University of Jordan
Faculty of Graduate Studies

Occurrence and Distribution of Alfalfa Mosaic Virus on Alfalfa (*Medicago sativa* L.) in Jordan

By

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Submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Protection / Plant Pathology, Faculty of Graduate Studies, University of Jordan

November, 1994.

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Didication

TO MY FAMILY

Acknowledgments

I express my thanks to Dr. Akel Mansour, my advisor, for his indispensable guidance, encouragement, and enthusiasm in establishing the manuscript.

Special thanks to Prof. Walid Abu-Gharbieh, Prof. Abdullah Al-Musa, Prof. Tawfiq Mustafa, and Dr. Mohammad Ababneh for their efforts in reviewing the manuscript. Sincere thanks are extended to Prof. T. Mustafa for confirming aphid species and Dr. Jamal Qasem for his help in weeds identification .

I am grateful to my colleagues, and especially, Mr. Ammar Haddad, and Mr. Abdullah Yousef for their helpful advice, and Mr. Abdel Shakoor Abu-Halawa for his help in glasshouse works. I am also, indebted to my friends, and particularly, Mr. Basheer Dawood for their sincere and tireless assistance in the arduous task of this work.

I would also like to take this opportunity to express my thanks and appreciation to the National Center for Agricultural Research and Technology Transfer (NCARTT) and to the Technical Assistance and Services Office (TASO) for partially funding my research activities through the National Agricultural Development Project which is financed by the United States Agency for International Development (USAID).

Last but not least, my deepest gratitude is due to my mother, sisters, and brothers to whom I express my profound appreciation. My special thanks to my brother, Fakher, and sisters, Azhar and Kamela, for their considerable and endless support and encouragement.

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Abstract

Occurrence and Distribution of Alfalfa Mosaic Virus on Alfalfa (*Medicago sativa* L.) in Jordan

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Two virus isolates were isolated from two hundred leaf samples collected from Alfalfa fields in the Jordan Valley and Al-Hallabat regions. Isolate (140/93) was identified as Alfalfa Mosaic Virus (AMV) based on host range, aphid transmission, physical properties, and serology, while isolate (43/93) was tentatively identified to be another isolate of the same virus depending on its reaction with diagnostic assay plants .

Transmissibility tests showed that the virus was non-persistently aphid-transmitted. Pea aphid (*Macrosiphum pisi*) and black bean aphid (*Aphis fabae*) were the most efficient vectors. Transmission through Alfalfa seeds was also possible, where the virus was detected serologically in 2-4% of seed samples tested.

Incidence studies of Alfalfa Mosaic Virus in Alfalfa fields with different ages in the Jordan Valley and Al-Hallabat regions, showed that there is a positive correlation between virus incidence and the age of plant. The incidence ranged from 11-43% in less than one year-old fields, 50-70% in 1-2 years-old fields, and 80-100% in 2-3 years-old Alfalfa fields.

INTRODUCTION

Alfalfa (*Medicago sativa* L), is probably the world's most important leguminous forage crop planted in the different irrigated areas for animal nutrition (1).

In Jordan, the crop is planted to 1480 dunums comprising approximately 9% of the total area planted to forage legumes and 0.2% of the total area planted to forages in the year of 1992 (2).Nowadays, alfalfa cultivation should be expanded in order to reach self-sufficiency in red meat production to cope with the rapid increase in population growth.

Many viruses were reported to infect alfalfa fields worldwide such as; alfalfa mosaic virus (AMV), alfalfa enation virus (AEV) (1), bean yellow mosaic virus (BYMV), clover yellow mosaic virus (CYMV), clover yellow vein virus (CYVV) (3), and pea streak virus (PSV) (4). Alfalfa mosaic virus is the most prevalent virus that causes reduction in the dry weight of mother plants (5,6). Moreover, AMV-infected alfalfa may act as a source of virus inoculum to other economic crops (4).

More than 300 plant species from different plant families have been known to be natural hosts for AMV (7). Some hosts have the ability to transmit the virus through their seeds which when coupled with aphid transmission, may play à critical role in virus survival and spreading (7,8).

LITERATURE REVIEW

Alfalfa Plant

Alfalfa, or lucerne as it is called in many European countries, is a herbaceous perennial legume originated in Southwest Asia. It is believed that it was first cultivated in Iran (17). Alfalfa planting has extended to all countries to became of worldwide distribution (1, 17).

Alfalfa has the highest feeding value of all commonly sown hay crops. It produces more protein per hectare than any other crop for livestock. It is high in mineral content and contains at least 10 different vitamins. These characteristics make alfalfa a desirable ration component for most farm animals. Alfalfa in combination with bacteria *Rhizobium meliloti* Dangead form a highly effective symbiosis for biological nitrogen fixation. Bacteria in the nodules convert atmospheric nitrogen into a form readily used by the plant. Alfalfa can increase subsequent crop productivity when it is used in crop rotations. Positive effect of rotation after alfalfa is due to improved water-holding capacity, increased soil organic matter, and reduction of some soil pathogens in addition to supplying nitrogen residues . The plant is the primary honey crop; as accounts for about one-third of the annual production by honeybees. Lucerne is a high-quality pasture for all classes of livestock. Alfalfa also helps minimize pollution by reducing water runoff and soil erosion. Studies also are underway to test the feasibility of developing a palatable protein product for human consumption (17).

Viruses of Alfalfa

Several viruses are involved in stunting and mosaic disease complex of alfalfa plants. These viruses differ in their physical properties, distribution, symptoms, and transmission. The most important viruses are alfalfa mosaic virus (AMV), alfalfa enation virus (AEV)(1), bean yellow mosaic virus (BYMV), clover yellow mosaic virus (CYMV), clover yellow vein virus (CYVV) (3), and pea streak virus (PSV) (4).

Alfalfa Mosaic Virus

Alfalfa mosaic virus has worldwide distribution. It is considered to be the most prevalent virus affecting alfalfa fields (5). Strains of this virus were reported to affect plants species in at least 73 plant genera, and the list continually increases. Alfalfa appears to be host to more strains than any other plant species (1).

The virus belongs to alfalfa mosaic virus group with bacilliform particles. The tripartite genome is composed of the virus's three larger molecular species of single stranded RNA. A fourth smaller species of RNA which is the coat protein messenger RNA is also present and each is separately encapsidated. The three larger RNA species contained in the large particles. These three, together with the forth RNA are required for infectivity. The large three particles measuring a diameter of 180 A^{o} with a length of 570, 430, and 350 A^{o}. The fourth is isometric measuring 180 A^{o} in diameter (18).

The virus is readily transmitted by sap inoculation. Mechanical transmission between or within fields by harvesting machines is a possibility but has not been demonstrated (1). Laboratory experiments showed that alfalfa could be infected in various stages of its growth by cutting tools freshly contaminated with AMV. Repeated cutting considerably increases the number of virus infected plants in the cultivars which were highly resistant during the seedling stage, where repeated cutting influenced the susceptibility of different alfalfa genotypes to viral infection (19). Vectors also may introduce the virus from nearby infected plants. At least, thirteen aphid species were reported worldwide to transmit the virus in non-persistent manner (7). The virus spreads in the field chiefly by pea aphid (*Macrosiphum pisi* Kalt.). Black bean aphid (*Aphis fabae* Scopoli), melon aphid (*Aphis gossypii* Glover), and green peach aphid (*Myzus persicae* Sulzer) may also play an important role in virus transmission (1).

Infected seeds are the most likely primary source of initial infection in the field. The virus was transmitted to alfalfa seeds at much higher frequency through male gametes (pollen) than through female gametes (ovules). The transmission frequency through pollen ranged from 0.5 to 26.5% and transmission through the ovules ranged from 0 to 9.5%. As net result of gametes transmission, AMV transmission through alfalfa seeds ranged from 0.8 to 28.5%. The variations in seed transmission frequency were due to effect of AMV strain, temperature, and variability among alfalfa clones. Laboratory experiments for AMV survival in seeds of alfalfa showed that the percentage of virus-infected seeds was not reduced significantly in a seed lot containing about 20% infected seeds after storage for 5 years at -18C°, 4C°, or 21-27C°. The relatively high incidence of AMV-infected seeds and the longevity of the virus in seeds suggest that infected seeds are

On bean cultivars, strains most commonly found in lucerne give necrotic lesions, other strains produce chlorotic local lesions or none at all but give systemic mild mottle, vein necrosis and leaf distortion (7).

On broadbean, it develops black necrotic lesions which may become systemic and gives rise to general necrosis causing the death of the plant. Occasionally, where necrosis did not develop, a mild chlorotic mottle may be present (7,9).

On cowpea, the response of most strains varies from sharply defined, small or large, black, irregular and immarginate local lesions to necrotic or chlorotic spots often ring-like in form (9). Others infect systemically (7).

On peas, the first symptom is a drying out of the inoculated leaves. This is followed by a general wilting and stunting of the plant with mild mottling of tip leaves. In some cases only the tip leaves may be wilted, and in severe cases the whole plant may be killed (9).

Incidence and Effect of AMV on Alfalfa

Incidence of AMV in alfalfa fields grown in different locations in Wisconsin State was found to be 63% in 2 year-old stands and 75% in older stands. Yields of alfalfa hay were significantly reduced to 30% in the first cutting, and the virus strains complex might be contributed to increased winter-killed plants. Crude protein content and degree of nodulation were also reduced (6,22).

