Comparative study on two thrips transmitted viruses *Tomato spotted wilt virus* (TSWV) *and Iris yellow spot virus* (IYSV) as a *Tospovirus*

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Abstract

Biological, biochemical and serological comparison between *Tomato spotted wilt virus* (TSWV) *and Iris yellow spot virus* (IYSV) a *Tospovirus* were studied. These two viruses were isolated from *Vinca rosae* (L). G. Don and onion (*Allium cepa* L.), respectively. The observed symptoms included systemic mosaic and yellowing for TSWV and necrotic eyelike spots and yellow tan spots on leaves, and flower stem for IYSV. Both TSWV and IYSV were mechanically and seed transmitted. TSWV was transmitted by two different thrips species, *Thrips tabaci* L (33.3%) and *Frankliniella occidentallis* Pergamde (60.9%) whereas the transmission of IYSV was obtained by *Thrips tabaci* L. only (45%). Adults of *T. tabaci* and *F. occidentallis* Pergamde as vectors of TSWV and IYSV were discussed. *Franklinella tritici* L. and *Gynaikothrips ficorum* Marchal did not play a role as vectors of these plant viruses. Both TSWV and IYSV had a wide host range the differences in host reactions were studied. TSWV differed in its stability properties from IYSV in dilution end point (DEP) and longevity in vitro (LIV) but the two viruses are heat-inactivated at 55 ℃. Purified TSWV and IYSV each migrated as a single zone in density gradient column. Ultraviolet absorbance of both TSWV and IYSV were typical of nucleoprotein with minimum and maximum at 247 and 260 nm for TSWV and IYSV, respectively. The ratios of $A_{260/280}$ and $A_{max/min}$ were 1.2 and 1.11 for TSWVand 1.2, 1.3 for IYSV. Electron microscopy of purified TSWV and IYSV showed the presence of spherical particles with 85 nm and 80-120 nm in diameter for TSWV and IYSV, respectively. Titer of the prepared antisera as determined using indirect ELISA were 1/3000 and 1/8000 for TSWV and IYSV, respectively. Authentic and induced antisera for both TSWV and IYSV were used for virus detection using different serological diagnostic methods such as indirect ELISA and dot-blot immunoassay (DBIA) on nitrocellulose membranes

Key words: *Tomato spotted wilt virus*, *Iris yellow spot virus*, *Tospovirus*, Host range, Seed transmission, Thrips transmission, Purification, Antisera, dot-blot immunoassay, Indirect ELISA

Introduction

Tomato spotted wilt virus (TSWV) and *Iris yellow spot virus* (IYSV) are the type species of *Tospovirus* genus in the family *Bunyviridae*, a large group of predominantly vertebrate and insect- infecting RNA viruses (**Francki *et al.*, 1991**). There are now at least twelve distinct viruses (species) in the *Tospovirus* genus. Later these *Tospovirus* species have been identified and distinguished to four distinct serogroup based on nucleoprotein (N) serology, N-protein sequences, and vector specificity (**Corte's *et al.*, 1998**) but now the current classification of *Tospovirus* based on amino-acid sequence identities (%) of the N-gene and include five serogroups so far, TSWV is the

representative of serogroup I (**De Avila *et al.*, 1993a**) *Groundnut ring spot virus* (GRSV) is placed in serogroup II, *Tomato chlorotic spot virus* (TCSV) in serogroup III (**De Avila *et al.*, 1993b**), *Impatien necrotic spot virus* (INSV) in serogroup IV (**Law and mayer 1990** and **Law *et al.*, 1991**); Watermelon silver mottle virus (WSMV) (**Heinze *et al.*, 1995 and Yeh *et al.*, 1992**) *Groundnut bud necrosis virus* (GBNV)) (**Satyanarayana *et al.*, 1996**) and *Iris yellow spot virus* (IYSV) in serogroup V (**Corte's *et al.*, 1998**).

The spotted wilt disease of tomato was first described in Australia in 1915 (**Brittlebank, 1919**) wherase IYSV was isolated for the first time from Iris and leek in the Netherlands (**Corte's *et al.*, 1998**). A serologically similar virus was isolated from onions in Israel (**Gera *et al.*, 1998**).

In Egypt, TSWV, the type member of the genus *Tospovirus,* have been reported in *Physalis peruviana* and tomato since 1999 (**Alkhazindar, 1999 and Abdel Nazeir, 1999**). Recently IYSV was isolated for the first time from onion and leek in Egypt (**Abdel Wahab 2004** and **Elnagar *et al.*, 2006**).

Tospoviruses are responsible for serious economic losses on a wide variety of different crops (**Goldbach and Peter, 1994**) and cause necrosis on several plant parts, chlorosis, ring pattern, mottling, silvering, stunting, and local lesions. Symptoms vary depending on the virus, host plant, time of the year and environment (**German *et al.*, 1992; and Mumford *et al.*, 1996**). While these viruses can generally be transmitted by thrips (Thysanoptera: Thripidae) in a propagative manner (**Ullman *et al.*, 1992**) and replicate in both the thrips vectors and the plant hosts. There are significant differences in *Tospovirus* and their relationship with specific thrips species, TSWV is known to be transmitted by nine species of thrips of which two, the western flower thrips (*Frankliniella occidentalis*) and the onion thrips (*Thrips tabaci*) were more efficient vectors in transmission of the virus (**Wifkamp *et al.*, 1995 and Nagata *et al.*, 2000**). IYSV can be transmit by thrips by, *Thrips tabac* (**Mullis *et al*, 2006**) but *Frankliniella occidentalis* and *F. schultzei* are not vectors (**Kritzman *et al.*, 2001**).

