

# EXPERIMENTAL RESULTS

## 1- Isolation and Symptomatology:

The isolated virus was isolated for the first time from pepper (*Capsicum annuum* L. Balady) crops, during this study, in Qalubia, Menofya, and Sharkia governorates. The virus causes severe mosaic whose main field symptoms are characterized by necrotic spots on the stems, fruits and leaves, followed by premature foliar abscission. The leaves were developing subsequently in the defoliated plants, and especially the top leaves, show severe mosaic (Fig., 1).

The crude sap of the virus isolate used in this study was obtained from naturally infected pepper plants showing severe mosaic symptoms and grown in different areas at Qalubia, Menofya, and Sharkia Governorates. The infectious sap was mechanically inoculated on 600 mesh-dusted carborundum *Chenopodium amaranticolor* Coste & Ryn. and *C. quinoa*, *Datura stramonium* Jimson, *Solanum tuberosum* L. "hybrid A6" as diagnostic host plants. Inoculated plants were kept in an insect-proof greenhouse. Chlorotic local lesions were observed on *C. amaranticolor* and *C. quinoa* (20 and 9 days of inoculation, respectively). Single lesions were cut out and macerated on a glass-slide and inoculated onto the former diagnostic hosts. Extracts obtained from the reformed local lesions were used to inoculate *Capsicum annuum* L. "California Wonder", which served as the source of virus inoculum throughout this study.

Symptoms induced by the virus isolate in experimentally inoculated pepper plants (*C. annuum* L. cv. "California Wonder") maintained in a greenhouse were similar to those expressed by filed plants (Fig. 2).



**Fig. (1):** Naturally infected leaves and fruits of pepper (*Capsicum annuum* L.) cv. Balady hot, used as initial source of the virus isolate.



**Fig. (2):** Symptoms induced by isolated virus in experimentally inoculated pepper leaves (*C. annuum* L. cv. "California Wonder").

## 2- Host range of the tested virus isolate:

The host range of isolated virus was shown in **Table (1)**. Of the 25 plant species or cultivars belonged 4 families were mechanically inoculated with this virus, 18 species or cultivars (belonging 3 families, i.e., *Amaranthaceae*, *Chenopodiaceae*, and *Solanaceae*) were infected under greenhouse conditions.

Five test plants per pot (30 cm in diameter) were inoculated mechanically at the cotyledonary or four- to eight-leaf stage (according to the species) and were kept in an insect-proof glasshouse. Plants without subsequent symptoms were inoculated again after 15-20 days. Uninoculated plants were included as controls.

Virus symptoms were observed for a long period at regular intervals. In order to check symptomless plants and the virus in plants with symptoms, back inoculations were made on *Chenopodium quinoa*.

Susceptibility of the tested hosts differed in their symptoms according to the species and cultivars as follows:

Necrotic or chlorotic local lesions without systemic infection appeared on *Amaranthus ascendens* Lois. *Chenopodium amaranticolor* Coste & Ryn., and *C. quinoa* Willd. (**Fig., 3, A, B, C & D**).

Whereas, *Nicotiana debneyi* Domin; *N. rustica* L., and *N. tabaccum* "White Burley" were reacted with local lesions followed by systemic infection.

On the other hand, systemic symptoms only (according to host plant species or cultivars) were appeared on *Capsicum annuum* L. cvs. "Balady Hot, Balady Sweet, California Wonder", *C. frutescens* "Tabasco" *Datura metel* L., *N. glutinosa* L., *N. clevelandii* Gray, *N. tabacum* "Samsun and Xanthi-nc" *Petunia hybrida* Vilm., *Physalis floridana* Rydi. and *Solanum nigrum* L. (Fig., 3 E, F, G & H).

On the contrary, hosts showing no symptoms with negative back inoculation tests were: *Gomphrena globosa* L. (*Amaranthaceae*), *Beta vulgaris* L. cv. "Raspoly", (*Chenopodiaceae*), *Phaseolus vulgaris* L. cv. "Pinto", *Vigna unguiculata* (L.) Waip. cv. "Blackeye" (*Leguminosae*), *Lycopersicum esculentum* Mill, *Datura stramonium* L. and *Solanum tuberosum* L. cv. hybrid A6 (*Solanaceae*).

Clear necrotic local lesions without systemic infection appeared on *Amaranthus ascendens* Lois this first record for this host may serve later as indicator or differential host plant.

**Table (1):** The host range of the isolated virus. Results of the inoculations onto selected indicator plants.

Families	Indicator species	Symptoms
Amaranthaceae	<i>Amaranthus ascendens</i> Lois. <i>Gomphrena globosa</i> L.	NLL NS
Chenopodiaceae	<i>Beta vulgaris</i> L. cv. Raspoly <i>Chenopodium quinoa</i> Willd. <i>C. amaranticolor</i> Coste & Ryn.	NS CLL CLL
Leguminosae	<i>Phaseolus vulgaris</i> L. cv. Pinto <i>Vigna unguiculata</i> L. cv. Blackeye	NS NS
Solanaceae:	<i>Capsicum annuum</i> L. cv. Balady hot cv. Balady sweet cv. California Wonder <i>C. frutescens</i> cv. Tabasco <i>Datura metel</i> L. <i>D. stramonium</i> L. <i>Lycopersicum esculentum</i> Mill. <i>Nicotiana clevelandii</i> Gray <i>N. debneyi</i> Domin. <i>N. glutinosa</i> L. <i>N. rustica</i> L. <i>N. tabacum</i> L. cv. Samsun cv. White Burley cv. Xanthi <i>Petunia hybrida</i> Vilm. <i>Physalis floridana</i> Ryd. <i>Solanum nigrum</i> L. <i>Solanum tuberosum</i> L. cv. Hybrid A6	VC, MM, LD, SG VC, SM, LD, SG SM, VB, LD, SG MM MM, LA NS NS VC, SM, SG NLL/SM M, LD CLL/SM  VC, M CLL/SM VC, M MM, LD MM M NS

MM=Mild mosaic, SM=Severe mosaic, LD=Leaf distortion, CLL=Chlorotic local lesions, NLL=Necrotic local lesions, SG=Stunting growth, LA=Leaf abscission, LD= Leaf deformation, VC=Vein clearing, and NS=No symptoms

### 3- Physical Properties:

The results of the *in vitro* virus property tests are shown in Table (2) as follows:

#### A- Thermal inactivation point (TIP):

Infectious sap extracted from *Capsicum annuum* L. "California Wonder" as a source of the virus, was used to determine thermal inactivation point of the present virus isolate. Inoculum was heated to 55, 60, 65, 70, 75, 80, 85, 90 and 95°C for 10 minutes. Treated and untreated sap was tested on leaves of *C. quinoa* as an indicator plant.

The obtained results were cleared that, the virus isolate was inactivated at 80°C but not at 75°C after 10 min.

#### B- Dilution end point (DIP):

Several dilutions up to  $10^{-9}$  was prepared from infectious sap of *C. annuum* L. "California Wonder" leaves. Each dilution was separately inoculated on leaves of *C. quinoa* as local lesion an indicator plant.

Results of trials showed that, the infectivity of the present virus isolate was preserved at dilution between  $10^{-6}$  and  $10^{-7}$ .

#### C- Longevity *in vitro* (LIV):

In regard to effect of storing at room temperature (25°C) on the infectivity of the virus isolate in crude sap extracted from *C. annuum* L. cv. "California Wonder", was determined. Obtained data indicated that, the present virus isolate kept its infectivity for a period between 28-35 days on *C. quinoa* plants.

