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Different Methods for Detection and Diagnosis of Tomato Spotted Wilt Tospovirus Infection of some Cucurbit Host Plants and its Main Vector of Thrips Species in Field and Greenhouse in Egypt



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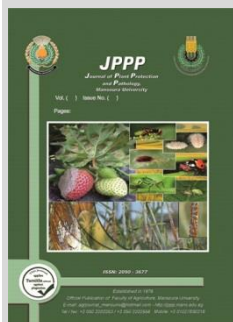
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ABSTRACT

The study recorded for the first time in Egypt the presence of tomato spotted wilt virus (TSWV) on cucurbits, which was confirmed using several serological tests, like double antibody sandwich ELISA (DAS-ELISA); Dot-blot ELISA assay and Immuno capture polymerase chain reaction (IC-PCR). By using Dot blot immunoassay test reaction against TSWV antisera, the ability of thrips species *Thrips tabaci* (Lind), *Thrips palmi* (Karny), and *Frankliniella occidentalis* (Pergande), on the transmission of the (TSWV) were asserted. In the greenhouse, eleven plant species were determined as the host range of virus and field samples showed the similar symptoms. The mechanical transmission of TSWV showed a positive reaction bearing symptoms on the leaves of Cucumber, zucchini, smoke, datura and tomato plants 15-20 days after inoculation with transmission rates of 46.5, 40, 46.5, 46.5 and 86.5%, respectively. DAS-ELISA using antisera of TSWV showed that the greenhouse leaves samples were infected; otherwise, an amplification of IC-PCR products of 500 bp was detected by agarose electrophoresis of total RNA isolated from infected leaves.

Keywords: Tomato spotted wilt virus (TSWV), Thrips, double antibody sandwich ELISA (DAS-ELISA), Dot-blot ELISA, Immuno capture polymerase chain reaction (IC-PCR), cucurbits, Egypt



INTRODUCTION

In Egypt, cucurbitaceous (cucurbit) plants, especially squash and cucumber, are important vegetable crops (Mohamed, 2003). They are attacked by many pests, especially piercing sap sucking pests causing serious damage to the host plants either directly by feeding on plant sap or indirectly through transmitting the causative agents of various diseases especially those belong to viral diseases (Abdel Salam *et al.*, 2008 and Ghallab *et al.*, 2011). The common diseases on cucurbit crops are varying causing approximately 16% decrease in yield, the additional loss caused by tomato spotted wilt virus (TSWV), (Roosjen *et al.*, 1998). TSWV alone ranks among the ten most detrimental plant viruses, causing more than one billion US dollars crop losses worldwide, (Goldbach and Peters, 1994). Moreover, it is known to infect more than 900 different plant species, belonging to more than 82 botanical families (Moyer, 1999). Nowadays, Tospoviruses are very economic importance of plant viruses all over the world including Egypt, the tomato spotted wilt virus (TSWV) used in the present study, belongs to the sole phytovirus tospovirus genus, in the family *Bunyaviridae* and transmitted by thrips species (Nakahara & Footit 2012). Until now, only eight thrips species are known as vectors of tospoviruses and they are members of the genera *Thrips* and *Frankliniella* within the family Thripidae (Nagata and Peters 2001). Thrips vectors and viruses have large, overlapping host ranges around the world, among which the solely onion thrips '*Thrips tabaci*' is recorded to be a vector of several Tospovirus diseases all over the world. Originating from North America, the western flower thrips (WFT), *F. occidentalis* become the most important main

vector of tospoviruses worldwide (Ullman *et al.*, 1997 & Nagata and Peters, 2001). In Egypt, thrips are known as efficient vectors of surveyed thrips species during 2010/2011 cucumber Tospoviruses (Abd EL-Wahab, *et al.*, 2012; Salah, 2016).. Symptoms caused by Tospovirus species varies greatly and have a little diagnostic value depending on the plant species or cultivar, plant age and growing conditions (Best 1968; Maris, 2004). TSWV can spread systemically through the susceptible plant and can produce a wide range of symptoms on different plants, such as necrotic and chlorotic lesions, systemic necrosis, systemic wilt, streaking, mosaic, mottling, brown spots, vein yellowing, ringspots and flower colour breaking (Goldbach and Peters, 1994).