Tospoviruses are one of only two known plant virus taxa whose virions are bounded by a membrane like envelope. The viral genome is divided among three segments of RNA which is contained within the envelope. Particles of TSWV are membrane – bound isometric, and about 80 nm in diameter (**Scot, 2000**).Whereas electron microscopic analysis of tissue from diseased iris

2000).Whereas electron microscopic analysis of tissue from diseased iris indeed confirmed the presence of spherical particles 80 to 120 nm in diameter (Kritzman *et al.*, 2001).

This paper was initiated to: (I) Study and compare the symptoms of TSWV (serogroup I) and IYSV (serogroup V) in various hosts. (II) Study the biological transmission of TSWV and IYSV, and identify the thrips vectors and its efficiency in transmitting the virus. (III) Produce specific antibodies from rabbit injected with whole virions which can be used for the rotine detection of both TSWV and IYSV, or a host range of *Tospovirus*.

Materials and Methods

Virus source and symptoms:

Samples of *Vinca rosea* (L). G.Don. plants showing typical systemic mosaic and yellowing symptoms of TSWV were collected from Agriculture Research Experimental Station (ARES).

Evidence of natural infection of onion (*Allium cepa*) by IYSV was found in samples collected from Experimental farm, Faculty of Agriculture, Cairo University. Infected plants were showing necrotic eyelike spots on leaves and flower stem.

Virus isolation and propagation:

The isolates understudy were serologically identified using two authentic polyclonals. The first one for TSWV was obtained from Dr. N. Katis (Agistotelian University, Faculty of Agriculture, Plant Pathology Laboratory Greece) and the second one for IYSV obtained from Dr. D. Peters (Department of Virology, Wageningen, the Netherland Agricultural University). The virus isolates were biologically purified through a single local lesion technique repeated two times on *Chenopodium amaranticolor Coste&Reyn* plants (Kuhn 1964). The two viruses were then transmitted mechanically to *Gomphrena globosa* L. and *Nicotiana rustica L.* These two hosts were used as a source and for virus propagation.

Host range

TSWV and IYSV were mechanically inoculated on 29 selected hosts including certain diagnostic hosts belonging to *Apocynaceae*, *Alliaceae*, *Amarnthaceae*, *Cucurbitaceae*, *Chenopodiceae*, *Fabaceae*, *Malvaceae* and *Solanaceae*, then, seedlings of each host plant were maintained for 45 days in the greenhouse for symptom development. An equal number of healthy seedlings of the same age and species were left as a control. Inoculated and healthy plants were serologically tested by indirect ELISA method using induced antisera for TSWV and IYSV.

Virus stability in crude sap:

Dilution- end point (DEP), thermal inactivation point (TIP) and Longevity in Vitro (LIV) were measured according to **Noordam (1973).**

Transmission studies:

1-Mechanical transmission:

Mechanical transmission tests were made by homogenized samples of TSWV and IYSV- infected plants separately in distilled water or 0.01 M sodium phosphate buffer, pH 7.0, containing, 0.1 % sodium sulfite. The sap was used to inoculate *G.globosa* and *N. rustica* predusted with carborundum. Plants were kept under greenhouse conditions, observed for symptom expression, and assayed by enzyme linked immunosorbent assay (ELISA) and DBIA techniques.

2- Seed transmission:

Mature seeds were harvested from each of infected *D. innoxia* for TSWV and *A. cepa* for IYSV grown in the greenhouse those showed severe necrotic symptoms. The presence of TSWV and IYSV in plants designated as seed sources were confirmed by indirect ELISA prior to virus-infected seed collection. Healthy seeds of *D. innoxia* or *A. cepa* were used as a control. Healthy and infected seeds were washed in running tap water and stayed for 48 hr. in Petri dishes with wet cotton before homogenized and assayed by dot-blot-immunobinding assay (DBIA).

3- Thrips transmission:

3.1. Maintenance of virus-free thrips culture

A virus-free thrips culture was established as collected adults *T. tabaci* L, and *F. occidentalis* Pergamde were individually confined on bean pods to lay eggs. Newly hatched larvae were collected and reared on bean pods (**Doane *et al.*, 1998 Murai and Loomans, 2001).** *F. tritici* L *and G. ficorum* were collested from wheat and ficus, the four thrips species were tested as larvae and adults as vectors of IYSV and TSWV.

3.2.The transmission efficiency of different forms.

Groups of virus-free thrips, newly hatched larvae and adults, were placed on the virus source plant for the tested acquisition access time (AAT) 24h. After acquisition, thrips were transferred in groups of 10 individuals/ seedling to healthy indicator plants and allowed 24h. inoculation access time (IAT), then the thrips were killed by malathion 0.01% and the plants were observed for symptoms appearance.

Two groups of *T. tabaci* L and *F. occidentalis* Pergamde adults; the first one was reared from larvae stage to adult stage on the virus

source for AAT of IYSV and TSWV separately then transferred to healthy indicators for 24h. IAT as groups of 10 adults per plant, another group of adults from the culture "virus- free" were tested as control. They allowed 24 h. AAT on virus source and then transferred to indicator plants for 24 h. IAT after that tested by DBIA as two groups one for viruliferous insects and the other for control.

