University of AL-Tahadi
Faculty of Agriculture
Department of Plant production

Identification of Viruses Causing Mosaic on Wild Tobacco (*Nicotiana glauca* Graham) in the Western Costal-belt of Libya

A thesis

Presented to the Faculty of Agriculture,
AL-Tahadi University in Partial Fulfillment
of the Requirements For the Degree of

MASTER OF SCIENCE

In

PLANT PATHOLOGY

Presented by

MAHJOUB ALI MOHAMMED EJMAL

Supervised by

Dr. Omar M. El-Sanousi **Prof. Salah S. El-Ammari**

2007

University of AL-Tahadi

Faculty of Agriculture

Department of Plant production

Identification of Viruses Causing Mosaic on Wild Tobacco (*Nicotiana glauca* Graham) in the western Coastal-belt of Libya

Presented by

MAHJOUB ALI MOHAMMED EJMAL

11-2- 2007

Committee members:

Dr. Omar M. El-Sanousi (Advisor):.....

Dr. Salah S. El-Ammari (Co-Advisor):.....

Dr. Hosny A. Younes (External Examiner):.....

Dr. Abdel-Nasser A. Galal (Internal Examiner):.....

Approved:

Dr. Atef S. Shahata

.....

Graduate Study Office
Faculty of Agriculture

Dr. Mohamed A. Alaib

.....

Dean of Faculty of Agriculture

ACKNOWLEDGEMENT

Praise is to ALLAH, the lord of the world. I praise him for his favours and ask him to give me more grace and generosity. Peace and blessing be upon our Prophet Mohammed, his servant and Messenger and all his companions.

I would like to express my sincere gratitude to Dr. Omar M. El-Sanousi my major advisor for his encouragement, active guidance and constant advice throughout my research work and preparation of this manuscript.

I am pleased to express my special thanks to Dr. Salah S. El-Ammari my co-advisor for his guidance, kindness and encouragement

I am also thankful to Dr. Ali Battaw the co-secretary of Omar Al-Mukhtar University and Dr. Omraun Abu-Salah the head of plant protection department at the Faculty of Agriculture, Omar Al-Mukhtar University for their great help and continuous encouragement.

My appreciation to all the staff members and graduate students at plant virology laboratory, Omar Al-Mukhtar University for their help, kindness, and valuable suggestions.

I am very pleased to thank Dr. Mohammed A. Alaib, the Dean of Faculty of Agriculture at Al-Tahaddi University for his great help during my study.

I am pleased to express my thanks to Mr. Mohammad A. Zaied, at plant virology laboratory, Al-Fateh University for his help, kindness and encouragement.

I am thankful to Dr. Amr S. Taha, Dr. Kryem G. Kryem, Dr. Ali O. Trina and Mr. Ali A. Esmew for their kindness, suggestions and help during antisera preparation.

My appreciation to Dr. Fatihi A. Swaid, Mr. Khalid M. El-Mansuri, Mr. Mukhtar O. Agoub, Mr. Amhemmed S. Al-Gallal, Mr. Ahmad R. El-Dala, Mr. Abdul-Hameed A. El-Dargash, Mr. Salem A. Safar, Mr. Abdel-Rahman S. Sharia, Mr. Ebrahim M. Al-Ferjani, Mr. Fathi M. Abu-Zaid, and Hassan B. Ben-Sauod For their kindness, help and encouragement.

Special thanks are extending to my mother, brothers, sisters, wife and kids for their love, patience, support and encouragement.

Finally, appreciation is extended to all the staff members and post-graduate students of Faculty of Agriculture at Al-Tahadi University for their kind cooperation and assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

About one fourth of all known viruses attack and cause diseases in plants. One virus may infect one or dozens of different species of plants and each species of plant is usually attacked by many different kinds of viruses. A plant may also be infected by more than one kind of virus at the same time (Agrios, 1997).

In Libya certain viruses on some host plants had been identified, Bean yellow mosaic virus was reported on Broad bean (Younes, 1984), Potato virus Y and Tomato mosaic virus were reported on pepper (El-Sanousi *et al.*, 1991a; El-sanousi *et al.*, 1991b), Zidan (1996) identified Broad bean stain virus, Pea seed-borne mosaic virus, Beet western yellows virus, Bean yellow mosaic virus and Alfalfa mosaic virus on Pea. Also Bean yellow mosaic virus, Alfalfa mosaic virus, Pea seed-borne mosaic virus, Fababean necrotic yellows virus, had been reported on Broad bean by Fadel (2001).

Tree tobacco *Nicotiana glauca* Graham is a solanaceous plant, naturalized in areas with a Mediterranean climate, it is an evergreen wild plant may reach 3 meter high, leaves are thick and light green colour, flowers tubular with yellow colour. All plant parts are poisonous

containing 2% of rutin, 1.16% of anabasine, and a little amount of nicotine. Its habitat rocks, walls, and road sides (www.pfaf.org).

N. glauca was first introduced into Tripoli in 1850 probably via Egypt and reported in Mallaha in 1906, it spreads along the coast of 1900 km long and its public names are: Accus Mussa, Asa Mussa (Keith, 1965).

N. glauca like many other evergreen plants has a very important value when it carries viruses that able to infect any economic crop. In several parts of the world *N. glauca* was reported to be infected by Artichoke yellow ringspot virus (Rana *et al.*, 1983), Cucumber mosaic virus (Vovlas and Di Franco, 2004), Tobacco mosaic virus (Fraile *et al.*, 1997), and Tobacco mild green mosaic virus (Mckinney, 1929).

In several regions of Libya mosaic symptoms were clearly observed on *N. glauca* and on the basis of symptomology and mechanical transmissibility it was suspected that the causal agent could be one or more viruses. The objective of this study is to identify and characterize the suspected virus or viruses that induce mosaic symptoms on naturally infected *N. glauca* plants.

LITERATURE REVIEW

There are some viruses reported to infect *Nicotiana glauca* naturally and cause mosaic in different regions of the world, these viruses are described with some characteristics in Table (1).

In 1927, McKinney collected a strain of tobacco mosaic virus (TMV) from *Nicotiana glauca* Graham in Gran Canaria, Canary Islands and named it mild dark-green mosaic strain of TMV (MDGTMV) because of the symptoms caused in Havana tobacco (McKinney, 1929). An isolate of this virus naturally infecting *Nicotiana glauca* was reported in India (Nariani & Singh, 1952) and in the Middle East (Faccioli, 1964).

An isolate of tobacco mosaic virus named strain U5 (TMV-U5) was originally isolated from *N. glauca* from southern California and diagnosed by its ability to induce necrotic local lesions on *N. sylvestris* Speg & Comes and by its failure to infect most cultivars of *Lycopersicon esculentum* Mill (Siegel and Wildman., 1954).

An Australian isolate of TMV (TMV-Y) was found commonly associated with *N. glauca* in nature and characteristically inducing a bright yellow mosaic symptom and causing necrotic local lesions on *N. tabacum* cvs White Burley and Xanthi-nc, *N. glutinosa*, *Datura*

Table 1: Some characteristics of viruses naturally causing mosaic symptoms on *Nicotiana glauca*.

Virus	AYRSV	TMV	TMGMV	CMV
Virus group	Nepovirus	Tobamovirus	Tobamovirus	Cucumovirus
Particle morphology	Isometric 30 nm	Rod-shaped straight;308 nm; 18 nm wide	Rod-shaped straight;308 nm; 18nm wide	Virions isometric; not enveloped; 29 nm in diameter.
Symptoms on *N. glauca*	Chlorotic to yellow rings, mottling and occasionally, malformation of leaves	Almost symptomless, Systemic infection	Bright yellow mosaic, mottling ringspots and cupping stunting.	Severe mosaic and leaf deformation
Transmission	Mechanical & by seed	Mechanical	Mechanical	By insect (Aphididae), Mechanical& by seed
Physical properties in the crude sap.	TIP: 60°C LIV: 2 days. DEP: 10^{-3}or 10^{-4}	TIP: >90°C LIV: for decades. DEP: 10^{-6}	TIP:85-90°C LIV: > 35000 days. DEP: 10^{-7}	TIP: 55-70°C. LIV:1-10 days DEP:10^{-3} to 10^{-6}
Reference	(Rana *et al.*, 1983). (Brunt *et al.*, 1996a)	(Zaitlin and Israel, 1975) & (Bald and Goodchild, 1960)	(Brunt *et al.*, 1996c)	(Brunt *et al.*, 1996b), (Vovlas and Di Franco, 2004)

stramonium, and *Chenopodium amaranticolor*, and a yellow mosaic on *Lycopersicon esculentum* cv. Rutgers and on *N. glauca* (Randles *et al.*, 1981). Other isolate of the virus was found naturally in *N. glauca* in Spain (Moya *et al.*, 1993).

In another study of an isolate of strain U5 (TMV-U5) obtained from naturally infected *N. glauca* plants found near Riverside, California, similar systemic symptoms of mosaic were produced on *N. tabacum* cv. Turkish, *N. benthamiana* Domin., *N. clevelandii* A.Gray, *N. glauca, Vinca rosea* L. and *Physalis floridana* Rydb. Plants that developed localized infection only were: *Chenopodium quinoa* Willd, *C. amaranticolor* Coste & Reyn., *Gomphrena globosa* L., *N. tabacum* cv.Xanthi-nc, *N. sylvestris*, *N. rustica* L., *Capsicum annuum* L., cv. Yolo Wonder, *Cucurbita pepo* L., cv. Early prolific and *N. glutinosa* L. (Valverde & Dodds, 1986).

An isolate of the virus was obtained from plants of *N. glauca* in southern California named tobacco mild green mosaic virus, which induced mild mosaic on *N. tabacum* cv. Xanthi, systemic mosaic on *N. clevelandii*, systemic mild chlorosis on *Eryngium planum*, mosaic-like patterns on *Chenopodium quinoa*, leaf cupping, wilting and death at last on *N. benthamiana* near symptomless systemic infection, older leaves may show teeth on there margins on *N. glauca*; symptomless systemic

infection on *Gomphrena globosa* and induces necrotic local lesions on *N. tabacum* cv. Xanthi-nc, *N. sylvestris*, *N. glutinosa*, *N. rustica*, *Datura stramonium*, *Capsicum annuum* and *Chenopodium amaranticolor*; but no infection was induced on *Lycopersicon esculentum* cv. Rutgers and *Vigna unguiculata* (Bodaghi *et al*,. 2000).

All the above reported isolates are considered as synonyms to tobacco mild green mosaic virus TMGMV (Brunt et al., 1996c; Wetter, 1989). TMGMV is a virus with RNA-containing tubular rod-shaped particles (308 x 18 nm), distributed throughout the world, no natural vector was known for the virus but it is easily transmitted by mechanical inoculation and by handling during cultivation. The virus has economic importance for tobacco crops (Wetter, 1989). Bright yellow mosaic symptoms occur in *N. glauca* plants naturally infected by the U5 type virus (McKinney, 1929; Bald & Goodchild, 1960). Mild green mosaic symptoms with oak leaf patterns occur in naturally infected Turkish tobacco cv. Samsun and cultivars of Burley tobacco grown in West Germany, most cultivars of pepper react with severe mosaic symptoms and necrosis followed by leaf drop with TMGMV synonyms. TMGMV occurs probably in all regions where *Nicotiana glauca* is distributed: North America, Australia and many European and African countries

including Madeira, the Mediterranean and Canary Island. The virus occurs much more frequently than type tobacco mosaic virus in field tobacco in West Germany and was found in field tobacco also in Wisconsin, USA. Cigarettes with light tobacco may contain both of these tobamoviruses and may play a role in their infection cycle. The virus was isolated from Brazilian cigarettes but not from Chinese cigarettes; Widespread in many species of cultivated gesneriads in USA; Found also in plants of the monocotyledonous plant *Rhoeo spathacea* in Florida, USA (Wetter, 1989).

No extensive host range studies have been made. The virus infects many solanaceous species and also infects species of the families Chenopodiaceae, Commelinaceae, Gesneriaceae and Umbelliferae; also causes mild mosaic or mottle symptoms in many cultivars of tobacco. In later stages of infection leaves show weak or no symptoms. Late systemic infection of *Eryngium planum* is symptomless. *Rhoeo spathacea* shows conspicuous mosaic symptoms; the virus is less stable than type tobacco mosaic virus. In tobacco sap it lost infectivity after 10 min at 85°C or after dilution beyond 10^{-7} with distilled water. In herbarium specimens of *N. glauca* it was still infective after 45 years, Absorbance at 260 nm (1 mg/ml, 1 cm light path): 1.16, and A260/A280: 1.22, A260

(max)/A248 (min): 1.1 (Wetter, 1989). It was also reported to remain stable more than 35000 days (more than 90 years), and having a thermal inactivation point of 85-90°C (Brunt *et al;* 1996c). No vector is known, but spread of the virus in stands of *N. glauca* by an unknown vector is assumed; spread along the roadsides where *N. glauca* is planted may result from mechanical transmission; the virus is not transmissible through seed of *N. glauca* (Randles *et al.,* 1981). Gene sequences from TMGMV isolates collected from *N. glauca* populations from Australia, California, Spain, and the east Mediterranean Basin were analyzed and very little variation was found (Fraile *et al.*, 1996).

Mixed infections of tobacco mild green mosaic virus and type tobacco mosaic virus occur frequently in *Nicotiana glauca* (Wetter, 1989). And in a study over a period of a century in Australia, isolates obtained from both living wild *N. glauca* plants and herbarium specimens that had been collected in New South Wales (NSW), Australia, it was found that most of the earliest samples of *N. glauca* were infected with both Tobacco mild green mosaic virus (TMGMV) and TMV the type member of tobamoviruses, and the later subsequently disappeared from the *N. glauca* population (Fraile *et al.*, 1997).

Tobacco mosaic virus type member of Tobamoviruses is a rod-shaped virus, containing a single-stranded RNA molecule of sense polarity. The virus causes diseases in a broad variety of plant species. It has no known true vectors, but on rare occasions it is transmitted inefficiently by chewing insects. It is normally transmitted mechanically. Soil-borne virus particles or fragments of infected tissue can serve as sources of infection via roots. Virus is very persistent on clothing and on glasshouse structures. Not transmissible via seed or pollen. The virus is distributed world wide; it infects at least 199 species from 30 plant families. Symptoms in *Nicotiana tabacum* cvs. Turkish, Turkish Samsun, Samsun (Samsoun), White Burley, Burley and Xanthi, are vein clearing appears in young systemically-invaded leaves, 3-4 days post inoculation, followed by a light green-dark green mosaic, often accompanied by distortion and blistering. Local lesion assays are most frequently performed with *N. glutinosa*, *N. tabacum* cvs. Xanthi nc, Xanthi NN, Samsun NN, *Phaseolus vulgaris* cv Pinto, *Chenopodium amaranticolor* or *C. quinoa*. Virus is very stable; preparations of "unpreserved plant juice" retained infectivity after 50 years. Very heat stable; some infectivity is retained after 10 minute exposures at over 90°C. Dilutions of 10^{-6} of expressed tobacco sap can be infectious.

Absorbance at 260 nm (1 mg/ml, 1 cm light path): uncorrected for light scattering, ranges between 2.7 and 3.5, a value of 3.0 is commonly used (Zaitlin and Israel, 1975; Zaitlin, 2000).

