during summer and autumn seasons (Nour El-Din *et al.*, 1969).

Tomato yellow leaf curl virus has been described in Egypt principally by symptoms indicated in tomato plants (Nour El-Din *et al.*, 1969 and Mazyad *et al.*, 1979). The genomic DNA molecules and the replicative form of *Tomato yellow leaf curl virus*-Egyptian isolate (TYLCV-Eg) were isolated by Aref *et al.* (1991).

The complete nucleotide sequences and genome organization of the DNA full-length clone of TYLCV-Eg was determined as a single component with 2784 nucleotides (Abdallah *et al.*, 1993).

Tomato yellow mosaic virus (TYMV) was recorded as one of the most serious disease of tomato in Venezuela. The area of tomato production was reduced by 50% due to yield losses caused by this disease (Salas and Mendoza, 1995). The virus has caused millions of dollars in losses over the past several years in commercial tomatoes plantation in Venezuela. By the time of flowering, 90-100% of tomato plants typically become infected by TYMV (Piven *et al.*, 1995). The virus was nominated by Uzcategui and Lastra in 1978 as *Tomato yellow mosaic virus* (TYMV). A yellow mosaic disease on tomato in India (Verma *et al.*, 1975) and a virus-like disease of tomato in Nigeria (Femilana and Wilson, 1976).

TYMV which belonging to genus *Begomovirus* of the Geminiviridae family was found to infect tomato in Venezuela, and is transmitted by the whitefly (*Bemisia tabaci* Genn.) (Murphy *et al.* 1995). *Tomato yellow mosaic virus* was reported in Venezuela based on biological and epidemiological studies (Uzcategui and Lastra, 1978), and by cytopathological properties (Lastra and Gil, 1981). In Egypt, it has not been recorded yet.

MATERIALS AND METHODS

The experiments of this study were conducted in the Molecular Plant Virology Laboratory at Agricultural Genetic Engineering Research Institute (AGERI). Agricultural Research Center (ARC), Giza, Egypt.

Virus sources and Isolation

Tomato plants (*Lecopersicon esculentum* Mill.) showing symptoms were collected from the open field in plastic pots (40 diam.) according to variability on the symptoms from two different locations, Giza and Qalubia Governorates in Egypt. Plants exhibited severe curling, yellowing and rolling were collected from Giza Governorate, while severe yellowing, curling and dwarfing in the young leaves were collected from Qalubia Governorate. The plants of each group were sprayed by Acetlic 1.5 ml/ L. Kept in muslin covered cages and held in insect proof greenhouse. Healthy two leaf- old CastleRock tomato plants were inoculated by virus-free

whiteflies *Bemisia tabaci* (Gennadius) of natural infected ones.

Differentiation between two isolates

Three diagnostic host species were used to differentiate between the whitefliy- transmitted Geminiviruses (WTGs) infecting tomatoes according to Brunt *et al.* (1990& 1996), including Tenderpod bean (*Phaseolus vulgaris* L.), winter cherry (*Physalis floridana* Rydb.) and tobacco (*Nicotiana clevelandii* A. Gray.). Healthy tested plants were inoculated by viruliferous whiteflies that were maintained on the infected tomato plants with the original symptoms. Symptoms were recorded periodically and results were confirmed for each isolate by molecular diagnostic tools. The back inoculation assay was done by whitefly transmission using healthy two leaf-old CastelRock tomato plants. The two isolates were differentiated on the base of diagnostic hosts. The isolate that obtained from Giza government was named as G- isolate and the other one that obtained from Qaluobia government was named as Q- isolate.

Mode of transmission

1- Whitefly transmission.

Whiteflies (*Bemisia tabaci* Gennadius.) were collected separately from different plants in the open field, and were reared on Giza 83 cotton plants (*Gossypium barbadense* L.) for one month in muslin covered cages (50x50x100 cm) in insect proof greenhouse (28°C ±2) to obtain virus-free whiteflies (Czosnek *et al.* 1988; Cohen 1990 and Aref *et al.* 1995). Whiteflies were kept on infected tomato plants obtained from back inoculation for 48 h to acquire the virus. Then, transferred to test plants for 2 days as inoculation feeding period followed by killing with Actelic 1.5 ml/L.

Virus- free whiteflies were kept on an equal number of healthy tomato plants as a negative control.

2- Mechanical transmission.

Seedling of *Lycopersicon esculentum* Mill. cv. S-BVf145 plants were used for the mechanical inoculation. Two different buffers were used to inoculate the healthy plants; the first one consisted of 0.1M phosphate buffer, pH 8.15, containing 0.02M Na_2SO_3, 0.02M EDTA and 1.5% Triton X-100 (Abdel-Salam, 1990). Whereas, the second one consisted of 1% suspension of magnesium tri-silicate in 0.1M phosphate buffer pH 8.5- 9 (1-3, w/v) (Piven *et al.*, 1995). Seedlings of *Nicotiana glutinosa* L. and *N. tabacum* L. cv. Samson were inoculated mechanically using Piven's buffer only as diagnostic hosts for TYMV according to Piven *et al.* (1995). Symptoms were recorded after 14- 16 days post inoculation, and were confirmed by molecular diagnostic tools.

3- Grafting transmission.

Healthy S-BVf145 tomato plants were inoculated by wedge grafting using a stem piece (Mansour and Al-Muss 1992). The plants were held in an insect proof greenhouse at 28 °C ±2. Symptoms were recorded after 18 days post inoculation, and confirmed by molecular diagnostic tools.

Symptomatology and host range

Thirty-four plant species and cultivars belonging to six families that used as experimental host range for the Q- isolate were inoculated by groups of 100- 200 viruliferous whiteflies. The whiteflies had been given an acquisition feeding of 48 h on virus-

infected tomato plants, which obtained by the back inoculation. Then, transferred to test plants for 2 days as inoculation feeding period followed by killing with Actelic 1.5 ml/L. Virus-free whiteflies were kept on healthy control plants of each species and cultivars for one month after inoculation. Three weeks later after symptom expression, the whole tested plants were confirmed by molecular diagnostic tools.