Virus purification:

TSWV and IYSV were purified according to the summation of several purification techniques for tospoviruses described by **Black *et al.* (1963)** and **Feldhoff *et al.* (1997)** with some modifications as follows. Hundred grams of freshly and/ or frozen infected leaves of *G.globosa* or *N. rustica* with TSWV or IYSV were used for virus purification. 0.1 M potassium phosphate buffer, pH 7, containing 0.01M Na_2 So_3 was used as extraction buffer (1:3W/V). The homogenates were filtered through two layers of cheesecloth. Clarification of supernatant was made using 10% of cold chloroform for TSWV, whereas 6 % of cold chloroform was used for IYSV and blended for 5 min, then subjected to low speed centrifugation (LSC) at 10,000 rpm/ 10 min /4 °C using Beckman centrifuge Model J.21 with JA-20 rotor. 8% (W/V) of polyethylene glycol (PEG, 6000 MW) and 1% (w/v) NaCl were used to concentrate the TSWV, whereas 6% (w/v) of PEG, 6000 MW and 0.8% NaCl were used to concentrated the IYSV. After LSC, the virus pellets were suspended in 0.01M Na_2 So_3, pH 7 and concentrated by high speed centrifugation (45,000 rpm/1 hr/4 °C) using Beckman 80TI rotor. Purified viruses were layered onto 10-50% sucrose gradient columns, centrifuged (50,000/1.30 hr) using SW 60,000 rotor. Virus bands were collected from the sucrose gradient using a syringe, diluted in 0.01 M potassium phosphate buffer, pH 7, centrifuged (45,000 rpm/1.30 hr/4 °C), then resuspended in 0.01 M potassium phosphate buffer and measured spectrophotometrically.

Electron microscopy

Purified TSWV and IYSV preparations were negatively stained with 2% phosphotungestic acid (PTA), pH, 7, as described by **Noordam (1973)** and examined with an electron microscope (JEOL-JEM-1200 EX II).

Serological Studies:

A- Antiserum production:

Two Newzealand white rabbit were used for the induction of two virus antisera. Each animal was given five subcuteneaus injections with purified TSWV or IYSV at weekly intervals emulsified with an

equal volume of Freund's incomplete adjuvant. The rabbits were bled once, 10 days after the last injection.

B- Titer of antiserum:

The titer of the induced antiserum was determined using indirect ELISA. Healthy and infected leaves of *G.globosa* infection with TSWV or IYSV were extracted (1:10 W/V) in 0.05 M corbonate buffer ($Na2Co_3/NaHCo_3$) and 0.02% NaN_3, pH 9.6, clarified with low- speed centrifugation. Antisera were diluted from a concentration of 1/250 to 10^{-9} in 0.01 M potassium phosphate buffer, pH 7.4, containing 0.14 M NaCl and 0.05% Tween-20 (phosphate-buffered saline (PBST). The antigens were detected with antirabbit –alkaline phosphates (AP) conjugate (Sigma Product number A/8025) diluted 1/7000 in conjugate buffer (1 L PBST) pH, 7.4 containing 2% (W/V) polyvinyl pyrrolidone (PVP) and 0.2% (W/V) ovalbumin. Absorbance readings were recorded at 405 nm.

C- Indirect enzyme linked immunosorbent assay (ELISA)

Induced antisera for TSWV and IYSV were used to detect the presence of both TSWV and IYSV in the greenhouse after mechanical or trips inoculation and in host range studies as described by **(Converrse and Martin, 1990).**

D-Dot-Blot immunobindinding assay (DBIA) on nitrocellulose membrances:

The DBIA on nitrocellulose membranes was assentially similar to those described **by (Hsu and Lawson, 1991)** for the serological detection of antigen using authentic and induced antisera for TSWV and IYSV with some modification as follows. Healthy and infected tissues and seeds with TSWV& IYSV, viruliferous and virus- free adult trips were ground in Tris buffer saline (TBS) (0.01M Tris- HCl, pH 8.0, 0.15 M NaCl containing 0.05% (v/v) triton x-100). Blotted membranes were blocked for 1 min in polyvinyl alcohol (1mg/1 ml) then incubated with the induced TSWV or IYSV polyclonal antibodies (PAB) or with the authentic antisera for TSWV or IYSV, both diluted 1/1000 in TBS-Tween (TBST).

After incubation (1hr/ 25°C), membranes were washed three times in TBST (10 min, each), then incubated (1hr/25 °C) with antirabbit-AP conjgate (Sigma Product number A 8025) for each, diluted 1/7000 in TBST. Membranes were wased three times in TBST, the stained with Nitrioblue tetrazolium/5- Bromo-4- chloro-3-indolyl phosphate (NBT/BCIB).

Results and Discussion

1- Isolation and symptomatology

The two virus isolates, TSWV and IYSV were isolated from infected *Vinca rosea* and onion plants. After biological purification through single lesion transfers on *C. amaranticolor* , the resulting viruses were propagated on either *N. rustica* for TSWV or *G. globosa* for IYSV. Such viruses produced typical symptoms for those caused by trips-transmitted *Tospovirus* **(Le, 1970; Gibbs, 1985 and Kritzman *et al.*, 2001).**

Both TSWV and IYSV were identified by DBIA test using authentic polyclonal antisera supplied by Dr. N.Katis for TSWV and Dr. D. Peters for IYSV and positive reaction were obtained as shown in Fig (1)

Host range

Result in Table (1) and Fig (2) show the reaction of TSWV and IYSV with several tested hosts.

In both viruses, inoculated plants showed a great variability in symptoms expression, such as local necrotic and chlorotic ring spots, mosaic, mottling, and pin point. This is agreement with several investigators who reported that tospoviruses cause necrosis on several plant parts, chlorosis, ring patterns, mottling, silvering, stunting, and local lesions, symptoms vary depending on the virus, host plant, time of year and environment conditions such as temperature, **(Hsu and Lawson, 1991, German *et al.*, 1992 and Mumford et al., 1996),** Because the TSWV and IYSV diseases are not easily identified visually in many hosts so, the two viruses after infection were detected using indirect ELISA.