A comparison of symptoms induced by mild dark green tobacco mosaic virus (MDGTMV) and tobacco mosaic virus type in some tested plants showed that MDGTMV induced systemic mosaic or mottle in *Eryngium planum* L., *Nicotiana tabacum* cv. Samsun, *Nicotiana glauca*, and *Capsicum annuum* L. and local lesions in *Datura stramonium* L., *Nicotiana glutinosa*, *Nicotiana sylvestris* Speg & Gomes, *Nicotiana tabacum* cv. White Burley; and no symptoms induced in *Lycopersicon esculentum* L. Whereas TMV induced systemic mosaic or mottle in *N. glauca*, *Nicotiana tabacum* cv. White Burley, *Nicotiana tabacum* cv. Samsun, *Lycopersicon esculentum*, and *Nicotiana sylvestris*, and induced local lesions in *Capsicum annuum*, *Datura stramonium*, *Nicotiana glutinosa*, and induced no infection in *Eryngium planum* (Wetter., 1984).

Artichoke yellow ringspot virus (AYRSV) was reported as natural infection on *N. glauca* (Harris *et al.*, 2002; Rana *et al.*, 1983). This virus was identified as a member of subgroup C of the genus Nepovirus, A virus with isometric particles 30 nm in diameter, containing two

functional RNA species. Readily transmitted by inoculation of sap to many herbaceous hosts and frequently transmitted through seed and pollen. No vector known; occurs in a range of cultivated and wild plants in Greece and Italy. The virus induces chlorotic to yellow rings, mottling and occasionally, malformation of the leaves in naturally infected *N. glauca*, and reddish local lesions followed by systemic yellow ringspots and line patterns in *Gomphrena globosa*, and Chlorotic or necrotic local lesions, systemic mottling and tip necrosis in *Chenopodium quinoa*, and reddish necrotic local lesions, systemic mosaic and leaf deformation in *Phaseolus vulgaris*, chlorotic lesions on cotyledons followed by mosaic, malformation and enations in non inoculated leaves in *Cucumis sativus*, and local and systemic chlorotic rings, lines and oak leaf patterns in *Nicotiana tabacum*. No vector known to transmit the virus, transmitted through seed at rates ranging from 15 to 100% in artificially infected plants. In expressed sap of *Phaseolus vulgaris* the virus was still infective after dilution to 10^{-3} or 10^{-4}. Infectivity was lost after heating for 10 min at 60°C or storing at 22-24°C for 48-72 h (Rana *et al.*, 1983).

Cucumber mosaic virus is the type member of the genus Cucumovirus of the family Bromoviridae (Murphy *et al.*, 1995), was reported to cause severe mosaic symptoms on naturally infected *N.*

glauca in Egypt by Eid *et al.*,(1984), inducing reddish local lesions with yellow halos on *Chenopodium amaranticolor*, and *Ch. quinoa;* few faint spots on inoculated leaves followed by yellowish diffused mottling on new leaves of *Cucumis sativus* cv. Long Gelferos; new leaves developed systemic mottling, leaf blades became smaller and yellowish on *Nicotiana glutinosa;* dark veins developed on inoculated leaves, and large brown or white necrotic lesions, with or with not faint mottling in *Petunia hybrida;* yellowish local lesions on *Datura stramonium;* Brown local lesions on *Vicia faba;* and no infection was induced on *Phaseolus vulgaris* cvs. Giza 3, Giza 4, Bountiful, Black Turle Soup and Pinto; the thermal inactivation point (TIP) was between 60°C and 65°C, dilution end-point (DEP) was between 10^{-4} and 10^{-5}, and longevity *in vitro* (LIV) between 5-6 days. CMV was also reported on *N. glauca* by Valverde and Dodds (1986) and by Moll *et al.*, (1987). In another study CMV from *N. glauca* was found in Israel (Gafny *et al.*, 1996). Cucumber mosaic virus isolate belonged to subgroup II was reported to cause severe mosaic and malformation of the leaves of *Nicotiana glauca* plants in Greece (Vovlas and Di Franco, 2004).

CMV was first reported as the causal of cucumber mosaic disease in the United States (Jagger, 1916). A virus with a three-component

genome of ssRNA of messenger sense. RNA is packaged in three different particles, 30.5 nm (maximum) in diameter, which sediment at the same rate; Particles are isometric. In nature it is transmitted by aphids in a non-persistent manner, and through the seed in some plant hosts. Experimentally, it is easily transmitted by mechanical inoculation of plant sap. It is distributed world-wide, causes economically important diseases in a large variety of crop plants, and has the widest host range of any known plant virus, including more than 1200 species in over 100 families of dicotyledonous and monocotyledonous. It causes mosaic of cucumber, melon and other cucurbits, blight of spinach; mosaic, fern leaf and systemic necrosis in tomato, mosaic and ringspot in pepper; mosaic and stunting in clover; lupins and lucerne stunting in soybean; mosaic, infectious chlorosis and heart rot in banana; and mosaic and dwarfing in many other species of dicotyledonous and monocotyledonous plants; mosaic of celery, woodiness of passion fruit. Diagnostically causes large chlorotic local lesions on *Beta vulgaris*; chlorotic or necrotic local lesions rarely systemic on *Chenopodium amaranticolor and C. quinoa*; systemic mosaic and stunting varying in severity with the virus strain in *Cucumis sativus*; systemic mosaic and stunting of varying severity in Cucurbita spp. mosaic and stunting with filiform leaves to different

extents, often extreme in *Lycopersicon esculentum*; mild to severe mosaic and stunting, depending on the virus strain, Some strains induce severe yellow chlorosis in *Nicotiana tabacum*; Small purple necrotic lesions in inoculated leaves in *Phaseolus vulgaris* cv. Pinto; Severe stunting, leaf curling and leaf deformation, some strains also produce necrosis along veins in *Nicotiana benthamiana*; Transmissible in a non-persistent manner by more than 80 species of aphids in 33 genera, *Myzus persicae* and *Aphis gossypii* are two important vectors. Seed transmission has been reported in more than twenty plant species with varying efficiencies from a fraction of 1% up to 50%. It is relatively unstable in plant sap. Thermal inactivation point (10 min) is 55-70 °C. Infectivity can be lost in a few hours or in a few (less than 10) days (Palukaitis and García-Arenal, 2003). Extinction coefficient E (0.1%, 1cm) at 260 nm: 5.0, A260/A280: about 1.7 (corrected for light-scattering) (Gibbs & Harrison 1970). It is also reported to have a thermal inactivation point between 55-70°C, longevity *in vitro* between 1-10 days, and dilution end point between 10^{-3} – 10^{-6} (Brunt *et al.*, 1996a).

MATERIALS AND METHODS

Field collection:

Leaves of naturally infected *Nicotiana glauca* plants showing mosaic symptoms (Fig. 1) were collected from five regions Sirte, Misurata, El-Khomus, Tripoli, and El-Zawia of Libya five samples from each region. Samples were tested for virus or viruses present by Indirect Enzyme-Linked Immunosorbent Assay (indirect ELISA), and then the samples were served as a source of virus or viruses for preliminary mechanical transmission tests.

Detection of viruses present using indirect ELISA:

Samples collected were tested to be infected with virus or viruses using tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) Antisera obtained from Bio-Rad) using indirect ELISA and an isolate of each virus detected was taken for each different region for further work. Indirect ELISA was conducted at the laboratory of plant virology, Omar AL-Mokhtar University according to the method described by Lommel *et al.*, (1982) and Voller *et al.*, (1977).

100 µl of the sample sap extracted 1 g/10 ml in coating buffer (0.05 M carbonate buffer, pH 9.6) were added to the determined wells (3 wells

Fig. 1: Symptoms observed on *Nicotiana glauca* Graham in nature.

for each sample as a replication) of a microtitre plate for each virus to be detected, plate was incubated at 37°C for 3 h, then washed three times with Phosphate buffered saline, pH 7.4 containing 0.05% Tween-20 (PBS-T). 100 µl of purified IgG of specific virus either TMV or CMV diluted in serum buffer (PBS-T containing 2% Polyvinylpyrrolidone (PVP); 0.2% Bovin Serum Albumin (BSA)) was added to each well of the microtitre plate. The plate was incubated overnight at 4 °C and washed three times. 100 µl of goat anti-rabbit conjugate to alkaline phosphate (Sigma # A 3937) diluted 1:20,000 in conjugate buffer (PBST containing 0.2% BSA) were added to each well, and incubated at 37 °C for 3 hours, the plate washed three times, and 100 µl of enzyme substrate (paranitrophenyl phosphate dissolved in substrate buffer (0.1M diethanolamine buffer, pH 9.8 (mg/ml)) were added to each well of the plate, which then left at room temperature for about 15 min or as long as necessary to obtain clear reactions. Results assessed by Spectrophotometric measurement of absorbance at 405 nm by Bio-Rad ELISA reader and sample considered positive only when its reading reached two times the negative control, the reaction was stopped by adding 50 µl of NaOH 3 M solution, positive and negative controls were used for both CMV and TMV.

I- Tobamovirus isolates

Using of indicator hosts for tobamoviruses:

Each sample collected and gave a positive reaction with tobacco mosaic virus antiserum were used alone as a separate source of the virus. All samples were indexed on *Nicotiana glutinosa* to detect tobamoviruses in general (local lesions), each of which was cloned from single local lesion passage through *N. glutinosa* then inoculated to few plants of six indicator hosts including *Capsicum annuum* L. cv. Cayenna, *Lycopersicon esculentum* Mill cvs. Call J, Heinz 1370 F, Midi A and Riogrande, *Nicotiana tabacum* L. cvs. Burley gold, Burley 21 & White Burley and *Phaseolus vulgaris* L. and used to differentiate between tobamovirus members. Indicator plants were put in insect proof cages in a greenhouse, an alternative insecticide spray with (Actelic® & Cyperkil®) was performed at 2 weeks intervals as well, observed symptoms were recorded and back inoculations were made from inoculated plants not showing any symptoms after about 5 weeks on *Nicotiana glutinosa* for reproduction of local lesions and *Nicotiana tabacum* cv. Turkish, for reproduction of mosaic symptoms.

Source of virus isolates:

Each of the five virus isolates was cloned from single local lesion passage through *N. tabacum* cv. White Burley and maintained in *N. tabacum* cv. Turkish plants and used as a source of each isolate (Sirte 1, Misurata 2, El-Khomus 1, Tripoli 2, and El-Zawia 3) for further work in a greenhouse.

Mechanical transmission:

Inocula of each of the five virus isolates were prepared by grinding approximately 1 g of fresh and young infected leaves of *Nicotiana tabacum* cv. Turkish in 1 ml of inoculation buffer (0·1 M potassium phosphate buffer, pH 7), with a mortar and pestle. Inoculations were made by rubbing celite-dusted leaves with the expressed sap using the fore-finger followed by tap water rinsing.

Host range and symptomology:

At least five plants of each test species for each isolate were sap inoculated and three plants of each species were rubbed with distilled water and served as control. Inoculated plants were put in insect proof cages in a greenhouse, an alternative insecticide spray with (Actelic® & Cyperkil®) was performed at 2 weeks intervals as well, observed

symptoms were recorded and back inoculations were made from inoculated plants not showing any symptoms after about 5 weeks on *Nicotiana glutinosa* for reproduction of local lesions and *Nicotiana tabacum* cv. Turkish, for reproduction of mosaic symptoms.

The following different plant species or cultivars from different families were inoculated for host range studies:

Amaranthaceae:

Gomphrena globosa L. (Globe amaranth).

Apocynaceae:

Vinca rosea L. (Vinca).

Brassicaceae:

Matthiola incana L. (Common stock).

Chenopodiaceae:

Chenopodium amaranticolor Coste & Regn.

Chenopodium quinoa Willd

Chenopodium sp.

Cucurbitaceae:

Cucumis melo L. cv. Melon Pineapple (Cantaloupe).

Cucumis sativus L cv. Beit Alpha (Cucumber).

Cucurbita pepo L. cv. White Birginian 3. (Squach).

Cucurbita maxima cv. Etampes Bright Red (Pumpkin).

Citrullus vulgaris cv. Klondike (Water melon).

Leguminosae:

Phaseolus vulgaris L. (Bean).

Vicia faba L. (Broad bean).

Malvaceae:

Alcea rosea L. (hollyhock)

Solanaceae:

Capsicum annuum L. cv. Cayenna (pepper).

Datura metel L.

Datura stramonium L (Jimson weed).

Lycopersicon esculentum Mill cvs. Call J, Heinz 1370 F, Midi A and Riogrande (Tomato).

Nicotiana benthamiana Domin.

Nicotiana glauca Graham

Nicotiana glutinosa L.

Nicotiana repanda L.

Nicotiana tabacum L. cvs. Burley 21, Burley gold, Local Variety, Turkish, White Burley, Xanthi, & Xanthi-nc.

Petunia hybrida Vilm.

Physalis floridana Rydb.(Ground-cherry).

Solanum melongena L. cv. Black Beauty (Eggplant).

Solanum nigrum L.

Properties in the crude extract:

Fresh sap was extracted from infected *N. tabacum* cv. Turkish leaves by homogenizing 60 g in distilled water (1:1 w/v) using a mortar and a pestle. This extract was used in studies of thermal inactivation point, dilution end-point and longevity *in vitro.*

a- Thermal inactivation point (TIP):

One ml of the sap was drawn into thin-walled test tubes. These tubes were heated in a water bath at constant temperature for 10 min. at the indicated temperatures (80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91), then quickly immersed in ice water for rapid cooling. *Datura stramonium* as a local lesions host was inoculated for bioassay.

b- Dilution end-point (DIP):

The extracted sap was diluted with distilled water on a logarithmic scale (1:1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10}) and rubbed on celite-dusted leaves of *Datura stramonium* as a local lesions host.

c- Longevity *in vitro* (LIV):

The remaining sap extracted from infected *N. tabacum* cv. Turkish was stored in plugged flask kept at room temperature and was used to inoculate *Datura stramonium* as a local lesions host at weekly intervals.

Purification:

Virus was propagated in *Nicotiana glauca* plants. Systemically infected leaves showing clear symptoms were harvested three to four weeks after inoculation. The virus was purified by using the method described by Gooding and Hebert (1967). The purification procedure was carried out at room temperature. One hundred grams of frozen systemically infected *Nicotiana glauca* leaves were homogenized for 5 minutes in 100 ml of 0.5 M $NaHPO_4$ –KH_2PO_4 buffer, pH 7.2 containing 1% 2-mercaptoethanol. Sap was passed through double layered of cheesecloth and the homogenate was clarified by adding 6 ml of n-butanol/100 of the extract under continuous stirring. After the chloroplasts were coagulated, the extract was stirred for additional 15 min and then was centrifuged at 10,000 g for 30 min under continuous stirring; 4.0 g of polyethylene glycol (PEG) (M.W. 6000) per 100 ml were added to the supernatant and centrifuged at 10,000 g for 15 min. The pellet was resuspended in 20 ml of 0.01 M KH_2PO_4 buffer, pH 7 for each

100 ml of the initial extract. The mixture was centrifuged at 10,000 g for 15 min. Further purification was obtained by a second precipitation with PEG. Sodium chloride (NaCl) 0.4 g and PEG 0.4 g were added for each 10 ml of virus suspension under continuous stirring. After dissolving NaCl and PEG, the mixture was centrifuged at 10,000 g for 15 min. The pellet was resuspended in 2 ml of 0.01 M KH_2PO_4 buffer, pH 7 for each 100 ml of initial extract and centrifuged at 10,000 g for 5 min. The supernatant containing the virus was tested by ELISA for the presence of the virus and saved for further studies after testing its infectivity in *Datura stramonium* plants.

Ultraviolet absorption spectrum of the purified virus:

The absorption spectrum of tobamovirus purified suspension was determined using PU 8620 UV/VIS/NIR Philips spectrophotometer by measure absorbance at a range of wave length 220-300 nm with 5 nm intervals. The concentration was calculated using the absorbance value at 260 nm and extinction coefficient of 3.16 (Wetter, 1989). The values of a minimum and maximum absorbance for the purified virus was determined as well as the ratio of A260/A280 and A(max)/A(min) were calculated. The yield of the purified virus per 100 g fresh weight of *N. glauca* was calculated as well.

