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Identification Studies of a Virus Disease Affecting Guava in Minia Governorate

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An isolate of cucumber mosaic virus (CMV) was obtained from naturally infected guava (*Psidium guajava* L. cv. Balady) plants grown in Minia Governorate, exhibiting systemic green island and characteristic mosaic symptoms associated with deformation pattern. The isolated virus infected thirty-two plant species, using different varieties and cultivars. The virus reacted systemically with mosaic alone on 9 test plants out of 32, while some other in combination with other showed symptoms, *i.e.* crinkling, vein clearing, vein banding, deformation, stunting, wilting, deformation, necrosis, yellowing and green island. The virus under investigation was inactivated in sap after heating for 10 min at 70^{o}C.The dilution end- point was between 10^{-4} and 10^{-5} .Virus infectivity *in vitro* was maintained more than 10 days storage at room temperature (25^{o}C). The identification of the virus under study was confirmed serologically by DAS-ELISA. Positive reaction obtained indicated that the tested virus was related serologically to Cucumovirus. Light microscope examinations revealed amorphous cytoplasm inclusions (X-bodies) in the epidermal-hairs cells of squash (after 21 to 42 days from inoculation) with the tested virus. Electron micrographs observations of ultrathin sections in the infected cell with the isolated virus were: some abnormalities in the cell wall, degradation and malformation and showed conspicuous alterations in shape, malformed mitochondria, swollen of chloroplast, virus particles were found in the cytoplasm, around and in the chloroplast, 3-par mural bodies were also seen in the chloroplast.

Keywords: Cucumber, guava and mosaic virus.

Guava (*Psidium guajava* L.), an evergreen, subtropical, shrubby tree, is widely cultivated for its sweet acid yellow fruit. The genus *Psidium* is a native to the American tropics and today is found in all subtropical and tropical regions. It is an important fruit in many parts of the world where the climate is suitable for its production (Kwee and Chong, 1990 and Gould and Raga, 2002). It is one of 50 genera in the family Myrtaceae. Guava a vitamin C enriches fruit plant and is grown throughout, Minia Governorate.

Despite the economic importance of guava, its production is limited by many biotic factors in humid regions (Rahman *et al.*, 2003). It is attacked by many pathogens as *Colletotrichum gloeosporioides* the causal agent of anthracnose (Merida and Palmatee, 2006), *Botryosphaeria dothidea* and *Phytophthora citricola* the causal agents of brown rot of fruits (Pane *et al*., 2001), *Fusarium* spp. and *Rhizoctonia* spp. the causal agents of root rot disease (Anggraeni and Suharti, 1997), *Meloidogyne incognita* the causal agent of root-knot (Moura *et al*., 1989),

Pseudocercospora psidii the causal agent of pseudocercospora leaf spot, *Armillaria tabescens* the causal agent of mushroom root rot, *Cephaleuros virescens* Kunz the causal pathogen of algal leaf spot (Merida and Palmatee, 2006), *Puccinia psidii* the causal agent of guava rust (Laundon and Waterson, 1965), *Guignardia psidii*, an ascigerous state of *Phyllosticta psidiicola* the causal agent of fruit rot (Gonzalez and Rondon, 2005), *Glomerella cingulata* the causal agent of anthracnose on fruits (Carranza *et al*., 2002) and *Pestalotiopsis* spp. causing scab disease of guava (Keith *et al*., 2005).

In the year of 2009-2010, symptoms of dark green islands and sever mosaic were observed on the young leaves of guava in Shosha region at Minia. The present research was initiated to investigate the causal agent, host range, symptomatology, physical properties, virus transmission and identification which confirmed by ELISA, and cytological studies by light and electron microscope.

Materials and Methods

Virus source and isolation:

During the year of 2009-2010, samples from naturally infected guava plants exhibiting characteristic mosaic symptoms consisted of leaf green islands with deformation and systemic green mosaic were collected from Shosha region (Minia Governorate). The infected leaves were ground in a sterilized mortar. Phosphate buffer saline (PBS), 0.1M, pH 7 was used in preparing the crude sap. The obtained fresh sap was used for mechanical inoculation of healthy plants, *i.e. Chenopodium amaranticolor* Cost & Reyn, *Ch. quinoa* Wild and *Vigna unguiculata* L. as diagnostic host plants. The inoculated and uninoculated seedlings were kept under observation in an insect proof greenhouse at 25^{o}C. The single local lesions technique described by Jiang *et al.* (1992) was followed to obtain pure virus isolate from the local lesions developed on the inoculated plants.