There are several methods for detection and diagnosis of tospovirus, the presence of thrips that can transmit tospoviruses is the first indicator, also, the indicator plants can be used for detecting tospovirus infection. TSWV has a tripartite single stranded RNA genome with spherical particles in shape with a lipid envelope containing two kinds of glycoproteins and its diameter between 70 and 110 nm Goldbach and Peters, 1994. Enzyme-linked immunosorbent assay (ELISA), direct tissue-blot assay, dot blot immunoassay, and direct examination of plant tissues for characteristic viral inclusions are employed (Daughtrey *et al.*, 1997 and Xia *et al.*, 1997). Polymerase chain reaction (PCR) is an accurate way of TSWV identification. RT (RT-PCR) technique is most accurate and can distinguish between various tospoviruses strains. The detection of differences between tospoviruses is based on restriction enzyme digestions of the N gene obtained from PCR product (Dewey *et al.*, 1996 and Jain *et al.*, 1998). The present study

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is aimed to define the host range of tospovirus, and study the role of the surveyed thrips species in tospovirus transmission by using the serological and molecular techniques.

MATERIALS AND METHODS

Plant pathogenic virus. The tomato Spotted Wilt Virus (TSWV) was used in present study, collected from natural infection in cucumber and squash field, during growing seasons of 2010/2011, in the Experimental Station at the Faculty of Agriculture, Cairo University, Giza, Egypt. The typical symptoms of TSWV infection was diagnosed on cucumber and squash plants, showing signs of yellowing, stunting, crinkled, vein banding, mottling and local lesions on leaves.

Indicator and host range of TSWV. In order to recover the virus from the collected field leaf samples, 11 indicator plants were grown in the greenhouse and were used to detect tospovirus infection. Some plants were used to determine the host range of virus, including recommended cucurbitaceous host plants that used for detecting the TSWV infection as indicators of tospoviruses. The indicator plants were *Cucumis sativa* L. (cucumber), *Cucumis pepo* L. (squash), *Cucumis melo* L. (melon), *Luffa aegyptiaca* Mill. (luffa), *Citrullus lanatus* (Thunb.) (watermelon), *Solanum lycopersicum* (tomato) and *Vicia faba* (broad beans), in addition to 4 diagnostic hosts, were used for maintaining the virus, as *D. stramonium*, *D. innoxia*, *N. benthamiana* and *G. globosa* L. (Gomphrena).

Sampling of viruliferous insects

Four thrips species belong to order Thysanoptera and family thripidae were collected from the cucurbit plant leaves and the associated weeds grown in the Experimental Agricultural Stations, identified and diagnosed by (Abd EL-Wahab, et. al., 2012; Salah, 2016). These species are *Thrips tabaci*, *Thrips palmi*, *Frankliniella occidentalis* and *Chirothrips texanus*. The indicator plants were cultivated in pots in the greenhouse to detect tospoviruses incidence, and the field-collected thrips were fed in groups (5-10 adults/ plant) on each indicator plant. Under greenhouse conditions, the treated indicator plants were sprayed routinely with 0.01% Malathion and followed up for any appearance of typical TWSV viral symptoms.

Field monitoring of TWSV viral syndromes and Laboratory studies

During the field survey of thrips species on the cucurbitaceous plants, parallel observations were made to detect the appearance of any viral symptoms (e.g., Salah, 2016). According to the visual syndromes, plants showing viral symptoms were labelled, weekly observed and categorized into groups according to the visual syndromes. Leaf samples were taken to the laboratory and greenhouse for sap and thrips inoculation tests and stored at -20 °C until serologically tested.

Mechanical inoculation of tospovirus

Detecting and diagnostic leaf samples were grinded in KH_2PO_4 - K_2HPO_4 buffer containing 0.01M Na_2SO_3 with pH 7. As illustrated by (Peters 2003), inoculation of the indicator leaves was conducted by directly rubbing the samples on carborundum dusted. The plants were rinsed with water after inoculation and kept for approximately two weeks for symptom development.