TSWV was found to infect a wide range or hosts resemble those reported by some TSWV isolates especially in the family *Amaranthaceae*,, Chenopodiaceae, *Cucurbitaceae*, *Solanaceae*, and inoculated plants with TSWV had symptoms typical to those produced by TSWV. This result was similar to the result obtained by **Le (1970), Best (1968) and Hayam *et al.* (2004).**

The solanaceae family contains the largest number of susceptible plant species to TSWV. This result is similar to

Table (1): Symptoms of *Tomato spotted wilt virus* (TSWV) *and Iris yellow spot virus* (IYSV) *Tospovirus* in various hosts.

Test plant	Observed symptoms		ELISA	
	TSWV (1)	IYSV (2)	1	2
Apocynaceae				
Vinca rosea L.	Sys.M, Y	No	+	-
Alliaceae				
Allium cepa L.	No	NES	-	+
Amaranthaceae				
Gomphrena globosa L.	CRS, NLL, Sys.Mot	Ch.LL, NLL, Ch.S	+	+
Chenopodiaceae				
Beta vulgaris L Pleno.	N.RS	N.RS	+	+
Chenopodium quinoa Wild	NLL	NLL	+	+
C. amaranticolor Coste&reyne	NLL	NLL	+	+
C. murle L.	Ch.LL	Mo	+	+
Cucurbitaceae				
Citrulls vulgaris L.Giza1	M.M	M.M+VY	+	+
Cucumis melo L. Palmera	M.M	M.M+VY	+	+
Cucumis pubescens L.	No	M.M	-	+
Cucurbita pepo L. Escandrani	No	M.M	-	-
Cucumis sativus L.Beta Alfa	Ch.LL	No	+	-
Fabaceae				
Phaseolus vulgaris L.Bronco	M	M	-	-
Pisum sativum L.Litel Marvel	M.M	VY	-	-
Vicia faba L.Giza 3	NLL	NLL	+	+
Vigna unguiculata Walp	Ch.LL,M,P.P	Ch.LL	+	+
Solanaceae				
Datura innoxia L.	Ch, RS, N.RS	N.L.L	+	+
Nicotiana glutinosa L	NLL,M	M.M	+	+
N. rustica L.	M,	Icl.M,	+	+
N. tobacum L. White Burley	Sys.M	.L.L	+	+
N. tobacum L *Samsun* L.	N.L.L	Sys.M	+	+
Lycopersican esulentum L. Casle Rock	Sys.M,NLL	SYS.M	+	+
Solanum melongena L. Balady	NLL	NLL	+	+
Capsicum annuum L.California Wonder	NLL	NLL	+	+
Solanum tuberosum L.Cara	NLL	N.RS	+	+
Malvaceae				
Malva parviflora Baladi	No	No	-	-

CRS = Chlorotic ring spot
Sys.Mot = Systemic mottling
NLL = Necrotic local lesion
Ch.RS = Chlorotic ring spot
VY = vein yellowing
Sys.M = Systemic mosaic
P.P = pin point
bb= brown bronzed area
Y= yellowing
Ch. LL = Chlorotic local lesion
Ch.s = Chlorotic spot
M = Mosaic
Mo = mottling
N.RS = Necrotic ring spot
NES = necrotic eyelike spots
M.M = Mild Mosaic
No = No Symptomless
Icl= Interveinal chlorosis
\+ = Positive
\- = Negative