The virus isolate was propagated in *Cucarbita pipo* L., *Ch. amaranticolor* and guava cvs. Balady and Roomy which served as the source of virus infection for the subsequent experiments.

Virus identification:

Host range and symptomatology:

The host range and symptoms expression was determined. Thirty two plant species, cultivars and verities, belonging to 7 families were mechanically inoculated with the isolated virus. An equal number of healthy plants of the same cultivar and age were left without inoculation to serve as control.

The plants were examined daily up to 30 days for symptoms developing. Symptoms on both inoculated and uninoculated leaves were recorded. Plants showed no symptoms were checked by back- inoculation to *Ch. amaranticolor*. In case of preparing guava seedlings, seeds from healthy fruits were harvested from guava fruits of Balady and Roomy cvs. washed and left dry, then sewing in a peat moss and transferred to clay pots, 20 cm. in diameter (with sterilized mixed soil peat and sand 2:1) inside isolators in the greenhouse. After one month (5 leaves age) they were ready to be inoculated.

Virus stability in crude sap:

Dilution end point (DEP) was measured by testing the infectivity of undiluted and diluted infectious sap with distilled water from 10^{-1} to 10^{-6}, (ten-fold dilution). In thermal inactivation point test (TIP), undiluted fresh infectious sap, contained in thin walled test tubes (2ml/tube) with a thermometer inside, were heated for 10 min in a thermostatically water-bath under different degrees of temperature (50 to 80^{o}C), (5^{o}C interval). Longevity of the virus isolate *in vitro* (LIV) were determined, according to the techniques described by Daniels and Campbell (1992).

Guava leaves cv. Balady, with symptoms of green island and mosaic, was used as a source for virus isolate, while *Ch. amaranticolor* was used as indicator host.

Serology:

Double sandwich immunosorbent assay (DAS-ELISA) technique was used for virus detection as described by Clark and Adams (1977) using specific gamma globulin for CMV and compared with those WMV (watermelon mosaic virus) and SqMV (squash mosaic virus) in pre-coated ELISA strips. All immuonoreagents were commercial kits and used according to the manufacturer's instructions using Dynatech immunoassay system (Agdia, US). Optical Density was measured at A_{405} nm. Samples with an absorbency of at least twice those of the healthy control were considered as positive.

Cytological studies:

Examination of virus inclusions by light microscope:

To study the chemical nature of the inclusion bodies, method described by Christie and Edwardson (1986) and El-Kewey *et al.* (2007) was carried out. Epidermal hairs taken from the upper surface of systemically infected squash plants (*Cucurbita pepo*, cv. Eskandarany) with the isolated virus were treated with 5% tritonx-100 for 10 minutes. Then, they were stained with mercuric bromophenol blue stain (immersing them in a stain containing 100 mg bromophenol blue and 10g mercuric chloride in 100 ml distilled water for 15 min) the treated strips were then placed in 0.5% acetic acid for 15 min, and washed then mounted in water as described. The strips were sampled at 3-day intervals, beginning from the 3rd day up to the 42nd day after inoculation, were microscopically examined to detect the protein contents of inclusion bodies in infected leaves of the tested virus.

Electron microscope examination:

Leaf samples from cv. Balady guava seedlings were prepared for examination by transmission electron microscope type (Toel-TEM 100CX) in EMU, Assiut University. The method of Allam *et al*. (2000) was carried out. One month after being mechanically inoculated with the virus isolate, tissue specimens, 1-2 mm^2, were taken from areas of the green island with mosaic formed on inoculated upper service of the leaf. Healthy leaves of each one cultivar were used also as control. Specimens were fixed in 1% phosphate buffered glutaraldehyde pH 7 and post fixed in 1% osmic acid then cleared and Epon 812 was used. The sections were cut with LKB Microtome Lika at 300 A^{o}, and the thickness was about 50 nm. The ultra sections were mounted and stained in LKB ultra strainer in 5% aqueous uranyl acetate for 30 min and lead citrate for 4 min.

Results and Discussion

Host range and symptomatology:

The virus under investigation was isolated from natural infected leaves of guava cv. Balady showing severe mosaic and dark green islands (Fig.1) which collected from Shosha region, west Minia Governorate. The tested isolate was propagated in *Ch. amaranticolor*, *Cucurbita pipo* cv. Eskandarany, and Balady and Roomy guava seedlings, which served as the source of the virus infection for the subsequent experiments. The virus was transmitted mechanically to *Ch. amaranticolor* and from this host to other host plants.