Virus purification

Inoculated winter cherry (*Physalis floridana* Rydb.) plants 2 weeks after symptom expression were used as a source for virus purification. The method described by Luisoni *et al.* (1995) was used. All steps in purification were carried out at 4°C after initial homogenization of tissues. Leaves were frozen in liquid nitrogen, crushed to a fine powder, and homogenized in a warring blender after adding ice- cold 0.5 M phosphate buffer pH 6.0 containing 2.5 mM EDTA, 10 mM Na_2SO_3, 0.1% 2-mercaptoethanol, 1% Triton X-100 and 0.1% Driselase (3-4 ml of buffer for each gm of material). Stirred overnight with gentle agitation, squeezed through two layers of cheesecloth, and clarified with 10 % cold chloroform, and centrifuged 10 minutes at 8000 rpm in Sorvall GSA rotor. Polyethylene glycol (PEG) (mol. wt. 8000 (8%) and 2% Nacl were added to the aqueous phase. After being stirred 1.5- 2 hours, the precipitate was collected by centrifugation for 15 minutes at 8000 rpm in Sorvall GSA rotor, and resuspended in one- tenth the original volume of resuspension buffer 0.1 M phosphate buffer pH 7.2 containing 0.002 M EDTA and 1% Triton X- 100. The virus suspension was stirred for a minimum of 1 hour, centrifuged for 10 minutes at 10000 rpm in a Sorvall SA-600 rotor, and the supernatant was ultracentrifuge for 1 hour at 50,000 rpm in a Beckman Ti 70.1 rotor. Pellets were resuspended in 10 ml resuspension buffer, given a low-speed centrifugation at 10000 rpm for 10 minutes in an Eppendorf centrifuge 5415 C. The final was ultra-centrifuged in a Beckman Ti 70.1 rotor at 35,000 rpm for 3 hours. Pellets were resuspended in 4 ml resuspension buffer, given a low- speed centrifugation at 6000 rpm for 5 minutes in an Eppendorf centrifuge 5415 C.

Cesium sulfate gradients (10-40%, 2ml / fraction) were prepared in the resuspension buffer. Virus suspension was layered on top of each gradient and centrifugation at 40,000 rpm for 5 hours in a Beckman SW 41 rotor. The virus band was collected and ultracentrifuge for 1 hour at 50,000 rpm in a Beckman Ti70.1 rotor.

Electron microscopy

Purified virus preparations were negatively stained with 2% uranyl acetate mounted as recommended by Morales *et al.* (1990), on carbon-stabilized formavar- coated grids and screened for virus under the electron microscopy (Jeol 100 CX II) in The Electron Microscope Unite of Faculty of Science, Ain Shams University, Cairo, Egypt.

Extraction of total plant DNA

Infected and healthy plants were used to isolate total DNA as described by Dellaporta *et al.* (1983). One g from plant tissue was ground in liquid nitrogen to fine powder and allowed to partially thawed in the presence of extraction buffer containing 100 mM Tris pH 8.0, 50mM EDTA pH 8.0, 500mM NaCl and 10mM β- mercaptoethanol with

rate of 1:15 (w/v). The suspension was transferred into a 30 ml Oak Ridge tubes. One ml of 20% sodium dodecyl sulfate (SDS) was added, mixed thoroughly by vigorous shaking, and the tubes were incubated at 65°C for 10 minutes. Five ml from 5M potassium acetate was added, followed by vigorous shaking and incubating at 0 ° C for 20 minutes. Then, the tubes were centrifuged at 12000 rpm for 20 minutes in Sorvall SA-600 rotor. The supernatant was taken through a miracloth filter (Calbichem) into a clean 30 ml tubes containing 10 ml isopropanol, mixed and incubated at -20 ° C for at least one hour. DNA was pelted at 12000 rpm for 15 minutes in Eppendorf centrifuge 5415C, and redissolved with 0.7 ml of 10 mM Tris, 1 mM EDTA pH 8.0 in Eppendorf tubes. DNA solution was centrifuged for 10 minutes in an Eppendorf centrifuge 5415 C to remove insoluble debris. The supernatant was transferred to a new Eppendorf tubes and 75µl of 3M sodium acetate was added followed by 500µl isopropanol and incubated at -20°C for 1 hour. The DNA was pelted by centrifugation at 15000 rpm for 10 minutes in an Eppendorf centrifuge 5415 C. The pellet was washed with 70% ethanol, air dried and redissolved in (100µl of 10 mM Tris, 1 mM EDTA pH 8.0). DNA was stored at -20°C.

Oligonucleotide synthetic primers

The primers were designed according to Abdallah *et al.* (1993) and Aref *et al.* (1994); the oligonucleotide primers were synthesized at the AGERI, ARC, Giza, Egypt, on an ABI 392 DNA/RNA synthesizer. The primer positions direction and sequence were listed in Table (1).

Polymerase chain reaction (PCR)

Reaction mixture (100 µ l) containing 0.2- 0.5 µg total DNA, 75p mol of each primer, 100µM of each dNTPs, 2.5mM $MgCl_2$, 2.5 units of the thermostable Taq polymerase, and 10µ l 10X reaction buffer included in the PCR kit. The reaction mixture was over laid with 50µl of mineral oil. PCR was initiated with one cycle at 94 °C for 4 minutes, followed by 30 cycles at 94°C for 1 minute, 52°C for 1 minute and 72°C for 2 minutes finally by one extension cycle at 72°C for 10 minutes, in a DNA thermal cycler model 480 form Perkin Elmer Cetus. DNA from the plasmid pTYNA100 by *Sph*I (pTYNA100 is a full length DNA clone of TYLCV-Eg as a one component in pUC118) used as a positive control in the PCR reactions. Then, the PCR products were resolved by 1% agarose gel electrophoresis. The sizes of PCR products were determined based on mobility of appropriate molecular weight markers (ϕ X174 / *Hae*III , λ *Hind* III DNA and / or 1 kb ladder molecular weight markers).

Determination of viral coat protein molecular weight

Using polyacrylamide gel electrophoresis purified preparation viral coat protein was denatured by heating in the presence of sample buffer (Laemmli, 1970) and the mixture was boiled in water bath for 5 min, immediately was put in ice and loaded on the gel. The denatured gels were prepared as 12% running gel and 4.5% stacking gel. The gels were prepared from monomer solution of 30% acrylamide and 0.8% Bis-acrylamide. Ammonium persulphate and TEMED were used as initiators for cross-linking and polymerization.

Table (1): The nucleotide sequence of the primers used to amplify the TYLCV-Eg viral genome (Abdallah *et al.*, 1993 and Aref *et al.*, 1994).