Table ( 2 ): Physical properties of isolated virus *in vitro* (under laboratory conditions).

Stability <i>in vitro</i>	Treatments and Results												
	45	50	55	60	65	70	75	80	85	90			
Thermal inactivation point (TIP) (°C)	+	+	+	+	+	+	+	--	--	--			
Longevity <i>in vitro</i> (LIV) (days)	1	2	3	4	5	6	7	14	21	28	35	45	
	+	+	+	+	+	+	+	+	+	+	--	--	
Dilution end point (DEP)	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-8</sup>	10 <sup>-9</sup>				
	+	+	+	+	+	+	--	--	--				



#### **4- Mode of Transmission:**

##### **A-Aphid transmission:**

The isolated virus was readily transmitted by *Myzus persicae* Sulzer, from infected pepper (*C. annuum* L. 'California Wonder') source plants to healthy *C. quinoa* in a non-persistent manner.

##### **B- Seed transmission:**

Seeds were harvested from seriously infected pepper plants (*C. annuum* L., 'California Wonder') with the isolated virus. No symptoms were observed in the obtained seedlings. To check for the presence of the isolated virus, inoculum were obtained from infected *C. annuum* L. cv. 'California Wonder' seedlings and inoculated to *C. quinoa* plants. No symptoms were observed on the tested plant.

#### **5- Virus purification:**

The final pellet, during highly purified preparations of the isolated virus, was resuspended in a minimum volume of the extraction buffer and was used for further studies.

The infectivity of the purified isolated virus was tested biologically using *Chenopodium quinoa* by using concentrated and diluted ( $10^{-1}$ ) suspensions. Clear chlorotic local lesions were

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observed 7-days after inoculation with highly purified isolated virus, either concentrated or diluted.

Fractions collected at the end of the rotor were monitored for absorbance at 280 and 260 nm using spectrophotometer to check the presence and concentration of virus. The results showed that, the ratio of  $A_{\min}/\max A_{260/280}$  was 1.23. While, the ratio of  $A_{\max}/\min A_{280/260}$  was 1.11 with average 1.17 (Fig., 4). This mean that high yield of purified homologous virus particles was obtained

## 6- Electron Microscopy:

Electron microscopic examination of the purified preparation of the isolated virus, negatively staining with uranyl acetate (2%) pH 4, showed the presence of filamentous flexuous virus particles. (Fig., 5).

Results of the estimation of the particle length and width showed that obtained particles measured averaged 760 nm length and 15 nm width (Fig., 6).

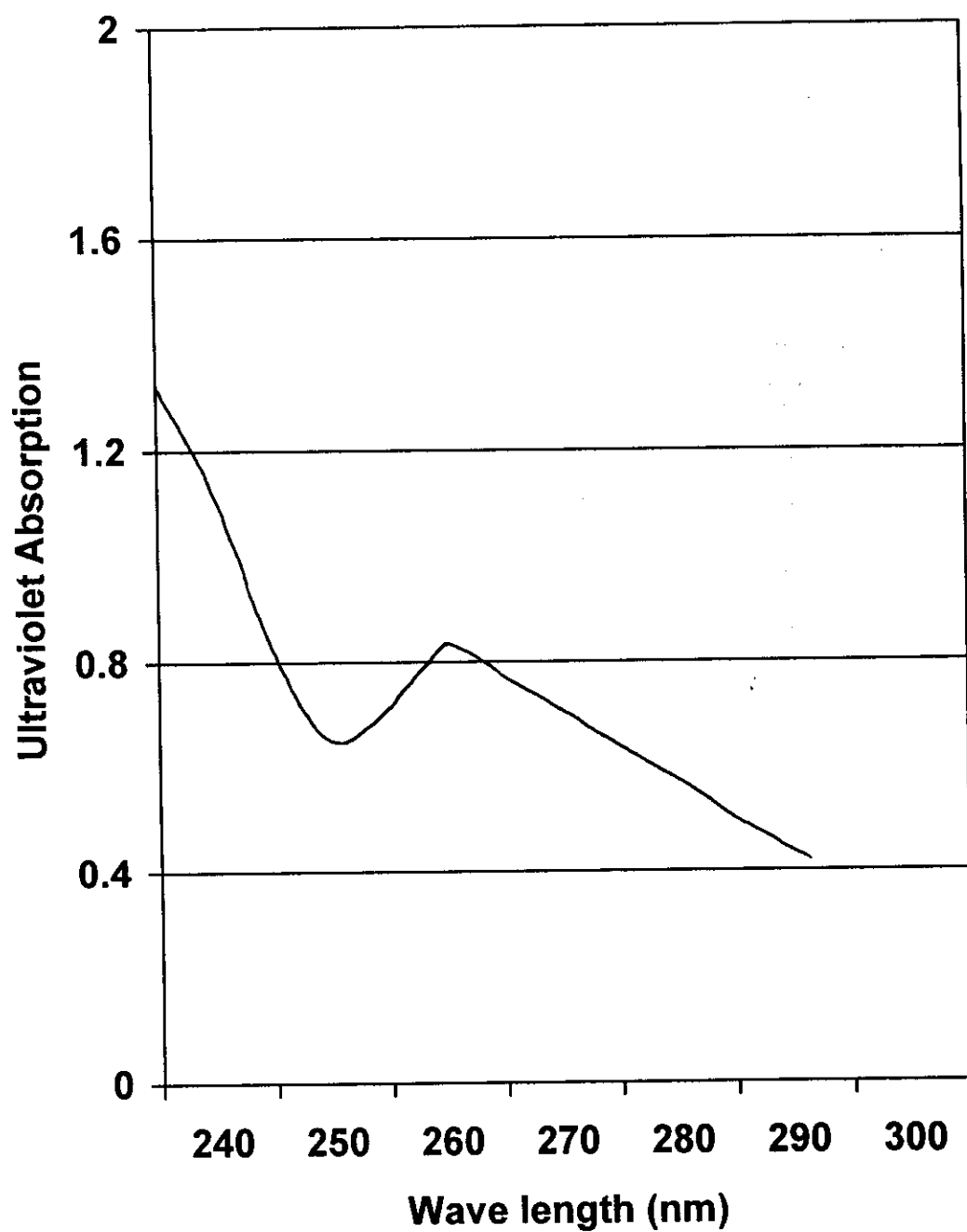
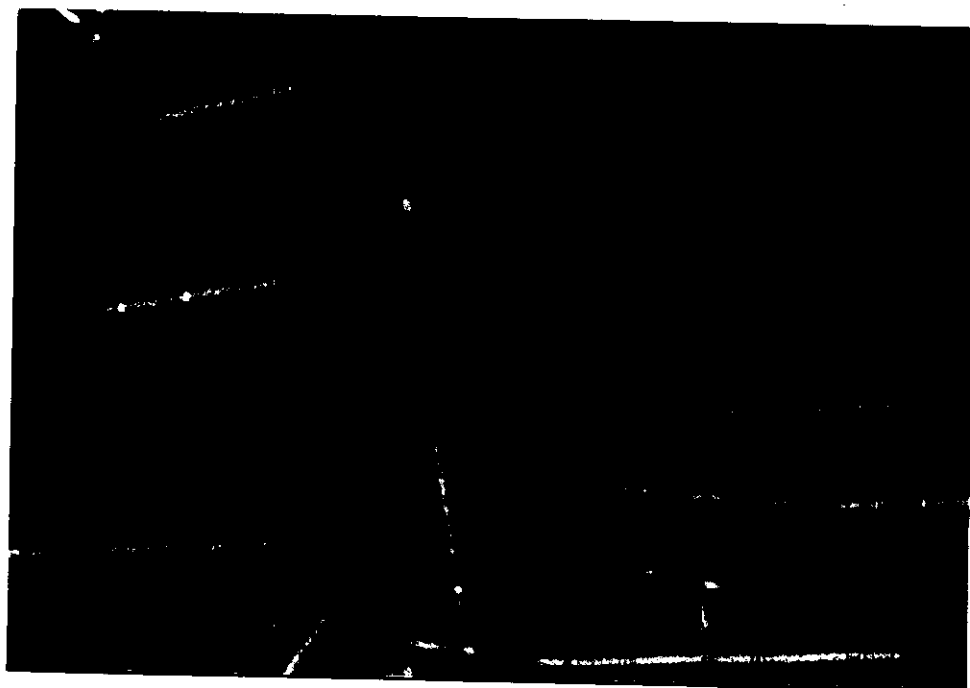
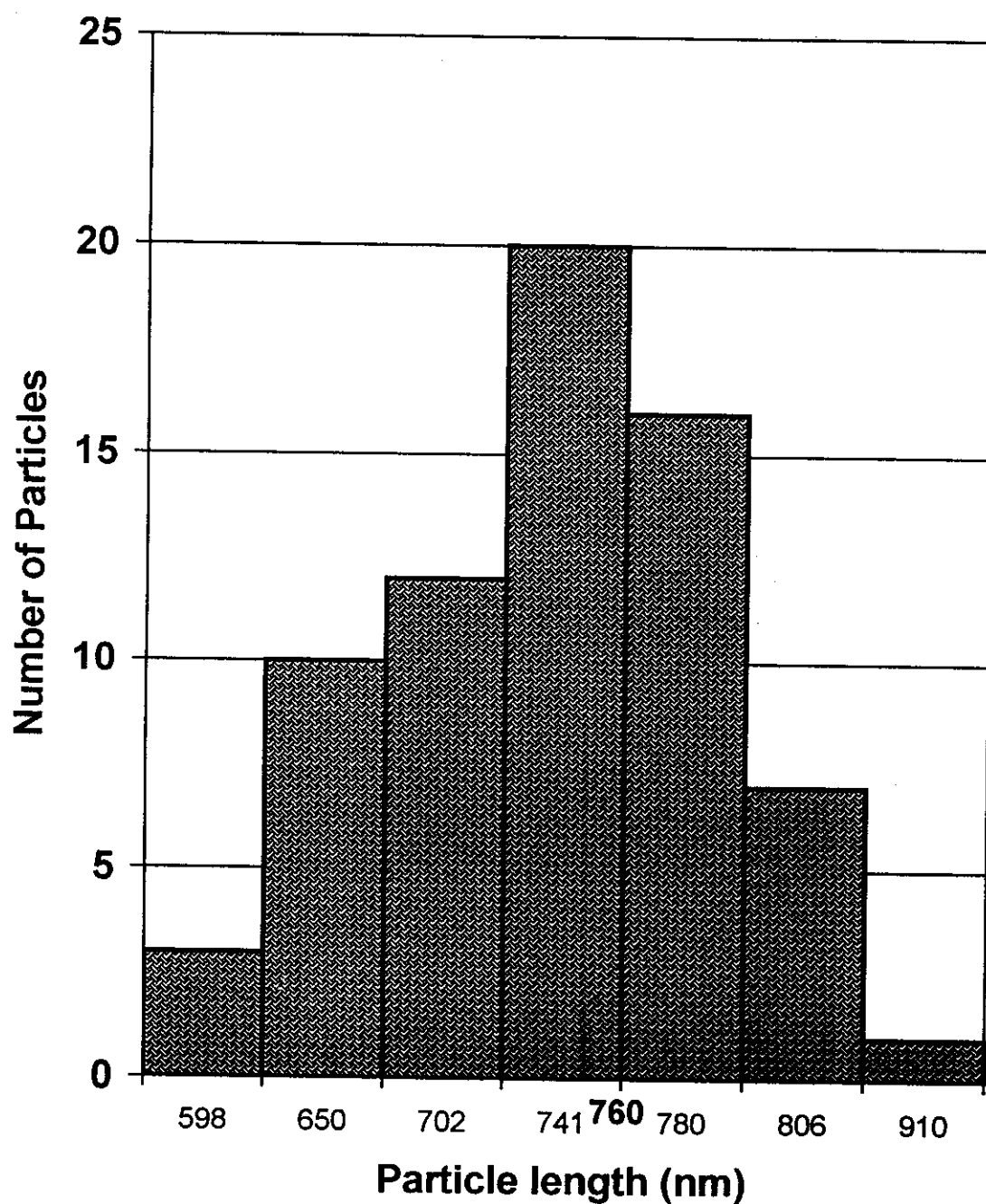