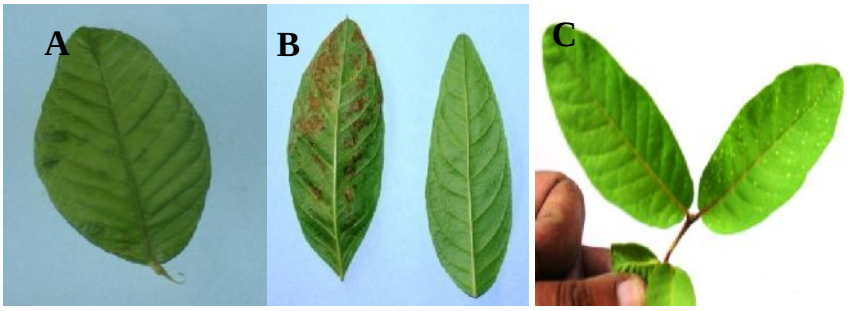

Fig. 1. Symptoms induced on guava seedlings: A) Naturally infected leaf of guava cv. Balady showing green islands during summer, B) Necrotic lesion in leaf lower-surface after mechanical inoculation in winter, C) Guava cv. Roomy with chlorotic lesions after mechanical inoculation.

Reaction and symptoms developed on the tested plants (Table 1) differed from on plant species to another. *Ch. amaranticolor* reacted with minute chlorotic lesion then turned to necrotic but no systemic infection as well as cowpea (*Vigna unguiculata*) that reacted with small brown lesion.

Symptoms ranged from local to systemic, local symptoms ranged between chlorotic to necrotic lesions which appears after 6 to 10 days from inoculation. Only one case (*Chenobodium album* L.) the symptoms appeared in the form of ring spots after 9 days of inoculation. The formation of water socking on leaves were observed, *i.e. Nicotiana clevelandii*, *Nicotiana debandii*, and *N. tabacum* var. White Burley after 7, 10 and 12 days respectively. In the case of *Ch. amaranticolor*, *Lantana amaranthus*, *Gossipium barbadens* cv. Giza 89 and *Psidium guajava* cv. Roomy, lesions appeared in the form of yellow local lesion after about 12 days. Systemic symptoms varied according to the host plant tested.

However, in most cases, they appeared in the form of mosaic, deformation, green-island, yellowing, stunting, vein clearing, crinkling, yellowing and necrosis. These symptoms appeared alone or in combination. The first disease symptoms appeared after the elapse of 15 to 20 days of inoculation except the symptoms of

Table 1. Symptomatology and host range of the isolated virus from guava

Family	Tested plant	Main Symptom*	
		L	S
Chenopodiaceae	*Chenopodium album* L.	Rs (9)**	-
	Ch. amaranticolor Cost.	Cl (12)	-
	Chenopodium murale L.	-	Cr (18)
	Chenopodium quinoa Wild.	Cs (7)	-
Compositae	*Conyza agyptiaca* L.	Cl (10)	M (20)
	Lantana amaranthus L.	Yl (10)	M (22)
Cucurbitaceae	*Cucamis sativus* L. var. Madina	Cs (7)	M (20)
	cv. Beta Alfa	Cs (6)	Gi (15)
	cv. Prense	-	Y (20)
	Cucamis melo L.	Cs (10)	Y (23)
	Cucurbita pepo cv. Eskandarany	Cs (8)	D+M (15)
	cv. Zucchini	-	M (18)
Solanaceae	*Capsicum frutescens* cv. Chilli.	Cs (7)	M (20)
	Datura stramonium L.	Cl(5)	Vc (22)
	D. tatula L.	-	Vc (20)
	L. esculentum cv. GS Nima.	Cl (6)	M (18)
	cv. Rutgers	Cs (7)	M (15)
	Nicotiana clevelandii L.	Wsok (7)	-
	Nicotiana debandii L.	Wsok (10)	Mt (22)
	Nicotiana glutinosa L.	Cs (6)	M (20)
	Nicotiana tabacum L. cv. Sumson	Ll (8)	Vb (22)
	cv. White Burley	Wsok (12)	M+Cr (20)
	cv. Xanthii-nc	Cl (12)	D+M (22)
	Petunia hybrida L.	Yl (7)	W (23)
Malvaceae	*Gossipium barbadens* L. cv. Giza89	Yl (12)	Vc (18)
	cv.Giza82	-	Vc (15)
	Hibiscus esculentus L.	Cl (5)	Gi+M (10)
Leguminose	*Phaseolus vulgaris* L.	Cs (7)	M (15)
	Vigna unguiculata L.	Bl (6)	Nl (20)
	Vigna sinensis cv. Black eye	Cs (7)	Gi (15)
Myrtaceae	*Psidium guajava* L. cv. Balady	-	-
	cv. Roomy	Ll (11)	

All test plants were back inoculated on *Chenopodium quinoa* leaves.