Open reading frames	Sequence 5' to 3'	Additional cloning sites	Length	Position from- to	Leng of amplified region
Cp- F Cp- R	CGGAATTCACTGTCGAAGCGACCAGG CGGGATCCTTAATTTGATAATGAATC	*Eco*RI *Bam*HI	27 mer 26 mer	476- 1253	778 bp
C1- F C1-R	GAAGATCTATGCCTCGTTTATTT GGGGTACCTACGCCTTATTGGTTT	*Bgl* II *Kpn* I	24 mer 24 mer	1710- 2781	1072 bp
WTG391 WTG1049	CGGAATTCGCCCACCAATAACTGTAGC CGGGATCCGCAGTCCGTTGAGGAAACTTAC	*Eco*RI *Bam*HI	27 mer 30 mer	391- 1049	659 bp
WTG1855 WTG2528	CGGGATCCAAACAGGTCAGCACATTTCC GGGGTACCTATATGAGGAGGTAGGTCC	*Bam*HI *Kpn* I	28 mer 27 mer	1855- 2528	674 bp
pCp-F pCp-R	CGCGGATCCATGTGGGACCCACTTCTA CGGAATTCTGAGGGCTTCGTCACATTC	*Bam*HI *Eco*RI	27 mer 27 mer	317- 664	348 bp
IPR-F IPR-R	CCCAAGCTTAGTCACGGGCCCTTACAA ACATGCATGCTGAAATGAATCGGTGTC	*Hind* III *Sph*I	27 mer 27 mer	1 - 475	474 bp
C4-F C4-R	CGGAATTCATGGGGAACCACATCTCCAT GATCCCGGGTTAATATATTGAGGGCCTCGG	*Eco*RI *Xma*I	28 mer 30 mer	2632- 2335	298 bp

The underlines are the sequence of the restriction sites

ELISA detection

The indirect- enzyme linked immunosorbent assay (indirect ELISA) was used for virus detection using (Immunogenic- oligopeptide antibody (Cp_3) "PQDFGEVFNMFDNEPS") specific for whitefly transmitted *Geminiviruses* {WTG} as described by Aref *et al.* (1995). which provided by C. Fauquet at the International Laboratory for Tropical Agricultural Biotechnology (ILTAB). the Scripps Research Institute. (N. Torrey Pines Road. La Jolla. CA92037. USA).

RESULTS AND DISCUSSION

Virus Isolation

The natural symptoms observed on infected tomato plants in the two locations are presented in Fig (1).

The causal agents were inoculated on tomato (cv. Castel Rock) plants by whitefly transmission. Produced symptoms were expressed within 3 weeks and they were similar to those observed on naturally infected plants. These symptoms are illustrated in Fig (2).

It was clear from the primitive results that the two obtained isolates were whitefly transmitted *Geminiviruses* infecting tomato plants and both isolates might contain the TYLCV viral genome. Similar to present external symptoms of whitefly transmitted *Geminiviruses* (WTGs) were described before in Egypt; and only TYLCV was recorded as a whitefly transmitted *Geminivirus* from different locations in Egypt (Nour El-Din *et al.*, 1969 and Mazyad *et al.*, 1979), in Fayoum Governorate (Abdel-Salam, 1990 & 1991 and Aref *et al.* 1991), from Nobarria (Abdallah *et al.*, 1993) and Qaluobia Governorate (Allam *et al.* 1994).

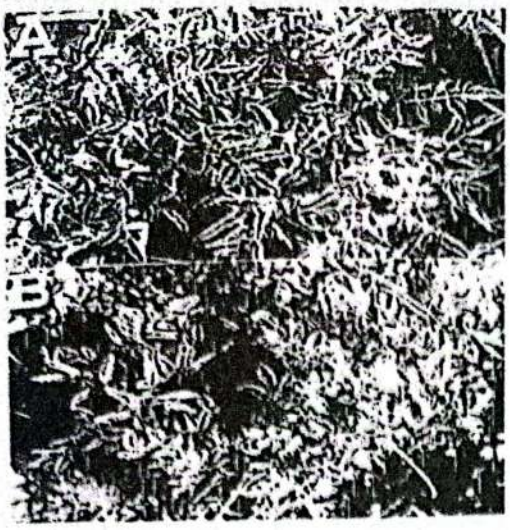

Fig. (1): Symptoms observed on tomato plants collected from open filed (A) showed severe curling, yellowing and rolling (B) showed severe yellowing, curling and dwarfing in the young leaves

Fig. (2): Tow types of symptoms on CastelRock tomato plants, 21 days post insect inoculation (A) showed severe curling, yellowing and rolling (B) showed severe yellowing, curling and dwarfing in the young leaves

Differentiation between the two isolates using diagnostic host species

Tenderpod bean plants (*Phaseolus vulgaris L.*) inoculated with G- isolate showed interveinal chlorosis and downward curling (Fig 3A) 22 days post inoculation.

Whereas, no symptoms were observed on Winter cherry and tobacco plants. Regarding the inoculation by Q-isolate, similar symptoms were found on Tenderpod bean plants, while on winter cherry (*Physalis floridana* Rydb.) showed mild yellow symptoms, which are illustrated in Fig (3B) 18 days post inoculation, however, no symptoms were found on tobacco (*Nicotiana clevelandii* A. Gray.)Plants.

The results were confirmed by PCR using two sets of old world whitefly transmission *Geminiviruses* (OWTG) specific primers (OWTG391 & OWTG1049). Positive amplified PCR products of 659 bp were obtained using DNA of the three diagnostic host species were illustrated in (Fig 4). According to the PCR products, it was obvious from the amplified fragment

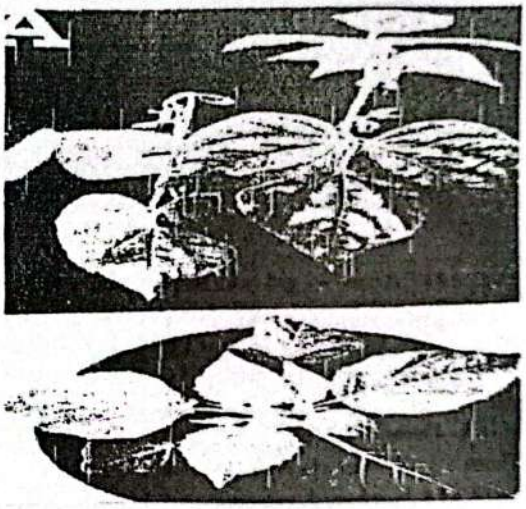

Fig. (3): Two types of symptoms on the diagnostic host species infected with two virus isolates (G& Q) 22 days post insect inoculation (A) Tenderpod bean *Phaseolus vulgaris* L. showing intervenal chlorosis and downward curling (B) Winter cherry *Physalis floridana* Rydb. showing mild yellowing symptoms

size that the bean plants could propagate both the isolates. In case of winter cherry plants inoculated with Q-isolate, positive PCR reaction was obtained. However no amplified fragment was detected with those inoculated with G- isolate.