Fig. (4): Ultraviolet spectrum of the purified preparation of the isolated virus obtained from systemically infected pepper (*Capsicum annuum* L. cv. California Wonder) plants.



**Fig. (5):** Electron micrograph of the purified preparation of the isolated virus from systemically infected pepper (*Capsicum annuum* cv. California Wonder) plants, after staining with 2% uranyl acetate, pH 4.0 (X 81420).



**Fig. (6):** Average particle length distribution of purified virus preparation from systemically infected pepper (*Capsicum annuum* cv. California Wonder) plants, stained with 2% uranyl acetate, pH 4.0

## **7- Serological Studies:**

### **A- Precipitation test:**

Tube precipitin test carried out to determine serological affinity of the isolated virus. Antisera of some Potyviruses, *i.e.*, pepper severe mosaic (PSMV) from Argentina, pepper veinal mottle virus (PVMV) from Nigeria, potato virus Y (PVY) from Germany, and tobacco etch virus (TEV) from Finland kindly obtained and used for serologically identification tests. Results indicated that, specific antiserum of the isolated virus strongly reacted with clear density precipitin with its antigen. Antigens of the isolated virus also reacted, with the same strong, with the antiserum of the PSMV (from Argentina). Reaction, less strong, was observed between isolated virus antigen and antisera of both PVY (Germany) and TEV (Finland). No precipitin observed between antigen of isolated virus and antiserum of PVMV (Nigeria).

### **B- Ouchterlony gel Immunodiffusion test:**

In this test the antibody-antigen reaction is carried out in a gel agarose plates. The reactants are allowed to diffuse through the gel and combine.

Results illustrated in Fig. (7) clearly showed that, precipitin bands between antigen of the isolated virus (partially purified

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virus), and specific antiserum, produced against isolated virus, were sharp and homologous without any cross in the end, meaning that, there were strong serological relationship between both. The same properties between isolated virus antigens and antibodies of PSMV imported from Argentina. Meanwhile, diffused and weak were induced between isolated virus antigen and antibodies of both PVY and TEV imported from Germany and Finland, respectively. On the other hand, No reactions observed between isolated virus antigen and antibodies of PVMV imported from Nigeria, or five Tobamoviruses imported from Holland.

Serological relationship confirmed between isolated virus (V) antigen and antisera of isolated virus (S), PSMV (P) PVY (Y), and TEV (H). No reaction observed between isolated virus antigen and antisera of PVMV (1), and Five Tobamoviruses [PMMV-P11 (2), PMMV-SL (3), ToMV (4), TMV-WU1 (5) and PepMV-3 (6)]. Central wells contained isolated virus antigen (V). No materials were added in (0) peripheral wells.