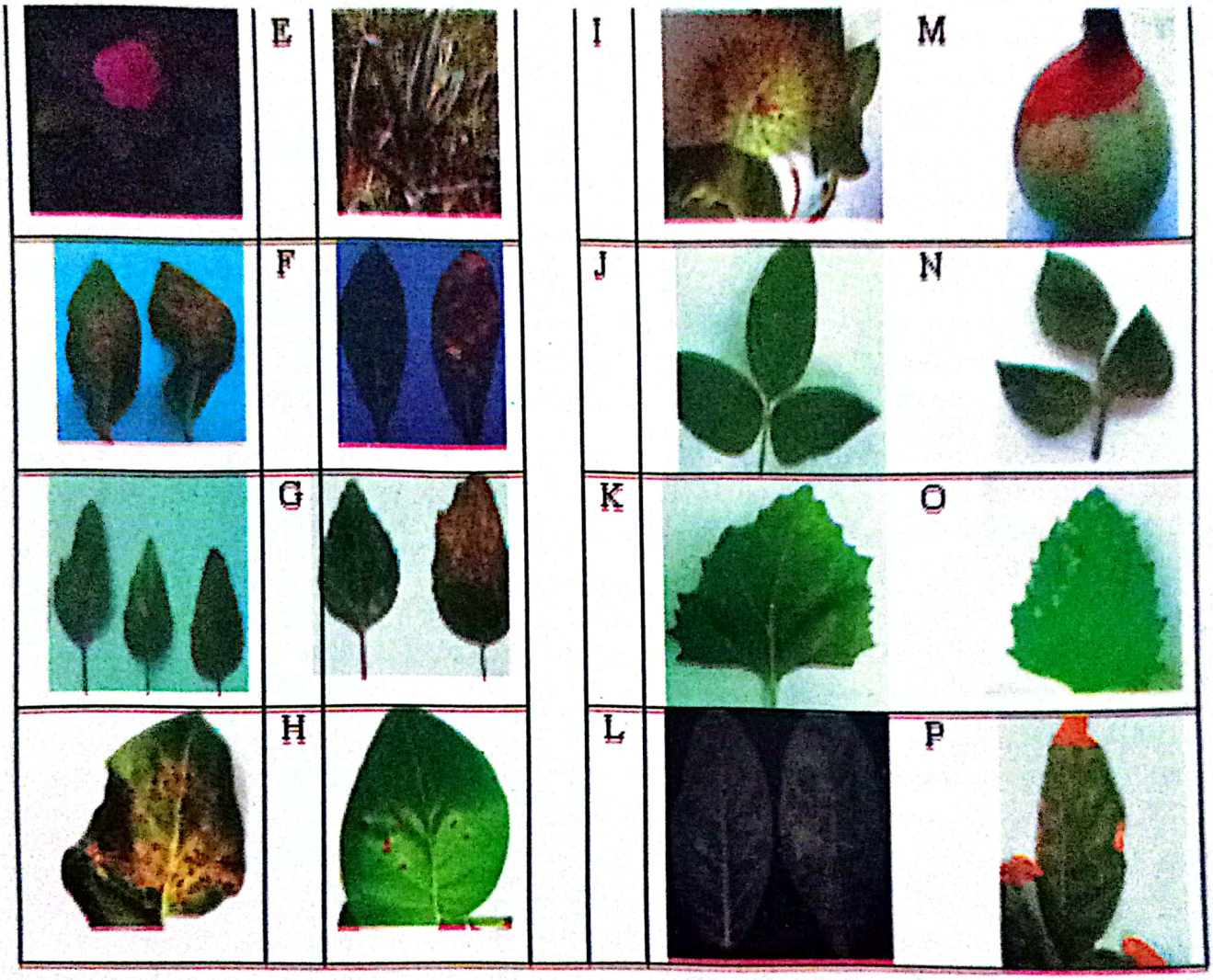

Fig (2) : Symptoms of **TSWV** infection on some hosts upon (A) V. rosa systemic mosaic and yellowing, (B) G. globosa Necrotic local lesions, (C) C. amaranticolor necrotic local lesion, (D , I) D. innoxia chlorotic and necrotic ring spot, (J) V. unguiculata pin point, (K) C.murle chlorotic local lesion, N.rustica (L) mosaic. Symptomatology and host range of **IYSV (E, M, P)** A. cepa necrotic eyelike spots, (F) G. globosa & (G) C. amaranticolor, (O) C.murle, and (H) D. innoxia necrotic local lesion, and (N) V. unguiculata mottling, and (P) N.rustica mosaic and brown bronzed area and interveinal chlorosis.

other result by **Prins and Kormelink (1998).** Results in Table (1) indicated that all tested hosts belonging to family solanaceae susceptible to infection with IYSV but some differences in host reactions were also observed, so these hosts can be used to differentiate between TSWV and IYSV, such as, *D. innoxia, N. rustica, N. tabacum* L. White Burley, *N. tabacum* cv. L Samsun, tomato (*Lycopersican esulentum*) , and potato (*Solanum tuberosum*), in addition of some hosts belonging to the families, *Amaranthaceae* ,*Cucurbitaceae*, and *Fabaceae*such as, *Gomphrena globosa* Hairy cucumber(*Cucumis pubescens*) cucumber (*Cucumis sativus*) Cowpea (*Vigna unguiculata*).

Moreover, the Egyption isolate of IYSV did not infect *Cucumis sativus, Cucurbita pepo, Phaseoluse vulgaris*, and *Pism sativum.* This result is similar to other results by **(Kritzman *et al.*, 2000, Kritzman *et al.*, 2001, and Corte's *et al.*, 1998).**

However, results of tested hosts when compared with the described hosts for some investigators indicate some differences in host reactions, revealed that the Egyption isolate of IYSV is different from other isolate like Isreal isolate, Brazil isolate and Netherlands isolate in reactions of host.

Virus stability in crude sap

Results in Table (2) showed that TSWV had DEP, TIP and LIV of 10^{-3}, 55°C, and 5 hours, repectively whereas IYSV had 10^{-4}, 55 °C and 5 days, respectively. TSWV differed in its stability properties from IYSV in DEP and LIV but these viruses are heat- inactivated at 55 °C, whereas this result is not agreement with those obtained by **Le (1970), Best (1946), Alkhazindar (1999), and Hayam *et al* (2004).**

Nagata and peters (2001) reported that Tospoviruses are heat inactivated at 56 °C within 10 min and sensitive to lipid solvents and detergents, and their instability is a first indication that the infecting virus might be *Tospovirus*. On the other hand, this result may be refered to the species of isolate and host adapted variants.

Table 2.	Stability of TSWV and IYSV in crude sap		
Tested Viruses	DEP	TIP	LIV
TSWV	10^{-3}	55	5 h.
IYSV	10^{-4}	55	5 D.

Transmission studies

1.Mechanical transmission

TSWV and IYSV were easily transmitted mechanically to *G. globosa* L. used as a propagative host for the two viruses as described by **(Le, 1970; and Cortes *et al.*, 1998).** Inoculated plants with TSWV showed

chlorotic and necrotic local lesions followed by systemic chlorosis (fig 1-B) within 5-7 days post inoculation as described by **Wang and Gonsalves (1990)** and **Bezerra *et al.* (1999)**, whereas most inoculated *G.globosa* plants reacted locally with chlorotic or necrotic lesion within 7 days and with chlorotic spot on younger leaves after 10 days post inoculation (Fig 1-F) as described by **Kritzman *et al.* (2001)**.

2- Seed transmission

Results in Fig (3) indicated that TSWV was found to be seed-transmitted through seeds harvested from infected *D .innoxia* plants as described by **(Le, 1970)**. Also, results in Fig (3) indicate that IYSV is seed borne transmitted through seeds which harvested from infected onion plants when tested using DBIA technique. This result is similar to those obtained by **(Toil *et al.*, 2004 and David *et al.*, 2006)**.

3-Thrips transmission

As shown in Table (3) one out four tested thrips species, *T. tabaci* was transmitted IYSV. This result goes online with that reported by **Kritzman *et al.* (2001)** they found that IYSV was efficiently transmitted by *T. tabaci* L from infected to healthy onion seedlings and leaf pieces. Two biotypes of *F. occidentalis* Pergamde, collected from two different locations in Israel, failed to transmit the virus.