Isolation and Effect of AMV on Other Crops

The effect of alfalfa mosaic virus on productivity of annual barrel medic (*Medicago trancatula* Geartn) planted in different locations in

Australia was studied by Dall *et al.* (23). They found that the herbage production was reduced by more than 50%. Root growth and root nodulation of infected plants was about one third less than that of healthy plants. Assessment of seedling mortality and seeds reduction of infected plants showed 27% and 15-30%, respectively. Burr medic (*Medicago polymorpha* L.) grown in the same country was also found to be susceptible host for AMV. Herbage yield of the infected plants decreased by 13-35% while seed yield was decreased significantly by 73% (24).

Black and Price (10) showed as early as 1940 that the virus producing potato calico in New Jersey is a strain of AMV. Potato calico virus and alfalfa mosaic virus were considered to be closely related based on similar but not identical reactions with *Nicotiana glutinosa* L., *Phaseolus vulgaris* L., *Vicia faba* L., and *Solanum tuberosum* L.. Recently, a new strain of AMV was isolated from older potato leaves at potato research station Gurshaiganj, and in the vicinity of Farukahbad. Losses due to this disease were reported to vary from 20 to 40% (25).

Alfalfa mosaic virus was isolated from mosaic disease complex of hyacinth bean (*Dolichos lablab* L.) and broadbean in Northern Sudan. A quantitative assessment of field infection on these crops was estimated at 19%. Infected bean leaves become malformed and puckered with bright yellow area covering the major portion of the leaf, while infected broadbean showed stem and leaf necrosis (26).

A strain of AMV was found as a naturally occurring pathogen of Wisconsin field grown tobacco. The virus causes intense systemic chlorotic mottle of various types and has the ability to be transmitted efficiently by aphids (27).

Alfalfa mosaic virus was widely distributed in most chickpea (*Cicer arietinum* L.) growing regions in Iran. The virus was blamed for low and erratic annual yields(28). Field inoculation with AMV at pre-bloom caused 82% mortality. Surviving plants were stunted and chlorotic (29).

In Egypt, a strain of AMV was isolated from naturally infected squash plants and found that 27 cucurbitaceous varieties and cultivars were susceptible to that strain. Estimates of virus incidence in a field at El-Minya revealed that 6-16% of squash plants were naturally infected with the virus (30).

Salama and Behadli (31) isolated strain of alfalfa mosaic virus from broadbean plants in the College of Agriculture Campus at Abu-Gharib, Baghdad. Greenhouse experiment showed that broadbean leaves developed necrotic lesions 4-5 days after inoculation. Preliminary surveys made in Iraq indicate that although the disease is not common in broadbean plant field, but it is considered of potential danger since infected plants usually die.

Alfalfa mosaic virus affected pepper in Israel. The virus contributed with other pepper viruses to affect the annual yeild (12). Indexing schemes of the grapevine viruses in Europe showed that AMV is one of the major viruses affecting grapevine yards in Bulgaria, Czechoslovakia, Germany, and Hungury (32).

In 1983, two strains of AMV were associated with a sèvere disease of tomatoes and reduced the survival of direct seeded tomato plants in fields adjacent to alfalfa in Imperial Valley of California. Disease symptoms in tomato plant are stunting, bronzing and necrosis followed by death. Two

strains of the virus were isolated and identified by serology, electron microscopy, and analysis of dsRNA. A necrosis-inducing strain that causes phloem necrosis and death of tomatoes was the most prevalent and was responsible for losses of tomato plants in the Imperial Valley. A chlorotic strain that caused non lethal, calico symptoms was present occasionally. Incidence studies showed that 60% of tomato plants were infected with AMV (11).

Alfalfa mosaic virus was the most common and widespread virus disease encountered in the Republic of Yemen. The virus was isolated from every alfalfa field tested. It was common wherever susceptible vegetables were grown near alfalfa. The widespread occurrence of AMV in alfalfa, susceptible vegetables and other crops in Yemen was attributed to the virus's high level of seed transmission in commercial seed stocks, and the traditional farming systems practiced in most villages. In these systems many small areas of mixed vegetables, herbs and alfalfa forage crops were grown close to one another (33).

MATERIALS AND METHODS

Sample Collection

Two hundred tip leaf samples of alfalfa plants showing virus-like symptoms (mosaic and/or stunting) were collected from the middle Jordan Valley, and Al-Hallabat areas. The survey was carried out at monthly intervals starting mid-February, 1993 till the beginning of June, 1994.

Virus Isolation

All samples were tested for AMV infection using diagnostic assay plants including *Chenopodium amaranticolor* Coste & Reyn, *Chenopodium quinoa* Willd., *Nicotiana glutinosa, Nicotiana tabacum* L. cv. Havana 423, *Phaseolus vulgaris* cv Kentacky, and Strike, *Pisum sativum* L., *Vicia faba,*and *Vigna unguiculata* (L.)Walp. . Inocula were prepared by macerating the collected tissues in 0.01 M neutral phosphate buffer (1gm tissue/1ml buffer) containing 0.01 M sodium diethyldithiocarbamate (Na-DIECA) and 0.01M cysteine hydrochloride (C-HCl) using sterilized pestles and mortars. Carborandum (6000-mesh) was dusted on cotyledonary or 3rd-4th leaf stage before thumb and forefinger inoculation. The plants were observed 6 weeks for symptoms development under glasshouse conditions.

Single lesion transfer was done for the representative isolate (140/93) by macerating single necrotic local lesion from *Phaseolus vulgaris* cv. Strike, and inoculated to two healthy plants of *Phaseolus vulgaris* cv. Strike which have been exposed to 24 hours darkness. After symptoms appearance, lesions were collected, macerated and inoculated to *Nicotiana tabacum* cv. Havana 423.

Properties in Crude Sap

Tip leaves of *Nicotiana tabacum* cv. Havana 423 infected with isolate (140/93) were extracted in 0.01 M neutral phosphate buffer containing 0.01M (Na-DIECA) and cysteine hydrochloride (1 gm tissue/1ml buffer). The extract was filtered through two layers of cheesecloth. The dilution end point (DEP) was determined by subjecting 1.0 ml of the extract to serial 10-fold dilutions using phosphate buffer. For longevity *in vitro* (LIV), 5ml of sap was stored at room temperature and assayed daily for infectivity. The thermal inactivation point (TIP) was determined by exposing 2.0 ml aliquots for 10 minutes heating to various temperature treatments; 40, 45, 50, 55, 60, 65, 70, 75, 80 C^{o}, using water-bath. All heated aliquots were immersed in an ice bath till the end of the experiment (36). All aforementioned treatments were assayed on *Nicotiana tabacum* cv. Havana 423. All assayed plants were kept 20 days in a glasshouse for symptom development.

Serology

Agar gel double-diffusion test was employed in identification procedure of the isolate (140/93). The test was performed in plastic petri dish with 0.85% purified agar in distilled water containing 0.85% sodium chloride (NaCl) and 0.2% (w/v) sodium azide (37,38). Antiserum for AMV was gifted by (T. Z. (Scott) , Clemson University, North Carolina). Antigen was prepared by macerating 1 gram of infected leaves of *Nicotiana tabacum* cv. Havana 423 showing typical AMV symptoms in 1 ml neutral phosphate buffer and strained through two layers of cheesecloth. Buffer, infected and healthy crude saps were charged into three peripheral wells bored in the gel to surround a central well that was charged with undiluted Antiserum.

Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA) was also employed in serological tests for AMV. Antiserum used in this test was the same one used in agar gel double-diffusion test. Goat Anti-Rabbit conjugate was supplied by HyClone Laboratories, Inc. Logan. Buffers adopted in this test were used by Koeing (39) [Appendix 1]. Antiserum and Goat Anti-Rabbit conjugate dilutions were selected through comparison preliminary ELISA test using sets of positive and negative controls.

Tip leaves of *Nicotiana tabacum* cv. Havana 423 infected with isolate (140/93) was macerated separately in grinding buffer (CEP) at 1:20 proportion containing 0.45% diethyldithiocarbamate (DIECA) and strained through two layers of cheesecloth. Wells of ELISA plate were separately charged with 0.2ml of extracted sap. Sap extract from healthy *Nicotiana tabacum* cv. Havana 423 and grinding buffer (CEP) were used as negative controls.

The plate was refrigerated overnight at 4C°. After the sap extract was discarded, the plate was washed three times with washing buffer (Phosphate Buffer Saline containing 0.05% Tween-20 (PBS-Tween). The wells were then charged with 0.2 ml of Antiserum diluted with (PEP) buffer to 1: 2000 proportion. After 1 hr incubation at room temperature, Antiserum discarded and the plate washed 3 times with PBS-Tween. 0.2ml of conjugate diluted to 1:3000 in (PEP) buffer were added to each well. The plate was then incubated for 3 hours at 37C°. After the conjugate was discarded and the wells were washed with PBS-Tween, 0.2 ml of the substrate which was prepared by dissolving 0.1 gm of *p*-nitrophenyl phosphate disodium in 100 ml in substrate buffer was added (40).

Result of the reaction was taken 30 minutes after substrate addition using ELISA-reader DENLEY WESCAN model. Positive results were considered when the value of absorbency at 405 nm were at least two times greater than the negative control (41).

Response of Bean Cultivars to Virus Isolate (140/93)

Ten cultivars of bean with different sources and origins, supplied by the Ministry of Agriculture were tested for their susceptibility to infection by the virus isolate (140/93) (Table 2). Five plants from each cultivars were inoculated mechanically at cotyledonary stage with virus inoculum. Plants were observed continuously for symptoms development. Tip and inoculated leaves of the tested cultivars were back-indexed on *Nicotiana tabacum* cv. Havana 423, and *Phaseolus vulgaris* cv Strike and/or Kentacky 30 days after inoculation.