* L=local symptoms and S= systemic symptoms, -=No symptoms, Vc=Vein clearing, Nl=Necrotic lesion, Ll=local lesion, Y=Yellowing, M=Mosaic, Mt=Mottling, Cr=Crinkling, Vb=Vein banding, D=Deformation, Cs=Chlorotic spots, Cl=Chlorotic lesion, Wsok=Water soaking, Bl=Brown lesion, W=wilting, Gi=Green island, Rs=Ring spots and Yl=yellow lesions.

** Figures between brackets refer to the period (days) required for symptoms appearance following mechanical inoculation.

green islands take only 10 days to appear, *i.e. Phaseolus vulgaris*. Only *L. esculentum* cv. GS-Nima reacted with mosaic (after 6 days) with narrowing of leaf laminas producing characteristic fern leaf symptoms. Also in case of squash, *Cucurbita pepo* cv. Eskandarany, infection occurred through cotyledonary leaves of seedlings and on the subsequent leaves in form of severe mottling, shoestring and malformation. Such symptoms began to appear after 15 days from inoculation.

In general, the host range studies showed that the virus under investigation has a wide host-range this comes in agreement with previous studies on cucumber mosaic virus (Hu *et al.*, 1995; Galal *et al*., 1996; Carrere *et al*., 1999 and Eiras *et al*., 2004).

Virus stability in crude sap:

It is clear that the strain of virus under investigation (CMV-Guava) is somewhat more stable than the ordinary and other strain of cucumber mosaic virus, particularly in respect of thermal inactivation point (TIP), and longevity *in vitro* (LIV). The virus was inactivated after heating for 10 min at 70^{o}C.

The dilution end point was between 10^{-4} and 10^{-5}. Virus infectivity *in vitro* was maintained more than 10 days storage at room temperature (25^{o}C). These results are in agreement with those reported by other workers (Ouf *et al*., 1991, Abdel Aziz *et al*., 1995 and Galal *et al*., 1996) and disagree somehow with Francki *et al*. (1979) and the early ones Noordam (1973) and Ouf *et al*. (1974) in longevity *in vitro*, which the first ones reported that the virus lost infectivity within a few days and in some instances hours at room temperature.

Serology:

The identity of the isolated virus as well as 4 test plants, which appear systematic infection, is confirmed by using DAS-ELISA. Table (2) show that the optical density at A_{405} obtained for the isolated virus from guava cv. Balady, cucumber cv. Beta alpha, squash cv. Eskandarany, been, and cowpea were 2.45, 2.15, 2.84, 2.31 and 2.09 respectively. While the healthy samples for the guava host as well as the healthy 4-test plants show negative reactions. Also, other viruses' AMV, WMV and SqMV obtained no O.D. values. This results were in agree with the previous studies by several investigations in different countries (Daniels and Campbell, 1992; Hu *et al*., 1995; Carrere *et al*., 1999; Fegla *et al*., 2001 and Eiras *et al*., 2004).

Table 2. DAS-ELISA values (A_{405}) for the tested virus compared with some other viruses as well as different test plants

Host	Sample tissues	A_{405}
Guava cv. Balady	Healthy	0.02
	Infected with the tested virus	2.45
Cucumber cv. Beta Alpha	Healthy	0.05
	Infected with the tested virus	2.15
Squash cv. Eskandarany	Healthy	0.04
	Infected with the tested virus	2.84
Been(*P. vulgaris*)	Healthy	0.02
	Infected with the tested virus	2.31
Cowpea (*V. unguiculata*)	Healthy	0.06
	Infected with the tested virus	2.09
AMV		0.00
WMV		0.00
SMV		0.00

Cytological studies:

The cytological effects of viruses have been a subject of interest ever since the early searchers with light microscopes for causative organisms in diseased tissues. A bout the beginning of the twentieth century, theses led to the discovery of two types of virus amorphous bodies or x- bodies and crystalline inclusions. Some workers erroneously considered that they were in fact the parasite or a stage in the life cycle of the parasite causing the disease. These early conclusions were not entirely wrong since many of the × bodies are in fact virus-induced structures in the cell where the components of viruses are synthesized and assembled (Hull, 2002).

