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FACULTY OF GRADUATE  
STUDIES

STUDY OF POTATO VIRUS Y ( PVY ) AND POTATO VIRUS A  
( PVA ) IN JORDAN VALLEY: INOCULUM SOURCES AND  
INCIDENCE.

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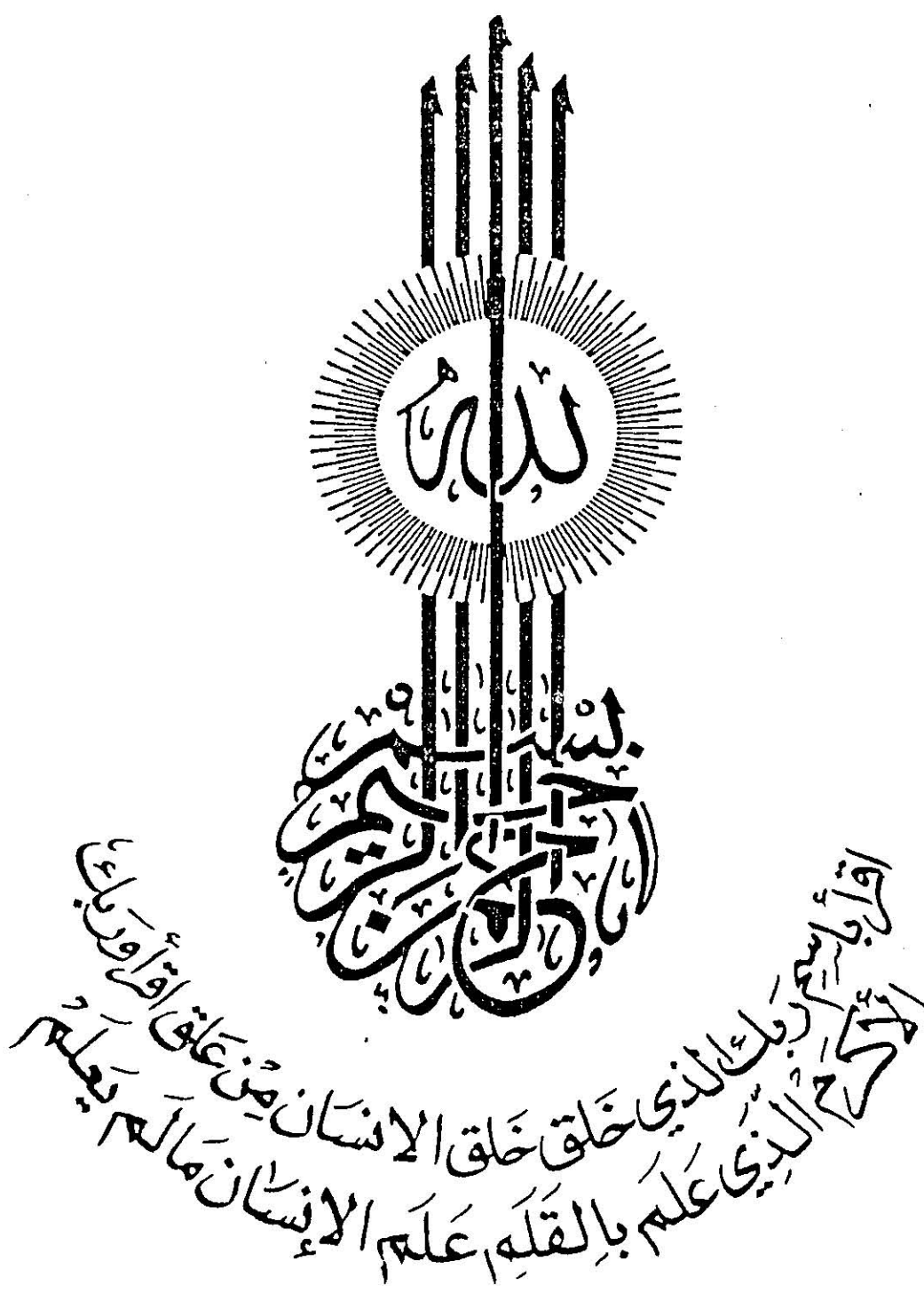
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## ABSTRACT

A study was conducted to identify inoculum sources and incidence of PVY and PVA in the Jordan Valley. Detection of these two viruses was done by ELISA and biological assay.

Incidence of virus infection in imported seed potatoes for 1991 / 1992 seasons was determined for each seed lot. In bioassay tests however, the average incidence reached 10.67 % for PVY and 5.75 % for PVA. The fact that PVY and PVA in seed potatoes occurred at a rate higher than that accepted by Jordanian quarantine regulation rules, may indicate their important role as inoculum source.

Three hundred and two weed samples were collected from different locations in the Jordan Valley, but *Solanum nigrum* L., a wide spread annual weed in the Jordan Valley is apparently the only weed found to be infected with PVY and PVA. About 26 % of the samples collected from this plant were infected with PVY, of which 42 % were mixedly infected with PVA.

One hundred and four samples of volunteer potato plants were collected from fields along the Jordan Valley. PVY and PVA were detected by bioassay 20.19 and 5.78 % of collected samples, respectively.

During 1991 fall growing season, incidence of the mosaic diseases affecting potatoes in the Jordan Valley reached 10 %, 12 % and 42 % for fields in Al-Karama, Dair-Alla and Wadi-Al-Yabis, respectively. However, incidence of the mosaic diseases during 1992 spring growing season, reached 31 %, 53 % and 62 % for fields in Kraimeh, Karama and Al-Arda, respectively.

A major flight activity period for the total winged aphids and *Myzus persicae* was detected in the spring season, whereas a second minor flight activity period was detected in the fall.

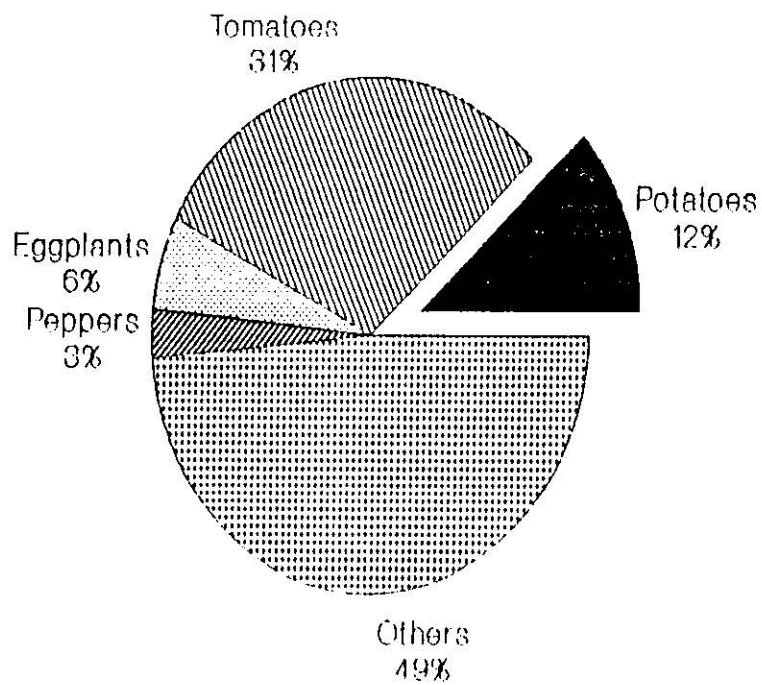
## INTRODUCTION

The potato ( *Solanum tuberosum L.* ) is one of the most important crops as it ranks the fifth major food crop next to wheat, rice, maize, and barley in the world ( 1, 2 ).

The world total potato production during 1990 had been estimated to be 269561 thousand metric tons, planted to 17854 thousand hectares, with an average yield production of 1,5098 kgs / ha. ( 3 ). Half of the world total potato production is used for human consumption and a third was still used for stock feed, mainly in Eastern Europe. Potatoes are also planted for starch production in Netherlands, Eastern Europe and Japan. The use of potatoes for alcohol production is negligible ( 4 ). In Jordan the area planted to potatoes increased from 10,000 dunum during 1979 - 1981 to 34,508 dunums during 1991 and the average yield increased from 1,687 to 1,783 kgs / dunum. During 1991 the area approached 12 % of the total area planted to vegetables and 23.1% of the total area planted to Solanaceous crops ( Fig.1). The Jordan valley is the main production area in Jordan where 71.3 % of the total area planted to potato is located ( 3, 5 ).

Potatoes are planted at two main growing seasons in the Jordan valley; the first season is in spring and the second is in fall. During 1991 / 1992, Jordan depends totally on imported seed tubers mainly from Syria and Netherland. There are many obstacles associated with importation process, among which transportation, storage, and the propability to be carrier for tuber-borne pathogens especially potato viruses are most important. In few cases some farmers store part of the spring crop to be used as seed potatoes for the following fall planting season.

The vegetative propagation of potatoes, enhance infection by viral and / or mycoplasma-like organisms and their passage from one generation to the next ( 6, 7 ). Potato crop is infected naturally with more than thirty three viruses and mycoplasma-like organisms not counting the many strains within some types ( 7 ). Virus multiplication occurs in the leaf cells and



**Fig. 1 : Percentage of vegetables planted areas in Jordan during 1991.**

when the concentration of the virus reaches a certain level it is transported<sup>3</sup> to tuber (7). The difference between a tuber infected with a virus and a healthy one can be assessed only through laboratory methods. Visual detection of viruses in field infected tuber is impossible ( 8 ).

Natural infection with potato virus Y ( PVY ), can reduce the yield up to 80 %, depending on virus strain and potato cultivars ( 8 ), Whereas Potato virus A ( PVA ) can decrease yield of infected potatoes up to 40 % (1).

Information on initial sources of potato viruses in Jordan is lacking. Nevertheless, weeds, volunteer potato plants and seed potatoes may constitute the primary sources of inoculum ( 1, 7 ). This study aims at extending information about the epidemiology of potato virus Y ( PVY ) and potato virus A ( PVA ) in the Jordan valley with regard to inoculum sources and incidence of the disease in the field. Identification of such sources, (imported tubers, weeds, and volunteer potato ) and incidence study will be helpful to construct proper management approach to control these diseases. Also to determine vector flight activity. Moreover, this study is expected to evaluate ELISA as a diagnostic technique for PVY and PVA in potato tubers.

## LITERATURE REVIEW

Potato (*Solanum tuberosum* L.) is infected naturally with more than thirty three viruses and mycoplasma-like organisms ( 7 ). Viruses that are most frequently encountered in potato fields are alfalfa mosaic virus (AMV ), andean potato latent virus ( APLV ), andean potato mottle virus ( APMV ), potato aucuba mosaic virus ( PAMV ), potato leaf roll virus ( PLRV ), potato mop-top virus ( PMTV ), potato virus A ( PVA ), potato virus M ( PVM ), potato virus S ( PVS ), potato virus X ( PVX ), potato virus Y ( PVY ), potato yellow dwarf virus ( PYDV ) and tobacco rattle virus ( TRV ) ( 1, 7, 9, 10 ). Mixed infection could be also encountered. Viruses which are less encountered include: Beet curly top virus ( BCTV ), cucumber mosaic virus ( CMV ), egg plant mottled dwarf virus (EMDV), potato black ringspot virus ( PBRV ), potato chlorotic stunt virus (PCSV ), potato virus T ( PVT ), potato virus V ( PVV ), potato yellow vein virus ( PYVV ), tobacco etch virus ( TEV ), tobacco mosaic virus ( TMV ), tobacco necrosis virus ( TNV), tobacco ringspot virus ( TRSV ), tobacco streak virus ( TSV ), tomato black ring virus ( TBRV ) and tomato spotted wilt virus ( TSWV ) ( 1, 7, 9, 10, 11, 12 ). Potato spindle tuber viroid ( PSTV ) could be also encountered ( 1, 7 ).

These viruses are mechanically transmitted viruses with the exception of potato leaf roll and beet curly top virus which are transmitted only by insect vector ( 13, 14 ).

Most potato viruses have achieved a worldwide spread in most potato growing areas ( 1, 7 ). PVY is one of the two most important viruses affecting potato crop on a worldwide basis ( 8, 15 ). In Sweden PVY<sup>O</sup> is the most important aphid-borne virus of potatoes, far more important than PLRV ( 14 ). During a survey conducted on potatoes in the Jordan valley and the high lands, Al-Mnayer ( 6 ) identified two mechanically transmitted viruses, namely PVX and PVY. Incidence of PVY was reported to approach 27 % for the fall growing season and 24 % for the spring season.