Incidence of Alfalfa Mosaic Virus in Alfalfa Fields

Field surveys were started during June, 1993 and continued through June, 1994. Four fields of alfalfa with different ages in the Jordan Valley, and Al-Hallabat regions were selected. The ages were ; newly established field and 2-3 years-old field in the Jordan Valley, and 1-2 years-old and 2-3 years-old fields in Al-Hallabat. Fourty tip leaf samples were collected randomly at monthly intervals from each field. Each sample was assayed for AMV infection serologically by ELISA and biologically using diagnostic assay plants including; *Chenopodium amaranticolor, Chenopodium quinoa* and *Nicotiana tabacum* cv. Havana 423, *Phaseolus vulgaris* cv. Strike and/or Kentacky, and *Vigna unguiculata.*

The percentage of infection was determined by dividing the number of infected samples over the total samples collected.

Table 2 : Bean cultivars* supplied by the Ministry of Agriculture, challenged with virus isolate (140/93).

Bean Cultivar	Origin
Contessa	France-graines/France
Femira	----------
Harvester	Sun Seed / America
Kentacky	Modesto Seed / America
Lolita	Valmorin / France
Mantra	Rijk Swaan / America
Selecta	Technism / France
Strike	Asgrow /America
Tema	Asgrow/America
Trophy	Marto Seeds/USA

* Information provided by The Ministry of Agriculture.
--------- : No information.

Field Virus Content

For *in Vivo* virus content determination, two 2-3 years-old alfalfa fields were selected in the Jordan Valley and Al-Hallabat regions. Fourty tip leaf samples were collected from each field at The at monthly intervals starting from June, 1993 till June, 1994. Each sample was tested biologically using diagnostic assay plants; *Chenopodium amaranticolor*,*Chenopodium quinoa*, and *Vigna unguiculata*. The numbers of necrotic local lesions which developed on inoculated leaves of cowpea were counted 20 days after inoculation. The average number of the local lesions was taken per one leaf.

Virus Content Variation in Tobacco Plants

For *in Vitro* virus content determination, two plants of *Nicotiana tabacum* cv. Havana 423 were inoculated mechanically at cotyledonary stage with the virus isolate (140/93). Samples were taken weekly from the tip leaves of inoculated and healthy plants, and symptoms development were recorded daily. Each sample was macerated in grinding buffer at (1:20) proportion for quantitative ELISA testing (42). Virus content was assessed with regard to absorbance value at 405 nm obtained by the ELISA-reader. ELISA plates were read 15 minutes after substrate addition.

Seed Transmission

American and Moapa 69 cultivars of alfalfa seeds provided respectively by A. Hafez and Miqdadi companies were sown in a polystyrene tray containing sterilized peatmoss. Three days after emergence, one hundred seedling plants of each cultivar were transplanted to plastic pots containing methyl bromide-fumigated soil and kept in an insect-free cages under glasshouse conditions. All plants were tested qualitatively and

RESULTS

Virus Isolation

Two hundred leaf samples showing mosaic and/or stunting were collected during the years 1993 and 1994 from alfalfa fields in the target regions. All samples were developed similar symptoms on most diagnostic assay plants. Isolate (140/93) was selected to represent other isolates based on its reaction with *Chenopodium amaranticolor*, *Datura stramonium*, *Lycopersicon esculentum* L., *Nicotiana tabacum* cv. Havana 423, *Ocimum basilicum*, *Phaseolus vulgaris* cv. Kentacky and Strike, *Pisum sativum*, and *Vigna unguiculata*. Isolate (43/93) was differentiated based on its reaction with *Chenopodium amaranticolor* (Table 3). The two representative isolates were tentatively identified as alfalfa mosaic virus.

Host Range and Symptomology

Isolate (140/93) was used for host range study. Thirty four host plants belong to several plant families were tested.

Host range studies showed that the virus was not restricted to legumenosae family. Other plant species from other plant families were found to be susceptible. Symptoms developed on the tested plants were:

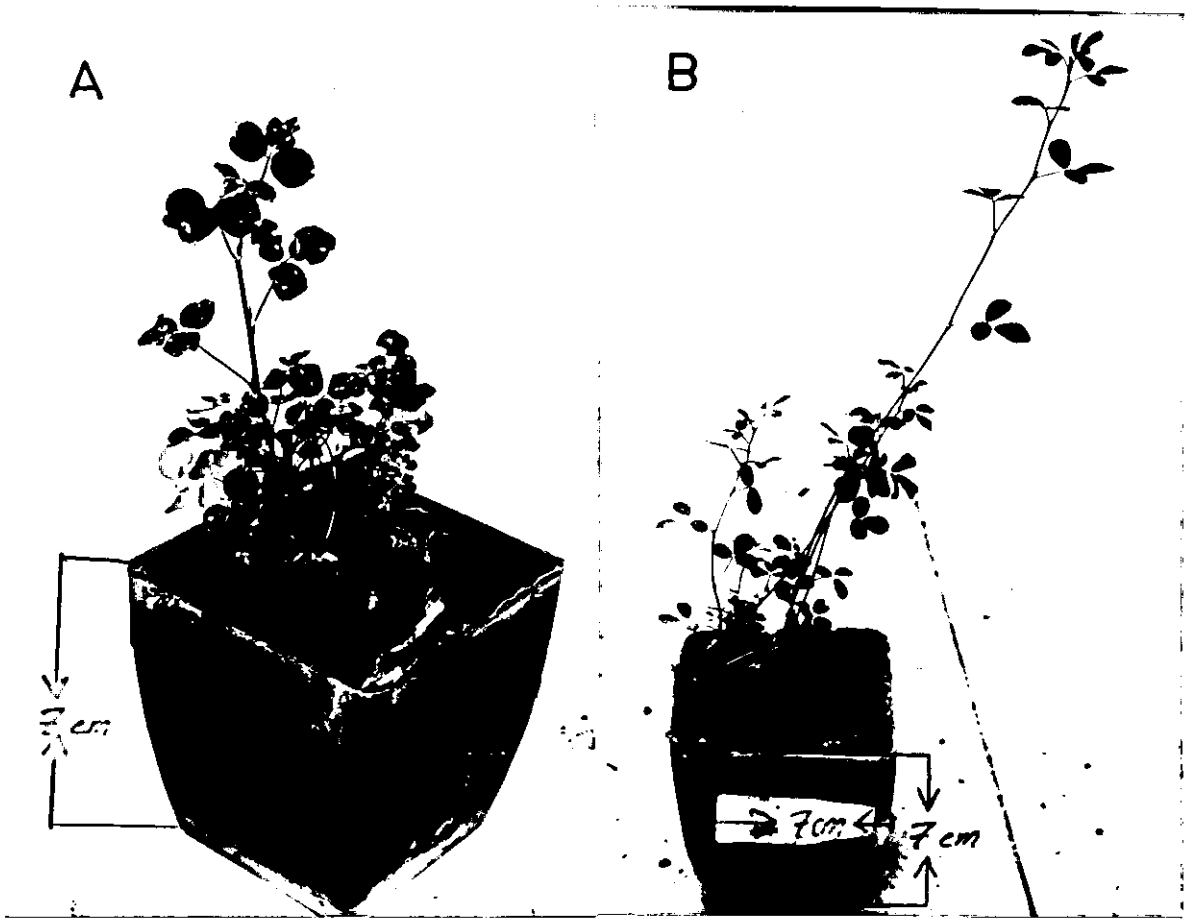
Chenopodium amaranticolor : Local lesions did not develop on inoculated leaves. Plants infected systemically by rapid invasion with yellow mosaic, and distortion of young leaves which had a gray mealy appearance [Plate 1].

[Plate 1] : Symptoms of yellow mosaic on tip leaves of *Chenopodium amaranticolor* infected with isolate (140/93).

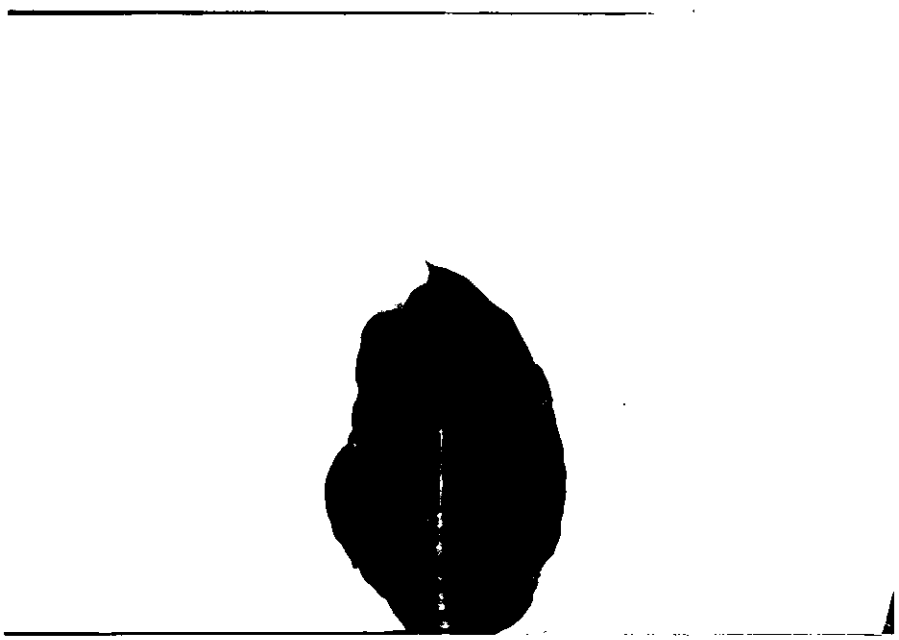
Table 3 : Preliminary plant species used in selecting the two isolates (43/93) and (140/93).