Detection of Tospovirus and Identification

Virus isolation of the symptomatic *Nicotians benthamiana* plants (collected from the greenhouse) was

conducted showing clear symptoms 15-20 days after inoculation with TSWV. Purification of TSWV ribonucleo capsids was conducted by adding 4-8 ml extraction buffer (0.1 M Tris/HCl [12.1 g/l]; 0.01 M EDTA [3.72 g/l], pH 8.0), per g of leaves and homogenize the leaves for 10 sec at low speed in a blender. The homogenate was filtered through two layers of cheesecloth [boiled in water with some EDTA]. The extract was centrifuged for 10 min at 5000g and the supernatant was centrifuge for 40 min at 30.000 rpm. The pellets was resuspended in 100 ml of re-suspension buffer (0.01 M Tris/HCl [1.21 g/l], pH 7.9; 0.01 M EDTA [3.72 g/l] 1 % Nonidet P-40 [after adjusting the Ph]), [4 ml per g of leaves] and stirred gently for 30 min and centrifuged 5 min at 5000 g. Centrifuge the supernatant on a 30% w/v sucrose foot in re-suspension buffer. Place 30 ml sucrose solutions below 30 ml virus suspension per R45 tube then centrifuge for 1.10 h at 40.000 rpm. The pellets were dissolved in citrate buffer 2 ml per 25 g of leaves and centrifuged for 5 min at 5000 g. Add 1.6 g CsSO_4 and 4.5 ml of the virus suspension to reach SW55 tube and the meniscus was 2 mm lower the rim than the rim of the tube. Centrifuged in SW55 rotor overnight at 36.000 rpm then the virus band was collected and dialyzes in citrate buffers (0.01 M Na-citrate [2.90 g/l], Ph 6.0) described by Cortez et al., 2001 (Fig. 1).

RNA purification

Viral RNA was extracted from both infected leaves and the vectors according to the Trizol method (Cortez et al., 2001). Macerate of leaf material was added to Trizol. The mixture was incubated for 5 min at room temperature. Chloroform was added and shaken by hand for 15 seconds, then incubated for 2-3 min at room temperature.

Centrifugation was done for 15 min at 11000 rpm under cooling (4°C). The water phase was collected with sterile pipettes in sterile eppendorf tubes. One volume of water phase of cold isopropanol was incubated overnight at -20°C or 30 min at -80 °C. The supernatant was discarded. Sterile ice cooled Ethanol 70% was used to wash the pellet by centrifugation for 5 min at 15000 rpm under cooling (4°C). The supernatant was very carefully removed. The pellet was dried by using speed vacuum for 5-7 min and stored until use in minus 80°C, then dissolved by gently pipetting in 10-15 μl sterile H_2O .

Double antibody sandwich ELISA (DAS-ELISA)

Following Clark & Adams (1977), basic procedure of (DAS-ELISA) was used to detect TSWV in infected cucumber and squash plants. Gamma globulin (200 μl) of a stock solution of 1 mg/ml (Dako Do 487) diluted 1000 times was added in coating buffer $\{(\text{Na}_2\text{CO}_3 1.59 \text{ g}; \text{NaHCO}_3 2.93 \text{ g}; \text{NaN}_4 0.2 \text{ g}; \text{pH } 9.6)\}$ to the wells of the microtitre plate $\{(0.14 \text{ M NaCl } 8.19 \text{ g} - 40.9 \text{ g}); (0.002 \text{ M KH}_2\text{PO}_4 0.272 \text{ g} - 1.36 \text{ g}); (0.008 \text{ M Na}_2\text{PO}_4 1.42 \text{ g} - 7.12 \text{ g}); (0.002 \text{ KCl } 0.149 \text{ g} - 0.745 \text{ g}; \text{pH } 7.2)\}$. This plate was incubated overnight at 4°C, followed by washing by PBS Tween {1L PBS buffer; 0.5 ml Tween- 20} and rapid upside down turning of the plate(s). The droplets were removed by blotting the plate carefully on a piece of tissue. Plant extract was added in sample buffer. The plate was incubated overnight in a cold room 4° C. The extract was prepared in fixed weight /volume ratios (diluted 1:10 folds). The plate was washed twice as mentioned above. Diluted gamma globulin conjugated 1mg/ml (1:1000) with alkaline phosphatase (Dako Do 487) was added to the wells of microtitre plate. The plate was incubated for two

hours at room temperature then washed by PBS Tween. Freshly prepared substrate solution AP 0.6 mg/ ml (alkaline phosphatase, p-Nitrophenyl Phosphate, Disodium Salt substrate, Powder (25g), was added to each well. {Substrate buffer: Diethanolamine 96 ml; Aqua dest 800 ml; Na₂CO₃ 0.2 g; adjust to pH 9.8 with 6 N HCl and making than up to 1 liter}). Further,, the plate was incubated at room temperature for 15-20 min depending on the color development. The optical density was determined by using an ELISA reader at the wave length 405 nm (Thermo Scientific™ Multiskan™ FC Microplate Photometer). In all experiments diluted virus samples were used in the proposed experiments or to be performed and healthy material in the same dilutions.