Examination of virus inclusions by light microscope:

Examination of epidermal strips of Eskandarany squash leaf infected with the isolated virus 24 days after inoculation with light microscopy revealed amorphous inclusions (x bodies) as well as crystalline inclusions stained with bromophenol blue and mercuric chloride aggregates in the cytoplasm near the nucleus in epidermal hair cell of infected leaf compared with healthy ones. This observation is agreed with other authors (Ouf *et al.*, 1991; Jiang *et al*., 1992; Matthews, 1993; Allam *et al*., 2000 and Hull, 2002) (Fig. 2).

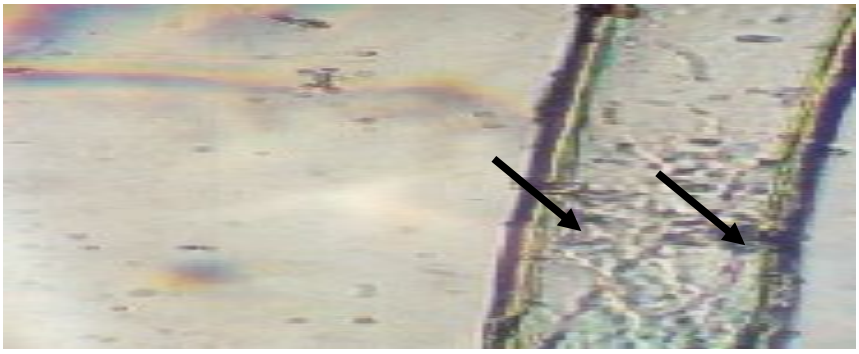

Fig. 2 Inclusion bodies (arrows) in epidermal hair-cells of infected leaf with the tested virus of Eskandarany squash leaf.

Electron microscope Examination:

Ultrathin sections of parts of cells from healthy and artificially-infected guava leaves are shown in figures 3A, 3B, 3C and 3D. Examination of mesophyll cells in ultra-thin sections prepared from infected leaves- samples from cv. Balady Guava seedlings revealed severe pathological changes in comparison with the healthy one. Observations of ultrathin sections of healthy leaf cell (Fig. 3A) revealed two small (normal cytoplasm (Cy) containing intact cell organelles: the nucleus (N) containing normal chromatin (Cr) and obvious nucleolus (Nuo) and nuclear membrane (Nm), the chloroplast (Ch) with normal grana (G) and lamellar membranes (Lm), the mitochondria (M), the vacuoles (V) and cell wall (Cw) were all seen obvious and without pathological changes. This observation is confirmed and in a good manor with Jiang *et al*. (1992); Matthews (1993); Hassan (1990); Paradies *et al*. (2000) and Hull (2002).

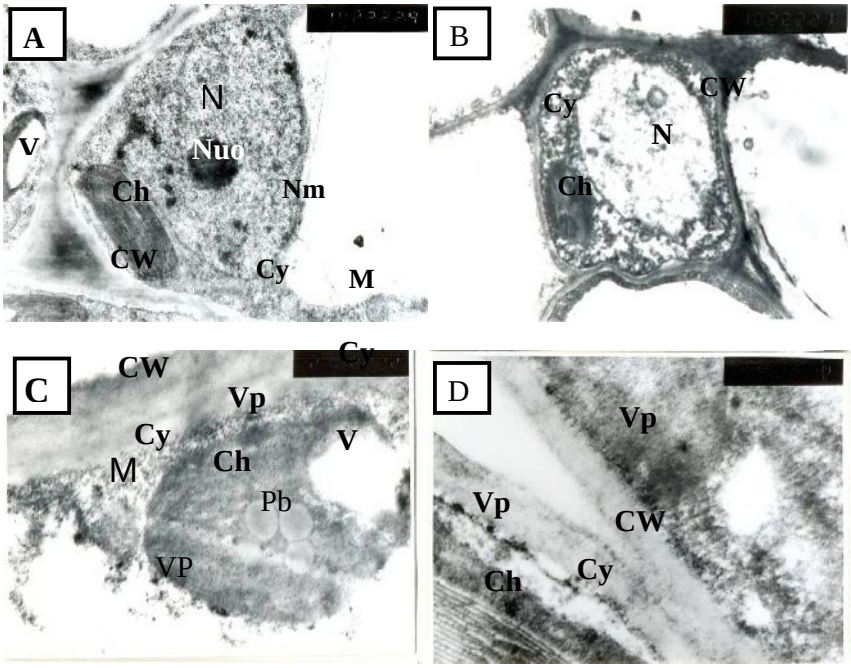

Fig. 3. Electron micrographs of ultra thin section of mature cell-leaf from guava cv. Balady, examined by transmission-electron microscope.