Serology:

1- Antiserum production:

A New Zealand white rabbit was injected with purified virus preparation. First intravenously with a purified virus diluted with normal saline solution (0.85% NaCl) to give the concentration of 3 mg/ml of purified virus (Younes, 1995). One week later the rabbit was injected intramuscularly with purified virus solution which was emulsified with an equal volume of Freund's complete adjuvant (Difco Lab. USA). The same intramuscular injection was given for another two times with an interval of one week. Two weeks after the last injection, the rabbit was bled and the blood was left for 2 hr at room temperature to clot, then stored in refrigerator overnight. The separated antiserum was clarified by centrifugation at 2,000 g for 10 min and kept frozen until needed for different serological tests.

2- Serological tests:

2.1- Preparation of Gel plates:

Gel mixture was prepared as described by Ball (1974) containing of 0.8 g agarose, 0.2 g sodium azide (NaN_3), and 0.05 g sodium dodecyl sulfate (SDS) per 100 ml distilled water. It was prepared by putting agarose and water in an Erlenmeyer flask stopped with cotton. Mixture was heated in a water bath until the agarose dissolved. Then mixture was cooled to about 40°C, sodium azide and SDS was added, mixed carefully, and then 15 ml of the solution was dispensed on each Petri dish, partially covered until gel solidified, condensed water was removed from lids.

2.2- Determination of Antisera titre using Ouchterlony test:

Three rows of seven wells with 3 mm spacing between rows were cut in four plates. Seven 2 fold dilutions of each week antiserum were made using normal saline 0.85% (dilutions at 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128); the same dilutions were made from the normal serum that was taken before the rabbit was injected with the virus. Purified virus was placed in the wells of the middle row, and the normal serum dilutions were placed in one side row and the antiserum were placed in the wells of the other different side. Plates were kept in a moist container at room temperature. Results of the reaction were taken 2 days later.

2.3- Determination of antiserum titre using indirect ELISA:

Leaf sap extracts from healthy and infected *N. tabacum* cv. Turkish plant were separately diluted at 1/10 (W/V) with coating buffer. Antiserum (against Sirte isolate) was diluted with antiserum buffer to the following dilutions: 1: $5x10^2$, 1: 10^3, 1: 2×10^3, 1: 4×10^3, 1: 8×10^3, 1: 1.6×10^4, and 1: 3.2×10^4.

2.4- Detection of virus isolates using Ouchterlony test:

One central well and six peripheral wells were cut in agar plate. 10 µl of prepared antiserum was placed in the central well, and 10 µl infected sap of each isolate was placed in a specific peripheral well, negative control sap was placed in one specific peripheral well. The test was repeated three times, and the plates were kept in a moist container at room temperature and results of the reaction were taken 2 days later.

2.5- Detection of virus isolates using indirect ELISA:

All five virus isolates were detected by indirect ELISA with the use of antiserum to Sirte isolate, and antibodies to TMV (Bio-Rad kit).

II- Cucumovirus isolates

Source of virus isolates:

Five samples of those gave positive reaction with cucumber mosaic virus antiserum were chosen to represent different regions (Sirte 2, Misurata 1, El-Khomus 4, Tripoli 1, El-Zawia 1) as a source of virus. Each extraction of sample was inoculated to *Nicotiana tabacum* cv. White Burley and *N. tabacum* cv. Local Variety (systemic hosts) until clear mosaic appeared, then inoculated on *N. glutinosa* (local lesions) to make sure no tobamovirus was present and then few plants of *N. tabacum* cv. Burley Gold and *N. tabacum* cv. Local Variety were reinoculated and used as a source for the five virus isolates for further work in a greenhouse.

Test of virus isolates using DAS-ELISA:

All virus isolates were tested by direct double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) using an antiserum to CMV from Bio-Rad. The test was conducted in accordance to the method described by Clark and Adams (1977) with some modifications.

Each well of the microtitre plate was coated with 100 µl of virus antibodies diluted in coating buffer (0.05M carbonate buffer, pH 9.6) and

incubated at 37°C for 3 hrs. After washing 3 times with PBS-T, 100 µl of the sample sap extracted in PBS-T (containing 2% soluble polyvinylpyrrolidone (PVP)) was added to each well and incubated at 4°C overnight. The plate was washed thoroughly and 100 µl of enzyme conjugate Y-globulin prepared in conjugate buffer (PBS-T containing 2% PVP; 0.2% BSA) was added and incubated at 37°C for 3 hrs. At last unreacted conjugates were washed and specific antibody-antigen reactions were detected by adding 100 µl of paranitrophenyl phosphate (mg/ml) prepared in substrate buffer (0.1 M diethanolamine buffer, pH 9.8) and left at room temperature for 15 min or as long as necessary to obtain clear reaction. The absorbance was measured at 405 nm in a Bio-Rad ELISA reader and sample considered positive when its value of at least double the negative control. The reaction was stopped by adding 50 µl of 3 M NaOH.

Mechanical transmission:

Inoculum from each of the five virus isolates was prepared by grinding approximately 1 g of fresh and young infected leaves of *N. tabacum* cv. Burley Gold or *N. tabacum* cv. Local Variety in 1 ml inoculation buffer (0·1 M potassium phosphate buffer, pH 7) with a mortar and pestle. Inoculations were conducted by rubbing celite-dusted leaves with the expressed sap using the fore-finger followed by tap water rinsing.

Host range and symptomology:

At least five plants of each test species for each isolate were sap inoculated and three plants of each species were rubbed with distilled water and served as control. Inoculated plants were put in insect proof cages in a greenhouse, 2 weeks interval alternative insecticide spray (Actelic® & Cyperkil®) was performed as well. Symptoms induced were observed, recorded and back inoculations on *Chenopodium amaranticolor* and *Nicotiana tabacum* cvs. Burley Gold & Local Variety were made from symptomless inoculated plants after about 6 weeks for reproduction of local lesions and mosaic symptoms respectively.

The following different plant species or cultivars from different families were inoculated for host range studies:

Amaranthaceae:

Gomphrena globosa L. (Globe amaranth).

Apocynaceae:

Vinca rosea L. (Vinca).

Brassicaceae:

Matthiola incana L. (Common stock).

Chenopodiaceae:

Chenopodium amaranticolor Coste & Regn.

Chenopodium quinoa Willd

Chenopodium sp.

Cucurbitaceae:

Cucumis melo L. cv. Melon Pineapple (Cantaloupe).

Cucumis sativus L cv. Beit Alpha (Cucumber).

Cucurbita pepo L. cv. White Birginian 3. (Squach).

Cucurbita maxima cv. Etampes Bright Red (Pumpkin).

Citrullus vulgaris cv. Klondike (Water melon).

Leguminosae:

Phaseolus vulgaris L. (Bean).

Vicia faba L. (Broad bean).

Malvaceae:

Alcea rosea L. (hollyhock)

Solanaceae:

Capsicum annuum L. cv. Cayenna (pepper).

Datura metel L.

Datura stramonium L. (Jimson weed).

Lycopersicon esculentum Mill. cv. Heinz 1370 F (Tomato).

Nicotiana benthamiana Domin.

Nicotiana glauca Graham.

Nicotiana glutinosa L.

Nicotiana repanda L.

Nicotiana tabacum L. cvs. Burley 21, Burley gold, Local variety, Turkish, White Burley, Xanthi, and Xanthi-nc.

Petunia hybrida Vilm.

Physalis floridana Rydb. (Ground-cherry).

Solanum melongena L. cv. Black Beauty (Eggplant).

Solanum nigrum L.

Properties in the crude extract:

Fresh sap was extracted from infected *N. tabacum* cv. Local Variety leaves by homogenizing 80 g in distilled water (1:1 w/v) using a mortar and a pestle. This extract was used in studies of thermal inactivation point, dilution end-point, and longevity *in vitro.*

a- Thermal inactivation point (TIP):

Two ml of the sap were drawn into thin-walled test tubes. These tubes were heated in a water bath at constant temperature for 10 min. at the indicated temperatures (40, 45, 50, 51, 52, 53, 54, 55, 60, 65, 70, 75), then quickly immersed in ice water for rapid cooling. *Chenopodium amaranticolor* as local lesion host was inoculated for bioassay.

b- Dilution end-point (DIP):

The extracted sap was diluted with distilled water on a logarithmic scale (1:1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) and rubbed on celite-dusted leaves of *Chenopodium amaranticolor* as local lesion host.

c- Longevity *in vitro* (LIV):

The remaining sap extracted from infected *N. tabacum* cv. Local Variety was stored in plugged flask kept at room temperature and was used to inoculate *Chenopodium amaranticolor* as local lesion host at half day intervals.

Purification:

Cucumovirus (Misurata isolate) was propagated in *N. tabacum* cv. Local Variety, and was purified by the method described by Walkey (1991); 100 g of infected leaves were placed in a glass jar of a kitchen liquidizer, 200 ml of 0.5 M sodium citrate buffer at pH 6.5, containing 0.2 ml thioglycollic acid, and 200 ml of chloroform were added to the infected tissue (all additives were precooled to 3°C, and all operations were carried out at 3°C and in a refrigerated centrifuge), the mixture was homogenized for several minutes until a fine homogenate produced. The

homogenate was then centrifuged at low speed (500 g) for 15 min., the aqueous supernatant was pipette off and retained, the pellet and chloroform were discarded. Polyethylene glycol (mol. wt. 6000) 10% (w/v) was added to the supernatant and shaked until dissolved and left to stand for 30 min., then centrifuged at 8000 g for 20 min. The pellet was resuspended in 0.05 M citrate buffer, pH 7.0, containing 2% Triton X-100 using 3.5 ml of buffer to each 35 ml capacity centrifuge tube, and left overnight. Insoluble material was removed by low centrifugation at 15000 g for 20 min. The clarified suspension was centrifuged at 75000 g for 150 min. in an ultracentrifuge (BECKMAN COULTER optima LE-80K). Pellet was resuspended in 1 ml of 0.05 M citrate buffer, pH 7.0 and left for several hours. Then centrifuged at 5000 g for 10 min to obtain virus suspension. The supernatant contains the partially purified virus was examined by ELISA for virus presence and also checked biologically by inoculation on *Chenopodium amaranticolor*.

Ultraviolet absorption spectrum of the purified virus:

The absorption spectrum of Cucumovirus purified suspension was determined (using PU 8620 UV/VIS/NIR Philips spectrophotometer) by measuring absorbance at a range of wave length 220-300 nm with 10 nm intervals. The concentration was calculated using the absorbance value at 260 nm and extinction coefficient of 5 (Gibbs and Harrison, 1970). The values of a minimum and maximum absorbance for the purified virus was determined, also the ratio of A260 /A280 and A(max)/A(min) were calculated. The yield of the purified virus per 100 g fresh weight of *N. tabacum* cv. Local Variety was calculated as well.

Serology:

1- Serological techniques:

1.1- Fixation of the virus with Formaldehyde:

In order to enhance immunogenicity, purified virus was fixed by adding formaldehyde (40% W/V Solution) to a final concentration of 0.2% (Francki and Habili, 1972), the mixture was left at 4°C for 1 hr, then dialyzed against 0.2% formaldehyde in three changes of 0.005 M borate buffer, pH 9, for 24 hrs and virus preparation was stored at 4°C.

1.2- Production of Antisera to the virus:

A New Zealand white rabbit was immunized using three subcutaneous and one booster injections, approximately 1 mg of fixed virus emulsified with an equal volume of Freund's complete adjuvant was injected subcutaneously at weekly intervals for three weeks (Wahyuni *et al.*, 1992). Booster injection was given subcutaneously with 1 mg of fixed virus emulsified in equal volume of Freund's complete adjuvant six weeks later. The rabbit was bled from the marginal ear veins at weekly intervals, starting four weeks after the priming injection. The serum was recovered after clotting and removal of red blood cells by centrifugation at 2000 g for 10 min.

2- Serological tests:

2.1- Preparation of Gel plates:

Gel mixture was prepared as described by Ball (1974) containing of 0.8 g agarose, and 0.2 g sodium azide (NaN3) per 100 ml distilled water. It was prepared by putting agarose and water in an Erlenmeyer flask stopped with cotton, mixture was heated in a water bath until the agarose dissolved. Then mixture was cooled to about 40°C and sodium azide was added, mixed carefully, 15 ml of the solution was dispensed

on each Petri dish, partially covered until gel solidified, condensed water was removed from lids.

2.2- Determination of Antisera titre using Ouchterlony test:

Three rows of six wells with 3 mm spacing between rows were cut in four plates. Six dilutions of twofold of each week antiserum and normal serum were made using normal saline 0.85% (dilutions at 0, 1/2, 1/4, 1/8, 1/16, 1/32). Infected sap from *N. tabacum* cv. Local Variety was placed in the wells of the middle row and the normal serum dilutions were placed in one side row and the antiserum were placed in the wells of the other different side. Plates were kept in a moist container at room temperature. Results of the reaction were taken 2 days later.

2.3- Determination of antiserum titre using indirect ELISA:

Leaf sap extracts from healthy and infected *N. tabacum* cv. Local variety plant were separately diluted at 1/10 (W/V) with coating buffer. Antiserum (against Misurata isolate) was diluted with antiserum buffer to the following dilutions: $1:5x10^2$, $1 : 10^3$, $1 : 2 \times 10^3$, $1 : 4 \times 10^3$, $1 : 8 \times 10^3$, $1 : 1.6 \times 10^4$, $1 : 3.2 \times 10^4$, $1 : 6.4 \times 10^4$, $1 : 1.28 \times 10^5$, $1 : 2.56 \times 10^5$, $1 : 5.12 \times 10^5$, $1 : 10.24 \times 10^5$, and $1 : 20.48 \times 10^5$.

2.4- Detection of virus isolates using indirect ELISA:

All five virus isolates were detected by indirect ELISA with the use of antiserum to Misurata isolate and antibodies to CMV (Bio-Rad kit).

2.5- Detection of virus isolates using DAS-ELISA:

All five virus isolates were detected by DAS-ELISA with the use of antiserum to Misurata isolate.

RESULTS

Detection of viruses present using indirect ELISA:

Viruses present in each of the 25 samples collected were detected using indirect ELISA (Table 2).

Table 2: Viruses of the 25 samples detected by indirect ELISA using antisera to CMV and TMV.

Sample	Virus present
Sirte (1)	TMV
Sirte (2)	CMV&TMV
Sirte (3)	CMV&TMV
Sirte (4)	CMV&TMV
Sirte (5)	CMV&TMV
Misurata (1)	CMV
Misurata (2)	CMV&TMV
Misurata (3)	CMV&TMV
Misurata (4)	CMV&TMV
Misurata (5)	CMV&TMV
El-Khomus (1)	CMV&TMV
El-Khomus (1)	CMV&TMV
El-Khomus (2)	CMV&TMV
El-Khomus (3)	CMV&TMV
El-Khomus (4)	CMV&TMV
El-Khomus (5)	CMV&TMV
Tripoli (1)	CMV
Tripoli (2)	CMV&TMV
Tripoli (3)	CMV
Tripoli (4)	CMV&TMV
Tripoli (5)	CMV&TMV
El-Zawia (1)	CMV
El-Zawia (2)	CMV&TMV
El-Zawia (3)	CMV&TMV
El-Zawia (4)	CMV&TMV
El-Zawia (5)	CMV&TMV

I – Tobacco mild green mosaic virus isolates

Using of indicator hosts for tobamoviruses:

The results of indicator host plants response to virus isolates are summarized in Table (3). All five isolates induced necrotic local lesions followed by severe necrotic mosaic (Fig. 2.A) and leaf drop (Fig. 2.B) in *Capsicum annuum* cv. Cayenna plants. Plants of *Nicotiana glutinosa* and *Nicotiana tabacum* cvs. Burley Gold (Fig. 3), Burley 21 (Fig. 4) & White Burley (Fig. 5) produced necrotic local lesions with all five isolates. But no infection was induced by any of the isolates on *Lycopersicon esculentum* cvs. Call J, Heinz 1370 F, Midi A & Riogrande and *Phaseolus vulgaris*.