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tubers ( 1, 7, 15 ). There is no consistent serological differences between strains of PVY ( 6, 15 ). PVY is serologically distantly related to tobacco etch, henbane mosaic, potato virus A, pepper veinal mottle and bidens mottle viruses, although the degree of relationship among these viruses is difficult to assess ( 15, 21 ).

PVYN strain is more stable and attains higher concentration than PVYO in infected plant tissue, which may lead to higher potential rate of transmission by aphids in the non-persistent manner of transmission ( 22 ). Translocation of PVYN into tuber after primary infection is faster than that of PVYO, while mature plant resistance develops later against PVYN than PVYO ( 14, 22 ). Cultural practices such as applications of nitrogen fertilizer could be important in determining the level of mature plant resistance against PVY ( 14 ).

PVY, is readily transmitted by mechanical inoculation, by stem grafting and core grafting ( 1, 15 ). The virus is transmitted in a stylet borne manner by at least 25 species of aphids ( 1, 7, 14, 15, 23, 24, 25, 26, 27 ). Among these the following aphid vectors are found in Jordan ( 28, 29, 30 ): *Aphis fabae* ( Scopoli ) ( 14, 15, 24, 25 ), *Aphis nasturtii* ( Kaltenbach ) ( 14, 24, 26, 27 ), *Acyrtosiphon pisum* ( Harris ) ( 14 ), *Brachycaudus helichrysi* ( Kaltenbach ), *Hyperomyzus lactucae* ( L ) ( 24, 27 ), *Macrosiphum euphorbiae* ( Thomas ) ( 14, 15, 20, 25, 26 ), *Metopolophium dirhodum* ( Walker ) ( 24 ), *Myzus persicae* ( Sulzer ) ( 1, 14, 15, 20, 24, 25 ), *Myzus certus* ( Walker ) ( 15 ), *Myzus ornatus* ( Laing ) ( 7 ), *Rhopalosiphum padi* ( L ) ( 14, 24, 25 ), *Sitobion avenae* ( Fabricius ) ( 27 ) and *Sitobion fragariae* ( Walker ) ( 27 ). *Myzus persicae* ( Sulzer ) is the most efficient vector; in many areas and seasons ( 1, 7, 15, 25 ). Other aphid species that are capable of transmitting PVY but were not reported in Jordan are: *Aphis frangulae* ( Kaltenbach ) ( 14, 24, 26 ), *Cryptomyzus galeopsidis* ( Kaltenbach ) ( 27 ), *Cryptomyzus ribis* ( Linnaeus ) ( 27 ), *Cavariella pastinacae* ( L ) ( 7 ), *Hyadophis foeniculi* ( Passerini ) ( 27 ), *Neomyzus*



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combination with PVA drastically reduced the crop yield. A systemic mosaic spotting and a slight curling of leaves were characteristic features in complex infection of PVA with PVX; while local systemic necrotic lesions and in some cases plant tip necrosis were such features of the PVA infection with PVY ( 7 ). When potato plants were infected with potato spindle tuber viroid ( PSTV ) and PVY or with PSTV prior to PVY infection, severe necrotic symptoms were reported ( 32 ). *Myzus persicae* can transmit potato aucuba mosaic virus only when the source plant also contains PVA or PVY ( 15, 21 ).

To study the epidemiology of PVY and PVA interaction among host, virus, vectors and environment must be taken into account ( 1, 14 ). Most potato viruses survive from season to season in infected tubers which produce systemically infected foliage, or remain in soil unharvested to produce infected volunteer plants that constitute reservoir hosts ( 1, 7, 34, 35 ). Seed tuber infection eliminates the requirement for movement of viruses from outside source into the potato fields, which is difficult step in dissemination of viruses transmitted by contact and by insect in nonpersistent manner (34) . Nienhaus *et al.* ( 36 ), found that volunteer potato plants in the Beqa'a plain showed heavy infection with PLRV, this may suggest that the early spread of this virus is most serious since the aphid vector ( *Myzus persicae* ) is most prevalent at the early stage of growing season . Volunteer potato plants were implicated as the main source of virus vectors and of inoculum accounting for the increases in incidence of PLRV and PVY ( 34 ). The time of tillage practices following potato harvest affected numbers of volunteer potato plants that emerged ( 37 ).

Weeds may also act as alternate hosts for potato viruses ( 1, 19, 34, 35, 38 ). Weeds play an important role as a reservoir for PVY ( 19 ). Perennials seldom act as reservoirs for PVY in nature ( 1 ). In Columbia Basin, native desert flora could not provide a source of primary inoculum because it is

dry or dormant during the period of potato virus dissemination ( 34 )<sup>9</sup>. A common Solanaceous weed, *Solanum gracile* link, nightshade, was an important host of PVY in Florida, and disease gradients were established in pepper fields away from a line-focus source of nightshade ( 39 ). *Solanum nigrum* var. *judaicum*, reported in Egypt to be naturally infected with PVY ( 38 ).

For the continuouance of disease, pathogens require not only means of survival but also effective method of spread. Aphid transmitted viruses depend on the build-up of the vector population, the stage of development of potato crop. Early infestation of *Myzus persicae* carrying PVY can almost destroy the crop ( 8, 35).

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*Myzus persicae*, which can live on a wide range of farm crops and weeds such as Shepherd's purse *Capsella bursa-pastoris* and black nightshade *Solanum nigrum* L., transmits several virus diseases such as PLRV and mosaic viruses ( 35, 40 ). *Myzus persicae* is seemingly the most potent vector for a number of potato viruses including PVY and PVA ( 1, 7, 15, 21, 25 ). The incidence of aphid transmitted viruses such as PVY and PVA increases to a greater degree, during the growing season in potatoes, compared to the incidence of viruses which are more dependent upon mechanical transmission such as PVX ( 7 ). Virus transmission to healthy plants during the growing season depends on the naturally occurring aphid population ( 41 ). Number of virus sources is of supreme importance and can be decreased by rouging. But if spring flight is very early, virus may spread before diseased plants show symptoms ( 7 ). Even low incidence of seed infection could be very important in the epidemiology of potato viruses when high aphid population is present in the area ( 34 ). The viruliferous aphids from the infected potato might spread by moving to next plant. Transmission rate of virus disease on potato was higher in the potato located near the virus source ( 2, 19 ). Alate aphids and not apterae are the important transmitter of PVY in potato fields ( 26, 42, 43 ). No correlation

was found between the occurrence of apterous aphids on potato foliage and spread of PVYO ( 14 ). Increasing accumulated vector pressure (AVP) and accumulated *Myzus* pressure ( AMP ) values resulted in a higher proportion of tubers infected with PVYN, AMP value being the major factor. Accumulated AVP and AMP calculated as a weighed sum of flight activity of aphid species in combination with a relative efficiency factor for each species ( 42 ). Counting of *Myzus persicae* on potato plants from emergence until the time that the traps caught a reliable number of aphids gave more information about early infection ( 42 ). The actual virus transmitting capacity of aphids in the fields depend on ecological and physiological factors, such as preference of aphids, distribution and concentration of viruses in the host ( 25 ). Relative humidity, and rainfall were primary factors influencing immigration of *Myzus persicae* ( 23 ). Flight activity of *Myzus persicae* in the Jordan valley showed two main peaks during 1986, 1987, and 1988, the first peak occurred in the spring and the second in the fall ( 40 ). When the vector numbers reach a critical level the haulms destruction is an important method to prevent virus infection or translocation from foliage to tubers ( 14, 25, 42 ). Insecticides treatment against aphids on potato foliage have little or no effect on the transmission of the non-persistently transmitted viruses ( 26, 44 ). The occurrence of viral infection in potato is commonly signified by the presence of symptoms ( 7, 31 ). Visual inspection of potato crop growing in the field is fully effective in detecting virus infection only when symptoms are reasonably conspicuous and characteristic. Detection of infection with inconspicuous or no symptoms require testing of potato leaf samples ( 7 ).

Serology is one of the most commonly used techniques for indexing potato viruses ( 6 ). Enzyme-linked immunosorbent assay ( ELISA ) was first applied to detection of plant viruses by Voller *et al.* ( 47 ). They showed that several nanograms of viral antigen per milliliter could be detected by ELISA. The ELISA has a number of advantageous properties

over the methods previously used for routine detection of plant viruses, above all high sensitivity, specificity, and easiness to apply in any moderately equipped research laboratory ( 47 ). ELISA sensitivity made it a widely usefull tool in diagnosis of plant viruses ( 9, 45, 46, 47, 48 ). Agglutination, microprecipitin, and radial immunodiffusion tests, are not sensitive enough for the routine detection of PVY in potato tubers or leaves ( 47 ). ELISA test was reliable for indexing PVX, PVY and potato leaf roll virus using potato foliage ( 46 ). ELISA has been successfully used for detection of PVY in primarily and secondarily infected tubers (48). At present, it is the only relatively rapid test method for the detection of PVY and PVA ( 47 ). In addition the ELISA test allows detection of latent viruses ( 49 ). Application of ELISA directly to tubers could not yet be envisaged for high quality material, since the method needs still to be improved for certain viruses ( PVA, PVS, PVM ) ( 49 ). Detection of PVY and PVA by ELISA was very difficult and unreliable in tubers after natural break of dormancy ( 48 ). While after break of dormancy with Rindite ( a mixture of anhydrous ethylene chlorohydrin, dichloroethane and carbon tetrachloride 7: 3: 1 by volume ), maximum concentration of PVY and PVA were detected at both tuber ends with generally higher values at the rose end than that at the heal end ( 48, 51).

Growing-on test is used to determine virus seed transmission ( 53 ). It allows assessment of tuber borne viruses after harvest for post-harvest inspection ( 31 ), which is compulsory for class S and SE. However, classes E, A and B are often not submitted to post-harvest control, provided that halum destruction was done in time ( 4 ).

Potato virus Y and PVA both infect potato, have particles indistinguishible by electron microscopy, and are difficult to distinguish by symptoms they produce in many test plants. However, they may be distinguished serologically ( 15, 21 ). However, PVA showed some cross reaction with PVY, and other potyviruses which may pose as a difficulty during seed-

potato testing by ELISA ( 21, 49, 52 ). Strain differentiation of PVY could<sup>12</sup> be achieved by ELISA when monoclonal antisera is employed ( 50 ). The potato clone A6 ( *Solanum demissum* X *Solanum tuberosum* Aquila ) developed local lesions after inoculation with many strains of PVA, PVY, including PVYC, PVYN and PVYO, and some severe strains of PVX ( 1, 7, 15, 21 ). Detached leaves of the clone A6 when inoculated with PVA develops local lesions, which might be distinguished from those of PVY under controlled environment ( 1, 7, 54 ). Detached leaves of *Solanum demissum* P.I. 230579 was homozygous for the local reaction of PVY and was a valuable aid for indexing aphid or mechanically inoculated potato for resistance to this virus (1, 21, 55 ). *Solanum demissum* P.I. 175404 reacted with local necrotic lesions upon infection with PVA ( 7, 21, 56 ). The detection of PVY by bioassay using *Solanum demissum* P.I. 230579 was more sensitive than ELISA ( 57 ). This is not surprising since ELISA test has been shown to be less sensitive than bioassay with certain other virus-host combinations. Detection of PVA in potato directly from tubers with primary or secondary infection on detached leaves of clone A6 ( *Solanum demissum* X *Solanum tuberosum* Aquila ), was not reliable in seed certification program compared with the common indexing test from potato leaves ( 54 ).

## MATERIALS AND METHODS

Before developing an efficient control method for any specific plant virus disease, epidemiology of the disease must be investigated and understood. Therefore, it is important to identify the initial sources of infection from which the virus spreads into or within a crop. Most potato viruses survive from season to season in infected tubers, which produce systemically infected foliage ( 1, 7, 34 ). Many viruses have weed or other alternative hosts, that provide a reservoir for virus from which economically important crop plants may become infected ( 1, 7, 9, 19, 34, 35 ). The volunteer plants arose from tubers missed in the previous harvest have been implicated as a primary source of inoculum in the epidemiology of potato viruses in many regions of the world ( 1, 7, 9, 34 ).

### **I- Inoculum Sources :-**

#### **I.1-potato seed-tubers:-**

Since almost all seed potatoes used in Jordan during 1991 / 1992 seasons were imported from Syria and Netherlands, it is important to assess infection levels by PVY and PVA in seed lots imported from these countries.