A) Healthy mature seedling leaf showing nucleus (N) with normal chromatin (Cr), nucleolus (Nuo), nuclear membrane (Nm), chloroplast (Ch), mitochondria (M), cell wall (CW), normal cytoplasm (Cy) containing intact cell organelles, large (V) and (CW) in a normal shape with magnification of X 10,000.

B) Infected leaf showing nucleus (N) with light dens of chromatin (Cr), small nucleolus (Nuo) and nucleolus membrane (Nm), granulated cytoplasm (Cy), swollen and degraded chloroplast with abnormal shape and size (Ch) containing virus particles (Vp) and as well as par mural bodies (Pb) with large vacuole (V), degraded mitochondria (Dm) surrounded by virus particles (Vp) , abnormal cell wall (CW) were shown in Fig. 3C with magnification of X 40,000.

C) Infected leaf showing part of chloroplast with grana (G) and small aggregates of virus particles, cytoplasm (Cy) containing virus particles (Vp) adhere to abnormal cell wall (CW), vacuole (V) in chloroplast (Ch), virus particles (Vp) as loose or in masses at different sites in the cytoplasm (arrow heads) having a diameter about 30 nm similar to that of CMV as shown in Fig. 3D with magnification of X 50,000.

Concerning the mosaic tissues, the following effects were noticed; 1-Granulation of the cytoplasm (Cy) with small cytoplasmic vacuoles (V) (Fig. 3 B &C) formed by imaginations of plasma lemma (Pl) and tonoplast (Tp) as shown in (Figs. 3B, 3C& 3D). 2-Malformation and degrading (M&Dm) of mitochondria (Fig 3C). 3-The chloroplasts (Ch) showed conspicuous alterations in shape and size. In internal structure destroying of grana (Gr) and thylakoid membrane (Tm) were noticed. Also 4- par mural bodies containing virus particles (Vp) were noticed. Changes in the grana (G) and lamellar membranes (Lm), or containing vesicles that surrounded virus like particles (Vp) were observed (Fig. 3 C and D). 5-the infected cell showed some abnormalities in the cell walls (CW), *i.e.* cell wall protuberance, depositions of electron dense materials near the walls (Fig. 3 C). 6- Virus particles were shown inside and around swollen chloroplast and cytoplasm as well as adhere to the cell wall (Fig. 3 B, 3 C and 3 D). Electron microscopic examination of ultra thin infected leaves revealed various cytological abnormalities which have been absent in healthy ones. These results are similar to those obtained by others (Allam *et al*., 2000; Paradies *et al*., 2000; El-Dougdoug *et al*., 2002 and Abo El-Ela *et al*., 2006).

R e f e r e n c e s

Abdel-Aziz, Nabila A. 1995. Occurrence of potato mosaic caused by a strain of cucumber mosaic virus in Minia, Egypt. *Assiut J. Agric. Sci.,* **26**: 37-50.

Abo El-Ela, Amal A.; Amer, M.A. and Eman, A.H.K. 2006. Cytological and molecular studies of an Egyptian isolate of carnation vein mottle potyvirus. *Egypt. J. Virol.* **3**: 1-18.

Allam, E.K.; El-Afifi, Sohair I. and Sadik, A.S. 2000. Inclusion bodies as a rapid mean for detection of some plant viruses. *Proc. 9th Congress the Egypt. Phytopathol. Soc.,* **11**: 7-141.

Anggraeni, I. and Suharti, M. 1997. Identification of some pathogens as causal agents of root rot disease on forest trees. *Buletin-Penelitian-Hutan.,* **610**: 17-35.

Carranza, M.; Larran, S. and Ronco, B. 2002. First report of *Glomerella cingulata* on common guava in Argentina. *Plant Dis.,* **86**: 440.

Carrere, I.; Tepfer, M. and Jacquemond, M. 1999. Recombinants of cucumber mosaic virus determinants of host range and symptomatology. *Archives Virol.,* **144**: 365-379.