Host range and symptomology:

Each one of the five virus isolates was mechanically inoculated to 36 plant species or cultivars representing several families. Results of symptoms and host range studies are summarized in Table (4).

Table 3: Response of indicator host plants to mechanical inoculation with TMGMV isolates:

Test plant	Response of indicator host plants to TMGMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
Capsicum annuum cv. Cayenna	NLL/ SNM,DF	NLL/ SNM,DF	NLL/ SNM,DF	NLL/ SNM,DF	NLL/ SNM,DF
Lycopersicon esculentum cvs. Call J, Heinz 1370 F, Midi A and Riogrande	0	0	0	0	0
Nicotiana glutinosa	NLL	NLL	NLL	NLL	NLL
Nicotiana tabacum cv. Burley Gold	NLL	NLL	NLL	NLL	NLL
Nicotiana tabacum cv. Burley 21	NLL	NLL	NLL	NLL	NLL
Nicotiana tabacum cv. White Burley	NLL	NLL	NLL	NLL	NLL
Phaseolus vulgaris	0	0	0	0	0

Abbreviation of symptoms: NLL= Necrotic local lesion, SNM= Systemic necrotic mosaic, DF= Defoliation, 0= No infection

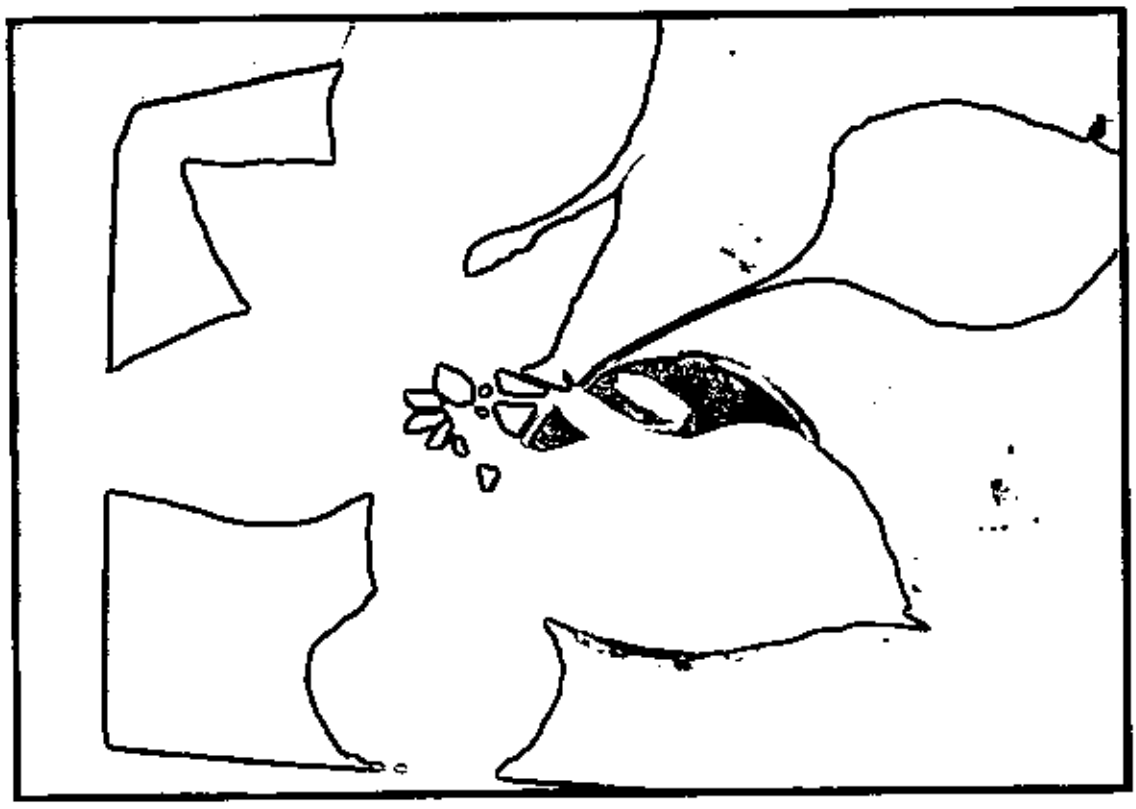

Fig. 2.A: Necrotic local lesion followed by severe necrotic mosaic in *Capsicum annuum* cv. Cayenna, induced by TMGMV isolates.

Fig. 2.B: Leaf drop following severe necrotic mosaic induced by TMGMV isolates on *Capsicum annuum* cv. Cayenna.

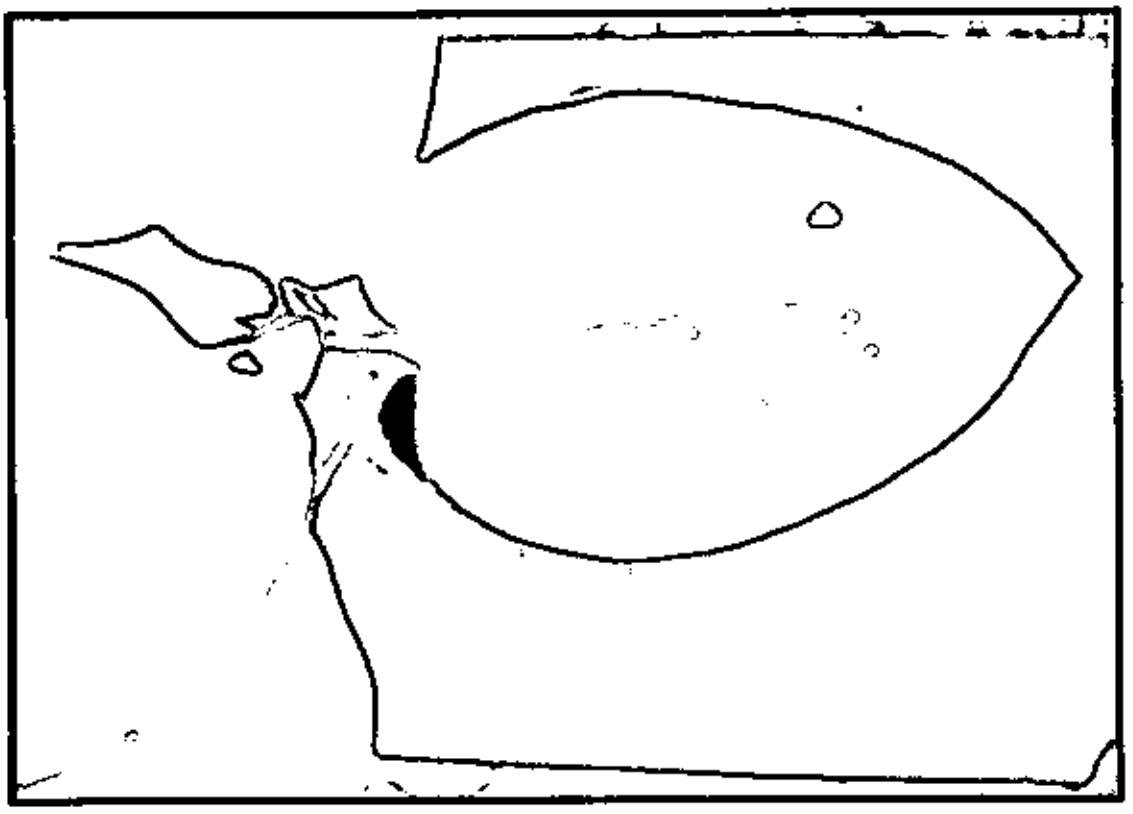

Fig. 3: Necrotic local lesions on N. tabacum cv. Burley Gold, induced by TMGMV isolates.

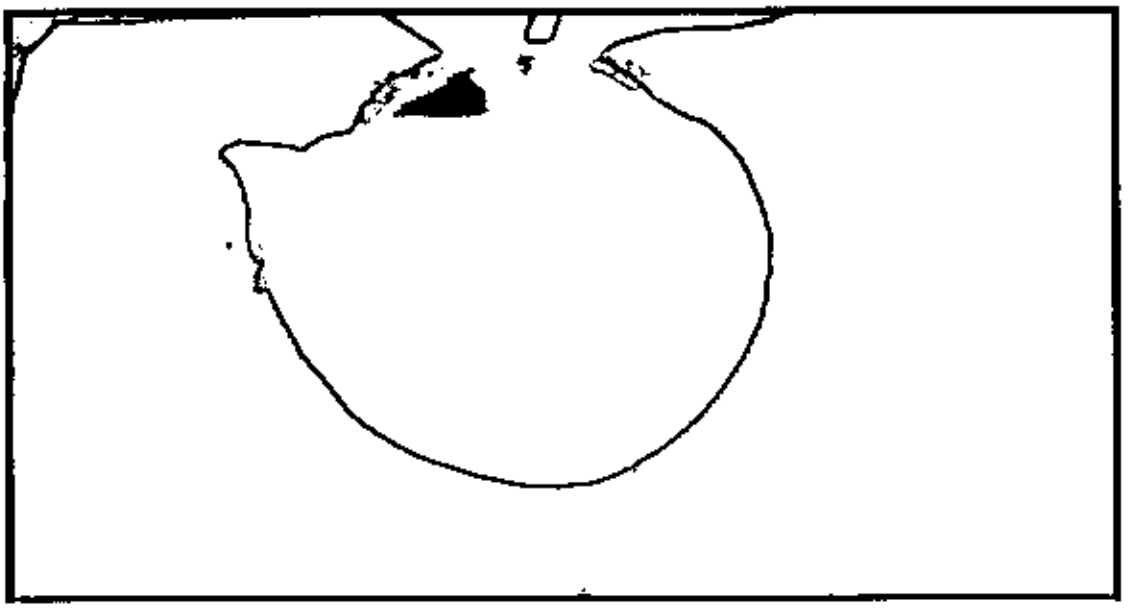

Fig. 4: Necrotic local lesion on *Nicotiana tabacum* cv. Burley 21, induced by TMGMV isolates.

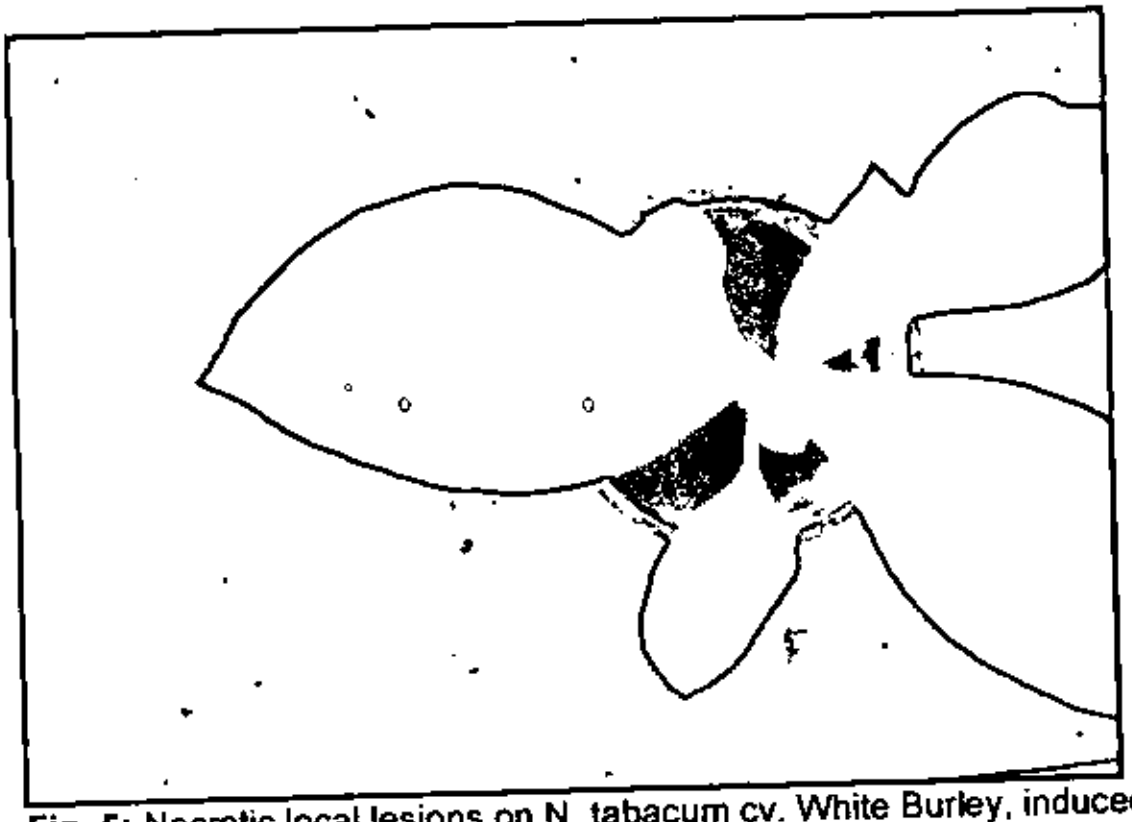

Fig. 5: Necrotic local lesions on N. tabacum cv. White Burley, induced by TMGMV isolates.

Table 4: Response of test plants to mechanical inoculation with TMGMV isolates:

Test plant	Response of test plants to TMGMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
Alcea rosea	0	0	0	0	0
Chenopodium amaranticolor	CLL	CLL	CLL	CLL	CLL
Chenopodium quinoa	NLL	NLL	NLL	NLL	NLL
Chenopodium sp.	NLL	NLL	NLL	NLL	NLL
Cucumis melo cv. Melon Pineapple	0	0	0	0	0
Cucumis sativus cv. Beit Alpha	0	0	0	0	0
Cucurbita pepo cv. White Birginian 3	0	0	0	0	0
Cucurbita maxima cv. Etampes Bright Red	0	0	0	0	0
Citrullus vulgaris cv. Klondike	0	0	0	0	0
Datura metel	NLL	NLL	NLL/SN	NLL	NLL/SN
Datura stramonium	NLL	NLL	NLL	NLL	NLL

Table 4: continued.

Test plant	Response of test plants to TMGMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
Gomphrena globosa	0	SYS	0	NLL/VN	SYS
Matthiola incana	0	0	0	0	0
Nicotiana benthamiana	SYS	LC/M	LW	SYS	W
Nicotiana glauca	M	M	M	M	M
Nicotiana repanda	NLL	NLL	NLL	NLL	NLL
Nicotiana tabacum cv. Local Variety	NLL	NLL	NLL/SNL, LDFR	NLL/SNL, LDFR	NLL
Nicotiana tabacum cv. Turkish	M	M	M	M	M
Nicotiana tabacum cv. Xanthi	NLL	NLL	NLL	NLL	NLL
Nicotiana tabacum cv. Xanthi-nc	M	M	M	SNL,M	M
Petunia hybrida	NLL/SNL, LDFR	NLL/M, LDFR	NLL/SNL,M	NLL/SNL,M	NLL

Table 4: continued.

Test plant	Response of test plants to TMGMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
Physalis floridana	M/SN,DF	M/SN,DF	M/SN,DF	M/SN,DF	M/SN,DF
Solanum melongena cv. Black Beauty	NLL	NLL	NLL	NLL	0
Solanum nigrum	SYS	MM	SYS	SYS	MM
Vinca rosea	0	SYS	0	SYS	0
Vicia faba	0	0	0	0	0

Abbreviation of symptoms: CLL= Chlorotic local lesions, DF= Defoliation, LC= Leaf cupping, LDFR= Leaf deformation, LW= Lethal wilting, M=Mosaic, MM= Mild mosaic, NLL= Necrotic local lesions, SN= Systemic necrosis, SNL= Systemic necrotic lesions, SNM= severe necrotic mosaic, SYS= Symptomless systemic infection, VN= Vein necrosis, W= Wilting, 0= No infection

Chenopodium amaranticolor produced chlorotic local lesions with all five isolates, whereas *Chenopodium quinoa, Chenopodium sp., Datura stramonium* (Fig. 3), *Nicotiana repanda, Nicotiana tabacum* cv. Xanthi produced necrotic local lesions.