Thrips Larvae transmitted the virus in a percentage of 45%, whereas the thrips adults were not able to transmit the virus as shown in all tests. In the present study TSWV was transmitted by two different thrips species *T. tabaci,* and *F. occidentalis.*

F. occidentalis was the most efficient vector of TSWV (60.9%) the lowest rate of transmission (36%) was obtained by *F. tritici.* This result is agree with that obtained by **Cho *et al.* (1986 and Yudin *et al.* 1986)**. They reported that TSWV is the only virus transmitted in a persistent manner with the western flower thrips (*F. occidentalis*), TSWV is also, vectored by other thrips species, yellow flower thrips *(F. schultzei),* onion thrips *(T. tabaci* L*),* and chili thrips *(Scirtothrips dorsalis).* The western flower thrips, however, is considered the most important vector. Both of *franklinella tritici* L. and *Gynaikothrips ficorum* Marchal were not able to transmit both viruses TSWV and IYSV. This result is agree with that obtained by **Assis *et al.* (2005),** they suggest that *F. tritici* does not transmit TSWV. Thus, *F. tritici* should still be considered as a

non-vector thrips species for TSWV. That was not found in the salivary glands of *F. tritici*, which is a prerequisite to virus transmission. Thus, movement to the salivary glands may determine vector incompetence of *F. tritici.* Parallel result was obtained by **Borbón *et al.* (2006),** they found that the five species thrips tested, *Frankliniella gemina* (first record), *F. occidentalis* and *F. schultzei* transmitted GRSV and TSWV. *F. schultzei* was a significantly more efficient vector of GRSV than *F. occidentalis.*

Table 3. Thrips species tested as a vector for both IYSV and TSWV.

Thrips species	Adults		Larvae	
	IYSV	TSWV	IYSV	TSWV
T. tabaci	0/20 (0.0%)	0/25 (0.0%)	9/20 (45%)	7/21 (33.3%)
F. occidentalis	0/18 (0.0%)	0/15 (0.0%)	0/16 (0.0%)	14/23 (60.9%)
F. tritici	0/15 (0.0%)	0/15 (0.0%)	0/13 (0.0%)	0/25 (0.0%)
G. ficorum	0/20 (0.0%)	0/17 (0.0%)	0/15 (0.0%)	0/15 (0.0%)

Acqusition access time 24 hr.
10 individuals/ seedling *Gomphrena globosaL* and *Datura innoxia* used as a test plant.

Thrips larvae were able to acquire and transmit both IYSV and TSWV, whereas Adults-virus-free of *T. tabaci* were not able to transmit the virus after (AAT) 24 on the virus source.

Data in Table (4) demonstrate that when larvae reared on virus source plant till adults, the developed adults were able to acquire and transmit the virus by percentage of 58.3% for (IYSV) and and 41.7 & 83.3% for TSWV by *T. tabaci* and *F. occidentalis* Pergamde respectively *T. tabaci* L adults were able to acquire IYSV (58.3%) and TSWV (41.7%), whereas *F. occidentalis* adults failed to transmit IYSV but *F. occidentalis* adults were able to acquire and transmit TSWV in a high percentage of (83.3). Although both viruses were detected in the tested adults (virus- free adults that have 24h. AAT and in the viruliferous adults produced from larvae that, reared on the virus source) by DBIA test Fig (3-5,6) for TSWV and Fig (3- 11,12) for IYSV. This result was agree with that discussed by **Ohnishi *et al.* (1998), Nagata *et al.* (1999b),** and **Nagata and Peters (2001).** Ingestion of virus by adults does not lead to transmission, the failure does not mean that the midgut epithelial cells not become infected, more severe infection in the midgut of thrips adults after feeding for 2 hours on infect source plant and this adults also did not transmit the virus.

Further study on the viruliferous vectors and its role in the epidemiology and incidence of *Tospovirus* were under taking,

also the serological detection of the virus in viruliferous vectors is going on now.

Table (4). Adults of *T. tabaci* and *F. occidentalis* tested as viruliferous of IYSV and TSWV.

Thrips species	*Virus*			
	IYSV (%)		TSWV (%)	
T. tabaci	7/12	58.3+	5/12	41.7+
**Control	0/12	0.0+	0/12	0.0+
*F.occidentalis	0/12	0.0+	10/12	83.3+
**Control	0/12	0.0+	0/12	0.0 +

*Tested thrips species reared on the virus source fro n larval stage to adults
**Thrips virus-free feed on the source 24h. AAP.
+Insect tested by DBIA. Was positive to the presence of IYSV, TSWV.

Virus purification

The modified protocol of virus purification of *Tospovirus* could be applied to members of serogroups I and V (TSWV and IYSV). *N. rustica* and *G.globasa* the favored host to propagate the two viruses.

A successful procedure to purify *Tospovirus*, is given when harvested systemically infected *N.rustica* or *G.globasa* leaves showing symptoms, one or two days after the development of the first systemic symptoms. The purification will be less successful when necrotic symptoms appear on the leaves.

Purified TSWV and IYSV migrated as a single zone, 3 and 4 cm respectively below the minsicus of the density gradient column. These zones were found infections when tested in the local lesion host plant and gave typical ultraviolet absorption spectrum of nucleoprotein with a maximum and a minimum at 260 and 247 respectively for both TSWV and IYSV (Fig .4 A, B).