Christie, R.G. and Edwardson, J.R. (1986). Light microscope techniques for detection of plant virus inclusions. *Plant Dis.,* **70**: 273-279.

Clark, M.F. and Adams, A.N. 1977. Characteristics of the micro-plates method of enzyme-linked immunisorbent assay for the detection of plant viruses. *J. Gen. Virol.* **34**: 475-483.

Daniels, J. and Campbell, R.N. 1992 .Characterization of cucumber mosaic virus isolated from California. *Plant Dis.,* **76**: 1245-1250.

El-Dougdoug, K.A.; El-Habbaa, G.M. and Abdel-Ghaffar, M.H. 2002. Mango malformation disease in Egypt: 2-Pathological and Histo-pathological changes in malformed inflorescences. *Egypt. J. Phytopathol.,* **30**(2): 97-113.

El-Kewey, S.A.; El-Kady, M.A.S.; Sidaros, S.A.; Emeran, A.A. and Abd El-Khalik, Samaa, 2007. Biological studies on pepper mild mottel virus affecting pepper plants in Egypt. *Egypt. Virol.,* **4**: 69-80.

Eiras, M.; Boari, A.J.; Chaves, A.R.; Briones, M.S.; Figueire, A.R. and Harakava, R. 2004. Characterization of isolates of cucumber mosaic virus Present in Brazil. *J. Plant Pathol.,* **86**(1): 59-67.

Fegla, G.I.; El-Samra, I.A.; Younes, H.A. and Abd El-Aziz, M.H. 2001. Comparative studies for detection of tomato mosaic tobamovirus (ToMV), cucumber mosaic cucumovirus (CMV) and potato Y potyvirus (PVY). *J. Adv. Agric. Res.,* **6**(2): 239-254.

Francki, R.I.B.; Mossop, D.W. and Hatta, T. 1979. Cucumber Mosaic Virus. CMI/AAB Descriptions of Plant Viruses. No. 213.

Galal, A.A.; Abdel-Aziz, Nabila A. and Saleh, O.I. 1996. Physiological changes of squash plants inoculated with cucumber mosaic virus. The 4th Arabic Conf. Hort. Crops, 25-29 March, Minia, pp. 1165-1178.

Gonzalez, M.S. and Rondon, A. 2005. First report of *Guignardia psidii*, an ascigerous state of *Phyllosticta psidiicola*, causing fruit rot on guava in Venezuela. *Plant Dis.,* **89**: 773

Gould, W.P. and Raga, A. 2002. Pests of guava. Pages: 295-313. In: *Tropical Fruit Pests and Pollinators: Biology, Economic Importance, Natural Enemies and Control*. J.E. Peña; J.L. Sharp and M. Wysoki (eds.). Wallingford, CAB, 430pp.

Hassan, Hanaa M.M. 1990. Studies of mosaic virus disease of grapevine in Minia. M.Sc. Thesis, Fac. Agric., Minia Univ., 123pp.

Hu, J.S.; Li, H.P.; Barry, K.; Wang, M. and Jordan, R. 1995. Comparison of dot blot, ELISA and RT-PCR assay for detection of two cucumber mosaic isolates infecting banana in Hawaii. *Plant Dis.,* **79**: 902-906.

Hull, R. 2002. *Matthew's Plant Virology*. 4th Ed. Architecture and Assembly of Virus Particles. Academic Press. Pp. 56-169.

Jiang, X.C., Cai, Z.N., Lin, Y. 1992. On carnation vein mottle virus 1. Biology ultrastructure and serology. *Acta Phytopathologica Sinica,* **22**(1): 10-23.

Keith, L.M.; Velasquez M.E. and Francis, T.Z. 2005. Identification and Characterization of *Pestalotiopsis* spp., causing scab disease of *Psidium guajava*, in Hawaii. *Plant Dis.,* **90**: 16-23.

Kwee, L.T. and Chong. K.K. 1990. Guava in Malaysia: Production, pests and diseases. Kuala Lumpur, Tropical Sdn., 260pp.

Laundon, G.F. and Waterson, J.M. 1965. *Puccinia psidii*. CMI Descriptions of Pathogenic Fungi and Bacteria No. 56. Commonwealth Mycological Institute, Kew, UK.

Matthews, R.E.F. 1993. *Diagnosis of Plant Virus Diseases*. CRC Press, Boca Raton, Ann Arbour, London, Tokyo, 374 pp.