Datura metel plant showed necrotic local lesions followed by systemic necrosis with El-Khomus & El-Zawia isolates, whereas it produced only necrotic local lesions with the other isolates.

Gomphrena globosa, plant differed in its reaction with the different isolates from no infection induced (Sirte & El-Khomus isolates) to symptomless systemic infection (Misurata & El-Zawia isolates) and necrotic local lesions with vein necrosis (Tripoli isolate).

Nicotiana benthamiana, response was symptomless systemic infection with Sirte and Tripoli isolates, wilting with El-Zawia isolate, lethal wilting with El-Khomus isolate and leaf cupping followed by mosaic with Misurata isolate.

Nicotiana tabacum cv. Local Variety, gave only necrotic local lesions with Sirte, Misurata and El-Zawia isolates but produced necrotic local lesions followed by systemic necrotic lesions and leaf malformation with both El-Khomus and Tripoli isolates.

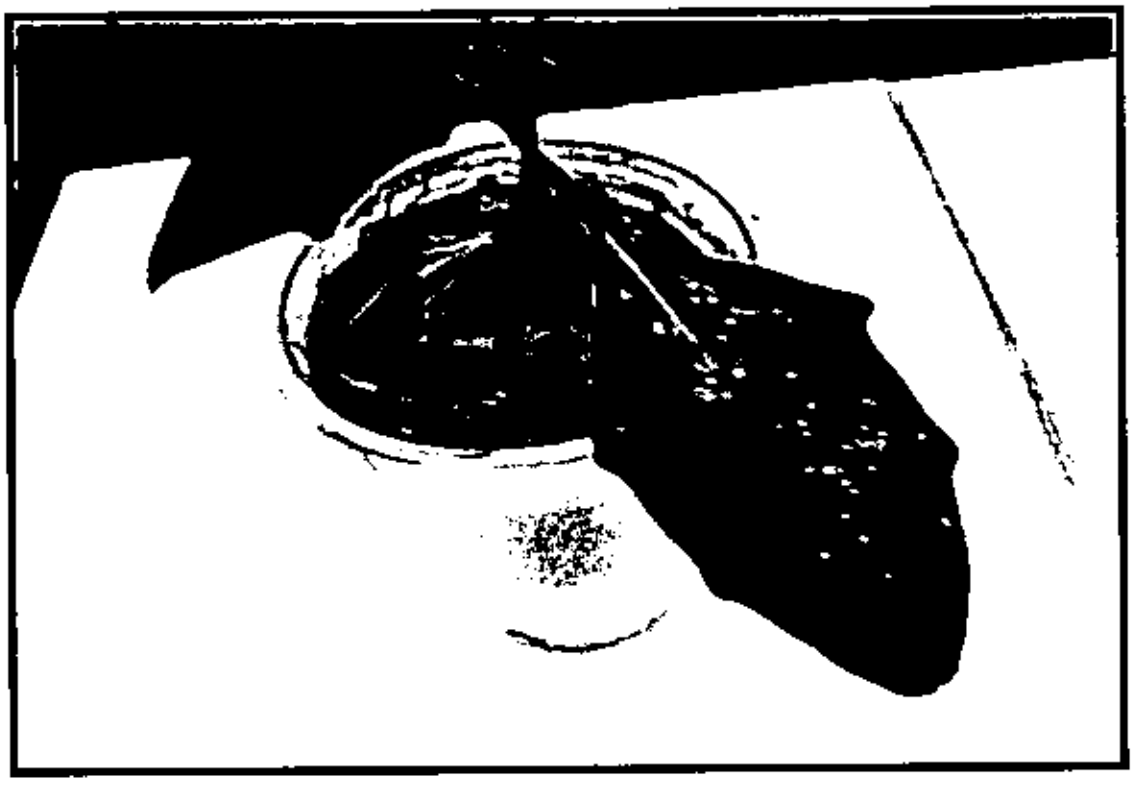

Fig. 6: Necrotic local lesions on *Datura stramonium* induced by TMGMV isolates.

Nicotiana glauca, and *Nicotiana tabacum* cv. Turkish, produced mosaic symptoms with the whole five isolates.

Nicotiana tabacum cv. Xanthi-nc, produced mosaic symptoms with the whole five isolates except with Tripoli isolate it produced systemic necrotic lesions with mosaic.

Petunia hybrida plant reacted in different response with the different isolates when it showed only necrotic local lesions with El-Zawia isolate; necrotic local lesions followed by systemic necrotic lesions and mosaic with El-Khomus & Tripoli isolates; necrotic local lesions followed by mosaic & leaf malformation with Misurata isolate and necrotic local lesions followed by systemic necrotic lesions & leaf malformation with Sirte isolate.

Physalis floridana plant produced mosaic with systemic necrosis and defoliation with all virus isolates.

Solanum melongena cv. Black Beauty, produced necrotic local lesions with all isolates except El-Zawia isolate which induced no infection.

Solanum nigrum plant produced mild mosaic with Misurata & El-Zawia isolates and produced symptomless, systemic infection with the other three isolates.

Vinca rosea plant produced symptomless systemic infection with Misurata and Tripoli isolates while no infection occurred with other isolates.

No infection was induced by any of the isolates on *Alcea rosea*, *Cucumis melo* cv. Melon Pineapple, *Cucumis sativus* cv. Beit Alpha, *Cucurbita pepo* cv. White Birginian 3, *Cucurbita maxima* cv. Etampes Bright Red, *Citrullus vulgaris* cv. Klondike, *Matthiola incana* and *Vicia faba*.

Properties in the crude extract:

Results showed that all five TMGMV isolates have thermal inactivation point (TIP) between 84-88°C (Table 5), dilution end point (DEP) between 10^{-5}-10^{-6} (Table 6) and Longevity *in vitro* (LIV) more than 13 months.

Table 5: Thermal inactivation point of TMGMV isolates:

Temperature	TMGMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
(°C)	No. LL*	No. LL*	No. LL*	No. LL*	No. LL*
80	>100	>100	90.6	79.6	67.3
81	92	83.3	77.6	72	56.3
82	42.6	67	58	35.3	16
83	13.6	48.3	23.3	11.3	5.6
84	3.3	39.6	7.6	3	1.3
85	0	14	2.3	0	0
86	0	6.3	0	0	0
87	0	1.6	0	0	0
88	0	0	0	0	0
89	0	0	0	0	0
90	0	0	0	0	0
91	0	0	0	0	0

No. LL* = Average of local lesions Number / three leaves of *D. stramonium* plant.

Table 6: Dilution end point of TMGMV isolates:

Dilution	TMGMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
	No. LL*	No. LL*	No. LL*	No. LL*	No. LL*
1:1	>100	>100	>100	>100	>100
10^{-1}	>100	>100	>100	91	>100
10^{-2}	91	81.6	87.3	42.6	85.6
10^{-3}	42.6	32.3	33	17	36
10^{-4}	11.3	9.3	13.3	6.3	10.6
10^{-5}	4	2.6	5.3	1.6	3.3
10^{-6}	0	0	0	0	0
10^{-7}	0	0	0	0	0
10^{-8}	0	0	0	0	0
10^{-9}	0	0	0	0	0
10^{-10}	0	0	0	0	0

No. LL* = Average of local lesions Number / three leaves of *D. stramonium* plant.

Purification:

The purified TMGMV preparation induced numerous necrotic local lesions when inoculated to *Datura stramonium*. Ultraviolet absorbance of the purified TMGMV suspension was typical for nucleoproteins with a minimum at 240 and a maximum at 260 nm (Fig. 7). The ratio of A260/A280 and A (max)/A (min) (uncorrected for light scattering) were 1.56 and 1.28 respectively. The yield of the purified TMGMV was 52.9 mg/100 g infected *N. glauca* leaves.

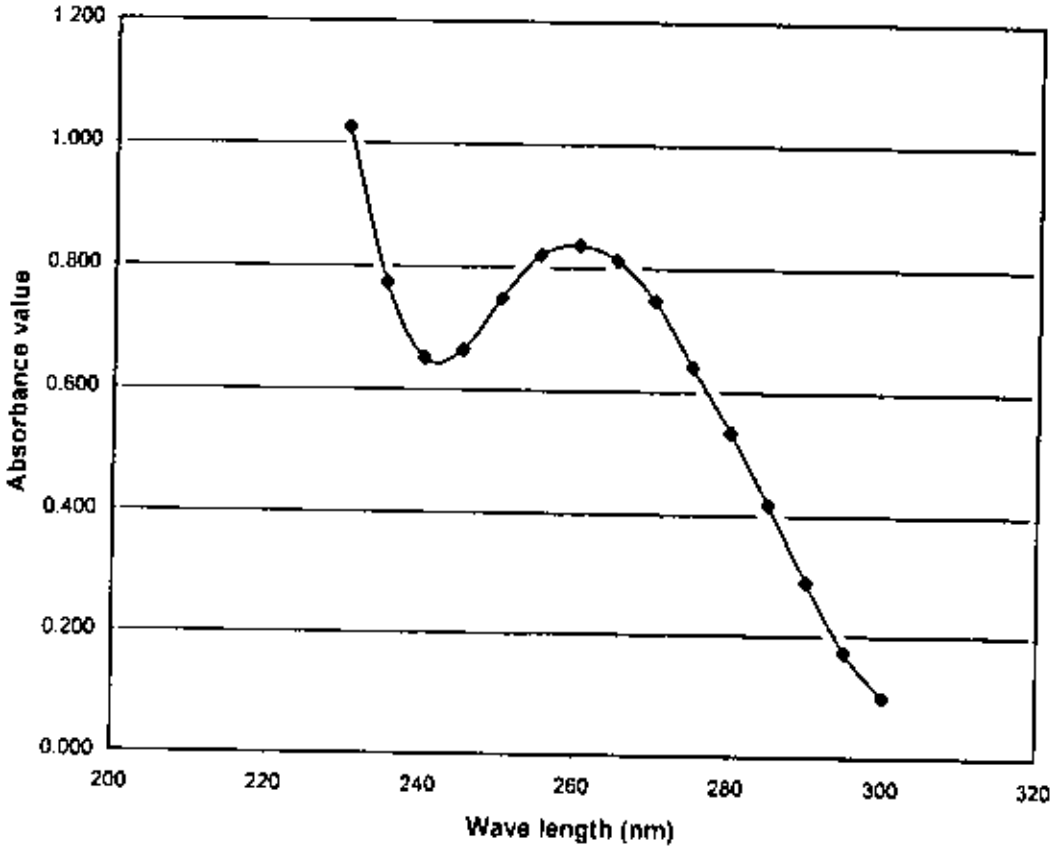

Fig. 7: Ultraviolet absorption Spectrum of the purified TMGMV

Serology:

1- Determination of Antisera titre using Ouchterlony test:

The bands were formed between the purified virus (Sirte isolate) and its homologous Antisera at the dilutions of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 for the first and second week Antisera (Fig. 8.A). But the bands were only formed at dilutions of 1/2, 1/4, 1/8, 1/16 for the third and fourth week Antisera (Fig. 8.B).

2- Determination of Antiserum titre using indirect ELISA:

The second harvesting week antiserum gave the highest reactive dilution in Agar double diffusion test, and so it was chosen to be tested by indirect ELISA. Results showed positive reaction of the antiserum with sap from infected *N. tabacum* cv. Turkish at dilutions up to 1: 8 x 103 (Table 7).

3- Detection of TMGMV isolates using Ouchterlony test:

The precipitin lines were formed between the antiserum to Sirte isolate and infected plant sap of all isolates (Fig. 9).

4- Detection of TMGMV isolates using indirect ELISA:

The whole five isolates reacted positively with the use of antiserum to Sirte isolate and also positively with antibodies to TMV (Kit from Bio-Rad).

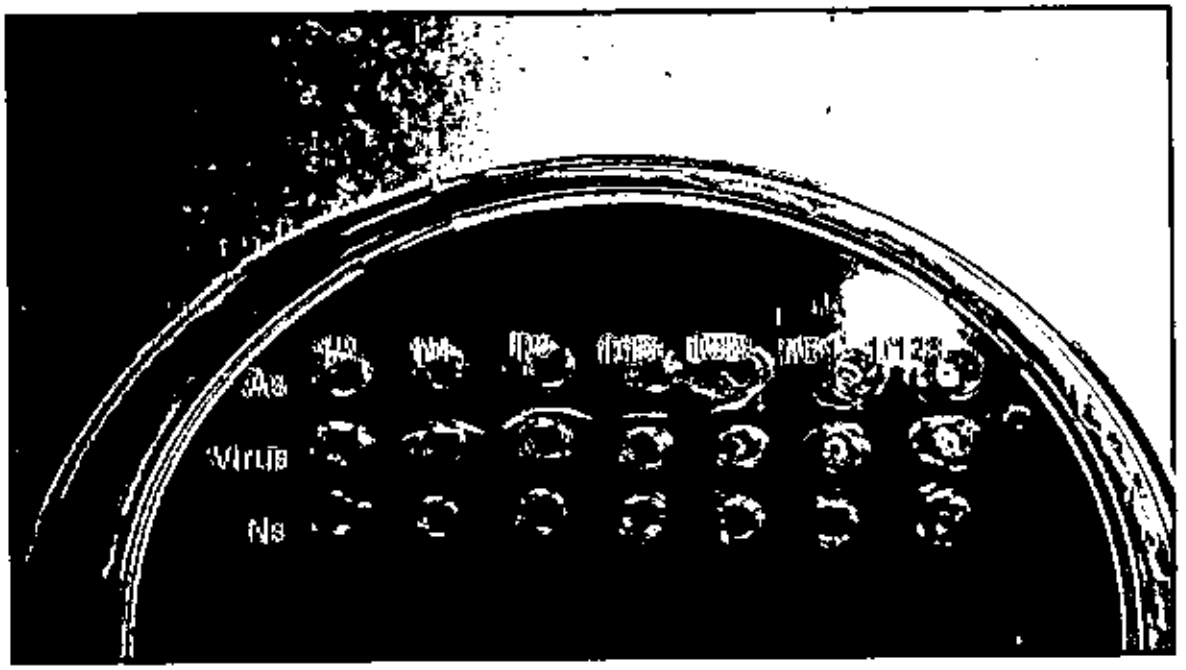

Fig. 8.A: Second week Antiserum titre using Ouchterlony test.
As = Antiserum, **Ns** = Normal serum.