Plant Species	Symptoms			
	(43/93)		(140/93)	
	Inoculated leaves	Tip leaves	Inoculated leaves	Tip leaves
C. amaranticolor	CHLL	YM	(-)	YM
Cucurbita pepo	(-)	(-)	(-)	(-)
Datura stramonium	(-)	MM	(-)	MM
L. esculentum	(-)	CL+CR	(-)	CL+CR
Nicotiana tabacum cv. Havana 423	(-)	CHM	(-)	CHM
Phaseolus vulgaris cv.				
Kentacky	NLL	(-)	NLL	(-)
Strike	NLL	(-)	NLL	(-)
Pisum sativum cv.				
Alderman	Wil	(-)	Wil	(-)
Onword	Wil	(-)	Wil	(-)
Ocimum basilicum	(-)	YM	(-)	YM
Vigna unguiculata	NLL	(-)	NLL	(-)

CHLL: Chlorotic local lesion
CL : Chlorosis
MM : Mild mosaic
YM : Yellow mosaic
(-) : No infection
CHM: Chlorotic mottle
CR : Curling
NLL : Necrotic local lesion
Wil : Wilting



[Plate 2]: A) Severe stunting and small leaves of *Medicago sativa* infected during the cotyledonary stage with isolate (140/93).
B) Healthy alfalfa



[Plate 3] : Chlorotic mottle on tip leaf of *Nicotiana tabacum* cv. Havana 423 infected with isolate (140/93).

[Plate 4]: A) Severe stunting of *Ocimum basilicum* infected with isolate (140/93).
B) Healthy *Ocimum basilicum*

Vicia faba: Inoculated broadbean plants developed few black necrotic local lesions 6-10 days after inoculation. Systemic symptoms occurred by invasion with mild mosaic.

Vigna unguiculata : Mechanically inoculated cotyledonary leaves of cowpea developed few, small, black-brownish irregular and immarginate ring-like necrotic local lesions. Back-indexing from the tip leaves on *Nicotiana tabacum* cv. Havana 423 and *Phaseolus vulgaris* cv. Kentacky and/or Strike showed that the plant was not systematically infected [Plate 5].

Reactions of tested plants inoculated with isolate (140/93) are shown in (Table 4).

Aphid Transmission

The virus isolate (140/93) was transmitted in the non-persistently by all aphid species tested (Table 5). Symptoms on the indicator plants developed 3-5 days after inoculation. Pea aphid (*Macrosiphum pisi*) was the most efficient aphid vector, since it was capable of 70% transmission efficiency. *Aphis fabae* stood in the second position in the efficiency of transmission, as 60% of the tested plants were infected. *Aphis gossypii*, and *Myzus persicae* showed to have low efficiency of transmission under glasshouse conditions.

Properties in Crude Sap

Isolate (140/93) was found to be infective at 60C° but not at 65C°. It was viable up to the dilution of 10^{-4} but not at 10^{-5}. The virus withstand aging up to 2 days in extracted crude sap.

[plate 5] : Small, black-brownish irregular and immarginate ring-like necrotic local lesions on cotyledon leaves of *Vigna unguiculata* inoculated with isolate (140/93)

Table 4 : Reactions of different plant species inoculated with virus isolate (140/93)

Plant species	Symptoms		Back-Indexing	
	Inoculated leaves	Tip leaves	Inoculated leaves	Tip leaves
Arachis hypogea L.	NS	NS	(-)	(-)
Carthamus tinctorius Light	NS	NS	(-)	(-)
C. amaranticolor	NS	YM	(-)	(+)
C. murale L.	NS	NS	(-)	(-)
C. quinoa	NS	YM	(-)	(+)
Citrullus vulgaris Schrad	NS	NS	(-)	(-)
Cucumis sativus	NS	NS	(-)	(-)
Cucurbita moschata L.	NS	NS	(-)	(-)
Cucurbita pepo	NS	NS	(-)	(-)
Datura stramonium	NS	M.MOT	(-)	(+)
Gomphrena globosa L.	NS	NS	(-)	(-)
Helianthus annus L.	NS	NS	(-)	(-)
Lens culinaris Med.	NS	YM	(-)	(+)
Lycopersicon esculentum	NS	CL+CR	(-)	(+)
Medicago noeana L.	NS	MM	(-)	(+)
Medicago Polymorpha	NS	MM	(-)	(+)
Medicago rigidula L.	NS	MM	(-)	(+)
Medicago rotata L.	NS	MM	(-)	(+)
Medicago sativa	NS	MM	(-)	(+)
Medicago truncatula	NS	MM	(-)	(+)
Nicotiana clevilandi Gray	NS	MM	(-)	(+)
Nicotiana glutinosa	NS	VC.+CH.M	(-)	(+)
Nicotiana rustica L.	NS	VC.+CH.M	(-)	(+)
Nicotiana tabacum	NS	VC.+CH.M	(-)	(+)
Ocimum basilicum	NS	YM+ST	(-)	(+)
Pisum sativum cv alderman	WIL	NS	(+)	(-)
Pisum sativum cv onward	WIL	NS	(+)	(-)
Ranunculus soncus	NS	NS	(-)	(-)
Trifolium alexandrimum L.	NS	MM	(-)	(+)
Vinca rosea L.	NS	NS	(-)	(-)
Vicia faba (cyprus)	NLL	MM	(+)	(+)
Vicia faba (mahali)	NLL	MM	(+)	(+)
Vicia faba (malti)	NLL	MM	(+)	(+)
Vigna unguiculata	NLL	NS	(+)	(-)
Zea mays L.	NS	NS	(-)	(-)

- CH.M: Chlorotic Mottle,
- CL: Chlorosis
- CR : Curling
- MM: Mild Mosaic.
- M.MOT: Mild Mottle
- NLL: Necrotic Local Lesion
- NS : No symptom
- St: stunting,
- VC: Vein Clearing.
- WIL: Wilting
- YM: Yellow Mosaic
- (+) : Virus detected
- (-) : Virus not detected

Table 5 : Efficiency of different aphid species in transmission of the virus isolate (140/93).

Vector	No.of infected plants/total	Percentage of infection
Aphis gossypii	5/20	25%
Aphis fabae	12/20	60%
Macrosiphum pisi	14/20	70%
Myzus persicae	5/20	25%

Serology

Agar gel double-diffusion test showed that isolate (140/93) reacted strongly with AMV Antiserum by formation of a sharp precipitin line. Such reaction line was not observed against buffer and healthy sap extract [Plate 6].

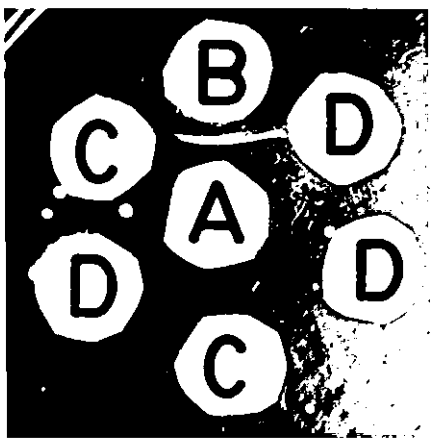
Indirect immunosorbent assay showed that isolate (140/93) reacted with Antiserum specific for AMV by developing darker yellow color. Wells charged with buffer and healthy sap extract of tobacco did not show any specific reaction [Plate 7].

ELISA-reader showed that the reading values at 405 nm of the wells charged with the target isolate were more than two times greater than the negative controls [Appendix 2].

Response of Bean Cultivars to Virus Isolate (140/93)

All bean cultivars tested against the virus isolate were not ystemically infected as proved by back-indexing on *Nicotiana tabacum* cv. Havana 423 and *Phaseolus vulgaris* cv. Kentacky and/or Strike (Table 6). However, the reaction of the inoculated leaves with the target virus isolate ranged from symptomless to severe infection. Inoculated cultivars of Femira, Tema, and Trophy showed no symptoms when observed for one month.

Contessa, Harvester, kentacky, Lolita, Mantra, Selecta, and Strike reacted by developing different types of local lesions on inoculated leaves. Contessa and Selecta cultivars developed pinpoint necrotic local lesions 6-10 days after inoculation, whereas Kentacky, Lolita, Mantra, and Strike



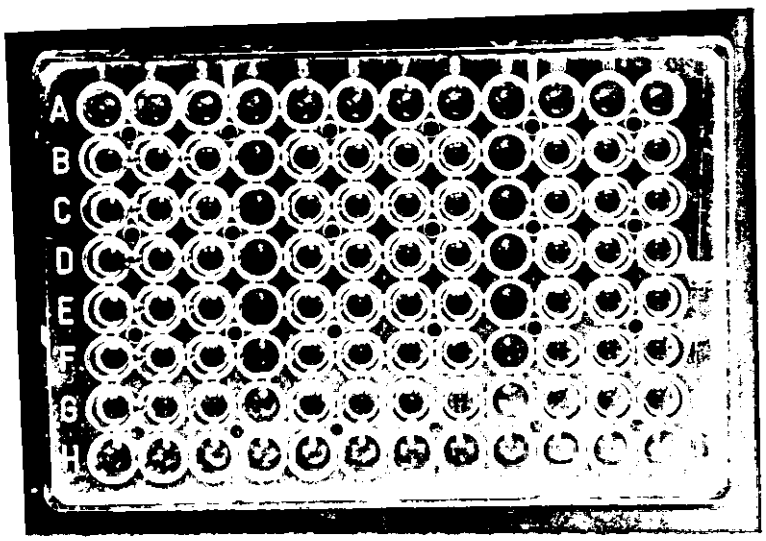
[Plate 6] Results of serological reaction in agar gel double-diffusion test.