Incidence of virus infection in imported seed potatoes was determined for samples of each seed lot. Samples were supplied by the Ministry of Agriculture. These samples represented seed lots destined to be sold in the Jordanian market as a commercial seed potatoes. Randomly chosen 90 potato seed tuber from each imported seed tubers lot were indexed for the presence of PVY and PVA by Enzyme-linked immunosorbent assay (ELISA). The results were then substantiated by biological assay.

### I.1.a-ELISA tests :

The indirect form of ELISA was used in all tests for seed potatoes. Antisera to PVY and PVA were supplied by Agdia inc. company ( Indiana, U.S.A. ). The test procedure adopted followed those described by Clark *et al.* ( 45 ) and Hill ( 31 ). Antisera and conjugate dilutions were compared in preliminary ELISA tests, using sets of positive and negative controls. The dilutions that gave the best reaction were selected in further tests. Several buffers were used in the various steps involved in the ELISA, according to Koeing ( 58 ) ( Appendix 1 ).

Tests were made in polystyrene flat-bottom microtitre plates, type T.M ( 2 ) ( Dynatech Immulon Ltd. U.S.A. ) with an array of 8 x12 wells.

One gram of potato peel and tissue from the heel and rose ends of each seed tuber were macerated at 1 : 5 dilution in grinding buffer, 0.02 M sodium phosphate pH 7.4 containing 0.15 M sodium chloride, 2.0 % polyvinylpyrrolidone ( PVP ), 0.2 % egg albumin and 0.05 % Tween 20 ( PEP ). ELISA plate wells were separately charged with 0.200 ml sap extract. Healthy sap extract and 0.02 M sodium phosphate pH 7.4 containing 0.15 M sodium chloride and 0.05 % Tween 20 ( PBS-T ) buffer were used as a control. The plates were then covered and then incubated overnight at 6 C°. After the sap extract was discarded, the plate was washed by flooding with PBS-T. This process was repeated three times. The wells were then loaded with 0.200 ml of antisera specific to PVY or PVA diluted in PEP buffer at 1 : 500 dilution. The plates were incubated 1 hr. at 37 C°, after which the antisera was discarded and the plates were washed as before. Then 0.200 ml aliquots of goat antirabbit conjugate diluted to 1 : 2000 in PEP buffer were added to wells that had been coated with PVY or PVA antisera. The plates were incubated 3 h at 37 C°, and then washed as before. The 0.200 ml aliquots of freshly prepared substrate were added. The substrate was p-nitrophenyl phosphate (Sigma) at a concentration of 0.20 mg / ml in substrate buffer. Plates were incubated at laboratory



temperature, until the hydrolysis reaction had progressed sufficiently to induce the production of a yellowish coloration ( p-nitrophenol ) ( plate 1 ). Results were taken after 30 minutes, using ELISA reader BIO-TEK model EL 308. The results were considered positive when the absorbance values at 405 nm of the two sample replicates exceeded the healthy control values by a factor of two ( 45 ).

### **I.1.b-Bioassay :**

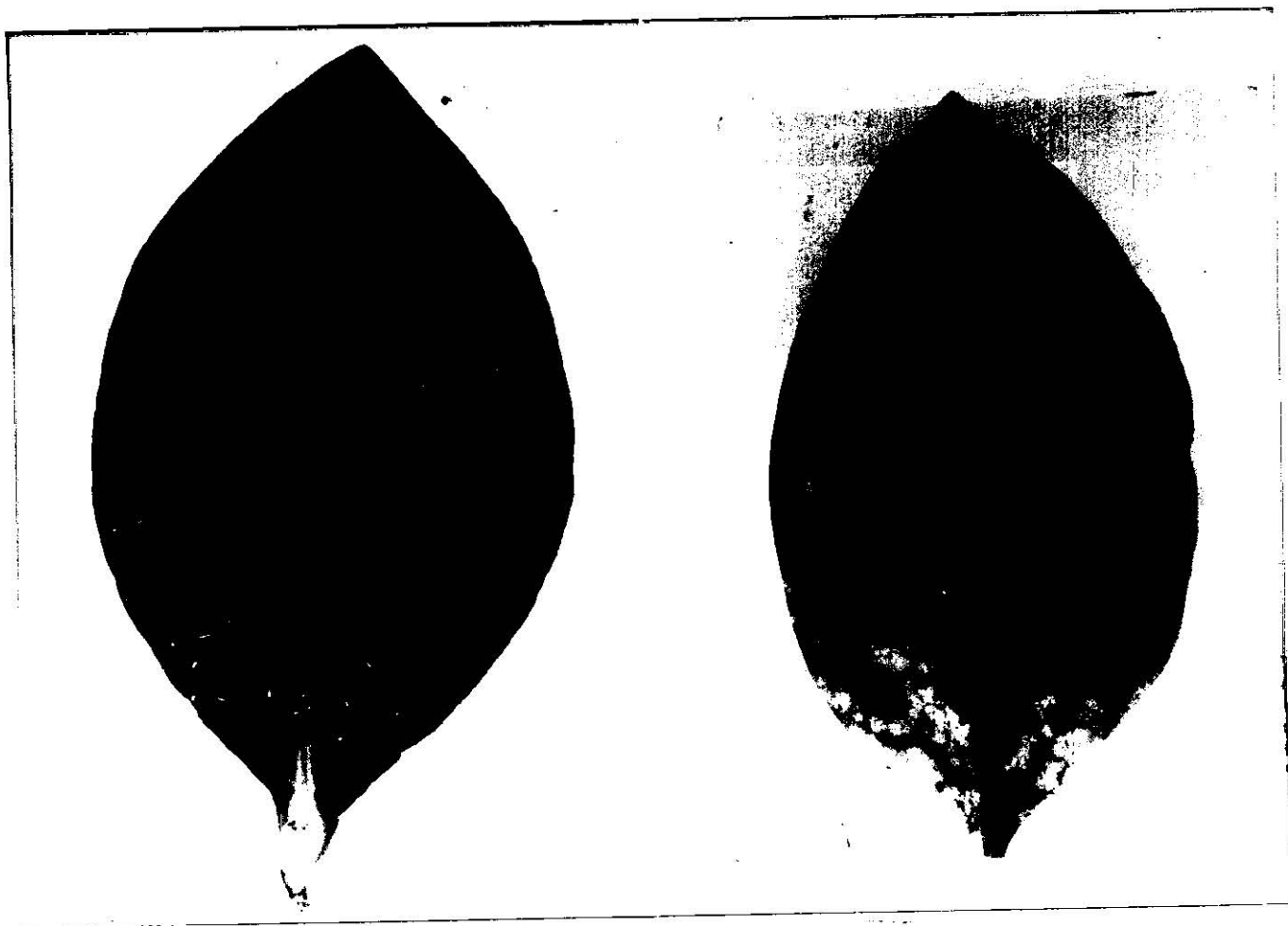
Labeled potato tubers that had been tested by ELISA were soaked in berelex ( Gibberellic acid [ GA3 ] ) at 50 ppm cocentration for 3 minutes, then grown in plastic pots filled with methyl bromide fumigated or pasturized soil under glass house conditions. At proper stage of growth ( 4-6 weeks after artificial break of dormancy ) leaf samples from potato sprouts were taken for biological assay.

Seeds of *Solanum demissum* P.I. 230579 and *Solanum demissum* P.I. 175404 were kindly supplied by Dr. Peter, E. Thomas ( United State Department of Agriculture, Agriculture Research Service ). Plants of P.I. 230579 and P.I. 175404 were grown in an aphid-free glasshouse. To improve compactness of seedling growth, daylength was extended to 18 hr with 36-Watt cool white fluorescent.

Excised individual leaves of *Solanum demissum* P.I. 230579 at 5 - 10 leaf stage, develop dark, irregular, slightly elongated local lesions 5 days following inoculation with potato leaves sap containing PVY ( plate 2 ) ( 55 ), whereas detached leaves of *Solanum demissum* P.I. 175404 at 4 - 6 leaf stage, develop small, bluish-black lesions characteristic of PVA infection, 4 days after inoculation ( plate 3 ) ( 56 ).

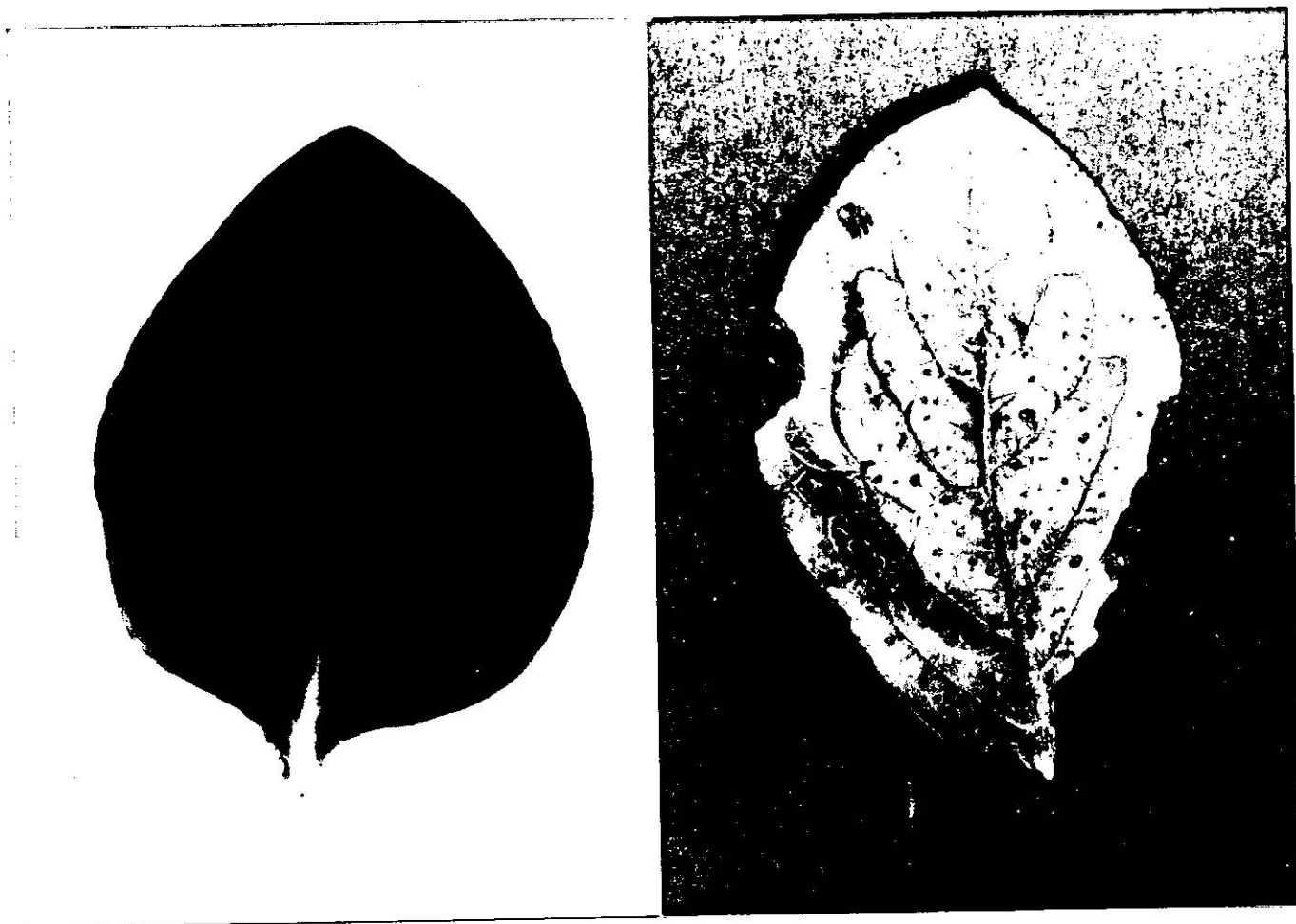
Detached leaves were dusted lightly with carborandum ( 600-mesh ) before inoculation. The leaves were inoculated with a preparation of potato sprout leaves macerated in a mortar using in a neutral phosphate buffer 0.01 M, containing ( Na-DIECA & Cystine ), by 1 : 1 ( w / v ) ratio. Inocula

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**Plate 2 : Symptoms of potato virus Y on *Solanum demissum***

**P.I. 230579.**



**Plate 3 : Symptoms of potato virus A on *Solanum demissum***

**P.I. 175404.**

were applied uniformly over the respective leaves with the index figure. The petioles of leaves were embedded in a paper towel wetted by liquid media ( Appendix 2 ). Inoculated leaves were placed in suitably sized plastic boxes ( 50 X 25 X 5 cm ). The boxes were covered with cling film to maintain humidity and to prevent leaves from wilting. PVY inoculated detached leaves were kept for 7 days at 21 C° and about ( 2000 - 3000 LUX ), whereas those for PVA were kept at 18 C° for 7 days.