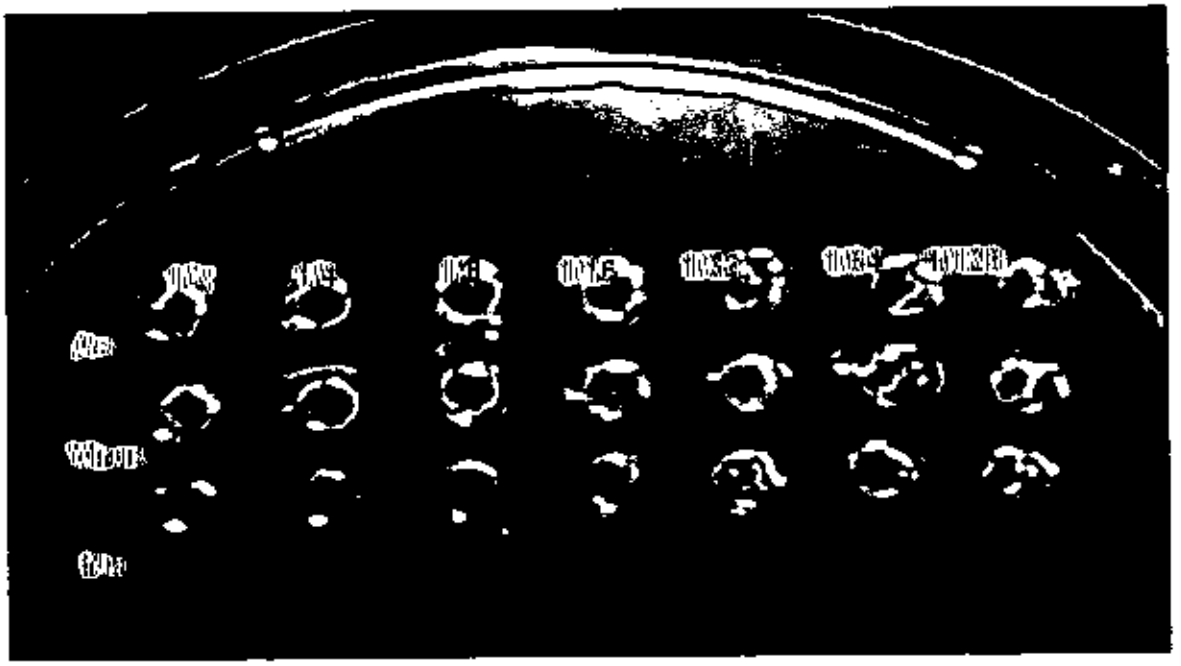

Fig. 8.B: Third week Antiserum titre using Ouchterlony test.
As = Antiserum, **Ns** = Normal serum.

Table 7: TMGMV antiserum titre determined by indirect ELISA as absorbance value at 405 nm.

Antiserum dilutions	Indirect ELISA absorbance value at 405 nm for TMGMV	
	Healthy control	Infected
1: $5x10^2$	0.891	2.182
1: 10^3	0.535	1.310
1: $2x10^3$	0.292	0.670
1: $4x10^3$	0.184	0.387
1: $8x10^3$	**0.114**	**0.228**
1: $1.6x10^4$	0.800	0.149
1:$3.2x10^4$	0.63	0.101

-ELISA absorbance values at 405 nm are average of two replicates each.

- Absorbance value of at least double that of the healthy control considered positive.

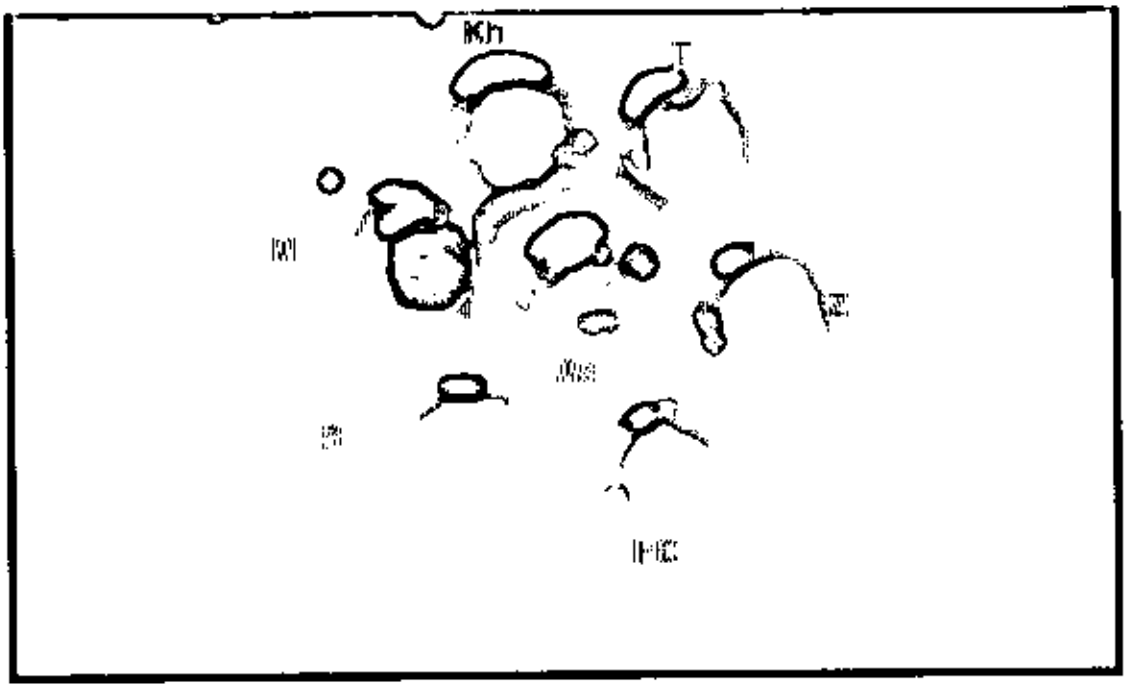

Fig. 9: Detection of TMGMV isolates in crude sap using Ouchterlony test: **As** = Antiserum to Sirte isolate.
HC = Healthy control; **Kh** = El-Khomus isolate.
M = Misurata isolate; **S** = Sirte isolate.
T = Tripoli isolate; and **Z** = El-Zawia isolate.

II – Cucumber mosaic virus isolates

Test of virus isolates using DAS-ELISA:

All virus isolates reacted positively in DAS-ELISA using an antiserum to CMV (Bio-Rad kit).

Host range and symptomology:

Each one of the five virus isolates was mechanically inoculated to 33 plant species or cultivars representing several families. Results of symptoms and host range studies are summarized in Table (8).

Capsicum annuum cv. Cayenna plant reacted in different ways with the CMV isolates, it produced mosaic with Sirte isolate, mosaic followed by leaf cupping with Khpmus and Tripoli isolates, necrotic local lesions followed by vein necrosis, systemic necrosis, and mosaic with Misurata isolate, but no infection was produced with El-Zawia isolates.

Chenopodium amaranticolor plants gave a response of chlorotic local lesions converted later into necrotic local lesions (Fig. 10).

Chenopodium quinoa, Chenopodium sp. Plants produced necrotic local lesions with all five isolates.

Table 8: Response of test plants to mechanical inoculation with CMV isolates:

Test plant	Response of test plants to CMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
Alcea rosea	0	0	0	0	0
Capsicum annuum cv. Cayenna	M	NLL/VN,SN	M/LC	M/LC	0
Chenopodium amaranticolor	CLL/NLL	CLL/NLL	CLL/NLL	CLL/NLL	CLL/NLL
Chenopodium quinoa	NLL	NLL	NLL	NLL	NLL
Chenopodium sp.	NLL	NLL	NLL	NLL	NLL
Cucumis melo cv. Melon Pineapple	0	0	0	0	0
Cucumis sativus cv. Beit Alpha	0	0	0	0	0
Cucurbita pepo cv. White Birginian 3	0	0	0	0	0

Table 8: continued.

Test plant	Response of test plants to CMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
Cucurbita maxima cv. Etampes Bright Red	0	0	0	0	0
Citrullus vulgaris cv. Klondike	0	0	0	NLL	0
Datura metel	0	0	0	0	0
Datura stramonium	0	0	0	0	0
Gomphrena globosa	M	M	RLL/M	0	M
Lycopersicon esculentum cv. Heinz 1370F	0	0	0	0	0
Matthiola incana	0	0	SYS	0	0
Nicotiana benthamiana	SYS	SYS	SC/N	SC/N	SYS
Nicotiana glauca	SYS	M	SYS	M	M
Nicotiana glutinosa	0	0	0	M,LDFR & S	M & S
Nicotiana repanda	0	0	0	0	0

Table 8: continued.

Test plant	Response of test plants to CMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
Nicotiana tabacum cv. Burley Gold	M	M	M & LDFR	M	M
Nicotiana tabacum cv. Burley 21	M	0	M & LDFR	M	M
Nicotiana tabacum cv. Local Variety	M	M	M,LDFR& S	M	SC/M
Nicotiana tabacum cv. Turkish	0	M	M	M	0
Nicotiana tabacum cv. White Burley	M	M	0	M	M
Nicotiana tabacum cv. Xanthi	SYS	M	M,S & SC	M	M
Nicotiana tabacum cv. Xanthi-nc	M	M	M	SYS	M
Petunia hybrida	0	NLL/SNL,M	0	0	0

Table 8: continued.

Test plant	Response of test plants to CMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
Phaseolus vulgaris	0	0	0	0	0
Physalis floridana	M & DF	M & DF	M	C,N & DF	0
Solanum melongena cv. Black Beauty	0	0	M	0	0
Solanum nigrum	0	0	0	0	0
Vinca rosea	0	0	0	0	0
Vicia faba	0	0	NLL	0	M

Abbreviation of symptoms: C= Chlorosis, CLL= Chlorotic local lesions, DF= Defoliation, LC= Leaf cupping, LDFR= Leaf deformation, M=Mosaic, N= Necrosis, NLL= Necrotic local lesions, RLL= Reddish local lesions, S= Stunting, SC= Systemic chlorosis, SN= Systemic necrosis, SNL= Systemic necrotic lesions, SYS= Symptomless systemic infection, VN= Vein necrosis, 0= No infection

Fig. 10: Chlorotic local lesions on *Ch. amaranticolor* induced by CMV isolates; later become necrotic.

Citrullus vulgaris cv. Klondike, plants produced necrotic local lesion only with Tripoli isolate and no infection was developed by any of the other four isolates.

Gomphrena globosa plants reacted in symptomless systemic infection with Misurata, Sirte, and El-Zawia isolates, necrotic local lesions followed by mosaic with El-Khomus isolate, and no infection was produced with Tripoli isolate.

Matthiola incana plant produced symptomless systemic infection with El-Khomus isolate and no infection was induced by any other isolate.

Nicotiana benthamiana plants produced systemic chlorosis followed by necrosis with El-Khomus, and Tripoli isolates, symptomless systemic infection with Misurata, Sirte, and El-Zawia isolates.

Nicotiana glauca plants produced mosaic with Misurata, Tripoli and El-Zawia isolates and symptomless systemic infection with El-Khomus and Sirte isolates.

Nicotiana glutinosa plants response was mosaic, leaf malformation, and stunting with Tripoli isolate; mosaic and stunting with El-Zawia isolate, but no infection was developed by any of the other three isolates.

Nicotiana tabacum cv. Burley Gold plants produced mosaic with all five isolates except El-Khomus isolate which induced leaf deformation with mosaic.

Nicotiana tabacum cv. Burley 21 plants produced mosaic with Sirte, Tripoli, and El-Zawia isolates; mosaic, and leaf deformation with El-Khomus isolate, and no infection with Misurata isolate.

Nicotiana tabacum cv. Local Variety plants produced mosaic with Misurata, Sirte, Tripoli isolates; mosaic, leaf deformation, and stunting with El-Khomus isolate; systemic chlorosis followed by mosaic with El-Zawia isolate.

Nicotiana tabacum cv. Turkish plants showed mosaic with El-Khomus, Misurata, and Tripoli isolates; but no infection with Sirte and El-Zawia isolates.

Nicotiana tabacum cv. White Burley plants gave mosaic with Misurata, Sirte, Tripoli, and El-Zawia isolates; but no infection was given with El-Khomus isolate.

Nicotiana tabacum cv. Xanthi plants induced mosaic with Misurata, Tripoli and El-Zawia isolates; mosaic, stunting and systemic chlorosis with Komus isolate; Symptomless systemic infection with Sirte isolate.

Nicotiana tabacum cv. Xanthi-nc plants developed mosaic with El-Khomus, Misurata, Sirte, and El-Zawia isolates; but symptomless systemic infection was produced with Tripoli isolate.

Petunia hybrida plants reacted positively only with Misurata isolate by producing necrotic local lesions, followed by systemic necrotic lesions, systemic necrosis, and mosaic later; but none of the other isolates gave an infection.

Physalis floridana produced mosaic with El-Khomus isolate; mosaic and leaf deformation with Misurata and Sirte isolates; chlorosis, necrosis, and leaf deformation with Tripoli isolate; and no infection was produced by El-Zawia isolate.

Solanum melongena cv. Black Beauty plants produced mosaic only with El-Khomus isolate; and no infection was produced by the other four isolates.

Vicia faba produced necrotic local lesions with El-Khomus isolate; mosaic symptoms with El-Zawia isolate; no infection was induced by other three isolates.

No infection was produced by any of the isolates in *Cucumis melo* cv. Melon Pineapple, *Cucumis sativus* cv. Beit Alpha, *Cucurbita pepo* cv. White Birginian, *Cucurbita maxima* cv. Etampes Bright Red, *Datura metel, Datura stramonium, Lycopersicon esculentum* cv. Heinz 1370 F, *Nicotiana repanda, Phaseolus vulgaris, Solanum nigrum, Vinca rosea.*

Properties in the crude extract:

Results presented in Table (9) showed that the thermal inactivation point (TIP) between 50°C-53°C; dilution end point (DEP) was between 10^{-2} - 10^{-3} (Table 10) and Longevity *in vitro* (LIV) was between ½ – 1 day (Table 11).

Table 9: Thermal inactivation point of CMV isolates:

Temperature	CMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
(°C)	No. LL*	No. LL*	No. LL*	No. LL*	No. LL*
40	21.6	24.3	22	22.3	23
45	14	15.3	14.3	17	18.3
50	9.3	1.6	2.3	4.6	6.6
51	5.6	0	0	1.3	2
52	1.6	0	0	0	0
53	0	0	0	0	0
54	0	0	0	0	0
55	0	0	0	0	0
60	0	0	0	0	0
65	0	0	0	0	0
70	0	0	0	0	0
75	0	0	0	0	0

No. LL* = Average of local lesions Number / three leaves of *Ch. amaranticolor* plant.

Table 10: Dilution end point of CMV isolates:

Dilution	CMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
	No. LL*	No. LL*	No. LL*	No. LL*	No. LL*
1:1	33.6	68.3	41.6	46.6	52.3
10^{-1}	6.3	11.6	10.3	13.3	14
10^{-2}	1.6	3	2.3	1.3	3
10^{-3}	0	0	0	0	0
10^{-4}	0	0	0	0	0
10^{-5}	0	0	0	0	0
10^{-6}	0	0	0	0	0
10^{-7}	0	0	0	0	0
10^{-8}	0	0	0	0	0
10^{-9}	0	0	0	0	0
10^{-10}	0	0	0	0	0

No. LL* = Average of local lesions Number / three leaves of *Ch. amaranticolor* plant.

Table 11: Longevity *in vitro* of CMV isolates:

Longevity *In vitro*	CMV isolates				
	Sirte isolate	Misurata isolate	El-Khomus isolate	Tripoli isolate	El-Zawia isolate
(day)	No. LL*	No. LL*	No. LL*	No. LL*	No. LL*
0	27.6	42	36.3	44	43.3
½	11	9	13	11.6	14.3
1	0	0	0	0	0
1 ½	0	0	0	0	0
2	0	0	0	0	0

No. LL* = Average of local lesions Number / three leaves of *Ch. amaranticolor* plant.

Purification:

The purified CMV preparation induced numerous chlorotic local lesions on *Chenopodium amaranticolor.* Ultraviolet absorbance of the purified CMV suspension was typical for nucleoproteins with a minimum at 240 and a maximum at 260 nm (Fig. 11). The ratio of A260/A280 and A(max)/A(min) (uncorrected for light scattering) were 1.53 and 1.67 respectively. The yield of the purified CMV was 12.5 mg/100 g infected *N. tabacum* cv. Local Variety leaves.