A. Central well containing AMV specific Antiserum

B. Antigen of isolate (140/93)

C. Healthy crude sap.

D: Buffer.



[Plate 7] : ELISA test plate showing the reaction of virus isolate (140/93) with AMV specific antiserum. The darker yellow color indicates positive reactions.

1) Columns from 1 to 3 and rows from B to G were charged with healthy tissue.
2) Columns from 5 to 8 and rows from B to G were charged with tissue infected with isolate (140/93)
3) Columns from 10 to 12 and rows from B to G were charged with (CEP) buffer.

Table 6 : Response of bean cultivars to virus isolate (140/93).

Bean cultivar	Symptoms		Back-indexing	
	Inoculated leaves	Tip leaves	Inoculated leaves	Tip leaves
Contessa	P.P.NLL	NS	(+)	(-)
Femira	NS	NS	(-)	(-)
Harvester	CHLL	NS	(+)	(-)
Kentacky	RNLL	NS	(+)	(-)
Lolita	RNLL	NS	(+)	(-)
Mantra	RNLL	NS	(+)	(-)
selecta	P.P.NLL	NS	(+)	(-)
Strike	RNLL	NS	(+)	(-)
Tema	NS	NS	(-)	(-)
Trophy	NS	NS	(-)	(-)

•CHLL : Chlorotic local lesions.
•NS : No symptom
•P.P NLL : Pinpoint necrotic local lesions.
•RNLL : Ring-like necrotic local lesions.
• (-) : No infection.
• (+) : Virus detected

developed ring-like necrotic local lesions 8-11 days after inoculation [Plate 8]. Inoculated Harvester cultivar produced diffused irregular chlorotic local lesions 5-9 days after inoculation .

Incidence of AMV in Alfalfa Fields

The incidence of AMV was studied in four fields of alfalfa with different ages in Al-Hallabat and the Jordan Valley. Incidence of the virus was highly correlated with the age of the plants. Maximum percentage of virus infection was obtained in older fields. The incidence of the virus in newly established field (one month-old) in the Jordan Valley was 11% at the beginning of the survey, and increased slowly to reach 42% one year later (Fig 1). One year investigation of the incidence of the virus in 1-2 years-old alfalfa field in Al-Hallabat region, showed that the percentage of virus incidence occupied greater value compared with the pervious field starting from 50% at the beginning of the survey up to 70% after one year monitoring (Fig. 2). The incidence of the virus in 2-3 years-old alfalfa fields in the Jordan Valley and Al-Hallabat regions revealed very high percentage of virus infection. The incidence of the virus in Al-Hallabat region was 80% at the beginning of the survey, and reached 95% after one year monitoring, while it ranged in the field with the same age in the Jordan Valley from 80% to 100% (Fig. 3).

Field Virus Content

Virus content was studied in 2-3 years-old alfalfa fields in Al-Hallabat and the Jordan Valley during the survey which carried out through the years of 1993-1994. Virus content was assessed based on the average a numbers of necrotic local lesions which developed on local lesion host *Vigna unguiculata*. Results showed that the trend of the virus content varied between Al-Hallabat and the Jordan Valley during the year.

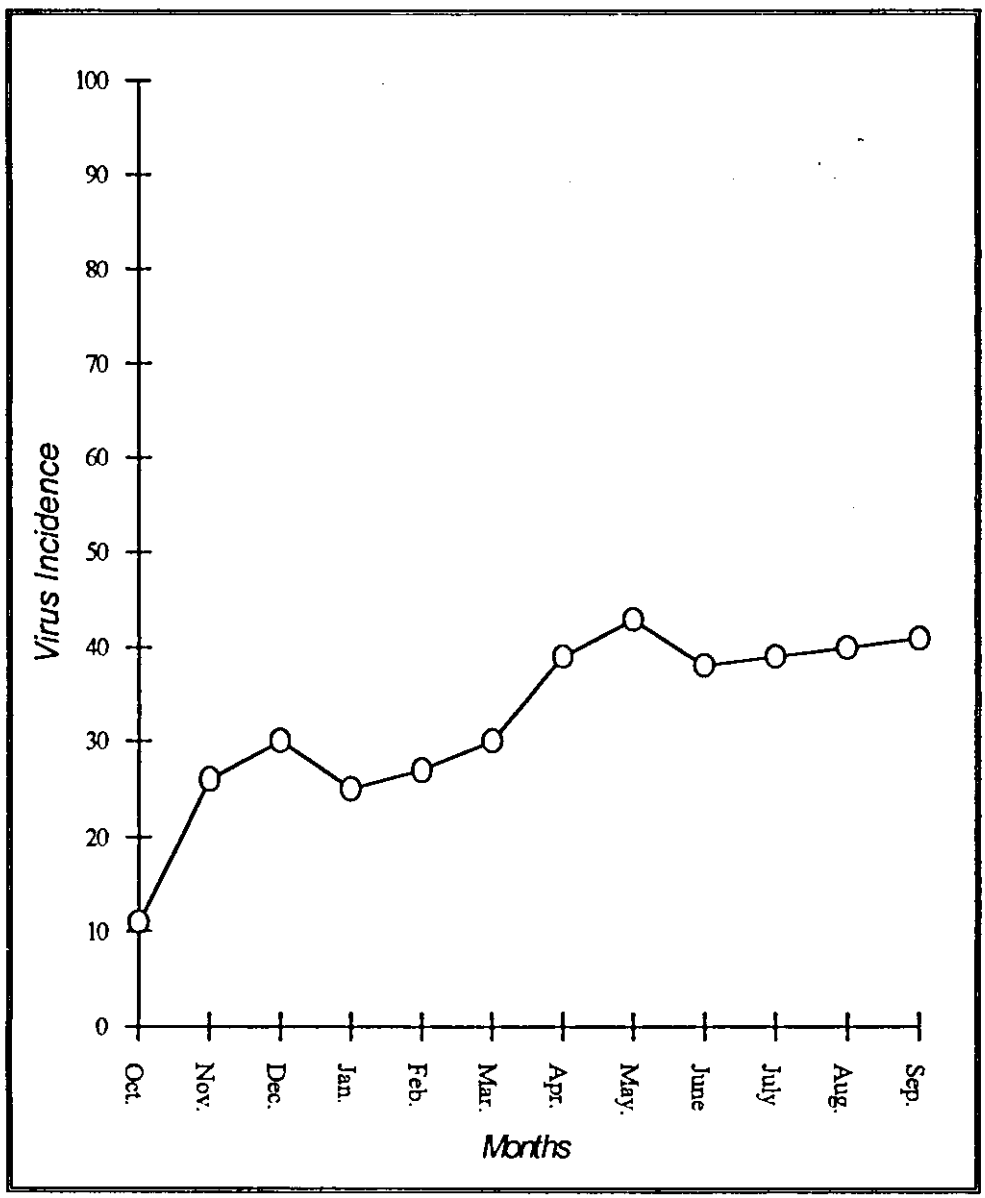


Figure 1: Incidence of AMV in less than one year-old alfalfa field in the Jordan Valley during 1993- 1994.