### I.2- Weeds :-

Detection of PVY and PVA from weed samples showing virus - like symptoms, was attempted. Three hundred and two samples ( Appendix 3 ) were collected from different locations and potato fields along the Jordan valley, during summer 1991 and 1992 when the Jordan valley was practically free from potatoes. Few samples were collected during potato growing seasons in these two years. The weed samples were identified either by Dr. B. Abu- Irmaileh ( University of Jordan ) or based on the descriptive weed identification hand books ( 59, 60, 61, 62 ).

Each sample was tested for the presence of PVY and PVA, using direct double antibody sandwich ( DAS ) form of ELISA. The test procedure adopted followed those described by Clark *et. al.* ( 45 ) and Hill ( 31 ) as modified by Bantari ( 63 ). Antisera and conjugate for PVY and PVA were supplied by ( Bioreba company AG ). Antisera and conjugate dilutions were used as recommended by the manufacturing company.

Buffers used in the various steps involved in the ELISA, were those suggested by Bantari ( Appendix 4 ).

Tests were made in polystyrene flat-bottom microtitre plate, type T. M [ 2 ] ( Dynatech Immulon Ltd., U.S.A ). Plates were coated with 0.200 ml aliquots per well of PVY gamma-globulin, diluted in coating buffer at 1 : 1000 ( v / v ). The plates were then covered and then incubated for 3 hours at 37 C°. Well contents were discharged and the wells were washed by flooding with PBS. This process was repeated four times. One gram from

each sample was macerated in extraction-conjugate buffer at 1 : 5 dilution, using electrical homogenizer ( Kinematica GmbH ). Healthy sap extract and PBS buffer were used as a control. The test samples were added as 0.200 ml aliquots to each well. Plates were then covered and incubated over night at 6 C°. The wells were washed as before , then 0.200 ml of Enzyme-labelled gamma globulin of PVY , diluted at 1 : 1000 ( v / v ) in extraction-conjugate buffer, was added to each well. The plates were covered and incubated for 4 hours at 37 C° , and then 0.200 ml of freshly prepared substrate was added. The substrate was p-nitrophenyl phosphate ( Sigma ) at a concentration of 0.75 mg / ml in substrate buffer. Plates were incubated for 30 minutes at laboratory temperature where the hydrolysis reaction had progressed sufficiently to induce the production of a yellowish coloration ( p-nitrophenol ). Results were taken using ELISA reader BIO-TEK EL 308 model. The results were considered positive if the absorbance values at 405 nm of the two sample replicates exceeded the healthy control by a factor of two.

The formentioned procedure was also used for PVA detection, using specific PVA gamma-globulin and Enzyme-labelled gamma-globulin of PVA.

Detection of these viruses was also carried out using local lesion host *S. demissum* P.I. 230579 for PVY, whereas P.I. 175404 for PVA as mentioned before.

### **I.3- Volunteer potatoes :-**

One hundred and four leaf samples of volunteer potato plants were collected from fields along the Jordan valley, starting from February after harvesting fall potato crop and extending to May. Detection of PVY and PVA was done by direct ELISA and by indexing on *Solanum demissum* P.I. 230579 for PVY, or P.I. 175404 for PVA, as outlined earlier in the materials and methods ( 1.2 ).

## II- Incidence Study :-

Incidence of mosaic diseases was determined in three potato fields selected randomly along the Jordan valley during two growing seasons. The fields were located in Al-Karama, Dair-alla and Wadi-Al-Yabis or in Al-Karama, Al-arda and Kraimeh for the fall season of 1991 and the spring season of 1992, respectively. In each field, four rows were chosen at random. Fifty plants from each row were inspected visually for mosaic symptoms at bi-weekly intervals. Plants showed mosaic symptoms were counted and the percentage of infected plants was calculated. To determine the prevalence of PVY and PVA, about 115 samples from plants showing mosaic symptoms were collected and tested against PVY and PVA gamma-globulin for the presence of the two viruses ( 19 ), using the direct ELISA as mentioned before in the materials and methods ( 1.2 ).

## III- Vector Flight Activity :-

Flight activities of both total aphids and *Myzus persicae* were monitored using an electrical centrifugal suction trap, developed by Taylor ( 7 ), located in the University of Jordan Experimental Station in the central Jordan valley, for the period from September, 1991 to August, 1992. The trap was 12 meters high. It consisted of 9.14 meter high tube, mounted on the top of 3 meter high box standing on a concrete base. The insects were concentrated into a bottle containing preservative fluid. The fluid was 45 % methylalcohol ( 95 % ), 22 % glycerol and 33 % distilled water.

Samples were collected bi-weekly and examined under a dissecting binocular microscope in plastic petridish to count the numbers of *Myzus persicae* and total aphids separately. Aphids were identified according to Samhan's key ( 40 ) and confirmed by Dr. T. Mustafa ( University of Jordan ).

#### **IV- Effect of Sprouting on Detection Levels by ELISA of PVY and PVA in Dormant Tubers:-**

ELISA tests were carried out for potato seed tubers of selected cultivars before and after they were allowed to sprout in compost in incubator at 20 C° in 24 hours light ( 31 ). Four potato cultivars were used in this experiment namely: Ajax, Diamont, Marfona and Spunta.

The sprouts were weighed and processed by direct ELISA as described earlier in the materials and methods ( 1.2 ).



## RESULTS

### I- Inoculum Sources :-

#### I.1- Potato seed-tubers :-

Assessment of infection levels in seed potatoes imported from Netherlands and Syria indicated that potato virus Y and potato virus A occurred in 57 % and 100 % of all imported seed lots, respectively ( Table 1 ).

All shipments of seed potato varieties imported from Syria were variably infected with PVY ( Table 2 ). Incidence of PVY as detected by ELISA ranged from 6.7 % in Krostar to 35.6 % in Spunta, while it ranged from 4.4 % in Krostar to 30 % in Spunta shipments as detected by biological assay. Discrepancy in detection levels of PVY using ELISA and bioassay varies with the varieties. In all variety except for Ferosa, detection level by ELISA was higher than that by bioassay. The difference ranged from 1.1 % in Diamont-1 to 6.7 % in Diamont-2 ( Table 2 ).

Potato virus Y occurred in 41 % of seed lots imported from Netherlands ( Table 1 ). Number of PVY infected Diamont seed lots as determined by either ELISA or bioassay were more than that in other cultivars. The two tests were equally sensitive in detecting PVY in Diamont seed lots except in lot 5-6374, where 4.4 % samples reacted positively only by ELISA. Incidence of PVY in Diamont seed lots ranged from 0 % in 5-5176 seed lot to 42.2 % in 5-6400 seed lot ( Table 3 ). Detection levels of PVY by ELISA or bioassay tests in Spunta seed lots reached 2.2 % in 6-3366 seed lot. In all other cultivars incidence of PVY as determined by ELISA and bioassay tests ranged from 0 % in Fambo-8-2698, Frisia-5-1789, Frisia-5-0530 and Gigant-8-1390 seed lots to 22.2 % in Ajax-1-2287 ( Table 3 ).

Detection of PVA by ELISA in seed potato varieties imported from Syria showed that the virus incidence ranged from 6.7 % in Lizita to 26.7 % in Diamont-2 ( Table 4 ). Incidence of this virus as determined by *Solanum demissum* P. I. 175404 reaction ranged from 5.6 % in Diamont-1 and

الصفحة غير موجودة من أصل المصدر

**Table ( 2 ) : Incidence of PVY in seed potato varieties imported from Syria.**

Variety	Seed lot*	Positive samples identified by			
		ELISA**		Bioassay***	
		No.	%	No.	%
Diamont -1	-	9	10	8	8.9
Spunta	-	32	35.6	27	30
Krostar	-	6	6.7	4	4.4
Fermosa	-	18	20	18	20
Lizita	-	30	33.3	26	28.9
Diamont-2	-	26	28.9	20	22.2

- Diamont- # : Number of shipment.

\* 90 samples were tested / seed lot.

\*\* Indirect ELISA.

\*\*\* *Solanum demissum* P.I. 230579.

**Table ( 3 ) : Incidence of PVY in seed potato lots imported from Netherlands.**

Variety	Seed lot*	Positive samples identified by			
		ELISA**		Bioassay***	
		No.	%	NO.	%
Diamont	5-5156	18	20	18	20
Diamont	5-6400	38	42.2	38	42.2
Diamont	5-5176	0	0	0	0
Diamont	1-0871	2	2.2	2	2.2
Diamont	5-6374	34	37.8	30	33.3
Diamont	1-6400	8	8.9	8	8.9
Spunta	8-3264	0	0	0	0
Spunta	5-0908	0	0	0	0
Spunta	8-3366	2	2.2	2	2.2
Spunta	5-0948	0	0	0	0
Spunta	5-9337	0	0	0	0
Spunta	5-8506	0	0	0	0
Ajax	1-2287	20	22.2	20	22.2
Fambo	8-2698	0	0	0	0
Frisia	5-1789	0	0	0	0
Frisia	5-0530	0	0	0	0
Gigant	8-1390	0	0	0	0

\* 90 samples were tested / seed lot.

\*\* Indirect ELISA.

\*\*\* *Solanum demissum* P.I. 230579.

Fermosa varieties to 24.4 % in Diamont-2 variety, respectively. The difference in incidence of PVA as determined by ELISA and bioassay ranged from 2.2 % in Diamont-2 to 8.8 % in Spunta, while no differences were observed for Krostar and Lizita varieties ( Table 4 ).

For seed lots imported from Netherlands, incidence of PVA in Diamont seed lots as determined by ELISA, ranged from 2.2 % in seed lot 5-6374 to 8.9 % in seed lot 5-6400, whereas the incidence as determined by bioassay test ranged from 2.2 % in 5-6374 and 5-5176 seed lots to 6.7 % in 5-6400 seed lot. The difference in detection levels of PVA using ELISA and bioassay ranged from 1.1 % in 5-5176 seed lot to 2.2 % in 5-5156 and 5-6400 seed lots, while no differences were observed in detection levels of PVA in 5-6374, 1-6400 and 1-0871 Diamont seed lots. Incidence of PVA as determined by ELISA in Spunta seed lots, ranged from 2.2 % in 8-3264 and 8-3366 seed lots to 6.7 % in 5-8506 seed lots, whereas it ranged from 1.1 % in 8-3264 seed lot to 5.6 % in 5-8506 seed lots by bioassay. The differences in incidence of PVA using the two tests, ranged from 1.1 % in 8-3264 and 5-8506 to 2.2 % in 5-0948. No variations were detected for other Spunta seed lots ( Table 5 ).

Potato virus A incidence in all other cultivars, as detected by ELISA ranged from 2.2 % in Ajax-1-2287, Fambo-8-2698 and Frisia-5-1789 seed lots to 13.3 % in Frisia-5-0530 seed lot, while it ranged from 2.2 % in Ajax-1-2287, Fambo-8-2698 and Frisia-5-1789 seed lots to 11.1 % in Frisia-5-0530 seed lot as determined by bioassay test. No differences were observed in detection of this virus in these seed lots, using ELISA and local lesion host assay, except for Frisia-5-0530 seed lot in which 2.2 % difference was detected ( Table 5 ).

Mixed infection of PVY and PVA was observed in 35 % of all seed lots imported from Syria and Netherlands ( Table 1 ). It occurred however, in 83 % and 18 % of seed lots imported from Syria and Netherlands, respectively ( Table 1 ).

**Table ( 4 ) : Incidence of PVA in seed potato varieties imported from Syria.**

Variety	Seed lot*	Positive samples identified by			
		ELISA**		Bioassay***	
		No.	%	No.	%
Diamont -1	-	9	10	5	5.6
Spunta	-	22	24.4	14	15.6
Krostar	-	8	8.9	8	8.9
Fermosa	-	9	10	5	5.6
Lizita	-	6	6.7	6	6.7
Diamont-2	-	24	26.7	22	24.4

- Diamont- # : Number of shipment.

\* 90 samples were tested / seed lot.

\*\* Indirect ELISA.

\*\*\* *Solanum demissum* P.I. 175404.