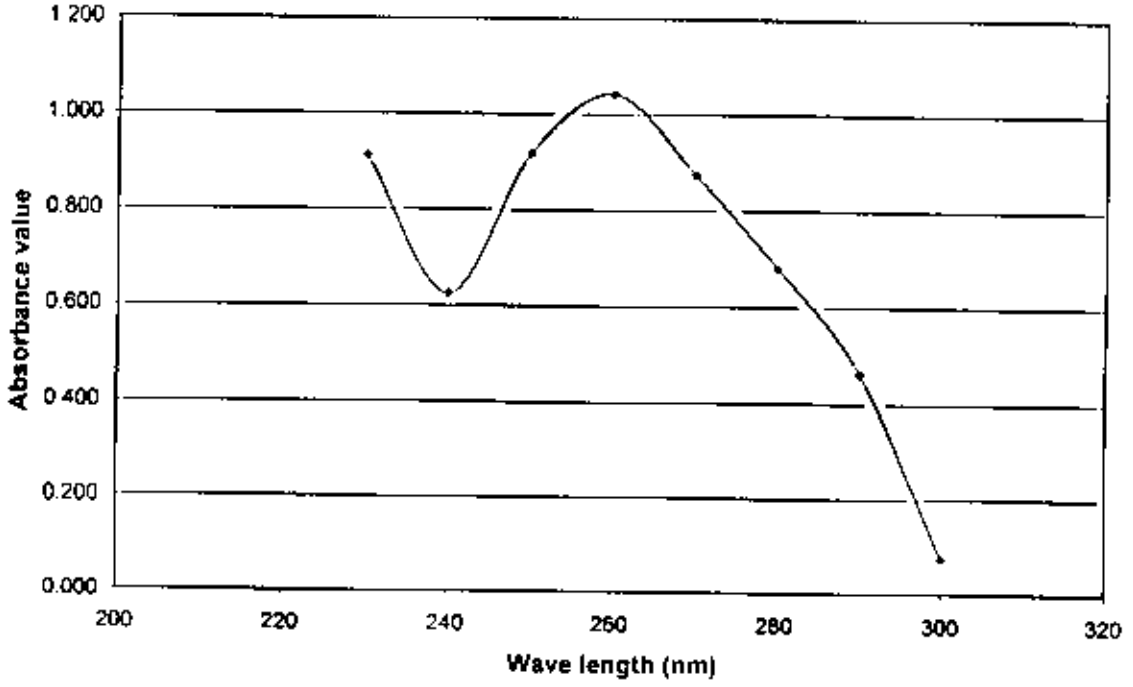

Fig. 11: Ultraviolet absorption Spectrum of the purified CMV

Serology:

1- Determination of Antisera titre using Ouchterlony test:

The bands were formed between sap of infected *N. tabacum* cv. Local Variety (Misurata isolate) and its homologous Antisera at the dilutions of 0, ½, and ¼ (Fig. 12).

2- Determination of Antiserum titre using indirect ELISA:

Antisera against CMV were produced. Antiserum titres were determined by indirect ELISA. Results showed that Antiserum reacted positively with sap from infected *N. tabacum* cv. Local Variety leaves when diluted up to 1: 10.24×10^5 Table (12).

3- Detection of virus isolates using indirect ELISA:

Indirect ELISA results showed that all five isolates reacted positively with the produced antiserum against Misurata isolate and with antibodies against CMV (Kit from Bio-Rad).

4- Detection of virus isolates using DAS-ELISA:

All the five isolates reacted positively with antiserum to Misurata isolate; when enzyme conjugate Y-globulin (Bio-Rad kit) was used.

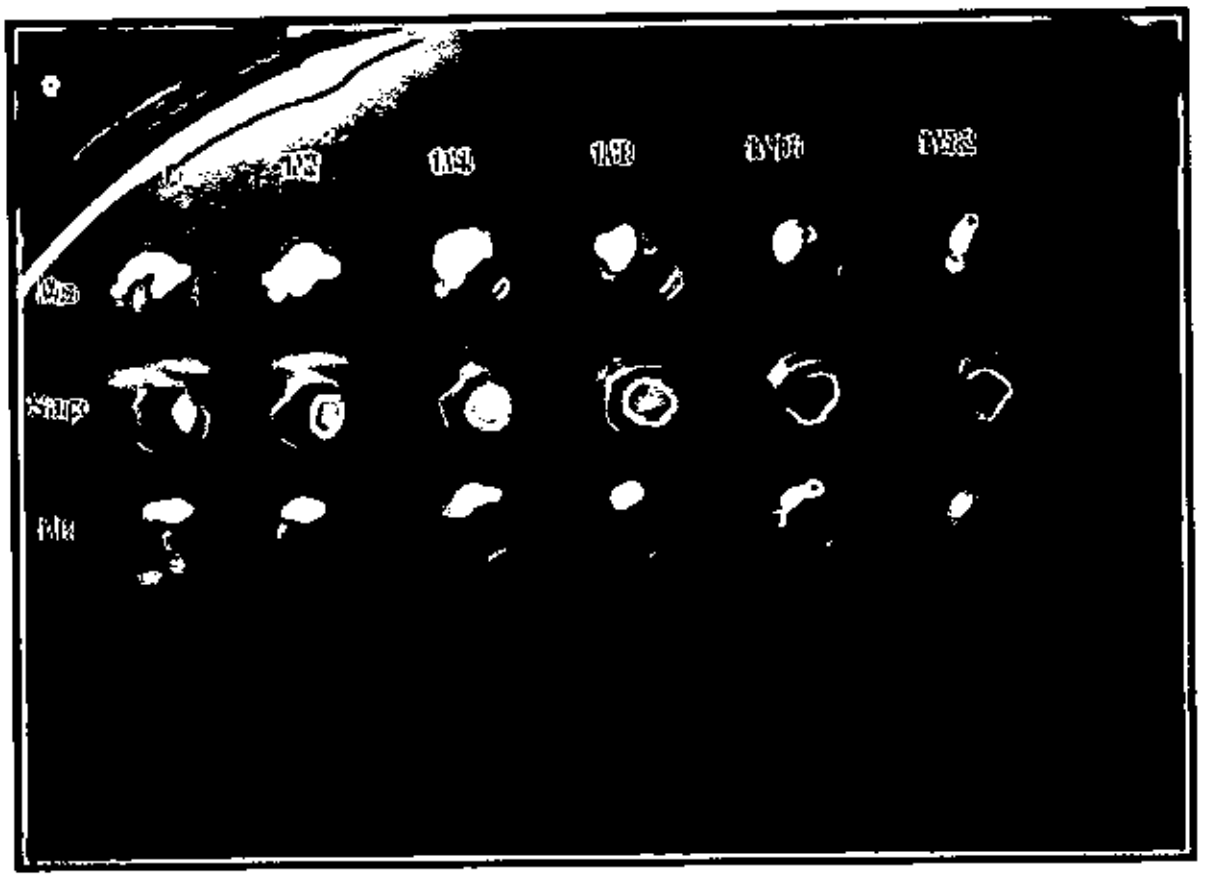

Fig. 12: Determination of CMV Antiserum titre using Ouchterlony test:
As = Antiserum; **Ns** = Normal serum.
Sap = Sap of Misurata isolate.

Table 12: CMV antiserum titre determined by indirect ELISA as absorbance value at 405 nm.

Antiserum dilutions	Indirect ELISA absorbance value at 405 nm for CMV	
	Healthy control	Infected
1: $5x10^2$	0.189	2.877
1: 10^3	0.135	2.262
1: $2x10^3$	0.092	1.473
1: $4x10^3$	0.215	2.735
1: $8x10^3$	0.161	1.634
1: $1.6x10^4$	0.135	0.907
1:$3.2x10^4$	0.114	1.185
1:$6.4x10^4$	0.074	0.844
1:$1.28x10^5$	0.063	0.447
1:$2.56x10^5$	0.053	0.189
1:$5.12x10^5$	0.045	0.123
1:$10.24x10^5$	**0.037**	**0.084**
1:$20.48x10^5$	0.029	0.043

-ELISA absorbance values at 405 nm are average of two replicates each.

- Absorbance value of at least double that of the healthy control were considered positive.

DISCUSSION

Wild tobacco *Nicotiana glauca* commonly shows mosaic symptoms in the coastal belt region of Libya and near the cultivated fields. No previous study has been done to determine the causal virus or viruses of this disease; therefore the objectives of this study are to characterize several isolates of each virus found from different regions in West of Libya. The presence of tobamovirus was confirmed by ELISA test using tobacco mosaic virus antiserum. Host range experiments of virus isolates showed that the whole five isolates induced necrotic local lesions on *Nicotiana glutinosa*, this result agree with that reported for tobamoviruses (Bodaghi *et al.*, 2000).

Also all isolates induced necrotic local lesions in *Nicotiana tabacum* cv. White Burley, the same symptoms was cited before to be induced by tobacco mild green mosaic virus (TMGMV) and not by tobacco mosaic virus (Randles *et al.*, 1981) this fact supports the probability that these isolates belonging to TMGMV and not to TMV.

The induction of local lesions obtained on *N. tabacum* cvs. Burley 21 & Burly Gold is not a characteristic for TMV (type strain) which reported to cause vein clearing on young systematically invaded leaves of Burley tobaccos (Zaitlin and Israel, 1975; and Zaitlin, 2000).

The failure of the five isolates of the virus to infect *Phaseolus vulgaris* is another evidence that the virus found associated with *N. glauca* in this study is TMGMV and not the type TMV because the latter was reported to induce necrotic local lesions on *Phaseolus vulgaris* (Zaitlin, 2000), while the former fails to infect it (Valverde and Dodds, 1986).

The failure of tobamoviruses to infect most cultivars of *Lycopersicon esculentum* was reported to be diagnostic for tobacco mild green mosaic virus (TMGMV) but not for the ordinary TMV which induced systemic mosaic or mottle on this host (Valverde and Dodds, 1986; Siegel and Wildman, 1954; Wetter, 1984; and Wetter, 1989). Similar result also had been reported with MDGMV (synonym of TMGMV) when the inoculation had failed to transmit the virus of *N. glauca* to tomato plants grown from Canary Island seed as well as from American seed (McKinney, 1929). The same failure of TMGMV to infect *Lycopersicon esculentum* cv. Rutgers was reported by Bodaghi *et al.*, (2000).

Several authors working with U2 and U5 strains (synonyms of TMGMV) reported *Lycopersicon esculentum* as a systemic host. This is quite unlikely because all cultivars of tomato tested so far have proved to be immune. It is more probable that tomato became infected because of contamination of the inoculums with type tobacco mosaic virus or tomato mosaic virus (Wetter, 1989).

The whole five isolates induced severe necrotic mosaic followed by leaf drop in *Capsicum annuum*, this result was reported to be induced by Tobacco mild green mosaic virus (Wetter, 1989; Brunt *et al.*, 1996c; & Li and Chang, 2004). And not induced by TMV which was reported to induce necrotic local lesions on this host (Wetter, 1984).

The thermal inactivation point were between 84°C-88°C for the five virus isolates which is almost the same range reported for TMGMV (85-90°C) by Brunt *et al;* (1996c) and Wetter (1989) and it is less stable than the type TMV which is very heat stable with some infectivity retaining after 10 minute exposures at over 90°C (Zaitlin, 2000).

The absence of type TMV in the whole 25 samples in the present study may confirm to an extent the fact that *N. glauca* has been reported to be a better host for TMGMV than for TMV (Bald and Goodchild, 1960). And also supports the fact that New South Wales *N. glauca* population was infected with tobacco mosaic virus (TMV) and tobacco mild green mosaic virus (TMGMV) before 1950 but only with TMGMV after that date and the accumulation level in *N. glauca* for all TMV isolates is much depressed in co-infections with TMGMV. Thus, the presence of TMGMV in *N. glauca* results in a decrease of its carrying capacity for TMV and in a reduction as large as 10-fold of TMV concentration in the population (Fraile *et al.*, 1997).

Depending on the above information, and results obtained it is proposed that this tobamovirus isolated from naturally infected *N. glauca* plants is TMGMV. However, further work involving the reaction of additional host plants, serology and amino acid analysis of the coat protein is needed to ascertain in a definite manner the identity of this virus isolates.

The Cucumovirus that reported to cause mosaic in naturally *N. glauca* plants is Cucumber mosaic virus which host range had not been studied enough yet; it was reported to infect *N. glauca* frequently (Valverde and Dodds, 1986; Moll *et al.*, 1987; Wetter, 1989; Gafny *et al.*, 1996 & Vovlas and Di Franco, 2004), but still no enough host range study done.

DAS-ELISA results confirmed that all the Cucumovirus isolates are belonging to Cucumber mosaic virus and not to any of the other Cucumovirus members since they reacted positively with known CMV antibodies (Bio-Rad kit) (Devergne *et al.*, 1981).

The experimental plants that used in the present study differed in their reaction with the five virus isolates. Although, *Chenopodium amaranticolor, Ch. quinoa Nicotiana glutinosa; Vicia faba; Phaseolus vulgaris* were reacted with one or more isolates similar to that previously reported by Eid *et al.*, (1984), *Cucumis sativus, Datura stramonium,*

Petunia hybrida were reacted differently. The thermal inactivation point (TIP), dilution end-point (DEP) and longevity *in vitro* (LIV) differed from that reported by Eid *et al.*, (1984), but still in agree with the range reported by Brunt *et al.*, (1996a); & Palukaitis and Garcia-Arenal (2003).

Virus isolates produced mosaic symptoms on different inoculated tobacco hosts including *N. glutinosa, N. tabacum* cv. White Burley and Xanthi when it was in a greenhouse of about 28°C. These results indicated that they probably belong to CMV subgroup I, since it had been reported that subgroup I and not II can produce such symptoms on tobacco at over 26°C (Palukaities *et al.*, 1992). However, further investigations involving the reaction of additional host plants, serology, and amino acid analysis of the coat protein is needed to ascertain in a definite manner the identity of this virus isolates.

SUMMARY

Mosaic symptoms on leaves of *Nicotiana glauca* plants were observed repeatedly along the costal region of Libya. Five samples from each region of different five regions (Sirte, Misurata, El-Khomus, Tripoli, and El-Zawia) were collected, tested for viruses present by indirect ELISA. Five isolates of each virus found (Cucumovirus & Tobamovirus) were mechanically inoculated to more than 30 plant species or cultivars representing eight plant families.

Tobamovirus isolates were successfully infected 23 out of the 36 plant tested in different responses. Thermal inactivation point of the tobamovirus isolates was between 84-88°C, dilution end point was between 10^{-5}-10^{-6} and longevity *in vitro* was more than 13 months.

Antiserum against Sirte isolate was produced and used to determine relationship between isolates which were related using Ouchterlony test and indirect ELISA. On the basis of serology these virus isolates belong to tobamovirus group and based on some diagnostic differential hosts, they could be isolates of tobacco mild green mosaic virus.

Cucumovirus isolates were successfully infected 20 out of the 33 plant tested in different responses. Thermal inactivation point of Cucumovirus isolates was between 50-53°C, dilution end point was between 10^{-2}-10^{-3} and longevity *in vitro* was between ½ - 1 day.

Antiserum against Misurata isolate was produced and used to determine relationship between isolates which all were positively reacted using indirect ELISA and DAS-ELISA (using enzyme conjugate Y-globulin kit from Bio-Rad). Indirect ELISA showed positive reaction between isolates and CMV antibodies (kit from Bio-Rad). DAS-ELISA revealed a close relationship between all isolates and CMV (Bio-Rad kit). On the basis of serology these virus isolates belong to Cucumber mosaic virus.

LITERATURE CITED

Agrios, G. A. (1997). Plant pathology, 4th edition. Academic press, New York.

Bald, J. G. and Goodchild, D. J. (1960). Tobacco mosaic virus in *Nicotiana glauca*. Phytopathology, 50: 497-499.

Ball, E. M. (1974). Serological tests for the identification of plant viruses. American Phytopathological Soc., 31pp.