These results might indicate that the Q-isolate has a mixed infection, as

it was able to propagate on both bean and winter cherry plants. So, the inoculated winter cherry plants were used as a source of infection for the Q-isolate in the back inoculation assay. In the case of G-isolate, inoculated bean plants were used as a source of infection in the back inoculation assay.

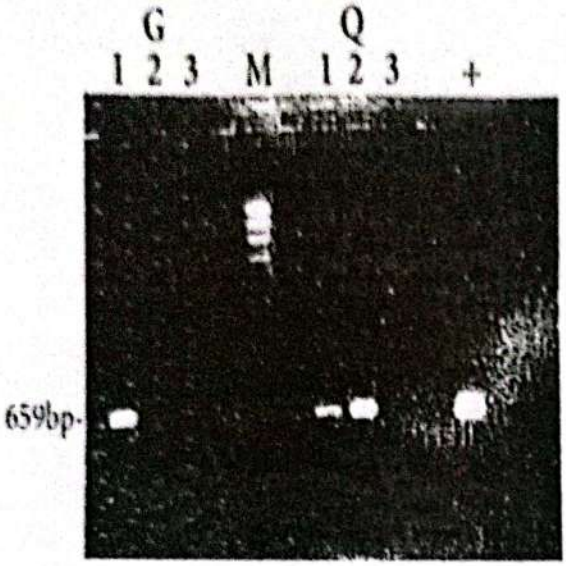

Fig. (4) Agarose gel electrophoresis of PCR amplified products for DNA of three diagnostic hot species infected with two virus isolates using primer pair (WTG 391 and WTG 1049) to detect the presence of the viral genome. The lanes are: (G) 1- Bean; 2- Winter cherry.; 3- Tobacco Q) 1- Bean ; 2- Winter cherry.; 3- Tobacco, and DNA of TYLCV cloned genome and M- λ *Hind* III DNA molecular weight marker.

The obtained results based on symptoms expression and PCR using OWTG-primers revealed that both isolates are belonging to whitefly transmitted *Geminivirus* group and are different in their ability to infect the indicator hosts. To be ensured that present results are in harmony with the conclusion of Brunt *et al.* (1991& 1996), back inoculations of infected Tenderpod bean with G- isolate and winter cherry infected with Q- isolate to healthy seedlings of CastleRock tomato plants were performed, and both types of symptoms are presented in Fig (5). G- isolate showed typical symptoms of TYLCV named G-gemini- isolate. Whereas, Q- isolate produced completely different symptoms from the symptoms on their original tomato plants (yellow mosaic symptoms). This expected to be due to a *Geminiviruses* infection and gave a positive result with PCR analysis named Q-gemini- isolate.

Based on the difference of symptoms on the main host, and the symptoms on diagnostic host and also, the confirmation PCR results. Regarding to these results it could be considered that the two isolates as two different viruses putative *Tomato yellow leaf curl virus* (G- isolate) and putative *Tomato yellow mosaic virus* (Q- isolate). These results are in agreement with those obtained by Cohen and Harpaz, (1964), Cohen and Nitzany (1966) for TYLCV while Uzcategui and Lastra (1978) and Piven *et al.* (1995) for TYMV.

Characterization of the two isolated viruses using molecular diagnostic tools

Seven primer pairs were tested for viral genome amplification to detect whitefly transmitted *Geminiviruses* in the infected DNA preparations from tomato plants cv. CastelRock inoculated by TYLCV-G and TYMV-Q. PCR results are presented in Fig (6). Two sets of TYLCV-Eg specific primers which flank the replicase ORF (C1 F& C1 R) and the coat protein ORF (Cp F & Cp R) gave a positive result with the TYLCV-G. C1 ORF amplified fragments, respectively 1072 bp and the Cp ORF amplified fragments, respectively 778 bp. However, negative results were obtained with the TYMV-K. Two sets of old world whitefly transmission Geminiviruses

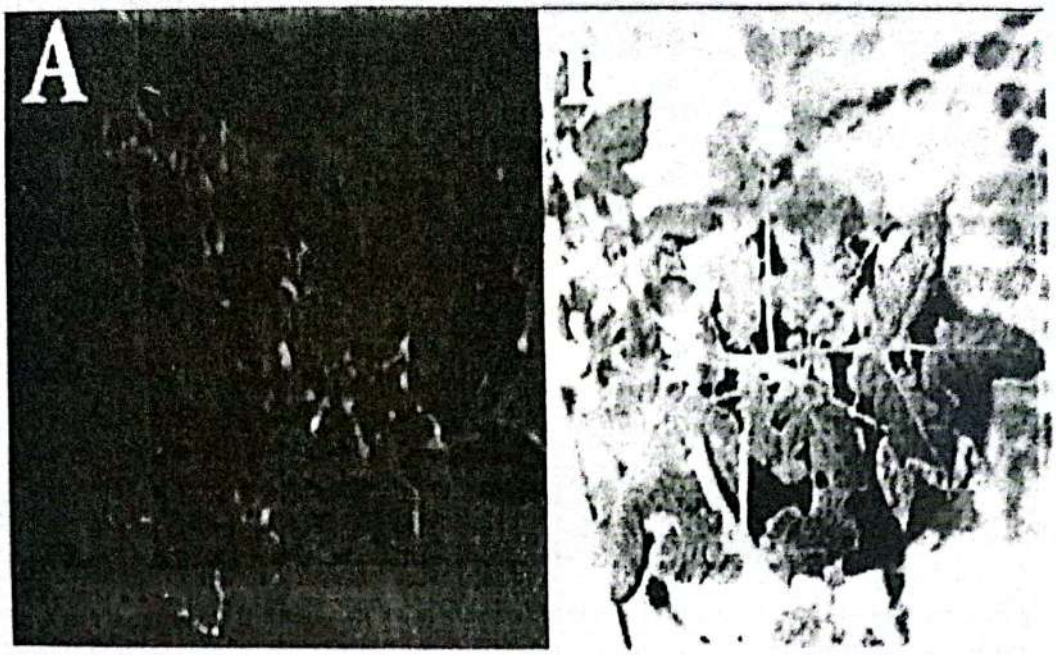

Fig. (5): Back inoculation from the diagnostic host species to healthy CastelRock seedling tomato using whitefly inoculation that showing (A) typical symptoms of TYLCV were obtained from *Phaseolus vulgaris* L. infected with G gemini- isolate. (B) showing yellow mosaic symptoms were obtained from *Physalis floridana* Rydb. infected with Q gemini- isolate.