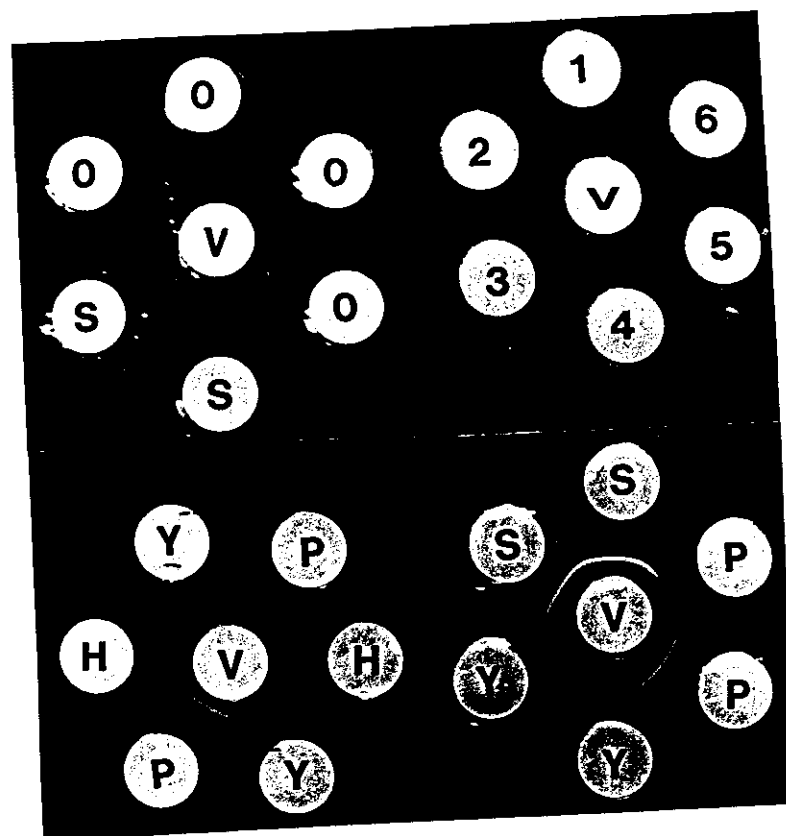
Obtained results indicated that, the isolated virus was related serologically to Potyviruses group. This may lead, to concede the isolated virus as a member of the plant Potyviruses.

**Table (3):** Serological tube precipitation reactions of isolated virus against specific antisera for PSMV, PVY, TEV, and PVMV.

Antigen Dilutions	Antibodies (specific antisera) of				
	Isolated virus	PSMV	PVY	TEV	PVMV
1 : 2	++++	++++	+++	+++	---
1 : 4	++++	++++	+++	+++	---
1 : 8	++++	+++	++	++	---
1 : 16	+++	+++	++	++	---
1 : 32	+++	+++	++	++	---
1 : 64	+++	++	+	+	---
1 : 128	+++	++	+	±	---
1 : 256	++	++	±	---	---
1 : 512	++	+	---	---	---
1 : 1024	+	±	---	---	---
Control (saline solution)	---	---	---	---	---

-- = No reaction; ± = Rarely visible precipitation; + = Slight precipitation; ++ = Moderate precipitation; +++ = Heavy precipitation and ++++ = Very heavy precipitation.





**Fig. (7):** Gel Immunodiffusion SDS-test showing the serologically relationships between antigen of the isolated virus (V) and antisera against of isolated virus (S), PSMV (P) PVY (Y), and TEV (H). No materials were added in (0) peripheral wells. Five Tobamoviruses (2, 3, 4, 5 & 6).

**8- Antiviral activity of some selected healthy medicinal plant extracts on the incidence of virus infection under greenhouse conditions:**

Leaves, flowers and fruits of 12 selected medicinal plant (Fig. 8) (belonging 10 families and containing different active integrated compounds) extracts were used to study the inhibitory activities (as antiviral agents), on systemic virus infection. Inhibition effect of tested plants was determined as the percentage of local lesion number on infected *N. debenyi* plants out of 12 healthy inoculated seedlings.

Four leaves of 3 test seedling plants (*Nicotiana debenyi* as local lesion host) were treated with crude sap of medicinal plants and inoculated with virus inoculum. One hundred and eight *N. debenyi* seedlings were divided into three groups and treated with sap of medicinal plants as follows:

a)- One day prior to the inoculation with the virus (pre-inoculation application).

b)- One day after inoculation with the virus (post-inoculation application).

c)- Crude sap mixed with virus inoculum and immediate inoculation (mixed application).

Equal number of *N. debenyi* seedlings plants were sprayed with distilled water and inoculated as mentioned with virus inoculum and kept to serve as control. Appropriately amount of distilled water was mixed with equal amount of virus inoculum for the mixed application.

**Figure (8):** Properties of selected medicinal plants used for antiviral activity against the isolated virus infection.



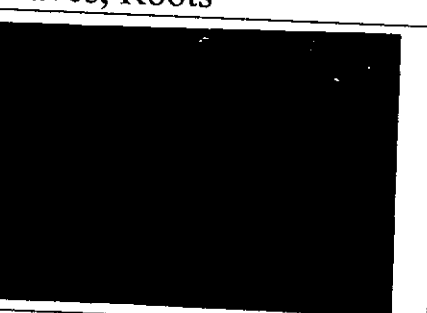
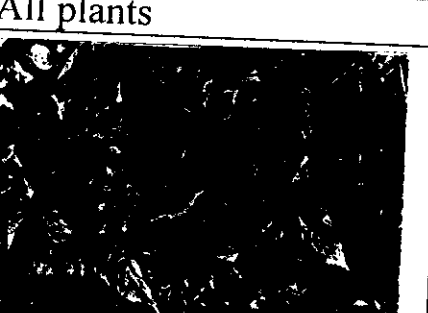
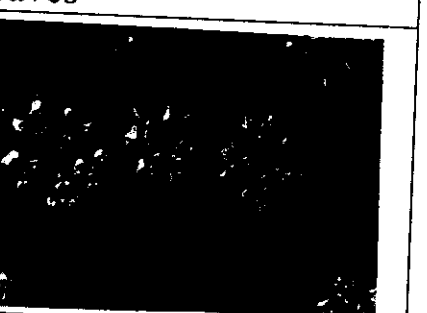
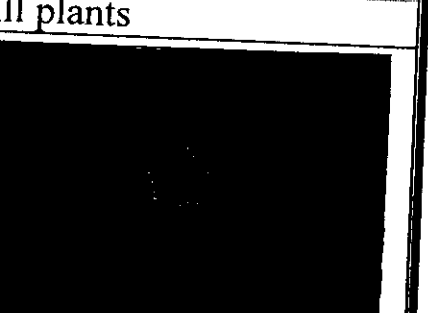