**Table ( 5 ) : Incidence of PVA in seed potato lots imported from Netherlands.**

Variety	Seed lot*	Positive samples identified by			
		ELISA**		Bioassay***	
		No.	%	NO.	%
Diamont	5-5156	6	6.7	4	4.4
Diamont	5-6400	8	8.9	6	6.7
Diamont	5-5176	3	3.3	2	2.2
Diamont	1-0871	4	4.4	4	4.4
Diamont	5-6374	2	2.2	2	2.2
Diamont	1-6400	3	3.3	3	3.3
Spunta	8-3264	2	2.2	1	1.1
Spunta	5-0908	4	4.4	4	4.4
Spunta	8-3366	2	2.2	2	2.2
Spunta	5-0948	4	4.4	2	2.2
Spunta	5-9337	4	4.4	4	4.4
Spunta	5-8506	6	6.7	5	5.6
Ajax	1-2287	2	2.2	2	2.2
Fambo	8-2698	2	2.2	2	2.2
Frisia	5-1789	2	2.2	2	2.2
Frisia	5-0530	12	13.3	10	11.1
Gigant	8-1390	4	4.4	4	4.4

\* 90 samples were tested / seed lot.

\*\* Indirect ELISA.

\*\*\* *Solanum demissum* P.I. 175404

Incidence of mixed infection as detected by ELISA and bioassay in Syrian seed potato lots reached 14.4 % in Spunta variety and 6.7 % in Diamont-2, respectively. Variation in incidence of mixed infection as determined by ELISA and bioassay ranged from 2.2 % in Diamont-1 to 8.8 % in Spunta, while no difference was detected for Krostar and Fermosa varieties ( Table 6 ).

For seed lots imported from Netherlands, incidence of mixed infection in Diamont seed lots as determined by either ELISA or bioassay ranged from 0 % in 5176, 1-0871 and 1-6400 seed lots to 4.4 % in 5-5156 seed lot. No discrepancy in detection levels were found in all Diamont seed lots, using both ELISA and bioassay, except for 5-6400 as 2.2 % differences level was detected. In all other cultivars no mixed infection was detected by either test ( Table 7 ).

PVY and PVA mixed infection occurred in seed potato varieties imported from Syria, at a rate higher than that in seed lots imported from Netherlands ( Table 8 ). For all varieties imported from Syria, average virus incidence as detected by ELISA reached 22.41 % for PVY, 14.44 % for PVA and 7.60 % for mixed infection by both viruses, while average incidence for these viruses using local lesion host reaction reached 19.08 % for PVY, 11.11 % for PVA and 3.9 % for mixed infection. Average incidence of virus infection as determined by ELISA in all potato seed lots imported from Netherlands was 8 % for PVY, 4.60 % for PVA and 0.65 % for mixed infection, while results of indexing on the differential host showed that PVY, PVA and mixed infection of both occurred in 7.70 %, 3.86 % and 0.52 %, respectively ( Table 8 ).

For all imported seed potatoes, average virus incidence as detected by ELISA was found to be 11.76 % for PVY, 7.17 % for PVA and 2.46 % for mixed infection. In bioassay tests, however, the average incidence reached 10.67 % for PVY, 5.75 % for PVA and 1.40 % for mixed infection ( Table 8 ).



**Table ( 6 ) : Incidence of PVY and PVA mixed infection in seed potato varieties imported from Syria.**

Variety	Seed lot*	Positive samples identified by			
		ELISA**		Bioassay***	
		No.	%	No.	%
Diamont -1	-	5	5.6	3	3.3
Spunta	-	13	14.4	5	5.6
Krostar	-	0	0	0	0
Fermosa	-	5	5.6	5	5.6
Lizita	-	6	6.7	2	2.2
Diamont-2	-	12	13.3	6	6.7

- Diamont- # : Number of shipment.

\* 90 samples were tested / seed lot.

\*\* Indirect ELISA.

\*\*\* *Solanum demissum* P.I. 230579 for PVY.

*Solanum demissum* P.I. 175404 for PVA.

**Table ( 7 ) : Incidence of PVY and PVA mixed infection in seed potato lots imported from Netherlands.**

Variety	Seed lot* #	Positive samples identified by			
		ELISA**		Bioassay***	
		No.	%	NO.	%
Diamont	5-5156	4	4.4	4	4.4
Diamont	5-6400	4	4.4	2	2.2
Diamont	5-5176	0	0	0	0
Diamont	1-0871	0	0	0	0
Diamont	5-6374	2	2.2	2	2.2
Diamont	1-6400	0	0	0	0
Spunta	8-3264	0	0	0	0
Spunta	5-0908	0	0	0	0
Spunta	8-3366	0	0	0	0
Spunta	5-0948	0	0	0	0
Spunta	5-9337	0	0	0	0
Spunta	5-8506	0	0	0	0
Ajax	1-2287	0	0	0	0
Fambo	8-2698	0	0	0	0
Frisia	5-1789	0	0	0	0
Frisia	5-0530	0	0	0	0
Gigant	8-1390	0	0	0	0

\* 90 samples were tested / seed lot.

\*\* Indirect ELISA.

\*\*\* - *Solanum demissum* P.I. 230579 for PVY.

- *Solanum demissum* P.I. 175404 for PVA.

**Table [ 8 ] : Average incidence of PVY and / or PVA in seed potatoes imported from Syria and Netherlands.**

Source	# of tested samples	% of positive samples identified by					
		ELISA*			Bioassay**		
		Y	A	MIX.	Y	A	MIX.
Syria	540	22.41	14.44	7.60	19.08	11.11	3.90
Netherlands	1530	8.00	4.6	0.65	7.70	3.86	0.52
Total	2070	11.76	7.17	2.46	10.67	5.75	1.40

\* Indirect ELISA

\*\* - *Solanum demissum* P.I. 230579 for PVY.

- *Solanum demissum* P.I. 175404 for PVA.

Average discrepancy in detection levels of these viruses by ELISA and bioassay was 1.09 % for PVY, 1.42 % for PVA and 1.06 % for mixed infection ( Table 9 ).

ELISA test was equally sensitive in detecting PVY and / or PVA in tissue taken from dormant tubers or from induced sprout growth of Ajax, Marfona and Spunta cultivars. In Diamont cultivar detection of these viruses was 2.2 % higher in tissue taken from dormant tubers than that in tissue taken from sprouts ( Table 10 ).

### **I.2-Occurrence in weeds :-**

Detection of PVY and PVA by ELISA and biological indexing was attempted in 302 samples, collected from different areas along the Jordan valley at different dates during summer and growing seasons of 1991, 1992 ( Appendix 3 ).

*Solanum nigrum L.*, a wide spread annual weed in the Jordan valley is apparently the only weed found to be infected with PVY and PVA . About 26 % of the samples collected from this plant were found infected with PVY, of which 42 % were mixedly infected with PVA ( Table 11 ). A sample from *Amaranthus retroflexus L.* reacted positively by ELISA for PVA, but it was not possible to detect the virus by bioassay on *Solanum demissum* P.I. 175404. Weeds from which PVY and / or PVA were not detected include, *Amaranthus blitoides*, *A. gracilis*, *A. hybridus*, *A. retroflexus*, *Calotropis procera*, *Chenopodium album*, *C. murale*, *Conyza bonariensis*, *Convolvulus arvensis*, *Cynanchum acutum*, *Datura innoxia*, *Limex spinosa*, *Euphorbia geniculata*, *Heliotropium europaeum*, *Lactuca serriola*, *Luffa cylindrica*, *Setaria verticillata*, *Solanum alatum*, *S. incanum*, *Withania somnifera* and *Xanthium strumarium*.

**Table ( 9 ) : Comparison between detection levels for PVY and / or PVA using different ELISA techniques and bioassay.**

ELISA technique	# of tested samples	Virus	Incidence as determined by		Average differences
			ELISA	Bioassay*	
Indirect	2070	PVY	11.76 %	10.67 %	1.09 %
		PVA	7.17 %	5.75 %	1.42 %
		MIX.	2.46 %	1.40 %	1.06 %
Direct	406	PVY	11.82 %	11.58 %	0.24 %
		PVA	4.43 %	4.19 %	0.24 %
		MIX.	3.20 %	3.20 %	0.00 %

\* - *Solanum demissum* P.I. 230579 for PVY.

- *Solanum demissum* P.I. 175404 for PVA.

**Table [ 10 ] : Effect of sprouting on detection of PVY and PVA by direct ELISA.**

<b>Cultivar*</b>	<b>% of infection in tissue taken from</b>			
	<b><u>Dormant tuber</u></b>		<b><u>Induced prouts</u></b>	
	<b>PVY</b>	<b>PVA</b>	<b>PVY</b>	<b>PVA</b>
<b>Ajax</b>	<b>2.2 a</b>	<b>2.2 *</b>	<b>2.2 a</b>	<b>2.2 *</b>
<b>Diamont</b>	<b>13.3 a</b>	<b>8.8 *</b>	<b>11.1 a</b>	<b>6.7 *</b>
<b>Marfona</b>	<b>6.7 a</b>	<b>2.2 *</b>	<b>6.7 a</b>	<b>2.2 *</b>
<b>Spunta</b>	<b>2.2 a</b>	<b>0 *</b>	<b>2.2 a</b>	<b>0 *</b>

\* 90 tubers were tested / Cultivar.

- Means followed by the same letter or asterisk within rows are not significantly different using " Paired t-test ".

**Table ( 11 ) : Occurrence of PVY and / or PVA in weeds during the summer and growing seasons of 1991 and 1992 in the Jordan valley :-**

Plant species	# of samples collected	# of samples identified by					
		ELISA*			Bioassay**		
		PVY	PVA	MIX.	PVY	PVA	MIX.
<i>Amaranthus blitoides</i> S.	1	0	0	0	0	0	0
<i>Amaranthus gracilis</i> Desf.	15	0	0	0	0	0	0
<i>Amaranthus hybridus</i> L.	18	0	0	0	0	0	0
<i>Amaranthus retroflexus</i> L.	19	0	1	0	0	0	0
<i>Galotropis procera</i> Ait.	2	0	0	0	0	0	0
<i>Chenopodium album</i> L.	3	0	0	0	0	0	0
<i>Chenopodium murale</i> L.	5	0	0	0	0	0	0
<i>Conyza bonariensis</i> L.	9	0	0	0	0	0	0
<i>Convolvulus arvensis</i> L.	5	0	0	0	0	0	0
<i>Cynanchum acutum</i> L.	7	0	0	0	0	0	0
<i>Datura innoxia</i> Mill.	19	0	0	0	0	0	0
<i>Emex spinosa</i> L.	4	0	0	0	0	0	0
<i>Euphrobia geniculata</i> O.	4	0	0	0	0	0	0
<i>Heliotropium europaeum</i> L.	2	0	0	0	0	0	0
<i>Lactuca serriola</i> L.	6	0	0	0	0	0	0
<i>Luffa cylindrica</i> L.	7	0	0	0	0	0	0
<i>Setaria verticillata</i> L.	3	0	0	0	0	0	0
<i>Solanum alatum</i> Monech.	18	0	0	0	0	0	0
<i>Solanum incanum</i> L.	12	0	0	0	0	0	0
<i>Solanum nigrum</i> L.	101	15	11	11	15	11	11
<i>Withania somnifera</i> L.	36	0	0	0	0	0	0
<i>Xanthium strumarium</i> L.	6	0	0	0	0	0	0

\* Direct ELISA.

\*\* - *Solanum demissum* P.I. 230579 for PVY.  
- *Solanum demissum* P.I. 175404 for PVA.

### **I.3-Volunteer potatoes :-**

Potato virus Y was detected in 20.19 and 21.15 % of the samples collected from volunteer potatoes by biological and serological tests, respectively. PVA however, was detected in 5.78 % of collected volunteer potato plants as determined by either biological indexing or by ELISA test ( Table 12 ).

### **II- Incidence of Mosaic Diseases :-**

The incidence of mosaic disease affecting potatoes (*S. tuberosum* L.) was studied in three fields, selected along the Jordan valley during two growing seasons. The incidence for 1991 fall growing season, was lower and increased slower over a long period of time compared to the 1992 spring growing seasons ( Fig. 2 & 3 ).

Incidence of mosaic disease in Wadi-Al-Yabis field was higher and increased more rapidly over the same period of time during the fall season compared to that in Dair-Alla and Al-Karama fields. Maximum disease incidence was observed in early January and reached 10 %, 12 % and 42 % for fields in Al-Karama, Dair-Alla and Wadi-Al-Yabis, respectively. The progress of the disease with elapse of time is shown in ( Fig. 2 ).

For 1992 spring growing season the incidence of the mosaic disease in Kraimeh field was lower compared to the other two fields in Al-Arda and Al-Karama. Maximum disease incidence was observed at harvesting date in early April and reached 31 %, 53 % and 62 % for fields in Kraimeh, Al-Karama and Al-Arda, respectively ( Fig. 3 ).