$A_{260/280}$ and Amax/min ratios were 1.20 and 1.11 for TSWV and 1.20, 1.30 for IYSV. These results are inagreement with several investigators **(Le, 1970 and Gibbs, 1985)**

Electron microscopy

. Electron microscopy (EM) analysis of purified TSWV and IYSV demonstrated the presence of spherical particles 85 nm and 80-120 nm in diameter for TSWV and IYSV (Fig .5 A,B) repectively . Such results agree with the diameter values reported for virions of TSWV and IYSV by **Kritzman *et al.*(2000), Kritzman *et al.*(2001), Bertaccini &Bellardi, (1990) and Scott (2000).**

Serologic studies

A- Antiserum production

Polyclonol antibodies raised against TSWV and IYSV were prepared. The antisera produced against TSWV and IYSV had titers of 1/3000 and 1/8000 repectively as determined by indirect ELISA test (Fig 6 A&B). Dilutions 1/500 and

1/6000 for TSWV and IYSV respectively were the best usable dilutions for producing high absorption values at 405 nm.

B- Indirect- enzyme linked immuno-sorbent assay (ELISA)

To confirm the presence of both TSWV and IYSV in the greenhouse after mechanical or trips inoculation and host range results, and to study the performance of the antisera in preliminary experiments the indirect ELISA was performed.

The results in Table (1) show that indirect ELISA technique was able to detect TSWV and IYSV in several hosts belonging different families. Such results are confirmed by several authors applying indirect ELISA test for *Tosposvirus* identification (**Gonsalves & Trujillo, 1986, Wang & Gonsalves, 1990; Daughtrey, 1996; corte's *et al.*, 1998; Kritzman *et al.*, 2000; Kritzman *et al.*, 2001, and Creamer *et al.*, 2004).**

C- Dot- Blot immunobinding assay (DBIA)

The technique of dot-blott on nitrocellulose membranes could be readily applied for detection of TSWV and IYSV in infected both tissues and seeds authentic and induced antisera for TSWV and IYSV (Fig.6).These results show the efficiency of DBIA for detection of *Tosposvirus*. Positive reaction were obtained with TSWV and IYSV infected tisssues and seeds as strong purple colour appeared.

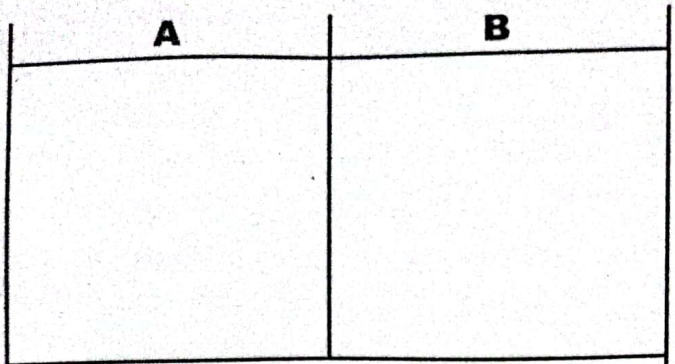

Fig (1) : TSWV (A) and IYSV(B) were identified by DBIA test using anthentic polyclonal antisera

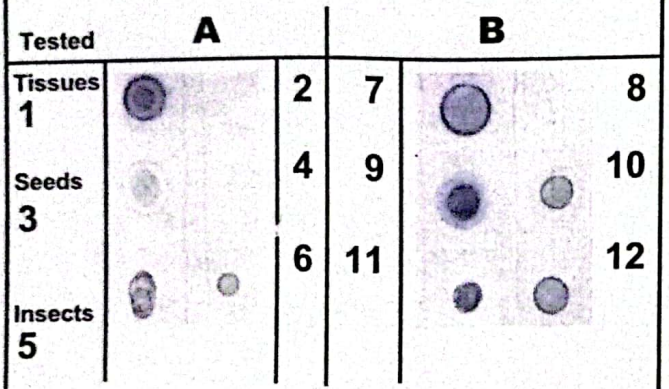

Fig (3) : TSWV (A) and IYSV (B) were identified by DBIA test using produced polyclonal antibodies for (1-A) infected tissue (2-A) healthy tissue (3-A) infected seeds, (4-A) healthy seed, (5-A infected insect, (6-A) healthy insect, (7-B) infected tissue, (8-B) healthy tissue (9-B) infected seeds, (10-B) healthy seeds, (11-B) adults virus free feed on the virus source anc (12-B) adults reacted on the virus source.

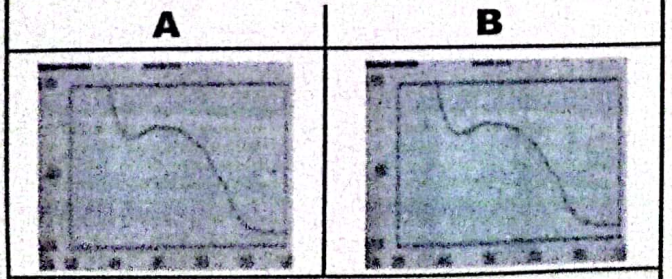

Fig (4) : Ulraviolet absorption spectra of purified A, TSWV; B, IYSV

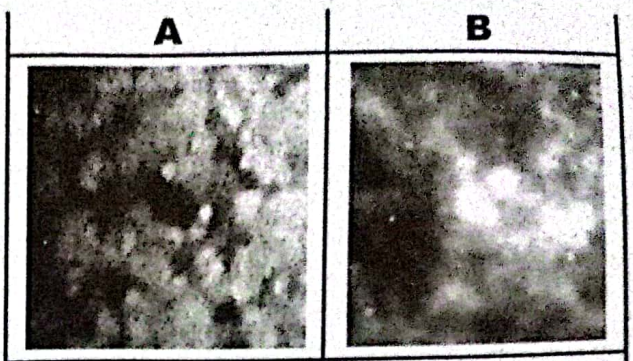

Fig (5) : . Electron microscopy testing of purified virus preparation from A, TSWV (mag40,000); B, IYSV (30,000) negatively stained with 2% phosphotungestic acid.