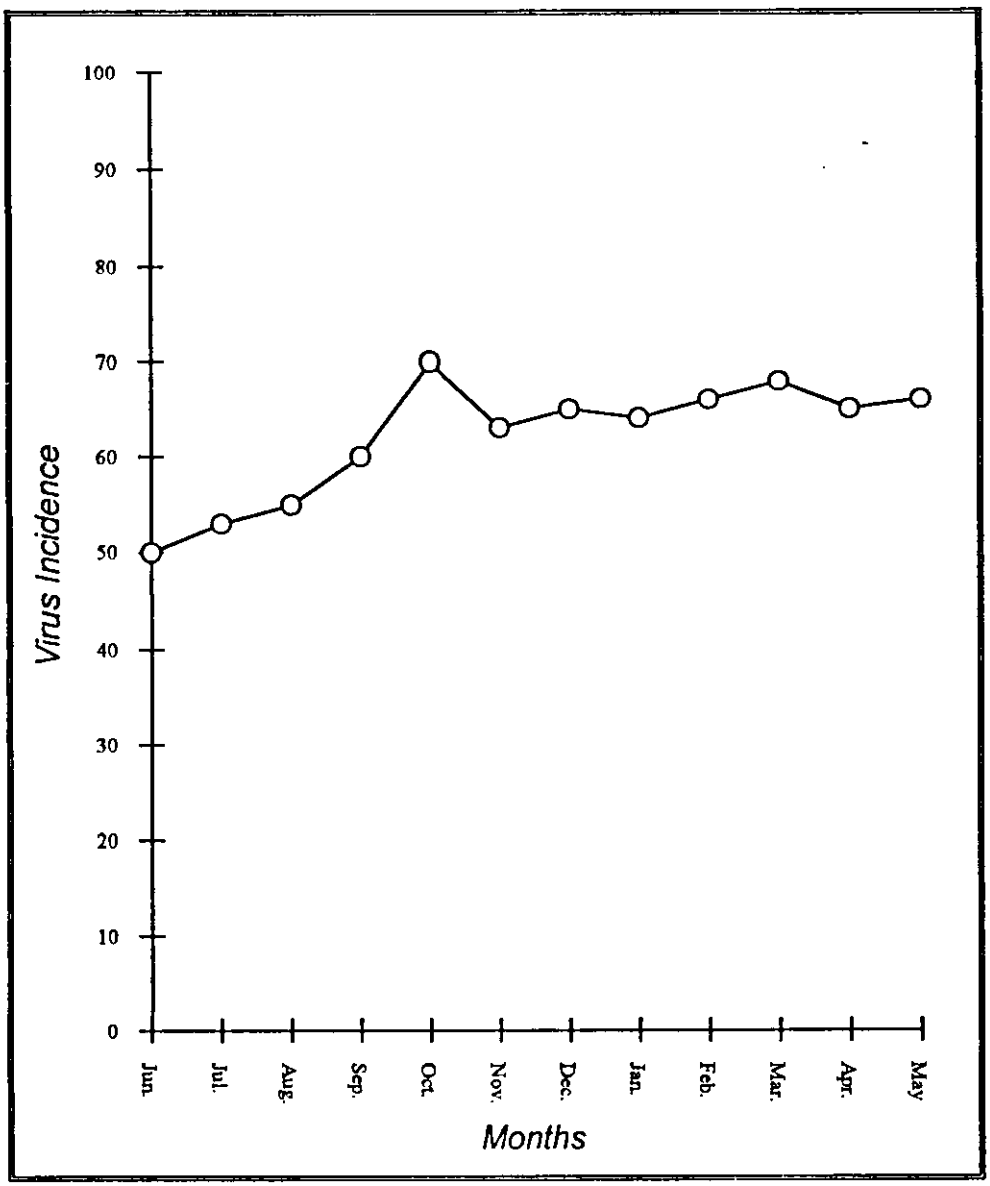


Figure 2 : Incidence of AMV in 1-2 years-old alfalfa field in Al-Hallabat region during 1993-1994

Lowest virus content in Al-Hallabat region was obtained between May and September. The magnitude of the virus content increased after September to reach the peak during October. Gradual decreasing started after October to reach its medium value between December and February, and the lowest value during May (Fig. 4).

In the Jordan Valley, alfalfa fields showed the lowest virus content between May and July. The virus content started gradual increasing after July to reach the medium value between August and November, and the peak at the end of December. After December, the content started gradual decrease to reach its lowest value during May (Fig. 4).

Virus Content Variation in Tobacco Plant

Virus content started increasing in tip leaves of *Nicotiana tabacum* cv Havana 423 two days after inoculation to reach the maximum value after two weeks. Then, the content started gradual decreasing until it reached the lowest value 10 weeks after inoculation. Symptoms development were coincided with virus content inside the plant. It started as a mild mosaic and vein clearing, and increased to become obvious chlorotic mottle during the maximum value of the virus content 2 weeks after inoculation, then the symptoms started gradual decreasing simultaneously with the virus content until they vanished during the lowest value of the virus content (Fig. 5).

Seed Transmission

Two seedlings out of one hundred seedling plants of American cultivar and four seedlings out of one hundred seedling plants of Moapa 69 cultivar of alfalfa were found AMV-infected. ELISA test was able to detect the virus at cotyledonary, unifoliate, and trifoliate leaf stages.

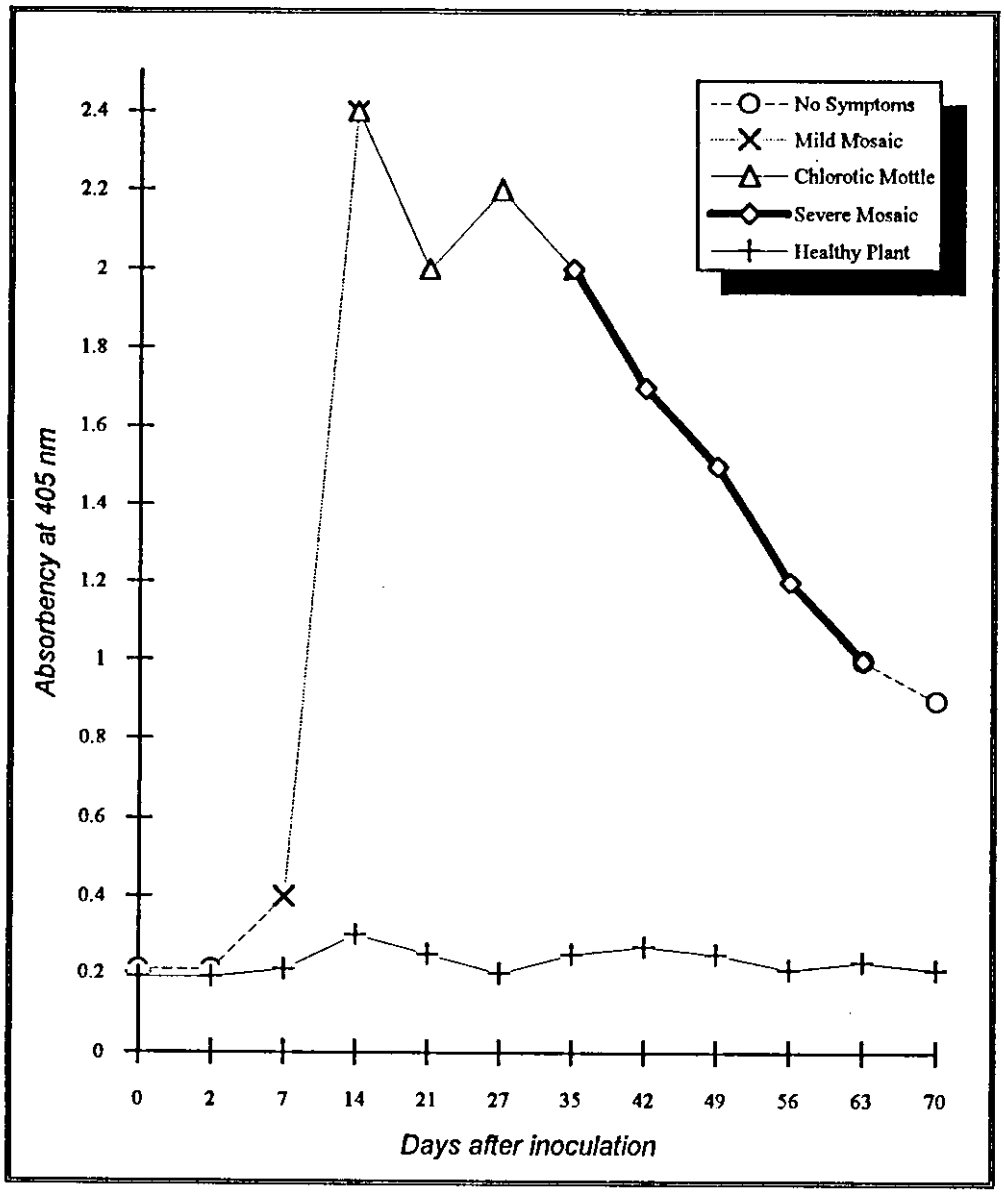


Figure 5 : Virus content variation, and symptom development in mechanically inoculated *Nicotiana tabacum* cv.Havana 423

Based on the ELISA tests, the virus content trend was fluctuated among the three stages. The lowest virus content occurred during the cotyledon leaf stage, and increased to reach the maximum value during unifoliate stage, then decreased to intermediate value during the trifoliate leaf stage (Fig. 6).

Detection of AMV in Other Crops

Different fields of bean, broadbean, carrot, chickpea, lettuce, pepper, potato, radish, squash, and tomato neighboring to alfalfa fields were selected for samples collection (Table 7).

Braodbean samples were found to possess the greatest possibility for AMV infection. ELISA tests detected the virus in five samples, while biological assay detected the virus in two samples which were collected during January and February from the Jordan Valley fields. According to ELISA tests, the virus contributed to 10% infection of the total samples collected from both regions.

Alfalfa mosaic virus was detected serologically in two samples of chilli pepper from open fields of Al-Hallabat region ,while the biological assay for the positive samples failed to detect the virus. All pepper samples collected from plastic houses in the Jordan Valley were found to be free from AMV infection. AMV-infected pepper samples comprised 6.6% of the total samples collected from both regions.

Two samples of tomato collected during February from the open fields in the Jordan Valley were found AMV-infected, while other samples from other fields established in the same area were found to be free from AMV infection.