Of all diseased samples collected from potato fields during the fall growing season, 69.4 % and 16.3 % were found infected by PVY and PVA, respectively. In the spring growing season, PVY and PVA were detected in 84.8 % and 25.8 % of collected samples, respectively ( Table 13 ).



**Table ( 12 ) : Incidence of PVY and PVA in potential inoculum sources :-**

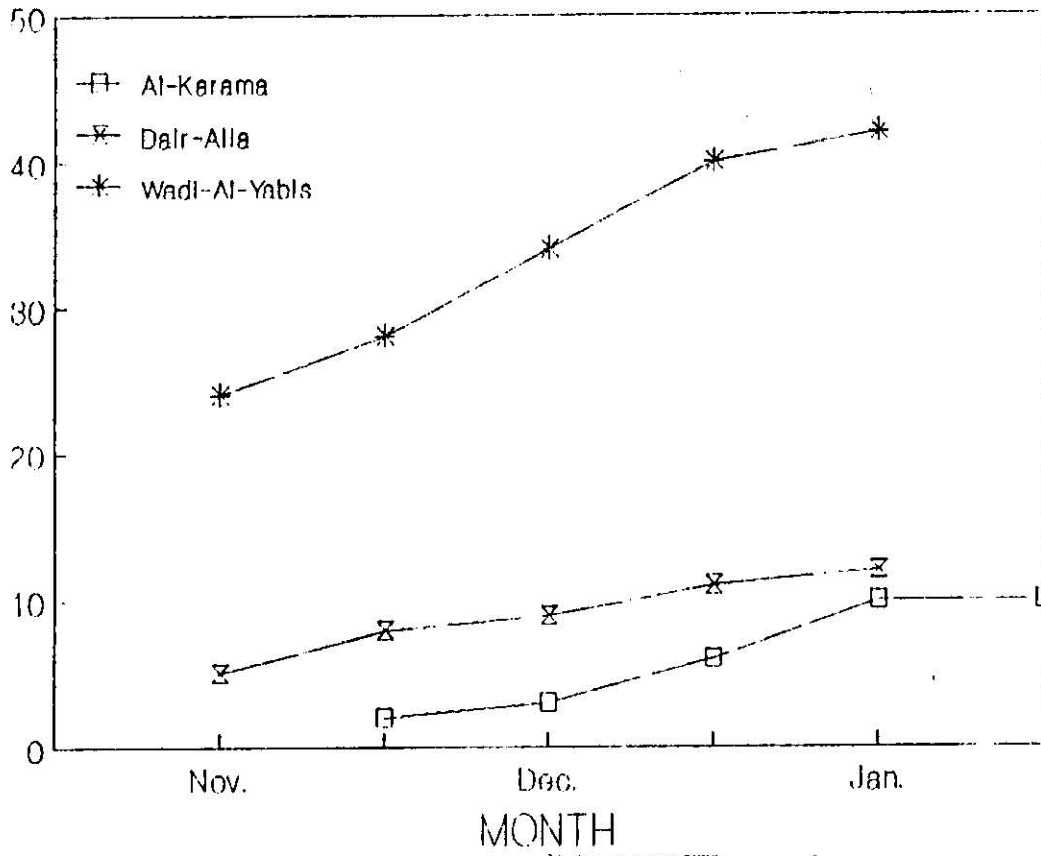
Inoculum source	# of collected samples	Incidence of			
		PVA		PVY	
		ELISA*	BIO.**	ELISA*	BIO.**
<b>Imported tubers</b>	<b>2070</b>	<b>7.17</b>	<b>5.75</b>	<b>11.76</b>	<b>10.67</b>
<b>Weeds</b>	<b>302</b>	<b>3.97</b>	<b>3.64</b>	<b>8.61</b>	<b>8.61</b>
<b>Volunteer potatoes</b>	<b>104</b>	<b>5.78</b>	<b>5.78</b>	<b>21.15</b>	<b>20.19</b>

\*- Indirect ( imported tubers ).

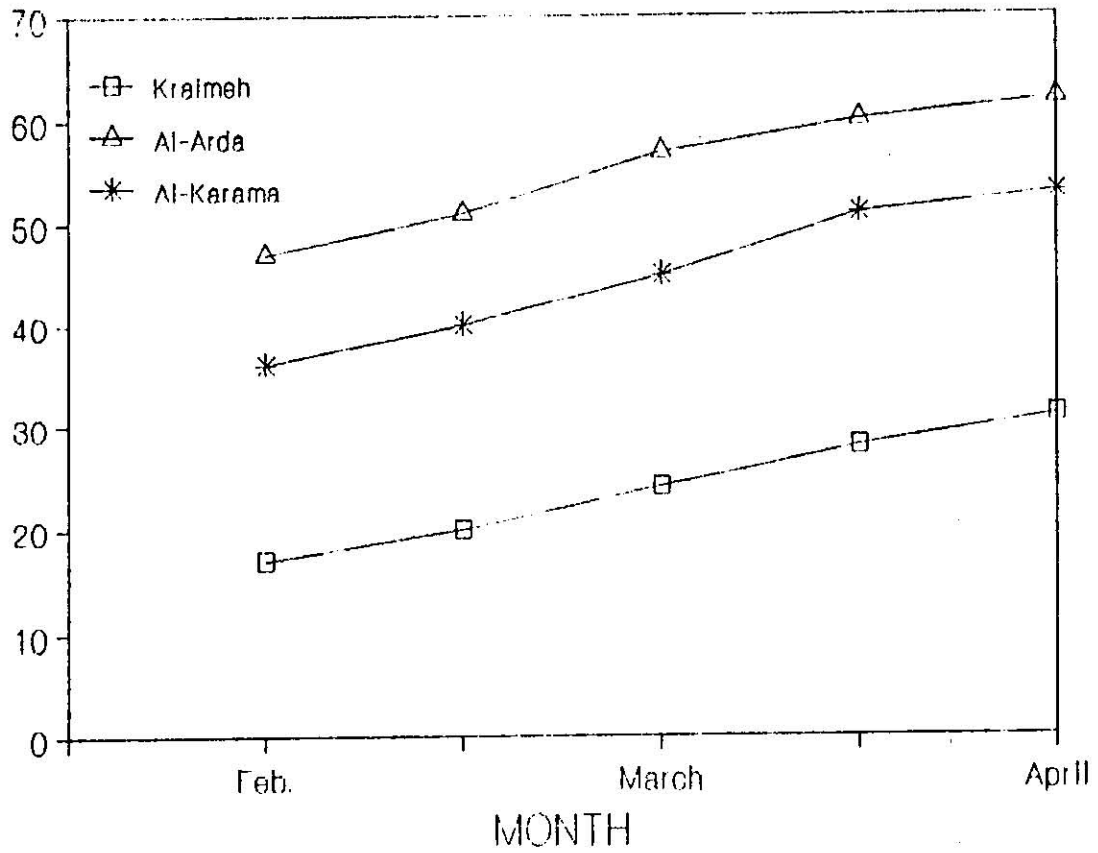
- Direct ( weed, volunteer potatoes ).

\*\* - *Solanum demissum* P.I. 175404 for PVA.

- *Solanum demissum* P.I. 230579 for PVY.



**Fig. 2 : Incidence of mosaic diseases in three potato (*Solanum tuberosum L.*) fields in the Jordan Valley during 1991 fall growing season.**



**Fig. 3 : Incidence of mosaic diseases in three potato ( *Solanum tuberosum* L. ) fields in the Jordan Valley during 1992 spring growing season.**

### III- Vector Flight Activity :-

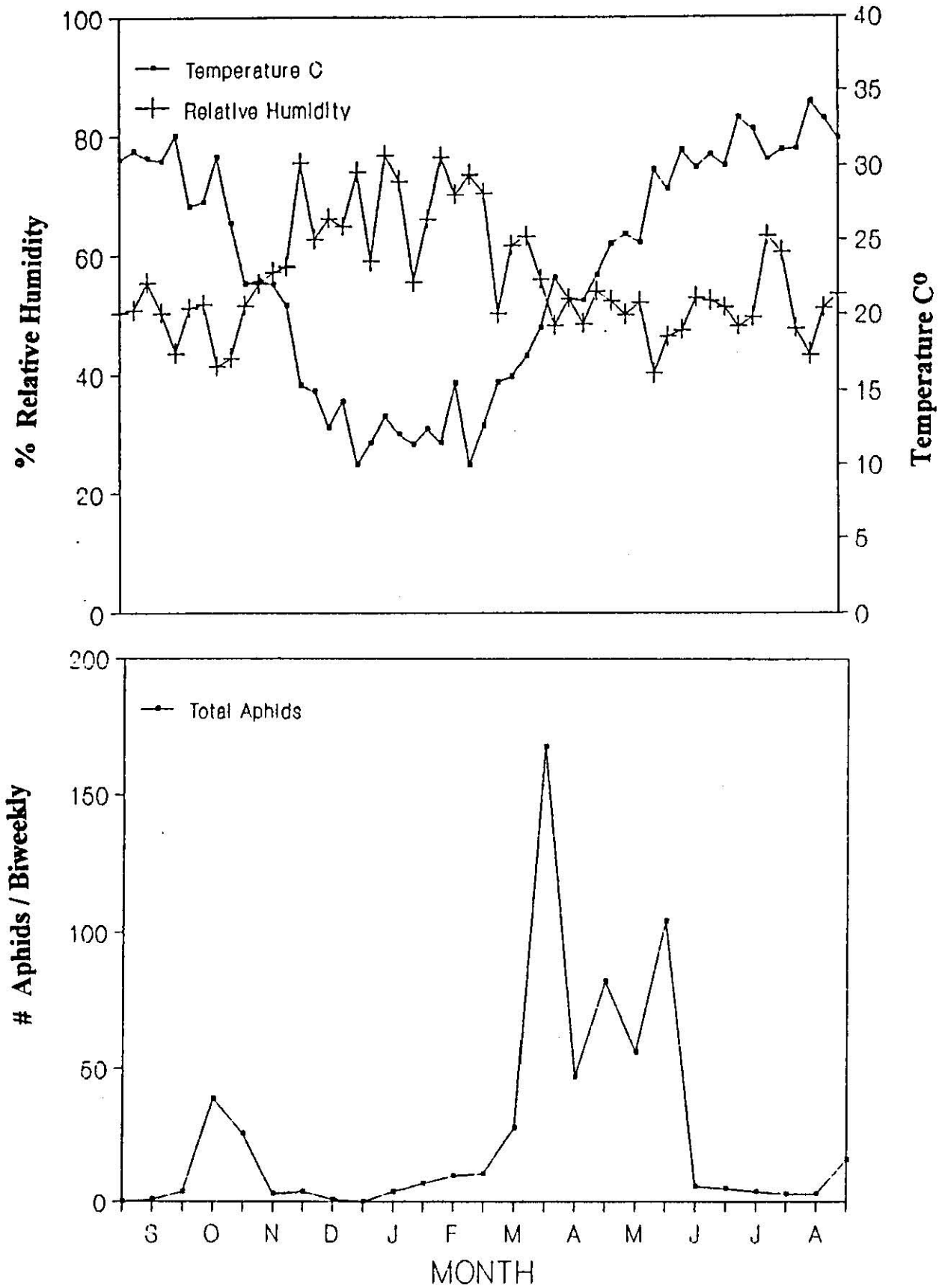
Flight activity of total winged aphids and *Myzus persicae* as monitored by a suction trap in the University of Jordan experimental station for the period, from September 1991 to August 1992 is shown in ( Figs. 4 & 5 ).

A major flight period for the total winged aphids and *Myzus persicae* was detected in the spring season, whereas a second smaller flight activity period was detected in the fall.