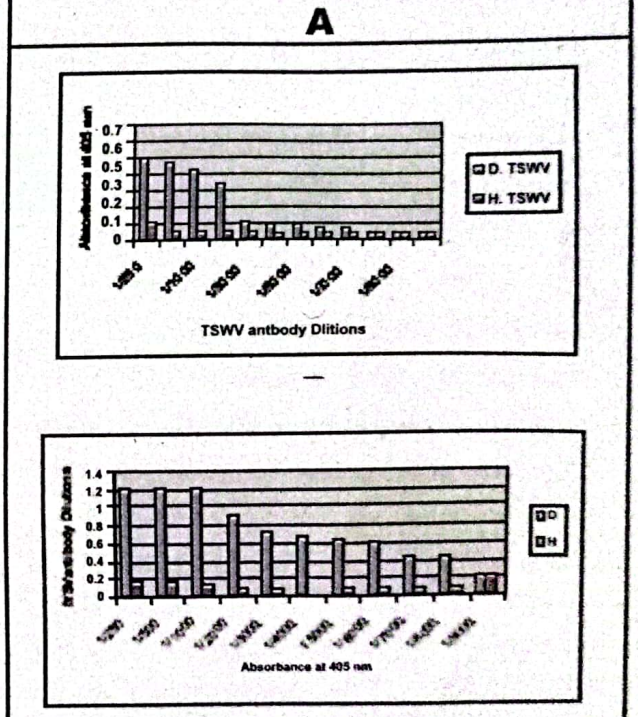

Fig (6) : Titer of TSWV (A) and IYSV (B) antisera measured with indirect ELISA D = Disease plant TSWV and IYSV H= Healthy

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دراسات مقارنة بين فيروس الذبول المنقط فى الطماطم والتبقع الأصفر فى السوسن

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تم إجراء دراسات مقارنة بيولوجية وبيوكيميائية وسيرولوجية بين فيروس الذبول المنقط فى الطماطم والتبقع الأصفر فى السوسن التابعين لمجموعة ال*Tospovirus*
وقد تم عزل هذين الفيروسين من نبات الونكا والبصل على التوالى والأعراض الظاهرية لتلك الفيروسين تتمثل فى أعراض موزيك وإصفرار جهازى على نباتات الونكا لفيروس الزبول المنقط فى الطماطم وبقع صفراء مغزلية الشكل على الأوراق والشمروخ الزهرى على الأبصال لفيروس التبقع الأصفر فى السوسن. وقد بينت دراسات النقل التجريبى لكلا من الفيروسين قدرتهما على الأنتقال ميكانيكيا وبالبذرة وكذلك بينت دراسات النقل الحشرى قدرة فيروس الذبول المنقط فى الطماطم على الإنتقال بواسطة نوعين مختلفين من التربس وهما *Thrips tabaci* بنسبة (33%) و *Frankliniella occidentallis* بنسبة (9و60%) بينما فيروس التبقع الأصفر فى السوسن ينتقل بواسطة نوع واحد فقط من التربس وهو *Thrips tabaci* بنسبة (45%) وقد تم مناقشة دور الحشرة الكاملة لكلا من نوعى التربس السابقين كناقل لكلا من فيروس الذبول المنقط فى الطماطم والتبقع الأصفر فى السوسن وقد أظهرت الدراسات أيضا أن كلا من نوعى حشرة التربس *Frankliniella tritici* و *Gynaikothrips ficorum* ليس لهم دور فى عملية نقل أى من الفيروسين .
وقد بينت دراسات المدى العوائلى أن كلا من الفيروسين ذو مدى عوائلى واسع وقد تم دراسة أختلاف الأعراض على العوائل المختلفة بالأضافة إلى ذلك وجد أن فيوس الذبول المنقط فى الطماطم يختلف عن فيروس التبقع الأصفر فى السوسن فى درجة التخفيف النهائية والبقاء خارج الخلية بينما أشترك الفيروسين فى درجة الحرارة المثبطة لكلاهما وكانت 55° م وذلك عند دراسة خواصهما الطبيعية.
وقد أمكن تنقية كلا من الفيروسين وقد وجد ان كلاهما يهاجر فى أعمدة السكروز المتدرج الكثافة منفصلا إلى مكون واحد. وقد أظهرت الدراسات الطيفية لكلا الفيروسين المنقيين أن أدنى وأقصى أمتصاص كان247 و 260 نانومتير على التوالى وكانت نسبة 260 / 280 و$A_{max/min}$ هى 1,2 ، 1,11 لفيروس الذبول المنقط فى الطماطم ، 1,2، 1,3 لفيروس التبقع الأصفر فى السوسن.
وقد أظهرت دراسات الميكروسكوب الألكترونى وجود جزئيات فيروسية كروية تتراوح مابين 85 نانومتير لفيروس الذبول المنقط فى الطماطم ، ومابين 80-120 نانومتير لفيروس التبقع الأصفر فى السوسن.
وقد تم أنتاج مصلين مضادين لكلا من الفيروسين وقد وجد أن درجة التخفيف النهائية للأنتسريم المنتج بأستخدام طريقة الأليزا غير مباشرة كانت 1/3000، 1/8000 لكلا من فيروسين الذبول المنقط فى الطماطم والتبقع الأصفر فى السوسن على التوالى.
وقد تم إستخدام كلا من الأنتسريم المنتج والأنتسريم المعروف أوالخاص بكلا من الفيروسين فى الكشف عنهما بأستخدام العديد من الطرق السيرولوجية مثل الأليزا غير المباشرة وأختبار الأرتباط المناعى على غشاء النتروسليلوز.