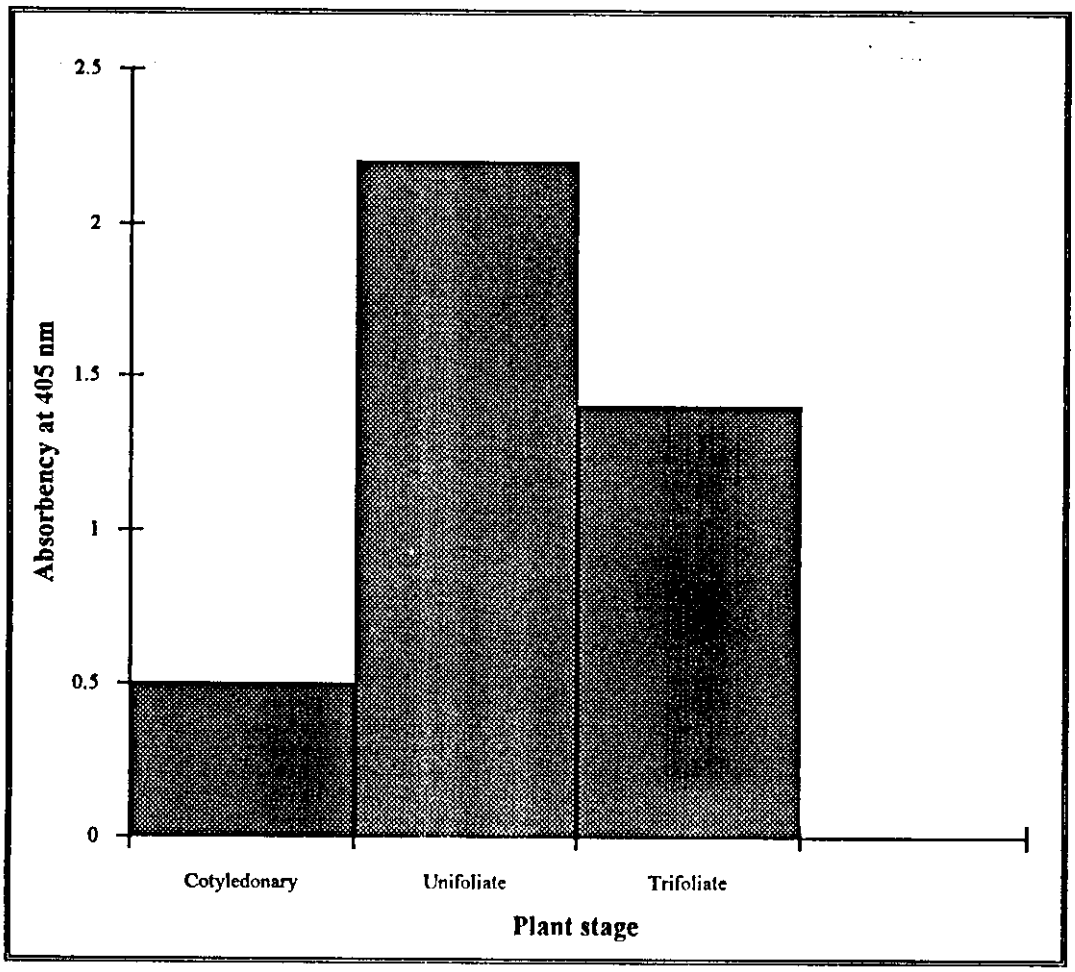


Figure 6 : Virus content development in alfalfa plants grown from virus-infected seeds.

All samples of bean, carrot, chickpeas, lettuce, potato, radish, and squash were found to be free from AMV infection regardless of their location and time of collection.

Occurrence of AMV in Weeds:

ELISA tests for weed samples which were collected from alfalfa fields in the Jordan Valley and Al-Hallabat showed that only two samples belonges to Malvaceae and Compositae families were infected with AMV,while the rest of the samples showed no virus infection (Table 8).

Table 7 : Crop, location, and collection times of samples tested for AMV infection.

Crop	Infected Plants / Total		Collection Time	Location•
	Serology	Biology		
Capsicum frutescens	2/15	0/15	24.10.93	Hall.
Lycopersicon esculentum	0/15	***	10.11.93	U.F.
Cucurbita pepo	0/15	***	10.11.93	U.F
Daucus carota L.	0/15	***	15.12.93	Hall.
Lactuca sativa L.	0/15	***	15.12.93	Hall.
Raphanus sativus L.	0/15	***	15.12.93	Hall.
Cicer arietinum	0/15	***	18.1.94	U.F.
Solanum tuberosum	0/15	***	18.1.94	Der.A.
Solanum tuberosum	0/15	***	18.1.94	U.F.
Solanum tuberosum	0/15	***	18.1.94	M.M.
Vicia faba	3/15	1/15	18.1.94	U.F.
Capsicum fructescens	0/15	***	25.2.94	U.F.
Cucurbita pepo	0/15	***	25.2.94	U.F.
Lycopersicon esculentum	0/15	***	25.2.94	U.F.
Phaseolus vulgaris	0/15	***	25.2.94	U.F.
Solanum tuberosum	0/15	***	25.2.94	Der.A.
Vicia faba	2/15	1/15	25.2.94	U.F.
Daucus carota	0/15	***	20.2.94	Hall.
Raphanus sativus	0/15	***	20.2.94	Hall.
Lycopersicon esculentum	2/15	1/15	25.2.94	U.F.
Cicer arietinum	0/20	***	20.4.94	Rab.
Cicer arietinum	0/30	***	20.4.94	Mosh.
Vicia faba	0/20	***	30.4.94	Hall.

- Der.A : Der Alla (Jordan Valley)
- Hall : Hallabat
- M.M. : Mothallath El-Masrie (Jordan Valley)
- Mosh.: Mushagar
- Rab. : Rabbah
- U.F. : University farm (Jordan Valley)

*** : No biological testing.

Table 8: Weed species and their locations tested for AMV infection .

Weed speices	Family	Location•	Result
Amaranthus gracilis Desf.	Amaranthaceae	U.F.	(–)
Apium graveolens	Umbelliferae	U.F.	(–)
Cardaria draba L.	Cruciferae	Hall.	(–)
Centaurea pallescens Del.	Compositae	U.F.	(–)
Chenopodium album L.	Chenopodiaceae	U.F.	(–)
Chenopodium murale	Chenopodiaceae	U.F.	(–)
Convolvulus arvensis L.	Convolvualceae	U.F.	(–)
Conyza bonariensis (L.) Cron.	Compositae	U.F.	(–)
Cynodon dactylon (L.) Pers.	Gramineae	U.F.	(–)
Heliotropium europaeum L.	Boraginaceae	U.F.	(–)
Hordeum liporinum Link.	Gramineae	U.F.	(–)
Malva sylvestris L.	Malvaceae	U.F.	(+)
Peganum harmala L.	Zygophyllaceae	Hall.	(–)
Prosopis fracta (Ban.) Mac.	Mimosaceae	U.F.	(–)
Silybum marianum	Compositae	U.F.	(–)
Sinapis arvensis L.	Cruciferae	U.F.	(–)
Sisymbrium irio L.	Cruciferae	U.F.	(+)
Sonchus oleraceus L.	Compositae	U.F.	(–)

- Hall. : Al-Hallabat region
- U.F. : University farm (Jordan Valley)

(–) : No infection.

(+) : Virus detected.

DISCUSSION

Isolate (140/93) was identified as AMV based on host range, symptomology, aphid transmission, physical properties, and serological tests. Isolate (43/93) was identified tentatively to be another isolate of AMV based on symptomology and reactions with diagnostic assay plants. *Chenopodium amaranticolor* is the most important diagnostic assay plant differentiating between the two isolates. Isolate (140/93) reacted systemically without developing local lesions on inoculated leaves of *Chenopodium amaranticolor*, while isolate (43/93) developed chlorotic local lesions followed by yellow mosaic.

Systemic invasion with or without local lesions on inoculated leaves of *Chenopodium amaranticolor, Chenopodium quinoa, Nicotiana glutinosa,* and *Nicotiana tabacum,* and local lesions with or without systemic invasion on different cultivars of *Phaseolus vulgaris* and *Vigna unguiculata* are good indication for alfalfa mosaic virus (7, 9).

The efficiency of aphid transmission for the isolate (140/93) was depending on aphid species. *Macrosiphum pisi* was found to be the most efficient aphid vector followed by *Aphis faba*. These aphid species are able to infest legumenosae (34), and so, they are expected to participate strongly *in Vivo* virus dissemination.

The virus needed one year to increase from 11% to 43% in newly established fields,while it needed 2-3 years to reach 80-100%. These results indicated that there was a positive correlation between virus incidence and the age of plants. Similar results, in some respects were obtained in Wisconsin by Crill *et al.* (6). They reported 63% virus infection from 2-

years-old and 78% from older alfalfa strands. In Western North America, Rahman and Peaden (5) reported 50-70% virus infection from 1-year-old, 40-90% from 2-years-old and 10-100% from older alfalfa strands.

The pattern of increase in the incidence of AMV in newly established alfalfa fields might be due to the infected seeds which act as a primary source of infection and to the subsequent transmission by the aphid vectors. In older fields of alfalfa, the disease incidence increased slowly and gradually. Hiruki and Miczynski (19) reported that once the virus is established in alfalfa field, aphid transmission and repeated cutting may lead to a gradual increase of AMV infection in subsequent regrowths.

Quantitative assessment of virus content in alfalfa fields revealed that maximum virus content occurred during the end of December and October in the Jordan Valley and Al-Hallabat regions, respectively. This finding may be due to the warm temperature that prevailed during these two months. The minimum virus content during spring and summer in both regions may be explained by the existence of high temperature during that time. In the Jordan Valley, the average monthly maximum temperature prevailed at the end of December and the beginning of January ranged from 23.3 to 20.5 C°, while the average monthly minimum temperature during the same period ranged from 14.5 to 13.4C°. After January, the temperature started gradual increasing until reached its maximum value during August. The average monthly maximum temperature which occurred during summer ranged from 36.6 to 39.6C°. Similar situation occurred in Al-Hallabat region, in which the average maximum and minimum temperatures occurred during October were 30.2 and 13.6 C°, respectively, while the average monthly maximum temperature prevailed during summer ranged from 34.2 to 36.2 C° [Appendix 3]. Crill *et al.*(44) obtained more virus content under

warm temperatures between 24-28C° and a suppression of the content at low temperature. Mayoral *et al.* (45) found that relatively high temperature causes deleterious effect in the virus structure thus hindering the virus replication capability. However, the differences in virus content between the Jordan Valley and Al-Hallabat regions might be attributed to differences in number of crop cutting and irrigation regimes. Sprinklers were the most dominant method of irrigation in the Jordan Valley, whereas surface irrigation was used in AL-Hallbat area.