(OWTG) specific primers were used to detect the genome of *Geminiviruses* in the inoculated plants with TYLCV-G or TYMV-K. Both isolates gave a positive results as they amplified 659 bp fragment by using the primer set OWTG 391 & OWTG 1049 and 674 bp fragment by using the primer set OWTG 1855 & OWTG 2528. However, three sets of primers designed from the TYLCV-Eg genome to amplify the pCp ORF, intergenic region (IPR) and Cp promoter (C_4) were able to give a positive result with the two viruses as the corresponding amplified fragments using the cloned TYLCV- Eg viral genome. The pCp primer pair amplified a 348 bp DNA fragment flank the pre- coat protein gene specific for TYLCV-Eg. The intergenic region primer pair amplified a 474 bp DNA fragment flank the coat protein promotor. The C_4 primer pair amplified a 298 bp DNA fragment flank the movement gene specific for TYLCV- Eg. In this investigation, it was clear that the coat protein and the replicase clean genes of TYMV are not identical to TYLCV resembled genes at least at the flanking regions of their ORFs. PCR product might be due to variability compared to biological properties in both the coat protein and the replicase clean genes. These result agreed with Padidam *et al.* (1995) who studied a phylogenetic tree obtained from the alignment of nucleotide sequences of 36 *Geminiviruses* and found that it may reflect the fact that allowed mutations in Cp are under the constraints of viral structure, vector transmission, host specificity and other unknown functions. Also, they proved that mechanical transmitted

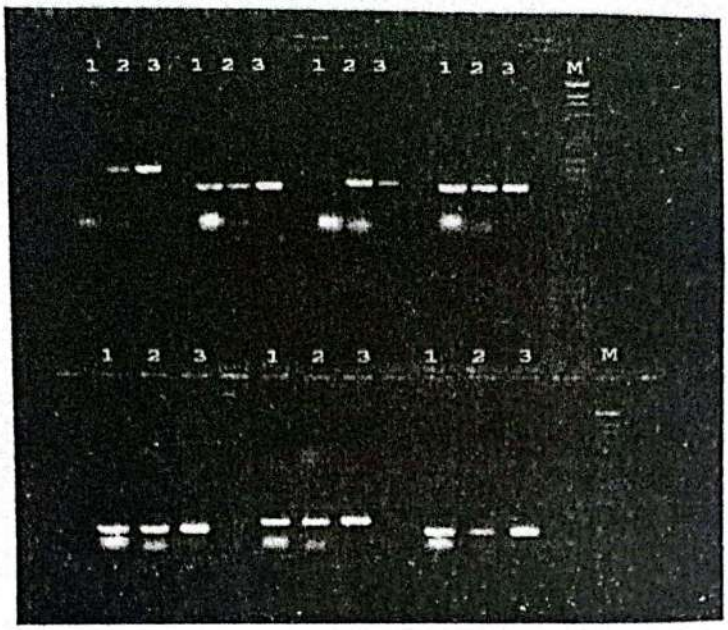

Fig. (6): Agarose gel electrophoresis for comparative study of PCR- amplified the infected DNA from tomato plants cv. CastelRock inoculated by TYLCV-G & TYMV-Q with using seven primer pairs. The lanes are (C1 F and C1 R) 1=Q; 2= G; 3= (+) amplification of 1072 bp DNA fragments with the replicase gene specific for TYLCV- Eg. (WTG1855 and WTG2528) 1=Q; 2= G; 3= (+) amplification of 674 bp DNA fragments with the whitefly- transmitted geminiviruses to detect and confirm the presence of the viral genome. (Cp F and Cp R) 1=Q; 2= G; 3= (+) amplification of 778 bp DNA fragments representing the coat protein gene specific for TYLCV- Eg. (OWTG391and OWTG1049) 1=Q; 2= G; 3= (+) amplification of 659 bp DNA fragments to detect and confirm the presence of the viral genome. (pCp F and pCp R) 1=Q; 2= G; 3= (+) amplification of 348 bp DNA fragments with the pre- coat protein gene specific for TYLCV- Eg. (IPR F and IPR R) 1=Q; 2= G; 3= (+) amplification of 474 bp DNA fragments with the Cp- promotor. (C_4 F and C_4 R)1=Q; 2= G; 3= (+) amplification of 298 bp DNA fragments with the movement gene specific for TYLCV- Eg; and M- 1 kb ladder DNA molecular weight marker.

Geminiviruses clustered in one group. In this respect, Abdallah *et al.* (1993), Abdallah *et al.* (2000), Aref *et al.* (1994) and Idriss *et al.* (1997) proved that, the universal old-world whitefly transmitted *Geminiviruses* primers (OWTG391 & OWTG1049 and OWTG1855 and OWTG2528) are located in a highly conserved regions. This could be used to investigate the genomic diversity of viruses based on the oligonucleotide primer set that are complimentary to the region flanking the DNA sequence to be amplified (Idriss *et al.*, 1997). Therefore, the OWTG primers should detect the two isolated viruses in their main host and other host range. What's more, the PCR products, which performed in this investigation, confirmed that there are two *Geminiviruses* depending on the different results obtained when using the coat protein and replicase primers specific for TYLCV- Eg. However, positive amplifications were obtained with the pCp ORF, intergenic region (IPR) and Cp promoter (C_4) primer sets designed from the TYLCV-Eg genome indicating that they are related. From this study it was clear that TYMV considered as a well identified virus and differed from TYLCV.

Symptomatology and Host range

The symptoms are presented in Fig (7: 1, 2) and tabulated in Table (2). The negative control plants did not show any symptoms.

The expressed symptoms after inoculating experimental host range with the TYMV recorded and confirmed by PCR using the primer set

OWTG 391 & OWTG 1049. The amplified PCR products with two set of old- world whitefly transmission *Geminiviruses* (OWTG) specific primers from thirty-four plant species and cultivars belonging to six families using as diagnostic tools to detect the genome of *Geminiviruses* in the inoculated plants with TYMV. The PCR results gave a positive result as they amplified 659 bp DNA fragment as expected. The PCR results were tabulated in Table (2). These results were completely agreed to the results recorded by Uzcategui and Lastra (1978) and Piven *et al.* (1995).

Obtained results indicated that TYMV appears to be able to infect only to some species of the family Solanaceae.