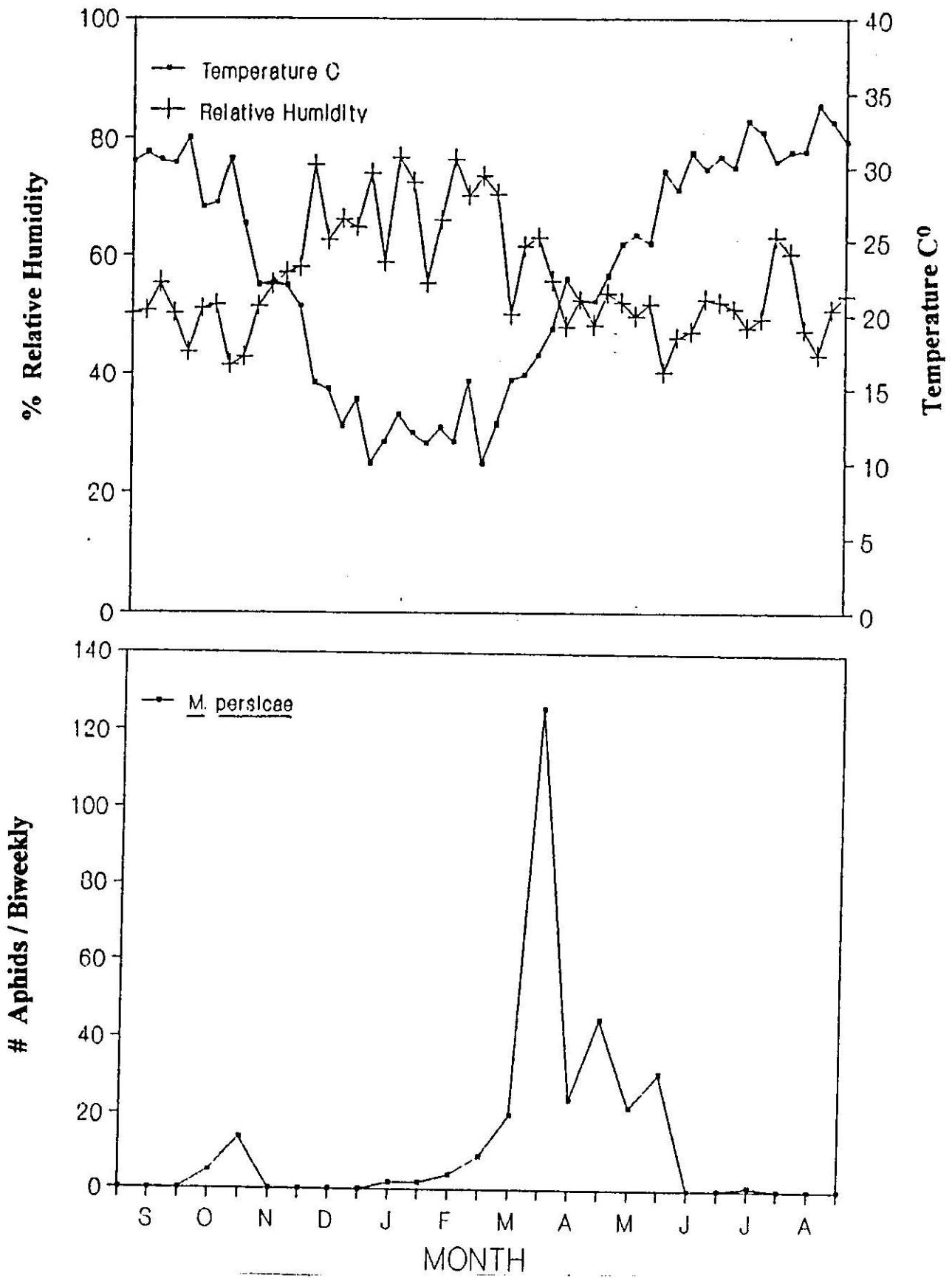
The main flight activity period for total winged aphids and *Myzus persicae* extended from mid-March to mid-June and approached a peak in late March. The second flight activity period for total aphids and *Myzus persicae* appeared during October and approached a peak in late October ( Figs. 4 & 5 ).

**Table ( 13 ) : Occurrence of PVY and PVA during growing seasons of  
Potato In Jordan valley ( 1991 / 1992 ).**

Season	# of samples collected	<u>Samples reacted positively by ELISA for</u>			
		PVY		PVA	
		#	%	#	%
Fall	49	34	69.4	8	16.3
Spring	66	56	84.8	17	25.8



**Fig. ( 4 ) : Flight activity of total aphids in the central Jordan Valley during the period from Sep. 1991to Aug.1992**



**Fig. ( 5 ) : Flight activity of *M. persicae* in the central Jordan Valley during the period from Sep. 1991 to Aug.1992**

## Discussion

Occurrence and increase of PVY and PVA depends on two important PVY and PVA are seed transmitted in potato tubers ( 1, 7 ), it is expected to be introduced to the field at early stages of growth acting as primary inoculum source especially in the absence of roguing practices . Moreover, tubers are means by which the PVY and PVA, passes from one season to another.

The fact that PVY and PVA in seed potatoes imported for the 1991 / 1992 seasons occurred at a rate higher than that accepted by Jordanian quarantine regulation rules ( Table 14 ), may indicate their important role as inoculum sources for consequent spread of the viruses in potato fields in Jordan Valley. This along with the availability of alternate hosts ( i.e., volunteer potatoes and weeds ) and abundance of vector activity had resulted in high incidence of both viruses in spring planting season. The relatively slow incidence in the fall may be attributed to availability of virus source, low vector population and / or short duration of activity of aphid vector .

Out of 23 seed lots imported from Syria and Netherlands, at least 11 seed lots were found to be infected with PVY and PVA at rates exceeding those allowed by quarantine regulations as set by Ministry of Agriculture. Seed lots imported from Syria were all found infected with PVY and PVA at an average incidence of 19.08 % and 11.11 %, respectively, as determined by bioassay test. These values exceeded allowable tolerance level at least by 5- folds. The high incidence of both viruses in Syrian seed-tubers might suggest that Syrian seed potato growers made little or no effort at roguing of PVY and PVA infected plants in the production fields.

Seed lots imported from Netherlands however, were infected with



**Table [ 14 ] : The maximum sum of allowable virus incidence to be accepted as seed potatoes, ( 64 ).**

VIRUS	GRADE			
	E	A	B	C*
PLRV, PVY & PVA.	2 %	6 %	8 %	10 %
Maximum allowable sum of virus incidence.				
PVX & PVS.	4 %	10 %	10 %	15 %
Maximum allowable sum of virus incidence.				

C\*: For local production.

PVY and PVA at a rate that exceeded allowable tolerance level, at least by a factor of 1.93. Seven out of 17 seed lots imported from Netherlands, were found to be infected with PVY at an average incidence of 7.7 % whereas all seed lots were infected with PVA at an average incidence of 4.6 %. This again might suggest that seed potato growers in Netherlands made little effort to control PVY and PVA.

In all varieties PVY and / or PVA were detected in higher numbers of samples by ELISA than that by bioassay. The average differences in seed-tuber transmission rates of PVY and / or PVA as determined by indirect ELISA, using agdia antisera, and bioassay was 1.09 % for PVY, 1.42 % for PVA and 1.06 % for mixed infection. Higher discrepancy level between bioassay and ELISA in the case of PVA might be due to a possible cross reaction of PVA with antisera for PVY. Thus a sample that was infected with PVY might react with antisera for PVA but failed to infect the differential host. Similar results have been reported in France in routine seed potato testing ( 49 ). The discrepancy in detection levels of PVY and / or PVA using ELISA and bioassay varied with the variety ( Table 15 ). The differences in detection levels of PVY using ELISA and bioassay ranged from 0.79 % in Spunta variety to 4.44 % in Lizita variety, while no discrepancy were found in Ajax, Fambo, Frisia, Gigant and Ferosa varieties. The differences in incidence of PVA using both tests, ranged from 1.11 % in Frisia variety to 4.44 % in Ferosa variety, while no variation were observed in Ajax, Fambo, Gigant, Krostar and Lizita varieties. However, in mixed infection discrepancy in detection levels of PVY and PVA ranged from 1.27 % in Spunta variety to 4.44 % in Lizita variety, while no differences were detected in Ajax, Fambo, Gigant, Krostar, Frisia and Ferosa varieties. Variation in detection level between ELISA and bioassay might be also dependent on the ELISA technique used. The results showed that the differences were lower when using direct instead of indirect ELISA ( Table 9 ). In addition differences in detection levels might be due

**Table ( 15 ) : Average differences in detection levels for PVY and / or PVA using ELISA and bioassay according to variety.**

VARIETY	# of tested samples	VIRUS	# of +ve samples by		Differences	
			ELISA	Bioassay	#	%
Diamont	720	PVY	135	124	11	1.53
		PVA	59	48	11	1.53
		Mix.	27	17	10	1.39
Spunta	630	PVY	34	29	5	0.79
		PVA	44	32	12	1.90
		Mix	13	5	8	1.27
Ajax	90	PVY	20	20	0	0
		PVA	2	2	0	0
		Mix	0	0	0	0
Fambo	90	PVY	0	0	0	0
		PVA	2	2	0	0
		Mix.	0	0	0	0
Frisia	180	PVY	0	0	0	0
		PVA	14	12	2	1.11
		Mix.	0	0	0	0
Gigant	90	PVY	0	0	0	0
		PVA	4	4	0	0
		Mix.	0	0	0	0
Krostar	90	PVY	6	4	2	2.22
		PVA	8	8	0	0
		Mix.	0	0	0	0
Fermosa	90	PVY	18	18	0	0
		PVA	9	5	4	4.44
		Mix.	5	5	0	0
Lizita	90	PVY	30	26	4	4.44
		PVA	6	6	0	0
		Mix.	6	2	4	4.44

Jordan Valley. So it is likely that destruction of alternate hosts of the pathogen alone may suppress disease by reducing the amount of initial inoculum.

Incidence studies of mosaic disease in three potato fields in 1991 fall growing season, showed relatively low infection level in two fields, Al-karama and Dair-Alla and high infection level in Wadi-Al-Yabis at the end of the study. The difference in the incidence of mosaic disease among the three fields might be due to the differences in health status of seed tuber used by the farmers, vector activity and / or population in the three locations. Since PVY and PVA were seed transmitted in potato tubers ( 1, 7 ), the stagnancy of the disease or lower incidence during the fall growing season corresponded to a lower level of aphid vector mainly *M. persicae* population, and / or to a shorter duration of their fall peak flights ( Fig. 4 & 5 ). Incidence of mosaic disease in three potato fields in the spring growing season, showed relatively high infection level. This situation could be explained by the spring rise in the aphid vector population which was responsible for initiation and the transmission of the disease and by abundance of virus sources, since *Solanum nigrum* weed and other PVY susceptible vegetables ( Tomato, Tobacco, Pepper ) were widely present in the open fields during that time. The peak of mosaic disease infection was found to be associated with the spring migration of winged aphids.

Two main flight activity periods for the total winged aphids and *M. persicae* were detected in the central Jordan Valley. The first flight activity period occurred in spring and the second in autumn. This agrees with the findings of Avidov ( 67 ) and Samhan ( 40 ). Numbers of *Myzus persicae* caught in the suction trap were low when high temperature prevailed (Fig.5). This might be attributed to the reduction in aphid ( *M. persicae* ) reproduction ( 67 ). The fact that alate *Myzus persicae* was caught in almost all winter months, might indicate that the mild winter temperatures prevailing in the Jordan Valley enable *Myzus persicae* to overwinter as

adults but not as eggs ( 68 ). In addition, relatively few reproduction might occur on secondary herbaceous hosts ( 67 ).<sup>52</sup>

Comparison between detection levels of PVY and PVA in tissues taken from dormant tubers or induced sprout growth showed no significant difference in assessment of both viruses in either tissue. Detection of these two viruses from induced sprout growth introduced undesirable delay ( 31 ).

## CONCLUSION

- 1- The fact that PVY and PVA in seed potatoes imported for the 1991 / 1992 seasons occurred at a rate higher than that accepted by Jordanian quarantine regulation rules, may indicate their important role as inoculum source.
- 2- The discrepancy in detection levels of PVY and / or PVA using ELISA and bioassay varies with variety and ELISA technique.
- 3- It is important to determine the cut-off point between positives and negatives in ELISA readings for each individual cultivar based on the results obtained from biological assay. Such standarization may be a prerequisite to accept abstract ELISA readings to implement quarantine regulations. Knowing that biological assay tests using *S. demissum* is more sensitive than ELISA.
- 4- PVY and PVA were encountered in a wide spread, annual Solanceous weed *Solanum nigrum*. It is expected that this weed might play an important role in the epidemiology of PVY and PVA as a potential inoculum source and oversummering reservoir for these two viruses.
- 5- Volunteer potato plants are implicated as a source of inoculum for these two viruses in the Jordan Valley.
- 6- The stagnancy of the disease or lower incidence during the fall growing sesason corresponded to a lower level of aphid vector mainly *Myzus persicae* population or to a shorter duration of their fall peak flights activity
- 7- Incidence of mosaic disease in potato fields during the spring growing season, showed relatively high infection level. This situation could be

explained by the spring rise in the aphid vector population and by abundance of virus sources.