Dot-blot Immunoassay (Dot-blot ELISA) for infected plants

Following Lin et al. (1990), Dot-blot ELISA assay was used. 1-3 µl of the plant sap was spotted on a nitrocellulose (Immunobilon-NC, Millipore) filter and completely dried. The filter was rinsed three times for 10 min with PBS-Tween, and was immersed in the primary antibodies solution 1 µg/ml in PBS-Tween with sap from a healthy plant (1g in 10 ml PBS-Tween). The filter was rinsed three times for 10 min in PBS-Tween, then was immersing in secondary antibodies solution (goat against rabbit conjugated, 1mg/ml (1:3000), with alkaline phosphatase (manufactured by Dako Do 487, Germany), and incubated for 2h at room temperature. The filter was rinsed two times for 10 min with PBS-Tween, and rinsed in AP buffer (12.1 g Tris-HCl, 5.8 g NaCl, and 10.2 g MgCl₂, pH 9.5) for 10 min. The filter was immersed in NBT/BCIP (10 µl/ml) nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3'-indolylphosphate p-toluidine salt) solution (Roche 001). Positive spots developed purple color. The enzymatic reactions were stopped by rinsing the filter 2-3 times with tap water and dried the filter paper and record the results.

Dot-blot Immunoassay to detect tospoviruses in the viruleferous thrips adults

A Dot-blot assay was conducted to confirm the presence of tospovirus in the tested thrips species. In total, 20 adults of the different species were collected from the mass rearing culture settled in the laboratory. These adults were transferred to the TSWV infected *Datura* plant leaf disks in Tashiro cages. Thrips adults were incubated for two days at 25°C. The cages were incubated at -20°C for 30 min. The adults were collected separately in Eppendorf tubes and labeled, and grinded in 10 µl of sample buffer (8g/l NaCl, 0.2g/l KH₂PO₄, 2.9g/l Na₂HPO₄ 12H₂O, 0.2g/l KCl and 0.5g/l Tween 20) and centrifuged in an Eppendorf centrifuge for 5 min. Several small droplets (2µl) were placed on a piece of nitrocellulose membrane and left to develop the reaction (Lin *et al.*, 1990), (Fig. 2).

Immuno capture polymerase chain reaction (IC-PCR)

Four steps simplified Immunocapture-PCR Protocol (Candresse, *et al.*, 1994), was used to detect the L-gene of TSWV. Coating of PCR eppendorff tubes (0,5 ml micro Amp PCR tubes, robbings scientific) by adding 100 µg/ml purified anti-virus IgG (Dako), in carbonate buffer (2.93 g/l of NaHCO₃, 1.59 g/l of Na₂CO₃ and pH adjusted to 9.6) to each tube and incubated for 3 hours at 37°C. The tubes were washed twice with 150 µl of PBS-Tween buffer (8 g/l of NaCl, 0.2 g/l of KH₂PO₄, 2.9 g/l of Na₂HPO₄ 12H₂O, 0.2 g/l of KCL and 0.5 g/l of Tween 20). The plant extract was prepared by grinding the plant leaves in sample buffer

similar to the one used for ELISA detection of the virus then centrifuged for 10 min in eppendorf centrifuge. The immunocapture step was done by adding 100 µl of clarified plant extract to a pre-coated tube and incubated for 2 hours at 37°C. The tube was washed twice with 150 µl of PBS-Tween buffer. The RT-PCR reaction was performed by adding 25 µl of reaction mix (Roche) to each tube (3 µl of 1,7 Triton X100, 2.5 µl of 10X reaction buffer (100 mM of Tris-HCl, pH 8.8, 15 mM of MgCl₂, 500 mM of KCl and 1% of Triton X100), 0.25 µl of 25 mM dNTP mix, 0.25 µl of 100 mM for each primers (forward primer) TSW1 corresponding to nucleotide position 762-781 (5'-ATGCTAAGGTTAAGCTC-3') and (reverse primer) TSW2 was complementary to nucleotide position 1064-1083 (5' -TTAAGCAAGTTCTGTGAG- 3') Jain *et al.*, 1997 were selected based on TSWV nucleoprotein gene (NP) sequence, located at the 3' end of RNA-S, 0.25U of AMV RTase, 0.5 U of Taq polymerase and water to 25 µl. The droplets were spin down by vortex well in eppendorf centrifuge. The reaction mix was over laid with 50 µl of mineral oil. The amplification reaction was carried out by using PCR thermal cycler (Thermo Scientific™, 2720 Thermal Cycler). Hard denaturation was performed at 95°C for 2 min followed by 35 cycles of amplification with denaturation at 94°C for 30 sec, annealing at 45°C for 45 sec, and extension at 72°C for 1 min. Finally, the amplification reactions were held at 4°C. PCR product was run on 1% agarose gel prepared in TAE buffer (Tris glacial acetic acid and EDTA) and pH was adjusted to 8.0 by using NaOH. Gels were stained by ethidium bromide solution (Fig.3) and photographed using gel documentation system (Biometra). All the materials and the apparatuses used in the serological and molecular assays were provided by *Prof. Dr. Dick Peters*, at The Virology lab, Plant Pathology Department, Wageningen University.