From the previous data. it could be concluded that some TYLCV-isolates in Egypt. considered as a mixed infection with TYMV.

Mechanical transmission

To study the possibility of mechanical inoculation of the TYMV, two extraction buffers were used. Results founf that. the TYMV is mechanically transmitted using Piven's buffer described by Piven *et al.* (1995). While the plants were tested mechanically using Abdel-Salam's buffer described to Abdel-Salam (1990) did not show any symptoms. The tomato plants inoculated mechanically using Piven's buffer showed mild yellow mosaic symptoms on 70 % of total tested ones reassemble those observed in the original. The symptoms are illustrated in Fig (8 C). Inoculated *Nicotiana glutinosa* L plants showed systemic yellow spots then mosaic and *Nicotiana tabacum* L. cv. Samson plants showed systemic mosaic and leaf deformation. the symptoms were shown after 14-16 days post inoculation and were illustrated in Fig (8A & B).

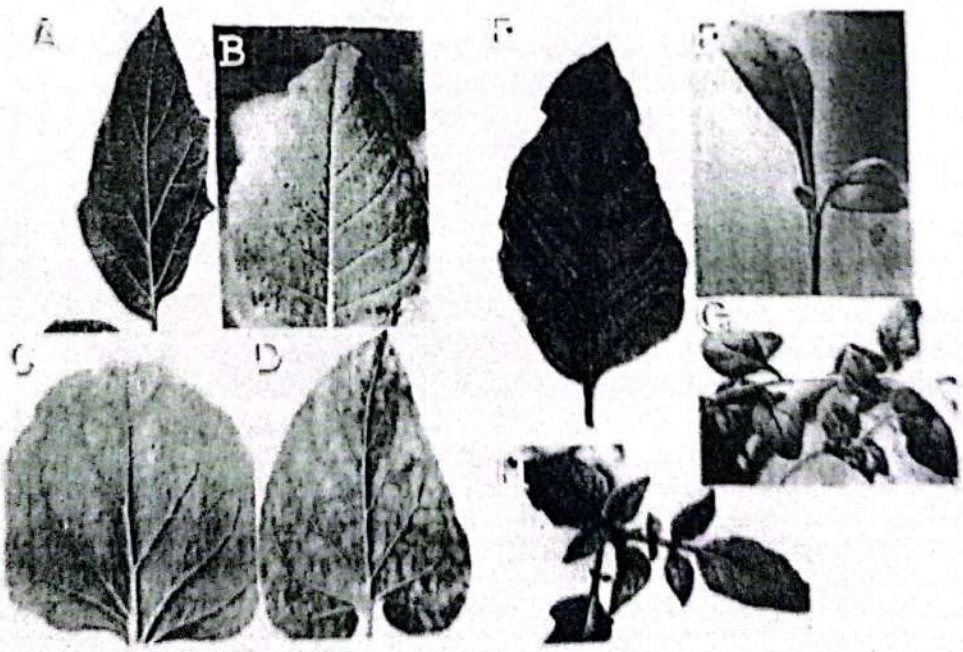

Fig. (7). Symptoms expression of tomato whitefly transmitted geminiviruses TYMV-Q on experimental host range as inoculated by viruliferous whiteflies. ; Plant samples are : (A) *Datura stramonium* L. showing systemic yellow mosaic; (B) *Nicandra physalodes* L. showing systemic mild yellow mosaic; (C) *Nicotiana benthemiana* L. showing systemic yellow mosaic; (D) *Nicotiana glutinosa* L. showing systemic yellow spots then mosaic. (E) *Nicotiana tabacum* L. cv. Samson showing systemic mosaic and Leaf deformation. (F & G) *Petunia hybrida* Vilm. showing systemic mild yellow mosaic and (H) *Solanum tuberosum* L. showing yellow mosaic.

Table (1): Experimental host range of TYMV after inoculation by viruliferous whitefly

Family Scientific name	Vars.	Visible Symptoms *	PCR Detection
Amaranthaceae			
Gompherena globosa L.		No Symptoms	**-
Chenopodiaceae			
Beta valgaris L.		No Symptoms	-
Chenopodium amaranticolor Coste& Reyn.		No Symptoms	-
Chenopodium quinoa Willd.		No Symptoms	-
Compositae			
Cucumis melo L.	Shahed	No Symptoms	-
	Ananas	No Symptoms	-
Cucumis sativus L.	Balady	No Symptoms	-
Cucurbita pepo L.	Eskandarani	No Symptoms	-
Leguminasae			
Phaseolus vulgaris L.	Tenderpod	No Symptoms	-
	Top crop	No Symptoms	-
Vicia faba L.	Giza 402	No Symptoms	-
	Giza 462	No Symptoms	-
Malvaceae			
Gossypium barbadense L.	Giza 83	No Symptoms	-
Hibiscus esculentus L.		No Symptoms	-
Malva parviflora L.		No Symptoms	-
Solanaceae			
Copsicum annuum L.	California wander	No Symptoms	-
Datura metal L.		No Symptoms	-
Datura stramonium L.		Systemic yellow mosaic	***+
Lecopersicon esculentum Mill.	BVF 145	yellow mosaic	+
	Castle Rock	yellow mosaic	+
	Floredada	yellow mosaic	+
	Peto 86	yellow mosaic	+
	Rotgers	yellow mosaic	+
	Supermarmand	yellow mosaic	+
	UC 82	yellow mosaic	+
	UC 97-3	yellow mosaic	+
Nicandra physalodes L.		Systemic mild yellow mosaic	+
Nicotiana benthemiana L.		Systemic yellow mosaic	+
Nicotiana glutinosa L.		Systemic yellow spot& mosaic	+
Nicotiana rustica L.		No Symptoms	-
Nicotiana tabacum L.	Samson	Systemic mosaic& Leaf	+
Petunia hybrida Vilm.		deformation	+
Physalis floridana Rybd.		Systemic mild yellow mosaic	+
Solanum tuberosum L.		Systemic mild yellow mosaic	+
		yellow mosaic	