Bodaghi, S., Yassi, M. N. A. and Dodds, J. A. (2000). Heterogeneity in the 3'-terminal untranslated region of tobacco mild green mosaic tobamoviruses from *Nicotiana glauca* resulting in variants with three or six pseudoknots. J. Gen. Virol., 81: 577-586.

Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J. and Watson, L. (1996a). Artichoke yellow ringspot nepovirus. In Viruses of plant descriptions and lists from the VIDE Database(ed), CAB international.

Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J. and Watson, L. (1996b). Cucumber mosaic cucumovirus. In viruses of plant descriptions and lists from the VIDE Database(ed), CAB international.

Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J. and Watson, L. (1996c). Tobacco mild green mosaic tobamovirus. In Viruses of plant descriptions and lists from the VIDE Database(ed), CAB international.

Clark, M. F. and Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J.Gen.Virol., 34:475-483.

Devergne, J. C., Cardin, L., Burckard, J. and Van Regenmortel, M. H. V. (1981). Comparison of direct and indirect ELISA for detecting antigenically related Cucumoviruses. Journal of Virological Methods, 3: 193-200.

Eid, S. A., Kishtah, A. A. and Abu-Zeid, A. A. (1984). *Nicotiana glauca* a natural host for Cucumber mosaic virus. Agricultural Research Review, 62 (2): 367-378.

El-Sanousi, O. M., Shagroon, M. A., and Khalil, J. A. (1991a). Isolation and identification of potato virus from pepper plants in Libya. Arab J. Plant Protection, 9 (1): 47-51.

El-Sanousi, O. M., Shagroon, M. A., and Khalil, J. A. (1991b). Isolation and identification of tomato mosaic virus from pepper plants in Libya. Arab J. Plant Protection, 9(1):52-56.

Faccioli, G. (1964). Su di un ceppo di virus del mosaico del tabacco isolato da *Nicotiana glauca* R. Grah. Phytopathologica Mediterranea, 3: 164-175.

Fadel, S. F. (2001). Identification of plant viruses infecting broad bean in western region of Libya. M.Sc. Thesis, AL-Fateh University.

Fraile, A., Fernando, E., Aranda, M. A., Malpica, J. M., Gibbs, A. J. and Garcia-Arenal, F. (1997). A century of tobamovirus evolution in an Australian population of *Nicotiana glauca*. J. Virol., 71(11):8316-8320.

Fraile, A., Malpica, J. M., Aranda, M. A., Rodriguez-Cerezo, E. and Garcia-Arenal, F. (1996). Genetic diversity in tobacco mild green mosaic tobamovirus infecting the wild plant *Nicotiana glauca*. Virology, 223:148-155.

Francki, R. I. B. and Habili, N. (1972). Stabilization of capsid structure and enhancement of immunogenicity of cucumber mosaic virus (Q strain) by formaldehyde. Virology, 48:309-315.

Gafny, R., Wexler, A., Mawassi, M. and Israeli, Y. (1996). Natural infection of banana by a satalite-containing strain of cucumber mosaic virus: nucleotide sequence of the coat protein gene and the satelite RNA. Phytoparasitica, 24(1):49-56.

Gibbs, A. J. and Harrison, B. D. (1970). Cucumber mosaic virus. CMI/AAB Description of Plant Viruses No. 1, 5 pp.

Gooding, G. V. and Hebert, T. T. (1967). A simple technique for purification of tobacco mosaic virus in large quantities. Phytopathology, 57: 1285

Harris, A., Gibbs, A. J. and Gibbs, M. J. (2002). Nepoviruses and their diagnosis in plants. A novel polymerase chain reaction diagnostic test for nepoviruses in nursery stock, Consultancy Report, Plant Biosecurity Australia.

Jagger, I. C. (1916). Experiment with the cucumber mosaic disease. Phytopathology, 6:148-151.

Keith, H. G. (1965). Libyan flora, Ministry of Agriculture, Libya.

Li, C. Y and Chang, Y. C. (2004). Identification of Tobacco mild green mosaic virus on *Capsicum annuum* in Taiwan. British Society of Plant Pathology, New Disease Reports, Vol. 10 (ed).

Lommel, S. A., Mc Cain, A. H., and Marris, T. J. (1982). Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. Phytopathology, 72: 1018-1022.

McKinney, H. H. (1929). Mosaic disease in the Canary Islands, West Africa, and Gibraltar. Journal of Agriculture Research, 39: 557.

Moll, J. N., Grech, N. M. and Vanvuuren, S. P. (1987). A lethal transmissible stem-pitting of avocados associated with Duke 6 rootstocks. South African Avocado Growers' Association, Yearbook, 10:122-123.

Moya, A., Rodriguez-Cerezo, E. and Garcia-Arenal, F. (1993). Genetic structure of natural populations of the plant RNA virus tobacco mild green mosaic virus. Molecular Biology and Evolution, 10: 449-456.

Murphy, F. A., Fauquet, C. M., Bishop, D. H. L., Ghabrial, S. A., Jarvis, A.W., Martelli, G. P. Moya, M. A. and Summers, M. D. (1995). Virus taxonomy, classification and nomenclature of viruses sixth report of the international committee on taxonomy of viruses. Springer-Verlag Wien New York.

Nariani, T. K. and Singh, N. (1952). A mosaic disease of *Nicotiana glauca* R Grah. Indian phytopathology, 5: 47-51.

Palukaitis, P. and García-Arenal, F. (2003). Cucumber mosaic virus. CMI/AAB Description of Plant Viruses, No. 400, 15 pp.

Palukaitis, P., Roossinck, M. J., Dietzgen R. G. and Francki, R. I. B. (1992). Cucumber mosaic virus. Advance Virus Research, 41: 281-348.

Rana, G.L., Kyriakopoulou, P.E. and Martelli, G.P. (1983). Artichoke yellow ringspot virus. CMI/AAB Descrip. of Plant Viruses, No. 271, 4 pp.

Randles, J. W., Palukaitis, P. and Davies, C. (1981). Natural distribution, spread, and variation, in the tobacco mosaic virus infecting *Nicotiana glauca* in Australia. Annals of Applied Biology, 98: 109-119.

Siegel, A. and Wildman, S. G. (1954). Some natural relationships among strains of tobacco mosaic virus. Phytopathology, 44: 277-282.

Valverde, R. A. and Dodds, J. A. (1986). Evidence for a satellite RNA associated naturally with the U5 strain and experimentally with the U1 strain of tobacco mosaic virus. J. gen. virol., 67:1875-1884.

Voller, A. Bidwell, and Bartlett A. (1977). The enzyme-immunosorbent assay. Flowline Press. Guernsey, U. K. 43 pp.

Vovlas, C. and Di Franco, A. (2004). Cucumber mosaic virus in *Nicotiana glauca* in Greece. Disease Note, Journal of Plant Pathology, 86 (1): 91-92.

Wahyuni, W.S., Dietzgen, R.G. Hanada, K. and Francki, R. I. B. (1992). Serological and biological variation between and within subgroup I and II strains of cucumber mosaic virus. Plant Pathology, 41: 282-297.

Walkey, D. G. A. (1991). Applied plant virology, 2nd edition, Chapman and Hall. London.

Wetter, C. (1984). Antigenic relationships between isolates of Mild Dark-Green Tobacco Mosaic Virus, and the problem of host-Induced Mutation. The American Phytopathological Society, 74 (11): 1308-1312.

Wetter, C. (1989). Tobacco mild green mosaic virus CMI/AAB. Description of Plant Viruses, No. 351, 7 pp.

WWW.pfaf.org/cgi-bin/pfaf/arr_html?*Nicotiana+glauca*

Younes, H. A. (1984). Identification of a virus causing mosaic disease in broad bean plants, in Tripoli area,Libya. M.Sc. Thesis,AL-Fateh University.

Younes, H. A. (1995). Studies on certain virus diseases affecting some vegetable crops under greenhouse conditions. Ph.D. Thesis, Alexandria University.

Zaitlin, M. (2000). Tobacco mosaic virus CMI/AAB Description of Plant Viruses, No. 370, 8 pp.

Zaitlin, M. and Israel H. W. (1975). Tobacco mosaic virus (type strain) CMI/AAB Description of Plant Viruses, No. 151, 6 pp.

Zidan, F.O.A. (1996). Identification of viruses infecting peas. M.Sc. Thesis, AL-Fateh University.

تعريف الفيروسات المسببة للتبرقش على نبات التبغ البري (*Nicotiana glauca* Graham) في الساحل الغربي من ليبيا

الملخص

لوحظ انتشار أعراض الموزاييك بشكل واسع على أوراق نباتات التبغ البري *Nicotiana glauca* النامية على طول الشريط الساحلي لليبيا ، ولغرض التعرف على المسبب تم جمع خمس عينات عشوائية من كل منطقة من المناطق التالية (سرت، مصراته، الخمس، طرابلس، الزاويه)، ومن ثمّ الكشف عن الفيروسات الموجودة بكل عينة باستخدام اختبار إليزا غير المباشر (Indirect ELISA) ، ثم أخذت خمس عزلات من كل فيروس عثر عليه بالعينات (Cucumovirus & Tobamovirus) واعديت ميكانيكيا إلى أكثر من 30 نوعاً أو صنفاً نباتياً تمثل ثمان عائلات نباتية.

وقد نجحت عزلات الفيروس التابع لمجموعة Tobamovirus في اصابة حوالي 23 نوعاً أو صنفاً من بين 36 نباتاً تمّت دراستها بردود أفعال مختلفة ، ولقد وجد أنّ درجة الحرارة المثبطة لنشاط عزلات الفيروس تقع مابين 84°م-88°م ، ودرجة التخفيف الذبائية تقع مابين 10^{-5} -10^{-6} ، كما أن بقاء الفيروس نشطا في العصارة الخام زادت عن 13 شهراً.

تم تحضير مصل مضاد لعزلة منطقة سرت واستخدم هذا المصل في تحديد العلاقة بين العزلات والتي وجد أنّ بينها علاقة واضحة وذلك باستخدام اختباري اشترلوني والليزا غير المباشر، واعتماداً على علم الامصال (Serology) فإن هذه العزلات تابعة لمجموعة التوبامو (Tobamovirus group) . وباستخدام بعض النباتات التشخيصية المفرقة بدا أن العزلات تابعة لفيروس (Tobacco mild green mosaic tobamovirus).

وأما عزلات الفيروس التابع لمجموعة (Cucumovirus) فقد نجحت في اصابة 20 نوعاً أو صنفاً نباتياً من بين 33 نباتاً تمّت دراستها بردود أفعال مختلفة ، وقد بينت الدراسة أن درجة الحرارة المثبطة لنشاط الفيروس تقع مابين 50-53°م، وأما درجة التخفيف النهائي فقد وقعت مابين 10^{-2}-10^{-3} ، ووقعت مدة بقاء الفيروس نشطاً في المعمل للعزلات الخمس بين نصف يوم إلى يوم كامل.

تمّ تحضير مصل مضاد لعزلة منطقة مصراته، والذي أستخدم لتحديد العلاقة بين عزلات الفيروس والتي أبدت جميعها تفاعلا موجباً وذلك باستخدام اختبار إليزا بنوعيه المباشر وغير المباشر (indirect & DAS-ELISA) استخدم جاما جلوبيولين المرتبط بالانزيم من (Bio-Rad kit) ، كما أبدت العزلات جميعها تفاعلا موجبا مع المصل المضاد لفيروس موزاييك الخيار(CMV) المنتج من قبل (Bio-Rad) باستخدام اختبار إليزا بنوعيه (Indirect & DAS-ELISA). وبناءً على علم الأمصال (Serology) فإن هذه العزلات تابعة لفيروس موزاييك الخيار (Cucumber mosaic virus).

V 54

SURVEY OF *CUCUMBER MOSAIC VIRUS* ON WILD TOBACCO (*NICOTIANA GLAUCA* GRAHAM) FROM DIFFERENT REGIONS IN WEST LIBYA. Mahjob A. Ejmal[1], Omar M. El-Sanousi[2] and Salah S. El-Ammari[3]. (1) Faculty of Agriculture, University of Al-Tahaddi, Sirte, Libya; (2) Faculty of Agriculture, University of Omar Al-Mukhtar, El-Beida, Libya; (3) Faculty of Agriculture, University of Garioums, Benghazi, Libya, Email: Omarelsanousi@yahoo.co.uk

Using DAS-ELISA, *Cucumber mosaic virus* (CMV) was identified as the main virus causing mosaic symptoms on *Nicotiana glauca* in 25 samples from five areas (Sirte, Misurata, El-Khoms, Tripoli and El-Zawia) in Libya. The CMV was found singly in some of the tested samples and with another *Tobamovirus* in most of them. The CMV in this study was successfully transmitted by sap to 19 species and varieties out of 32 tested. Those species were *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste & Regn., *Chenopodium quinoa* Willd., *Citrullus vulgaris* cv. Klondike, *Vicia faba* L., *Capsicum annuum* L. cv. Cayenna, *Nicotiana benthamiana* Domin, *Nicotiana glauca* R.C.Graham, *Nicotiana glutinosa* L., *Nicotiana tabacum* L. (cvs. Burley 21, Burley gold, Turkish, White Burley, Xanthi and Xanthi-nc), *Petunia hybrida* Vilm., *Physalis floridana* Rydb., *Solanum melongena* L. cv. Black Beauty, and *Solanum nigrum* L.

V 55

ISOLATION AND STUDY OF DIFFERENT ISOLATES OF A *TOBAMOVIRUS* ON WILD TOBACCO (*NICOTIANA GLAUCA* GRAHAM) IN LIBYA. Mahjob A. Ejmal[1], Omar M. El-Sanousi[2] and Salah S. El-Ammari[3]. (1) Faculty of Agriculture, University of Al-Tahaddi, Sirte, Libya; (2) Faculty of Agriculture, Omar Al-Mukhtar University, El-Beida, Libya; (3) Faculty of Agriculture, University of Garioums, Benghazi, Libya, Email: Omarelsanousi@yahoo.co.uk

Nicotiana glauca commonly shows mosaic symptoms along the coastal region of Libya. To identify the causal virus, 25 samples were randomly collected from five different areas in the western part of the coastal belt region. Using ELISA, one isolate showed the presence of *Tobacco mild green mosaic virus* alone, whereas in several samples this virus was found together with CMV. Symptomology and host range studies supported the identification of TMGMV by ELISA.

جامعة التحدي
كلية الزراعة
قسم الإنتاج الباتي

تعريف الفيروسات المسببة للتبرقش على نبات التبغ البري (*Nicotiana glauca* Graham) في الساحل الغربي من ليبيا

مقدمة من الطالب
محجوب علي محمد إجمال

نوقشت هذه الأطروحة بتاريخ 11-02 – 2007 وأجيزت

أعضاء اللجنة:

د. عمر موسى السنوسي (مشرفا):.....

د.صلاح سعيد العماري (مشرفاً مساعداً):.....

د. حسني علي يونس (ممتحناً خارجياً):.....

د.عبد الناصر عبد العال جلال (ممتحنا داخلياً):.....

يعتمد:

د. محمد الدراوي الغايب
أمين اللجنة الشعبية

د. عاطف سيد أحمد شحاته
.....
مكتبة الدراسات العليا
كلية الزراعة

جامـــعـــة التـــحـدي
كـــلـيـــة الـــزراعـــة
قـــسم الإنتاج النبـــاتي

تعريف الفيروسات المسببة للتبرقش على نبات التبغ البري (*Nicotiana glauca* Graham) في الساحل الغربي من ليبيا

مقدمة من:

محجوب علي محمد إجمال

اشراف:

د. عمر موسى السنوسي **أ. د. صلاح سعيد العماري**

قدمت هذه الأطروحة استكمالا لمتطلبات الإجازة العالية (الماجستير) في **أمراض النبات**

2007