RESULTS AND DISCUSSION

Symptoms of TSWV naturally infected cucurbit plants

Natural leaves infection symptoms show a silver etching of the veins, chlorotic spots, which later develop into yellow and necrotic spots. The oldest leaves showed a severe chlorosis along the leaf edges and a strong vein yellowing. However, the younger leaves show green mottling (Fig.4). Infected squash plants showed similar symptoms of TSWV as sever yellow to tan narrow, slight curling, green mottling on the younger leaves, ringed lesions on leaves contributing to early leaf senescence and a strong vein yellowing earlier than the diseased cucumber plants (Fig.5)

Sap (Manual) transmission, host range and diagnostic hosts

All the diagnostic plants and the test plant species that were either used for maintaining the virus or used to determine the host rang of the virus, (mechanically scratched and inoculated with TSWV by using carborandum) showed the symptoms as observed on the plants collected from the field. Transmission test recorded on some plants local lesions indicated a positive reaction on cucumber, squash, tomato, Nicotiana, and Jimson weed as systemic symptoms after 15-20 days' post inoculation (Figs. 6), with transmission rates 46.5%, 40%, 46.5%, 46.5% and 86.6% respectively Table (1). In order to assert that the tested plants are became infected with TSWV, Dot plot technique was used (Fig. 7).

Table 1. Mechanical (sap) transmission rates of TSWV on some plants.

Test plant species	Exp. No.	No. of tested	No. of infected	% of infection
<i>Cucumis sativus</i> (Cucumber)	1	5	2	
	2	5	3	
	3	5	2	
	Total	15	7	46.5
<i>Cucurbita pepo</i> (Squash)	1	5	2	
	2	5	2	
	3	5	2	
	Total	15	6	40
<i>Nicotiana benthamiana</i>	1	5	3	
	2	5	2	
	3	5	2	
	Total	15	7	46.5
<i>Datura stramonium</i>	1	5	2	
	2	5	2	
	3	5	3	
	Total	15	7	46.5
<i>Solanum lycopersicum</i> (Tomato)	1	5	5	
	2	5	5	
	3	5	3	
	Total	15	13	86.6

Confirm the presence of tospovirus in the tested thrips species

The three different thrips species were able to transmit TSWV within their lasted life-time and the Dot-blot Immunoassay technique confirmed that they were the virus vector (Fig. 8). However, *Ch. Texanus* larvae was not a vector of TSWV.

Detection of the presence of TSWV in different host plants by Double antibody sandwich ELISA (DAS-ELISA)

Leaves samples of the different infected plant species, cucumber and squash plants showed positive reactions by DAS-ELISA using TSWV antisera compared with the control (Table 2) and (Fig. 9).

Table 2. Schematic diagram showing the absorbance values of DAS- ELISA measured at 405 nm for determination of TSWV IgG against sap extracts from cucumber & squash leaves infected with TSWV

Dilutions	O.D. values of infected tissues			
	Cucumber		Cucumber	
	H	I	H	I
1/ 250	0.064	0.394	0.064	0.450
1/ 500	0.030	0.124	0.030	0.243
1/1000	0.026	0.039	0.026	0.055
1/ 2000	0.027	0.030	0.027	0.026
1/ 4000	0.025	0.023	0.025	0.023

H: Healthy (calculated mean of 2 wells); I: Infected (calculated mean of 2 wells).