* Symptoms appeared in 18 days post inoculation

** - : negative result for amplified PCR products

*** +: positive result for amplified PCR products

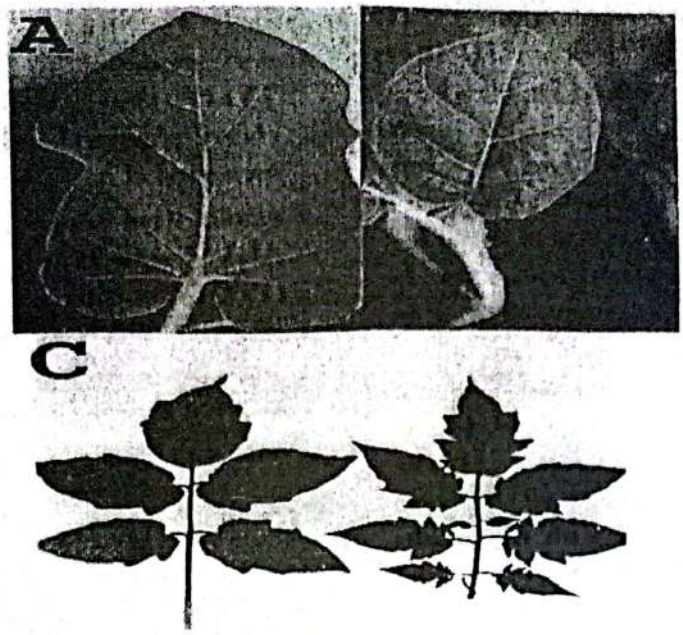

Fig. (8): Symptoms expression on the deferent host species infected with the TYMV by mechanical inoculation. (A) = *Nicotiana glutinosa* L. showing systemic yellow spots then mosaic. (B) = *Nicotiana tabacum L.* cv. Samson showing systemic mosaic and leaf deformation. The symptoms were shown after 16 days post inoculation and (C) *Lycopersicon esculentum* Mill. cv. 8Vf145 showing mild yellow mosaic, which were produced after 14 days post inoculation.

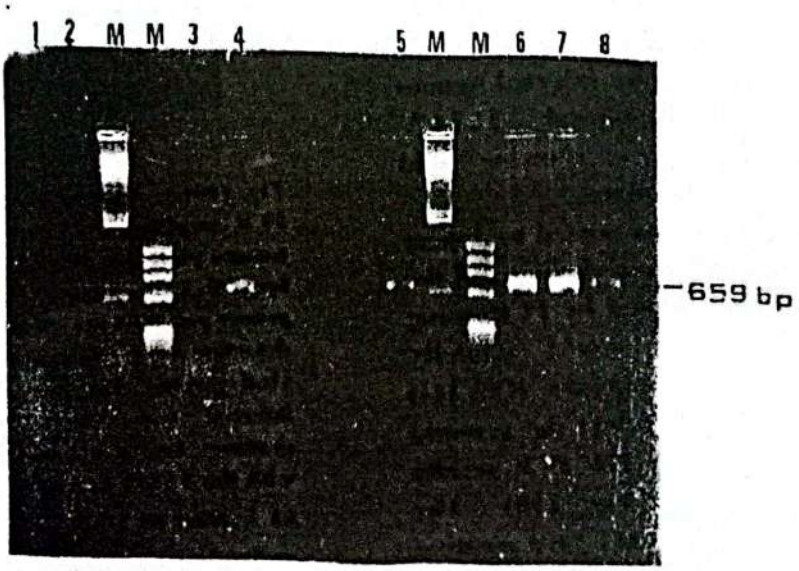

Fig. (9): Agarose gel electrophoresis of (PCR)-amplified DNA products from the results of the mechanical inoculated tomato plants cv. BVf145 of the two virus isolates and some plants as a host range amplification of 659 bp DNA fragment representing using primer pair (OWTG391and OWTG1049) to detect and confirm the presence of the viral genome. The lanes are 1= tomato plants-TYLCV- isolate. 2 =TYMV- isolate inoculated with Abdel-salam's buffer; 3= tomato plants- TYLCV- isolate. 4= TYMV- isolate inoculated with Piven's buffer; 5=tomato plants TYMV- isolate, 6= *Nicotiana glutinosa*, and 7=*Nicotiana tabacum L.* cv. Samson inoculated with Piven buffer and 8= (+) . M, λ *Hind* III DNA and ϕ X174 / *Hae*III molecular weight markers.

These results were confirmed by PCR using one set of old world whitefly transmission Geminiviruses (OWTG) specific primers to detect the genome of Geminiviruses in the mechanical inoculated plants with TYMV. The PCR results are illustrated in Fig (9). The inoculated

plants gave a positive result as they amplified 659 bp fragment using WTG primers (OWTG 391 & OWTG 1049) to detect the genome of Geminiviruses in the inoculated plants. These results were completely agreed to the results recorded by Goodman (1981) and Bock (1982) which reported that a few numbers of *Geminiviruses* can be transmitted experimentally by mechanical inoculation and these results confirmed with the previous data described by Piven *et al.* (1995) and Uzcategui and Lastra (1978). However, these results were completely disagreed with the results recorded by Abdel-Salam (1990) who reported that, TYLCV could be transmitted mechanically using special buffer.

Transmission by grafting

Healthy seedling of tomato plants cv. S-BVf145 were inoculated by grafting using tomato plants cv. CastelRock grafted by infected tomato plants showing typical TYLCV symptoms and others showing mild yellow mosaic symptoms.

The symptoms started to appear after 18 days post inoculation, and the results were confirmed by PCR using one set of old world whitefly transmission *Geminiviruses* (OWTG) specific primers to detect the genome of *Geminiviruses*. The PCR results are illustrated in Fig (10). The inoculated tomato plants gave positive results as they amplified 659 bp fragment using WTG primers (OWTG 391 & OWTG 1049). These primers were used to detect and confirm the presence of the viral genome. Present results are in agreement with those obtained by Uzcategui and Lastra (1978) and Brunt *et al.* (1990 & 1996) where they reported that *Tomato yellow mosaic virus* (TYMV) was transmitted by grafting.

Virus purification

The yield of the virus particles was measured as ~ 3 mg/Kg for TYMV virus particles from Winter cherry plants. The purity of the virus measured at A 260/280 was 1.4. These results were completely different with Abdel-Salam (1990) and Aref *et al.* (1995). But it's confirmed with Allam *et al.*, (1994) who reported that the purified virus yield was 3.09 mg/ kg of infected tomato tissue and A 260/280 ratio was 1.46.