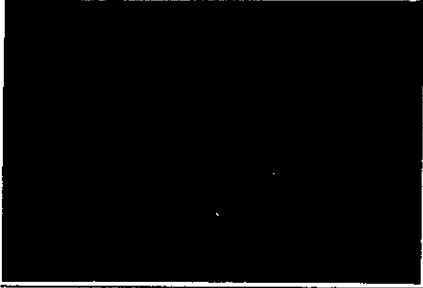




Picture		
<b>Family:</b>	Acanthaceae	Apocynaceae
<b>S. N.:</b>	<i>Adhatoda vasica</i>	<i>Vinca rosea</i>
<b>C. N.:</b>	Malabar nut tree	Vinca, Periwinkle
<b>A. I.:</b>	Alkaloids, volatile oils	Alkaloids (Vincristine)
<b>U. P.:</b>	Leaves, Roots	All plants
Picture		
<b>Family:</b>	Chenopodiaceae	Euphorbiaceae
<b>S. N.:</b>	<i>Chenopodium amaranticolor</i>	<i>Acalypha fruticosa</i>
<b>C. N.:</b>	Worm seed	Copper leaf
<b>A. I.:</b>	Alkaloids (Ascaridole)	Pigments (Hisbidine)
<b>U. P.:</b>	Leaves	All plants
Picture		
<b>Family:</b>	Euphorbiaceae	Euphorbiaceae
<b>S. N.:</b>	<i>Euphorbia peplus</i>	<i>Euphorbia pulcherrima</i>
<b>C. N.:</b>	Wild purslane	Easter flower
<b>A. I.:</b>	Toxic (Phorbol)	Flavonoids (Kaempferol)
<b>U. P.:</b>	All plants	All plants

Figure (8): Continued

Picture		
Family:	Geraniaceae	Labiatae
S. N.:	<i>Pelargonium zonale</i>	<i>Salvia officinalis</i>
C. N.:	Horse-shoe geranium	Common sauge
A. I.:	Essential oil (Geraniol)	Terpene hydroxide
U. P.:	All plants	Leaves
Picture		
Family:	Malvaceae	Meliaceae
S. N.:	<i>Hibiscus rosa sinensis</i>	<i>Azadirachta indica</i>
C. N.:	Shoe flower	Margosa tree
A. I.:	Glycosides (Saponins)	Limonoid (Azadirachtin)
U. P.:	Leaves, Flowers	Leaves, Fruits, Cortex
Picture		
Family:	Solanaceae	Verbenaceae
S. N.:	<i>Datura metel</i>	<i>Lantana camara</i>
C. N.:	Downy thorn apple	Lantana
A. I.:	Alkaloids (Hyoscyamine)	Triterpenes
U. P.:	Leaves	All plants

S.N.= Scientific Name, C.N.= Common Name, A.I.= Active ingredients,  
and U.P.= Used Parts.

Treated plants were kept under insect proof house and observed for the appearance of local lesion formation.

Percentages of the inhibitory effect were transformed to the arcsine. The arcsine percentages of the inhibitory effect of medicinal plant extracts were subjected to the proper analysis of variance.

Data presented in **Table (4) and Fig. (9)**, showed that leaves extract of *Chenopodium amaranticolor* gave the superior antiviral activity either applied pre- (99.3%), mixed (98.9%) or post-inoculation (97.1%) with isolated virus. Anterior petals extract of *Hibiscus rosa sinensis* was second with the same applications (98.3% pre-, 97.7% mixed & 94.5% post-inoculation with isolated virus). Strong inhibitory effect was induced by the leaf extract of *Vinca rosea* (95.3%, 94.7% & 90.0% for pre-, mixed & post-inoculation with isolated virus).

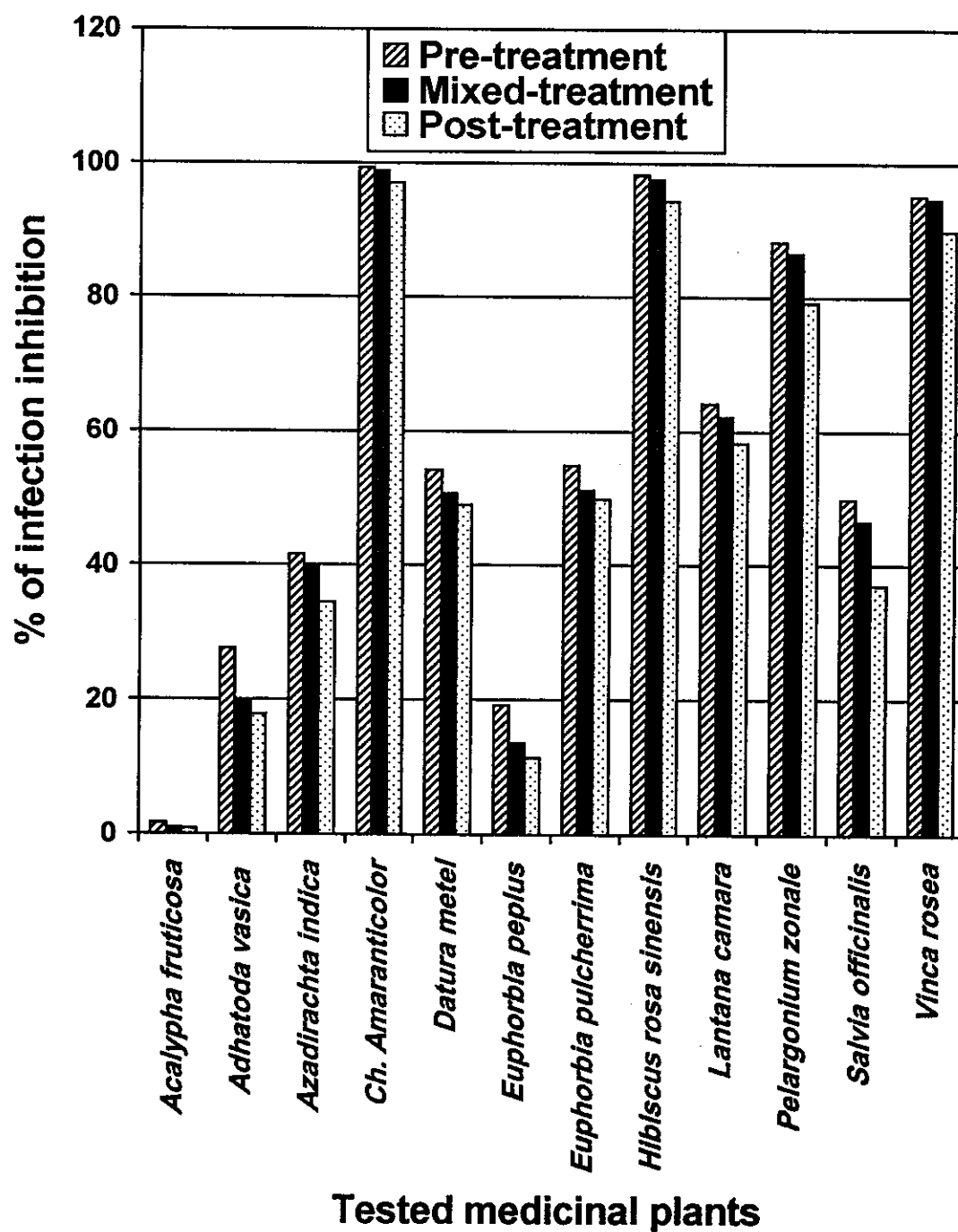
Tip and expanded leaves extract of *Pelargonium zonale*, *Lantana camara*, *Euphorbia pulcherrima*, *Datura metel*, *Azadirachta indica*, and *Adhatoda vasica* gave antiviral activity as descending manner, while applied pre-, mixed or post-inoculation with isolated virus (88.4, 86.7, 79.2%; 64.2, 62.1, 58.3%; 54.9, 51.1, 49.9%; 54.2, 50.6, 48.9%; 41.6, 39.9, 34.4% and 27.6, 20.1, 17.8%, respectively).

Extract of expanded leaves of *Acalypha fruticosa* showed the lowest inhibition effect while applied as mentioned before (1.7, 0.9 and 0.8%, respectively).