8- Two main flight activity periods for the total winged aphids and *Myzus persicae* were detected in the central Jordan Valley. The first flight activity occurred in the spring and the second in the fall.

9- Detection of these two viruses from induced sprout introduced undesirable delay.

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**Appendix 1. ELISA BUFFERS\*****PBS-TWEEN BUFFER**                      pH 7.4

32.0 g	NaCl
00.8 g	KH <sub>2</sub> PO <sub>4</sub>
11.6 g	Na <sub>2</sub> HPO <sub>4</sub> .12H <sub>2</sub> O
00.8 g	KCl
00.8 g	NaN <sub>3</sub>
2.00 ml	Tween-20 ( Polyoxyethylene sorbitan monolaurate ).
4.00 L	Distilled water.

**PEP BUFFER**

1200 ml	PBS-Tween
24.0 g	Polyvinylpyrrolidone ( PVP ) [ M. Wt. approx. 44000 ]
2.40 g	Egg albumen

**SUBSTRATE BUFFER**

97.0 ml	Diethanolamine
800 ml	Distilled water
0.2 g	NaN <sub>3</sub>

Full up to 1 liter with H<sub>2</sub>O ; add HCl to give pH 9.8

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\* Koeing. R. ( 58 ).



## Appendix 2. Liquid media for detached leaves in bioassay\*:-

Costituent	Concentration mg / L
( A ) Macronutrients	
NH <sub>4</sub> NO <sub>3</sub>	825
KNO <sub>3</sub>	950
CaCl <sub>2</sub> .2H <sub>2</sub> O	220
MgSO <sub>4</sub> .7H <sub>2</sub> O	185
KH <sub>2</sub> PO <sub>4</sub>	85
( B ) Iron	
Na <sub>2</sub> EDTA	18.63
FeSO <sub>4</sub> .7H <sub>2</sub> O	13.93
( C ) Micronutrients	
MnSO <sub>4</sub> .4H <sub>2</sub> O	11.15
ZnSO <sub>4</sub> .4H <sub>2</sub> O	4.30
H <sub>3</sub> BO <sub>3</sub>	3.1
KI	0.42
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.13
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.013
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.013

\* Dodds, J. ( 66 ).

## Appendix 3 :-

Location, date of collection and number of weed samples collected from different areas along the Jordan valley during the summer and growing season of 1991 and 1992:-

<i>Plant species*</i>	<i># OF SAMPLES</i>	<i>FAMILY**</i>	<i>LOCATION</i>	<i>DATE M.DD.Y.</i>
<i>Amaranthus blitoides</i>	1	Amaranthaceae	S.Shuneh	8.5 .1991
<i>Amaranthus gracilis</i>	1	Amaranthaceae	S.Shuneh	8.5. 1991
<i>Amaranthus gracilis</i>	1	Amaranthaceae	S.Shuneh	8.26.1991
<i>Amaranthus gracilis</i>	1	Amaranthaceae	U.J.farm	9.16.1991
<i>Amaranthus gracilis</i>	1	Amaranthaceae	U.J.farm	10.6.1991
<i>Amaranthus gracilis</i>	3	Amaranthaceae	Dair-Alla	7.19.1992
<i>Amaranthus gracilis</i>	1	Amaranthaceae	S.Shuneh	7.29.1992
<i>Amaranthus gracilis</i>	6	Amaranthaceae	Al-Arda	8.12.1992
<i>Amaranthus gracilis</i>	1	Amaranthaceae	Um-Alzekan	8.12.1992
<i>Amaranthus hybridus</i>	2	Amaranthaceae	S.Shuneh	8.12.1991
<i>Amaranthus hybridus</i>	1	Amaranthaceae	U. J. farm	8.20.1991
<i>Amaranthus hybridus</i>	1	Amaranthaceae	Abu-Sidu	8.20.1991
<i>Amaranthus hybridus</i>	1	Amaranthaceae	U.J.farm	8.26.1991
<i>Amaranthus hybridus</i>	2	Amaranthaceae	S.Shuneh	9. 4. 1991
<i>Amaranthus hybridus</i>	1	Amaranthaceae	Um-Alzekan	9.4. 1991
<i>Amaranthus hybridus</i>	1	Amaranthaceae	Dair-Alla	9.10.1991
<i>Amaranthus hybridus</i>	1	Amaranthaceae	U.J.farm	9.16. 991
<i>Amaranthus hybridus</i>	1	Amaranthaceae	Kraimeh	10.6.1991
<i>Amaranthus hybridus</i>	2	Amaranthaceae	Dair-Alla	3.10.1991
<i>Amaranthus hybridus</i>	1	Amaranthaceae	U.J.farm	4.15. 991
<i>Amaranthus hybridus</i>	2	Amaranthaceae	S.Shuneh	7.19.1992
<i>Amaranthus hybridus</i>	1	Amaranthaceae	S.Shuneh	7.29.1992

الصفحة غير موجودة من أصل المصدر

<i>Solanum incanum</i>	5	Solanaceae	Um-Alzekan	9.4.1991
<i>Solanum incanum</i>	3	Solanaceae	Um-Alzekan	9.16.1991
<i>Solanum incanum</i>	4	Solanaceae	Um-Alzekan	8.12.1992
<i>Solanum nigrum</i>	1	Solanaceae	U.J.farm	8.12.1991
<i>Solanum nigrum</i>	2	Solanaceae	S.Shuneh	8.12.1991
<i>Solanum nigrum</i>	1	Solanaceae	Al-Arda	8.12.1991
<i>Solanum nigrum</i>	2	Solanaceae	U.J.farm	8.20.1991
<i>Solanum nigrum</i>	5	Solanaceae	U.J.farm	8.26.1991
<i>Solanum nigrum</i>	2	Solanaceae	S.Shuneh	8.26.1991
<i>Solanum nigrum</i>	1	Solanaceae	S.Shuneh	9.10.1991
<i>Solanum nigrum</i>	3	Solanaceae	S.Shuneh	9.16.1991
<i>Solanum nigrum</i>	3	Solanaceae	U.J.farm	9.16.1991
<i>Solanum nigrum</i>	3	Solanaceae	U.J.farm	10.6.1991
<i>Solanum nigrum</i>	1	Solanaceae	Kraimeh	10.6.1991
<i>Solanum nigrum</i>	5	Solanaceae	Dair-Alla	7.19.1992
<i>Solanum nigrum</i>	15	Solanaceae	U.J.farm	7.19.1992
<i>Solanum nigrum</i>	7	Solanaceae	S.Shuneh	7.19.1992
<i>Solanum nigrum</i>	8	Solanaceae	U.J.farm	7.29.1992
<i>Solanum nigrum</i>	11	Solanaceae	S.Shuneh	7.19.1992
<i>Solanum nigrum</i>	1	Solanaceae	Al-Arada	8.12.1992
<i>Solanum nigrum</i>	6	Solanaceae	U.J.farm	8.12.1992
<i>Solanum nigrum</i>	11	Solanaceae	U.J.farm	8.24.1992
<i>Solanum nigrum</i>	13	Solanaceae	Al-Arada	8.24.1992
<i>Withania somnifera</i>	5	Solanaceae	S.Shuneh	8.5.1991
<i>Withania somnifera</i>	2	Solanaceae	S.Shuneh	8.12.1991
<i>Withania somnifera</i>	2	Solanaceae	Abu-Sidu	8.20.1991
<i>Withania somnifera</i>	5	Solanaceae	S.Shuneh	8.26.1991
<i>Withania somnifera</i>	1	Solanaceae	S.Shuneh	9.4.1991
<i>Withania somnifera</i>	3	Solanaceae	S.Shuneh	9.10.1991
<i>Withania somnifera</i>	3	Solanaceae	Kraimeh	10.6.1991

<i>Withania somnifera</i>	10	Solanaceae	S.Shuneh	7.19.992
<i>Withania somnifera</i>	5	Solanaceae	S.Shuneh	7.29.1992
<i>Xanthium strumarium</i>	2	Compositae	S.Shuneh	9.16.1991
<i>Xanthium strumarium</i>	2	Compositae	Dair-Alla	9.10.1991

\* The weeds were identified with the help of Dr. B.Abu-Irmcileh

\*\* ( 59, 61, 62 ).

**Appendix 4. ELISA BUFFERS\*****COATING BUFFER**                      pH 9.6

1.59 g	Na <sub>2</sub> CO <sub>3</sub>
2.93 g	NaHCO <sub>3</sub>
0.50 g	NaN <sub>3</sub>
1.00 L	Distilled water

**PBS BUFFER**                                      pH 7.4

8.0 g	NaCl
1.0 g	KH <sub>2</sub> PO <sub>4</sub>
14.5 g	Na <sub>2</sub> HPO <sub>4</sub> .12H <sub>2</sub> O
00.5 g	NaN <sub>3</sub>
1.00 L	Distilled water.

**EXTRACTION-CONJUGATE BUFFER\*\***                      pH 7.4

1.00 L	PBS-Tween
20.0 g	Polyvinylpyrrolidone ( PVP ) [ M. Wt. approx. 44000 ]
60.0 g	Urea
1.00 ml	Tween-20 ( Polyoxyethylene sorbitan monolaurate ).

**SUBSTRATE BUFFER**

97.0 ml	Diethanolamine
800 ml	Distilled water
0.5 g	NaN <sub>3</sub>

Full up to 1 liter with H<sub>2</sub>O ; add HCl to give pH 9.8

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\* Koeing, R. ( 58 ).

\*\* Banttari, E.( 63 ).

دراسة فيروس البطاطا "واي" وفيروس البطاطا "أ" في وادي الأردن:

### مصادر العدوى والانتشار

#### ملخص

أجريت الدراسة لتحديد مصادر العدوى والانتشار لفيروس البطاطا "واي" وفيروس البطاطا "أ" في وادي الأردن بالاعتماد على الفحوص المصلية والبيولوجية. درست مصادر العدوى الأولية لفيروس البطاطا "واي" وفيروس البطاطا "أ" في ثلاثة مصادر هي: درنات البطاطا المستوردة، الأعشاب، ونباتات البطاطا المتطوعة النامية في وادي الأردن. حيث فحصت عينات ممثلة لدنرات البطاطا المستوردة من سوريا وهولندا والمراد استخدامها في زراعة هذا المحصول لموسم عام 1991 \ 1992، وتبين أن هذه العينات مصابة بفيروس البطاطا "واي" بنسبة 10.7 % وفيروس البطاطا "أ" بنسبة 5.8 %، اعتماداً على الفحوص البيولوجية. وتعتبر هذه النسب أعلى بكثير من النسب المسموح بها في قانون الحجر الزراعي الأردني.

تم جمع ( 302 ) عينة من أعشاب ظهر عليها اعراضاً فيروسية. ونتيجة للفحص البيولوجي والسيرولوجي وجد ان فيروس البطاطا "واي" وفيروس البطاطا "أ" موجودان فقط في نبات واحد من العائلة الباذنجانية وهو عنب الديب ( *Solanum nigrum* )، حيث وجد أن 26 % من العينات المجموعه من هذا النبات حاملة لفيروس البطاطا "واي". في حين وجد فيروس البطاطا "أ" في 42 % من مجموع العينات الحاملة لفيروس البطاطا "واي" كأصابة مشتركة.

وجمعت ( 104 ) عينات من نباتات البطاطا المتطوعة فوجد انها تحتوى على فيروس البطاطا "واي" بنسبة 20.2 % وفيروس البطاطا "أ" بنسبة 5.8 %، حسب الفحص البيولوجي. كذلك تم إجراء دراسة لمعرفة انتشار أمراض الموزيك الفيروسي التي تصيب البطاطا، في ثلاثة حقول مختلفة في الكرامه، ديرعلا، و وادي اليابس للعروة التشرنية لعام 1991 وكنتيجه لهذه الدراسة وجد ان نسبة الاصابة قد بلغت في الحقول الثلاث 10 %، 12 %، و 42 % على التوالي. أما بالنسبة للعروة الربيعية لعام 1992 فقد تمت الدراسة في ثلاثة مواقع مختلفة هي الكريمة، الكرامه و مثلث العارضه، حيث بلغت نسبة الاصابة في الحقول الثلاث 31 %، 53 %، و 62 % على التوالي.

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أظهرت نتائج دراسة النشاط الطيراني للمن المجنح و من الدراق الأخضر في غور الأردن الأوسط وجود فترتين رئيسيتين له. كانت الأولى في الخريف والثانية في الربيع.