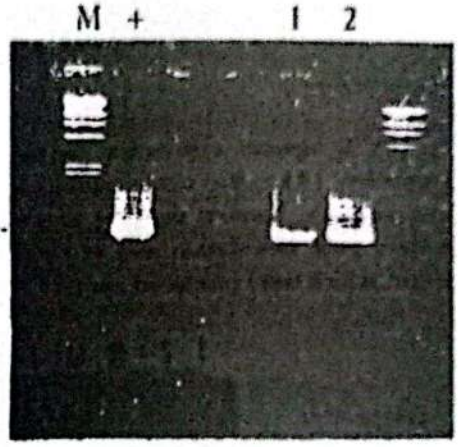

Fig. (10) : Agarose gel electrophoresis of PCR -amplified DNA products using grafted tomato plants cv. BVf145 amplified of 659 bp DNA fragments representing using primer pair (OWTG391and OWTG1049) to detecting and confirming the presence of the viral genome. The lane (1) tomato plants infected with the TYLCV-isolate by grafting; lane (2) tomato plants infected with the TYMV-isolate by grafting and (+) M λ *Hind* III DNA and ϕ X174 *Hae*III molecular weight markers.

Electron Microscopy

Electron Microscope of TYMV virus particles purified preparation stained with 2 % (w/v) uranyl-acetate showed typical geminate virus particles (18- 20 X 30nm) as a whitefly transmitted *Geminiviruses* (Fig 11). These results confirmed the previous data performed by Lastra and Gil (1981).

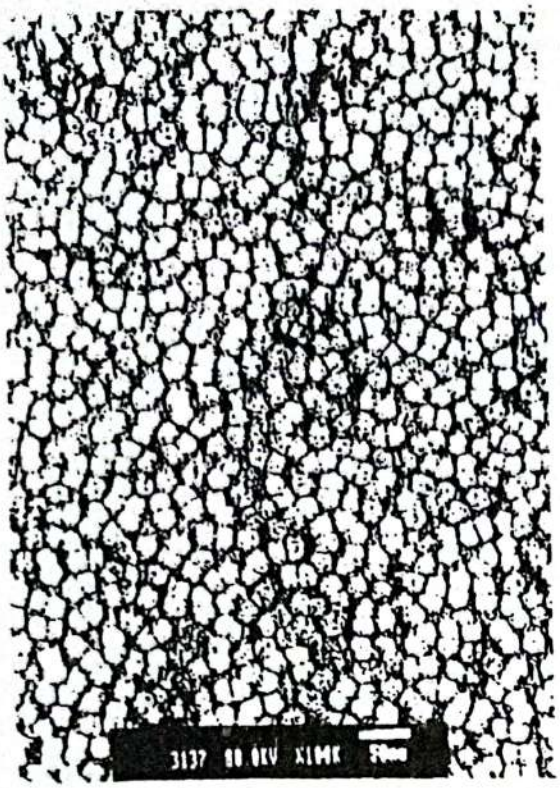

Fig. (11): Electron micrograph of putative TYMV purified by centrifugation in a cesium sulfate gradient and stained with 2% uranyl acetate. (X100K)

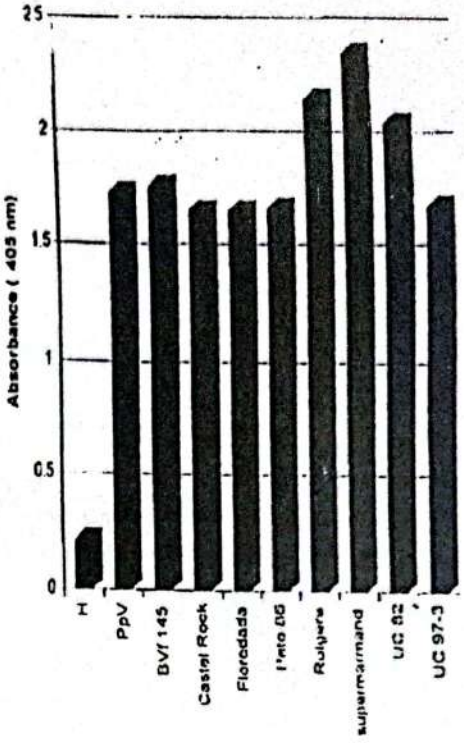

Fig. (12): Detection of TYMV in tomato cultivars with the antisera specific to the C- terminal (Cp3) using indirect ELISA.

ELISA detection

The ELISA test results were illustrated in Fig (12) using the C- terminal (Cp-3) oligo-peptide antibody.

The tomato cvs. Peto 86. Floridada and CastleRock were the lower titer for TYMV. Whereas supermarmand showed high sensitivity. Serological techniques. ISEM. ELISA. *etc.* based on the antigenic properties of the viral coat protein were inadequate for whitefly transmitted *Geminivirus* identification. They lack both the specificity and sensitivity required for diagnosis (Pico *et al.*, 1996).

These results confirmed the previous data performed by Murphy *et al.* (1995) who stated that serological tests for all subgroup III Geminiviruses seemed to be relatively closely related.

These results prove that two Geminiviruses in this study are different viruses having similarity in their antigenic properties of viral coat protein. The homology of the two viruses was high because they are whitefly transmitted *Geminiviruses* according to Pico *et al.* (1996). 10- Determination of molecular weight of TYMV coat proteins:

The capsid protein of TYMV migrated as a single band from virus purified preparation. with a molecular mass of ~ 28 KD as shown in Fig (13). The obtained results are in agreement with previous results reporting that *Geminiviruses* have small single-stranded DNA (2.5- 3.0 kb) in 1 or 2 parts. encapsidated by a shell consisting of two incomplete icosahedra built from a single species of polypeptide of M_r between 27-34x10^3 (Harrison *et al.* 1977 and Bock 1982).

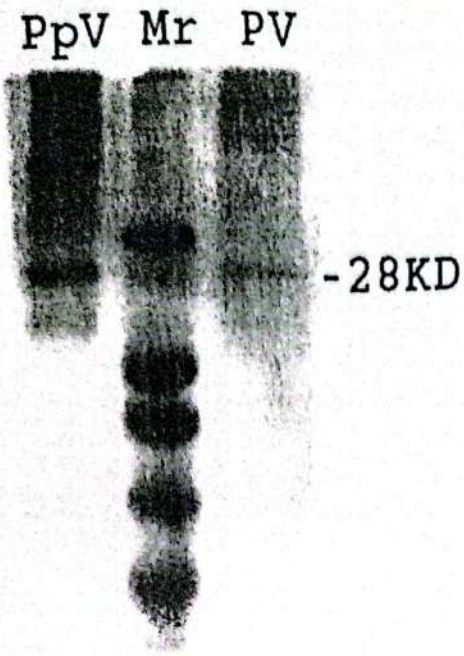

Figure (13): Determination of molecular weight of TYMV coat proteins by SDS-polyacrylamide gel electrophoresis.
PpV : partial purified virus
PV : purified virus
Mr : promega mid range protein marker

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