Merida, M. and Palmatee, A.J. 2006 .Guava diseases caused by fungi and stramenopiles. UF/IFAS, Florida Univ., Pp 232.

Moura, R.; Moura, A. and Marinho, D. 1989 .Root-knot on guava; a severe disease in Pernambuco State, Brazil. *Nematology-Brasileira,* **13**: 13-19.

Noordam, D. 1973. Identification of plant virus methods and experiments. Centre agricultural publishing and documentation. Wageningen, Netherlands, 207pp.

Ouf, M.F.; Rushdi, M. and Younis, F.A. 1974. Comparative studies on three strains of cucumber mosaic virus. *Res. Bull.,* **2**: 1-13.

Ouf, M.F.; Soliman, H.N. and Hassan, Hanaa M.M. 1991. Histopathological changes in tobacco leaf cells caused by tomato ringspot virus (Grapevine strain). *Assiut J. Agri. Sci.,* **22(**2): 53-64.

Pane, A.; Nicosia, M.G. and Cacciola, S.O. 2001. First report of *Phytophthora citrophthora* causing fruit brown rot of Feijoa in Italy. *Plant Dis.*, **85**: 97.

Paradies, F.; Finetti, M.S.; Gallitelli, D.; Castellano, M.A.; Franco, A.D.; Digiaro M.; Martelli, G.P. and Yilmz, Z.M.A. 2000. Partial characterization of cucumber mosaic virus isolates from citrus and grapevine. *J. Plant Pathol.,* **82**, 133-145.

Rahman, M.A.; Ansari, T.H.; Meah, M.B. and Yoshida T. 2003. Prevalence and pathogenicity of guava and anthracnose with special emphasis on varietal reaction. *Pak. J. Biol. Sci.,* **6**: 234-241.

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عزل وتعريف فيروس يصيب الجوافة في محافظة المنيا

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تم الحصول علي عزلة من فيروس تبرقش الخيار من اشجار الجوافة صنف بلدي النامية في محافظة المنيا والتى أظهرت جزر خضراء وتبرقش وموزايك وتشوة للاوراق بصورة جهازية. أثبتت الدراسه ان الفيروس المعزول يصيب ٣٢ صنف أو نوع نباتي ينتموا الي ٧ عائلات. أصاب الفيروس جهازياً ٩ نباتات أختبارمن الـ ٣٢ وصاحبت هذة الاصابة بعض الاعراض مثل التجعد وشفافية العروق والتشوة والقزم والذبول والتشوة الورقى والجزر الخضراء والاصفرار علي باقي النباتات المختبرة.

واظهرت بعض نباتات الاختبار الاصابة بصورة موضعية مثل أصناف الدخان كلافيلانديا وديبانديا ووايت بيرلي و لانتانا أمارنسس والبيتونيا. كما ظهرت البقع الحلقية فقط على جنس الكنوبيديم ألبم وأعطت اللوبيا انجيوكيولاتا بقع بنية اللون. يفقد الفيروس نشاطة في العصير الخام بعد التسخين علي ٧٠°م لمدة ١٠ دقائق وكانت درجة التخفيف النهائية بين ١٠$^{-٤}$ – ١٠$^{-٥}$ وقد فقد الفيروس نشاطة علي درجة حرارة الغرفة (٢٥ درجة مئوية) بعد ١٠ أيام.تم تأكيد هذا التعريف باستخدام الاليزا المباشرة حيث أظهر الفيروس المعزول علاقة مع فيروسات الكيوكيمو . من الفحص الميكروسكوبي اتضح أن الفيروس يكون أجسام أمورفية في الشعيرات الموجودة علي بشرة أوراق الكوسة المعدية بالفيروس بعد ٢١-٤٢ يوم من العدوي عند الفحص بالميكروسكوب الضوئي. وأظهر الفحص بالميكروسكوب الالكتروني للقطاعات فائقة الدقة لجزء من النسيج الوسطي لورقة الجوافة المصابة بالفيروس المختبر بعض التغيرات مثل المظهر الغير طبيعي للجدار الخلوي وتحلل وتشوة الميتاكوندريا وانتفاخ البلاستيدة واحاطتها بحبيبات الفيروس. كما شوهد أيضا بداخل البلاستيدة ٣-أجسام بارميلية الشكل .أظهرت القطاعات ايضا جزيئات الفيروس المنتشرة في السيتوبلازم بالقرب من الجدار الخلوي.