Generally, pre-inoculation applications gave the best protection against infection with isolated virus. Mixed application was the second, while post-inoculation applications were less effective in the same trend.

**Table ( 4 ):** Effect of the extract of some medicinal plants (as antiviral agents) on the inhibition of isolated virus infection (percent of inhibition).

Test plants	Part used	Relative inhibition (%)		
		Pre-treatment	Mixed-treatment	Post-treatment
<i>Acalypha fruticosa</i> Forsk	Leaves	1.7	0.9	0.8
<i>Adhatoda vasica</i> Ness	Leaves	27.6	20.1	17.8
<i>Azadirachta indica</i> A. Juss	Fruits	41.6	39.9	34.4
<i>Chenopodium amaranticolor</i> Coste & Reyn.	Leaves	99.3	98.9	97.1
<i>Datura metel</i> L.	Leaves	54.2	50.6	48.9
<i>Euphorbia peplus</i> L.	Leaves	19.2	13.6	11.5
<i>Euphorbia pulcherrima</i> Willd.	Leaves	54.9	51.1	49.9
<i>Hibiscus rosa sinensis</i> L.	Flowers	98.3	97.7	94.5
<i>Lantana camara</i> L.	Leaves	64.2	62.1	58.3
<i>Pelargonium zonale</i> L'Hérit	Leaves	88.4	86.7	79.2
<i>Salvia officinalis</i> L.	Leaves	49.8	46.4	36.9
<i>Vinca rosea</i> L.	Leaves	95.3	94.7	90.0



**Fig. (9):** The inhibition percentages of virus-infection by isolated virus as affected by the extract of some medicinal plants (as antiviral agents).

percentages of infection of each cultivar were calculated, transformed to arcsine then subjected to the proper analysis of variance.

Data obtained were recorded in **Table ( 5 ) and Fig. (10)**. The results showed that, all tested pepper cultivars were susceptible, in different categories, to mechanical inoculation with the present virus isolate. Statistical analysis revealed that there were highly significant differences between cultivars.

Yellow Banana cv. (Sweet pepper) among tested cultivars was more susceptible to infected with isolated virus (96.25%), then California Wonder (93.75%), Marconi (85.00%) and Gedion (58.75%). On the other hand, Serrano cv. (Hot pepper) showed lowest rate of infection with isolated virus (6.25%), then Cayenne Large (13.75%), Anheium (15.00%), Pical (22.50%).

Generally it could be concluded that, sweet pepper cultivars are more susceptible to artificial infection with the isolate tested virus than hot ones.

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**Table ( 5 ) : Response of some commercial hot and sweet pepper plants to infection with isolated virus under greenhouse conditions**

Replicates	Percentages of infection									
	Hot pepper cultivars					Sweet pepper cultivars				
	Anheium	Cayenne Large	Pical	Serrano	California Wonder	Gedeon	Marconi	Yellow Banana		
Block 1	10	10	25	5	95	65	85	95		
Block 2	15	20	25	10	90	60	90	95		
Block 3	20	15	20	5	95	50	85	100		
Block 4	15	10	20	5	95	60	80	95		
Mean	15.00	13.75	22.50	6.25	93.75	58.75	85.00	96.25		

L.S.D. at 0.05 = 5.555

L.S.D. at 0.01 = 7.557

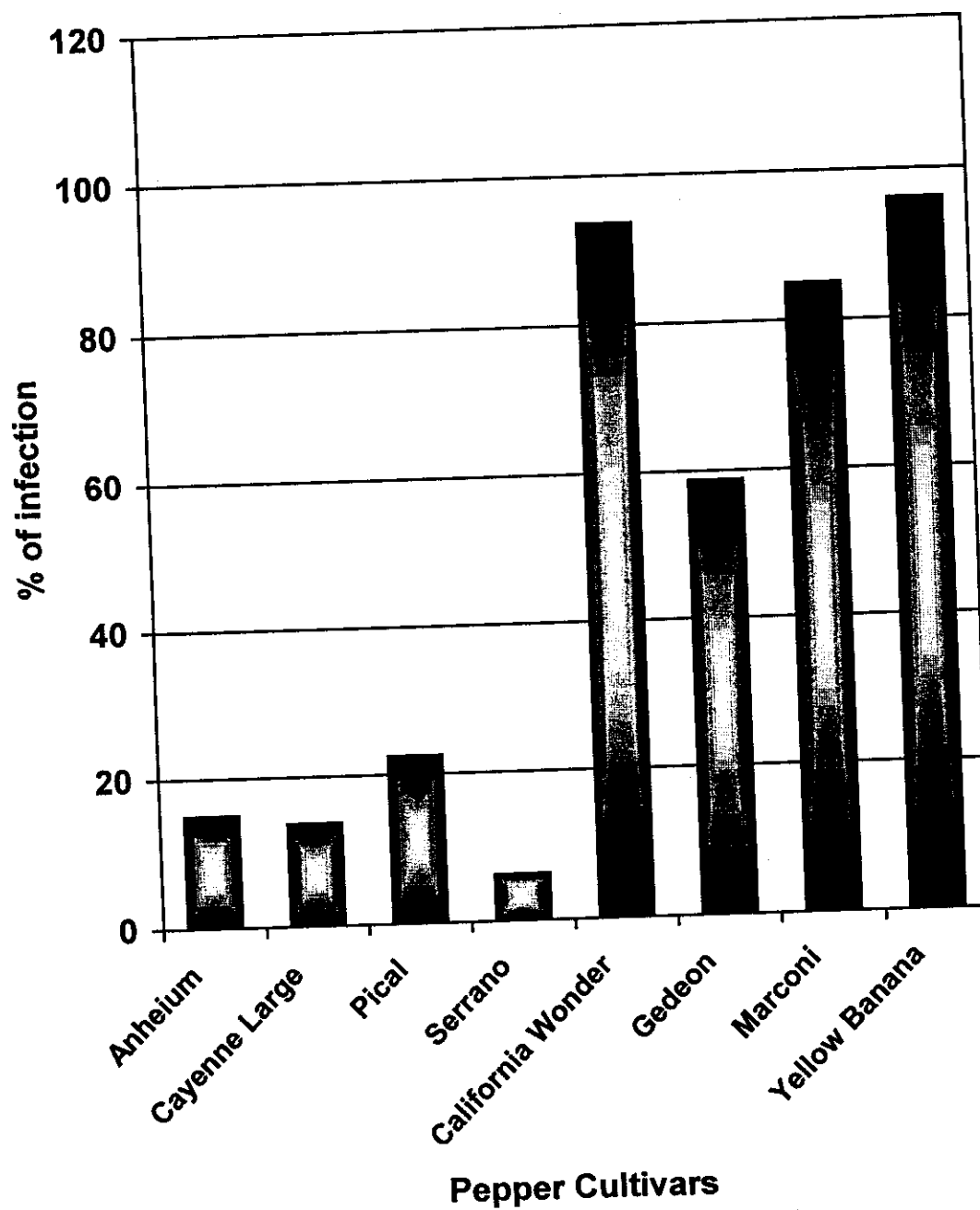


Fig. (10): Response of some tested pepper cultivars to infection with the isolated virus under greenhouse conditions.

## **10- Determination of the distribution and severity of natural infection with the seemed like tested virus in some Governorates:**

Screening for distribution and severity of natural infection with the tested isolated virus was carried out during summer season (July-September), 1997 including some Northern Egyptian Governorates (*e.g.*, El-Behera, Dakahlia, Domiat, Gharbia, Giza, Ismailia, Kafr El-Sheikh, Menofyia, Qalubia, and Sharkia). Upper Egypt governorates are not included in this screening because there is not enough cultivated *Capsicum* plants. Young leaves and fruits of pepper plants (commercial lines and other *Capsicum* species and varieties, sweet and hot) naturally-infected with typical potyvirus symptoms from different fields were collected and investigated for the virus isolate. Three samples from different ten locations for each governorate were taken.