Alfalfa seeds and virus content investigations in the developing alfalfa seedlings showed that AMV is seed transmissible, and the virus content reached maximum concentration during unifoliate leaf stage. The results obtained in this case correspond to those previously reported by other researchers (8,20). They found that the infected seeds ranged from 0.8-28.5%, and the maximum virus content occurred during primary leaf stage. Such investigation showed that checking of alfalfa seedlings infection with AMV in their early growing stages was methodologically very important. With considerable number of infected seedlings the presence of AMV was detected in stage of primary leaf. The results pointed out the possibility of relatively early AMV detection in germinated alfalfa seedlings.

This study showed that the virus rarely occurred in the fields of vegetables and other economic crops outside the legumenosae. These results could be explained by the limitation of alfalfa cultivated area, low flight activity of the efficient vectors from alfalfa fields to other fields, and protected planting system of certain crops. In the Jordan Valley, leaf samples of chilli pepper and bean were collected from plastichouses adjacent to alfalfa fields. This planting system may explain their AMV-free position. Absence of AMV in chilli pepper planted in the Jordan Valley agreed with the results of Batarseh (14)who isolated potato virus Y (PVY) and tomato aspermy virus (TAV) from sweet and chilli pepper planted in

different locations in the Jordan Valley. Similar results, to some extent, had been obtained in Jordan by other researchers. Al-Musa and Mansour (46) found that tomatoes were infected by tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), (PVY), and the predominant tomato yellow leaf curle virus (TYLCV). Takrouri (16) identified the viruses causing fruit necrosis of tomatoes in the Jordan Valley to be only CMV and TMV.

Assessment of virus content in *Nicotiana tabacum* cv. Havana 423 grown under glasshouse conditions showed that there was a positive correlation between virus content and flushes of severe symptoms. Virus content and symptom flushes coincided with a period of more rapid host growth (47, 48, 49).

CONCLUSIONS

1- Alfalfa mosaic virus was found to be widely distributed in alfalfa fields.

2- Incidence of AMV in alfalfa fields was correlated with the age of plants. Leaving alfalfa fields for long time without renewing will maximize the virus incidence, and increase chances for infection of other crops.

3- This study showed that AMV is seed and aphid-transmissible. Infected seeds act as a primary source of virus inoculum in newly established alfalfa fields which when coupled with aphid transmission play an important role in virus epidemiology. And therefore, adequate ELISA-indexing schemes for imported seeds is highly recommended.

4- Although, alfalfa mosaic virus is known for its wide range on legume and non legume plants, this work indicated the scarcity of this virus on economic crops neighboring to alfalfa fields. This situation might be due to limited alfalfa planting. However, larger scale planting of alfalfa is expected to cope with high demand of animal production. In this case, as it is in Yemen (33), the probability of widespread infection of AMV to economic crops planted in the surroundings will increase, and therefore isolation of alfalfa forage crops from other susceptible crop fields may strongly contribute to reduction of disease incidence.

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APPENDICES

Appendix 1. Buffers used in indirect ELISA tests

Washing buffer :

Phosphate buffer saline (PBS-Tween) PH = 7.4

NaCL	=	32 gm
KH_2PO_4	=	0.8 gm
Na_2HPO_4	=	11.6 gm
KCL	=	0.8 gm
NaN_3	=	0.8 gm
H_20	=	4 Liters
Tween-20	=	2 ml

Grinding buffer (CEP) PH = 9.6

Na_2CO_3	=	3.13 gm
$NaHCO_3$	=	5.86 gm
NaN_3	=	0.4 gm
PVP	=	40 gm
Egg albumin	=	4.0 gm

- All are dissolved in 2 liters H_2O

PEP buffer for antiserum and conjugate dilutions :

Phosphate buffer saline (PBS-Tween) = 1200 ml
Polyvinyl pyrrolidone (PVP) = 24 gm
Egg albumin = 2.4 gm

Substrate buffer PH = 9.8

Diethanol amine	=	97 ml
H_2O	=	800 ml
NaN_3	=	0.2 ml

- Fill up to 1 liter with H_20, add HCL to give PH = 9.8

Appindix 2: Absorbence value taken after 30 minutes at 405 nm of ELISA plate charged with buffer, infected and healthy leaves of *Nicotiana tabacum* cv. Havana 423.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.006	0.008	0.005	0.002	0.001	0.000	0.000	0.004	0.001	0.000	0.001	0.005
B	0.059*	0.054*	0.065*	0.000	2.293■	1.745■	2.068■	1.909■	0.011	0.054●	0.044●	0.040●
C	0.049*	0.041*	0.058*	0.003	1.902■	2.023■	1.994■	2.178■	0.005	0.046●	0.038●	0.037●
D	0.067*	0.064*	0.067*	0.001	2.399■	1.765■	1.925■	2.068■	0.010	0.060●	0.054●	0.036●
E	0.069*	0.053*	0.063*	0.003	2.000■	2.076■	1.988■	2.236■	0.007	0.049●	0.041●	0.043●
F	0.072*	0.070*	0.075*	0.001	2.192■	2.071■	2.098■	2.042■	0.007	0.055●	0.055●	0.043●
G	0.086*	0.078*	0.083*	0.005	1.991■	2.199■	2.198■	2.291■	0.006	0.053●	0.048●	0.052●
H	0.000	0.000	0.000	0.006	0.003	0.000	0.000	0.003	0.007	0.006	0.013	0.005

* : Healthy sample
■ : Infected leaves with isolate (140/93)
● : Buffer

الملخص

حدوث فيروس تبرقش الفصة وتوزيعه على نباتات الفصة في الأردن

إعداد

حازم ذباب عارف صوالحة

إشراف

الدكتور عقل منصور

جُمعت مِئَتا عينة من نباتات الفصة التي ظهرت عليها أعراضٌ شبيهة بالأعراض الفيروسية من منطقتي الحلابات وغور الأردن على مدى سنة كاملة بين عامي ١٩٩٣م و ١٩٩٤م. ولدى فحص هذه العينات بيولوجياً باستخدام النباتات المخبرية الكاشفة، تم تمييز عزلتين فيروسيتين، عُرِّفت إحدى هاتين العزلتين تعريفاً نهائياً باستخدام المدى العائلي والناقل الحشري والخصائص الفيزيائية والمصلية على أنها فيروس تبرقش الفصة. بينما عُرِّفت الثانية تعريفاً مبدئياً على أنها عزلة ثانية من الفيروس نفسه.

وأثبتت الدراسات التي أجريت على مدى انتشار هذا الفيروس في حقول الفصة أنّ ثمة علاقةً إيجابيةً تقضي بزيادة مدى انتشار الفيروس بتقدم عمر النبات. إذْ وجد أنّ الإصابة تصل إلى حوالي ٤٣٪ في الحقول التي لا تزيد أعمارها على سنة واحدة، ومن ٥٠-٧٠٪ في الحقول ذات الأعمار التي تتراوح من سنة إلى سنتين، في حين تصل الإصابة من من ٨٠-١٠٠٪ في الحقول التي تتراوح أعمارها من سنتين إلى ثلاث سنوات.

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وبينت الدراسة أنّ التركيز الفيروسي يصل إلى أعلى قيمة له في نهاية شهر كانون الأول في منطقة الحلابات، أيضاً بينما يصل إلى أعلى قيمة له في منطقة غور الأردن في نهاية شهر تشرين الأول .

وأثبتت الدراسة التي أجريت لمحاولة عزل الفيروس من النباتـات الإقتصاديـة المزروعـة في مناطق مجاورة أو قريبة من حقول الفصة أن معظمها خالٍ من الإصابة. إذ تّم الكشف عن وجود الفيروس في بعض عينات الفلفل والفول والبندورة فقط.

Appendix 3: The average values of maximum and minimum monthly temperatures (C^{O}) in Deir Alla and Wadi Dhuleil during between January, 1993 to June, 1994.

	Deir Alla		Wadi Dhuleil	
Month	Max. Temp.	Min. Temp.	Max. Temp.	Min. Temp.
January	17.3	9.5	12.5	0.1
February	17.2	8.7	13.4	0.9
March	22.7	11.6	19.4	4.0
April	29.9	15.3	26.4	8.1
May	31.7	18.1	28.5	12.0
June	37.5	22.0	34.6	15.6
July	38.2	23.6	35.5	17.0
August	39.2	24.5	36.2	17.5
September	36.6	22.9	34.2	15.1
October	34.9	23.3	30.2	13.6
November	25.8	16.8	20.5	6.1
December	23.3	14.5	18.7	4.8
January	20.6	13.4	15.3	4.7
February	20.5	11.8	15.5	3.4
March	23.3	12.5	20.0	5.8
April	32.4	18.1	28.4	10.4
May	35.4	19.6	32.1	13.5
June	36.5	21.9	33.9	15.3

Information provided by the nearest Meteorological Data Research Station to the Jordan Valley and Al-Hallabat areas, respectively (50).