Data including pepper cultivated area (feddan), production quantity (ton) and productivity (ton/feddan) during 1997 for the ten screened governorates were obtained from the Agriculture Economic Reports (1997), Ministry of Agriculture, Cairo, Egypt.

Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) technique in indirect mode used for further indexing and identification of the concerned virus in crude sap extracted from infected leaves of the diseased pepper plants were performed at Agricultural Genetic Engineering, Research Institute (AGERI), Giza, Egypt.

DAS-ELISA detection obtained results could be correlated with those data to study the effect of virus distribution in the Northern Egyptian Governorates on the productivity of pepper crop.

Results recorded in the Table ( 6 ) revealed that there were reduction in the productivity of pepper crop in some surveyed governorates may be due to severity of isolated virus infection in these governorates. Meanwhile, there were no relation between severity infection and productivity in the other governorates.

Although El-Behera governorate gave the highest quantity of pepper fruiting (42999 ton), but reduction in its productivity (5.64 ton/feddan) was noticed and may be due to severity of isolated virus infection. On the contrary, the productivity of Kafr El-Sheikh and Dakahlia governorates was best (7.16 and 6.99 ton/feddan), with no detection to the isolated virus.

Qalubia governorate gave the highest productivity in the Egypt (10.93 ton/feddan), but showed low level of isolated virus infection. In this trend, Gharbia and Giza governorates were came in the next (8.5 and 7.69 ton/feddan).

**Table ( 6 ):** Results of survey for PSMV in some Northern Egyptian provinces using DAS-ELISA test compared with the pepper productivity (ton/feddan) during summer season (1997).

Governorates	1997 (summer season)			
	ELISA results	Ton	Feddan	Productivity (ton/feddan)
El-Behera	++++	42999	7627	5.64
Dakahlia	—	930	133	6.99
Domiat	—	268	57	4.70
Gharbia	+	2091	246	8.50
Giza	++	20254	2633	7.69
Ismailia	++++	13511	2125	6.36
Kafr El-Sheikh	--	1390	194	7.16
Menofyia	+++	2588	295	8.77
Qalubia	+	8562	783	10.93
Sharkia	+++	22495	3492	6.44
Total of Egypt		240034	37833	6.34

++++ = Severe infection

+++ = High infection

++ = Moderate infection

+ = Low infection

-- = No infection

### **11- Determination of Capsiacin and Vitamin C in the natural infected pepper plants:**

Vitamin C (ascorbic acid) content was determined in both sweet and hot green mature fruit samples naturally virus-infected and healthy fruit samples (mg/100g fresh-weight) collected from field in the surveyed governorates using 2,6-dichlorophenol indophenol dye titration method.

Capsaicinoids (pungent alkaloids) content, as the major compound of them "capsaicin", was determined in hot green mature naturally virus-infected fruit samples (mg/100g dry-weight) collected from field in the surveyed governorates using liquid chromatographic method.

Obtained resulted clearly showed that, there were the inverse correlation between virus-infection and both ascorbic acid and capsaicin content, i.e. there were markedly to slight reduction in the content of ascorbic acid and capsaicin observed in the samples according to severity to slight infection.

Slight significant reduction observed in the ascorbic acid content of hot or sweet fruit samples collected from infected plant. But, non-significant recorded in the ascorbic acid content of hot or sweet fruit samples collected from healthy ones (**Table, 7) and Fig. (11).**

Capsaicin content showed significant reduction in the hot fruit samples collected from infected plants. However, capsaicin content were significantly high in fruit samples collected from healthy ones (**Table, 8) and Fig. (12).**

**Table (7):** Vitamin C content (mg/100 g fresh weight base) in the healthy and naturally infected hot and sweet pepper fruit samples collected from different ten Northern Egyptian Governorates.

Governorates	Hot peppers		Sweet peppers	
	Healthy	Infected	Healthy	Infected
<b>El-Behera</b>	<b>1221*</b>	<b>802.0*</b>	<b>1475</b>	<b>890.1</b>
<b>Dakahlia</b>	<b>1234</b>	<b>932.4</b>	<b>1532</b>	<b>1007.4</b>
<b>Domiat</b>	<b>1051</b>	<b>781.2</b>	<b>1356</b>	<b>985.6</b>
<b>Gharbia</b>	<b>1164</b>	<b>756.3</b>	<b>1485</b>	<b>891.3</b>
<b>Giza</b>	<b>1321</b>	<b>801.8</b>	<b>1652</b>	<b>811.5</b>
<b>Ismailia</b>	<b>1335</b>	<b>851.6</b>	<b>1541</b>	<b>586.7</b>
<b>Kafr El-Sheikh</b>	<b>1033</b>	<b>764.3</b>	<b>1530</b>	<b>1128.3</b>
<b>Menofya</b>	<b>1021</b>	<b>771.1</b>	<b>1428</b>	<b>472.0</b>
<b>Qalubia</b>	<b>1258</b>	<b>791.1</b>	<b>1489</b>	<b>655.4</b>
<b>Sharkia</b>	<b>1135</b>	<b>799.5</b>	<b>1387</b>	<b>460.3</b>
<b>L.S.D. at 0.05</b>	<b>N.S.</b>	<b>71.897</b>	<b>N.S.</b>	<b>57.521</b>
<b>L.S.D. at 0.01</b>	<b>N.S.</b>	<b>95.500</b>	<b>N.S.</b>	<b>76.410</b>

\*Mean of 10 replicates as samples collected from 10 different locations for each governorates.  
N.S. = Non significant.

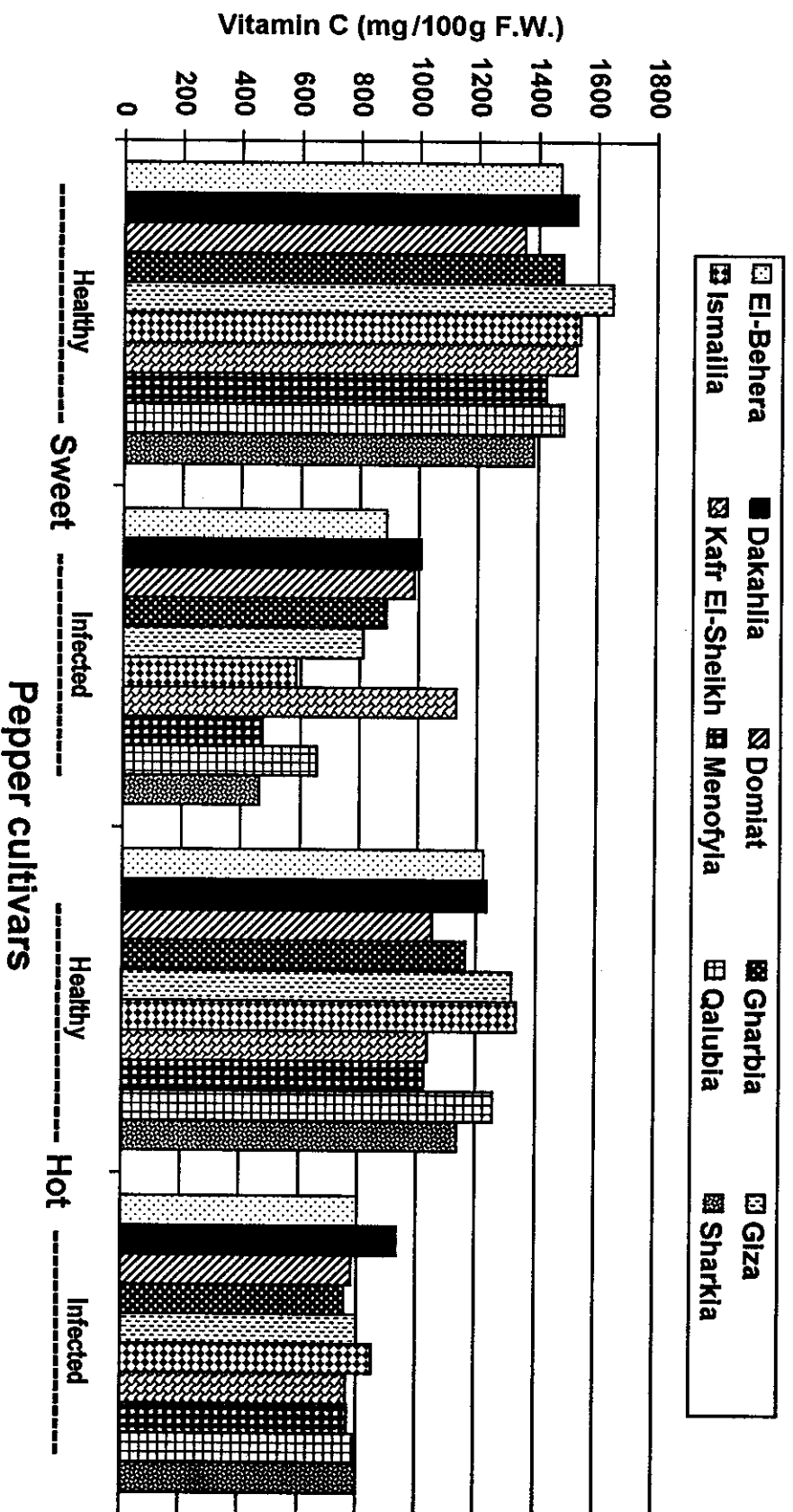


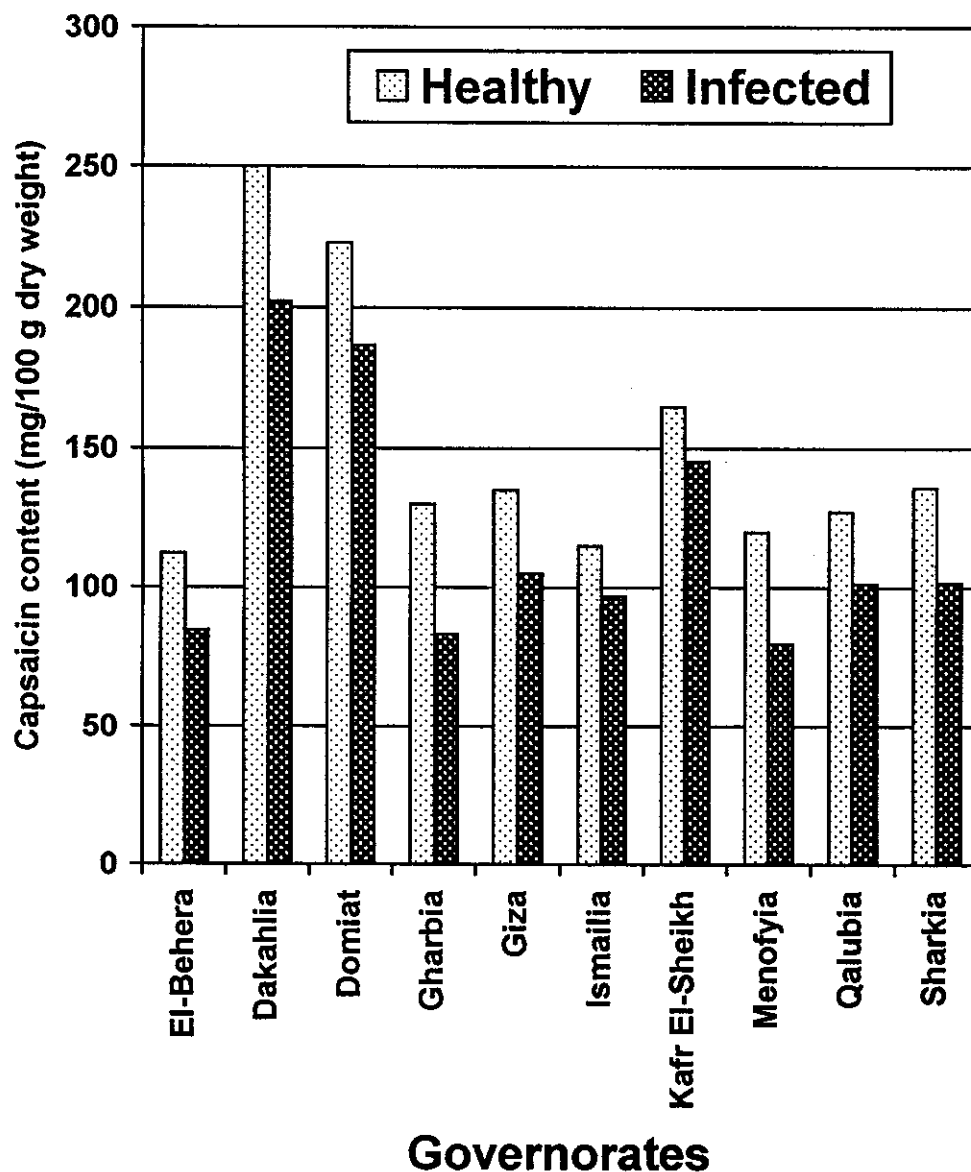
Fig. (11): Vitamin C content (mg/100 g fresh weight base) in the healthy and naturally infected hot and sweet pepper fruit samples collected from different ten Northern Egyptian Governorates.



**Table ( 8 ):** Capsaicin content (mg/100 g dry weight base) in the healthy and naturally infected hot pepper fruit samples collected from different ten Northern Egyptian Governorates.

Governorates	Hot peppers	
	Healthy	Infected
<b>El-Behera</b>	<b>112*</b>	<b>84.7*</b>
<b>Dakahlia</b>	<b>250</b>	<b>202.0</b>
<b>Domiat</b>	<b>223</b>	<b>186.8</b>
<b>Gharbia</b>	<b>130</b>	<b>83.3</b>
<b>Giza</b>	<b>135</b>	<b>105.1</b>
<b>Ismailia</b>	<b>115</b>	<b>96.6</b>
<b>Kafr El-Sheikh</b>	<b>165</b>	<b>145.4</b>
<b>Menofya</b>	<b>120</b>	<b>79.5</b>
<b>Qalubia</b>	<b>127</b>	<b>101.5</b>
<b>Sharkia</b>	<b>136</b>	<b>101.7</b>
<b>L.S.D. at 0.05</b>	<b>3.131</b>	<b>10.183</b>
<b>L.S.D. at 0.01</b>	<b>4.294</b>	<b>13.530</b>

\*Mean of 10 replicates as samples collected from 10 different locations for each governorates.



**Fig. (12):** Capsaicin content (mg/100 g dry weight base) in the healthy and naturally infected hot pepper fruit samples collected from different ten Northern Egyptian Governorates.