Immunocapture polymerase chain reaction (IC-PCR)

IC-PCR was performed using the specific primers for the amplifications of a fragment of 500 bp of the entire N gene of TSWV. Total RNA samples isolated from the two plants (cucumber and squash) leaf material and TSWV infected thrips species (*T.tabaci*, *T.palmi*, *F.occidentalis* and *Ch. texanus*) were used for the amplification. After gel electrophoresis, the two plants, the thrips species, and positive control gave the expected PCR band but no such band was present in the negative control and the thrips species, *Ch. texanus* (Fig. 10). The amplification results confirm that cucumber and squash plants in the filed were infected with TSWV. The results also confirm that the three species of thrips were vectors for TSWV.

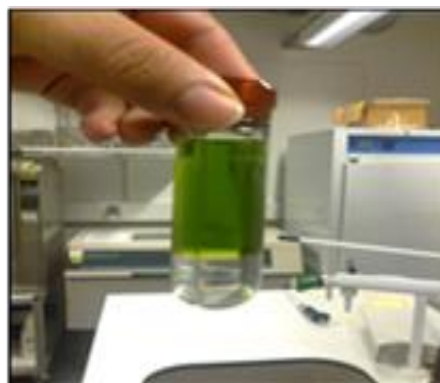


Fig. 1. TSWV purification by sucrose density gradient

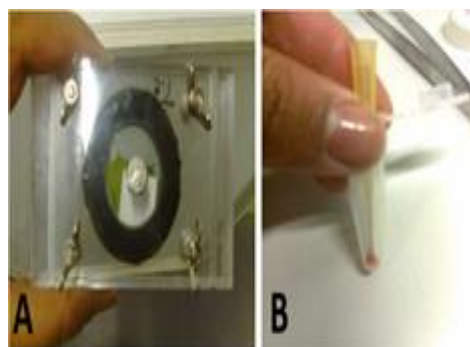


Fig.2. Tools used in dot-blot of immunoassay to detect tospoviruses in the viruleferous thrips adults: A=Tashiro cage B= Eppendorf with modified tips

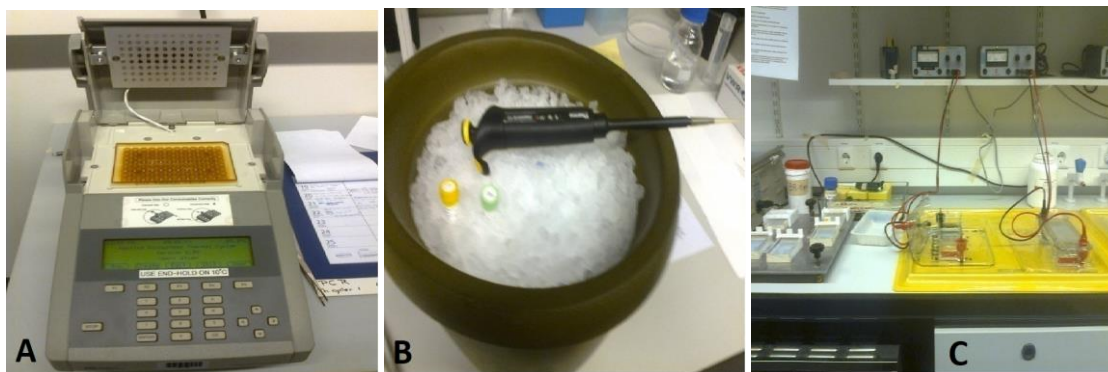
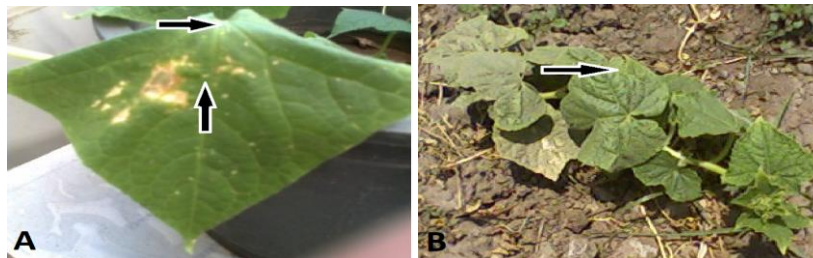


Fig. 3. Equipment used in molecular identification of Tospovirus A= PCR machine. B= PCR kits in crushed ice C= Electrophoresis apparatus.



Fig. 4. TSWV on natural squash plants showed a) Visible symptoms; b) Early natural infection with the severe silver vein etching; and c) Naturally diseased with the symptoms of yellow leaf edge due to senescens.



Figs. 5. cucumber leaves, a) Mechanically infected; Note many chlorotic spots with silver colored area; b) Naturally infected present infected top.

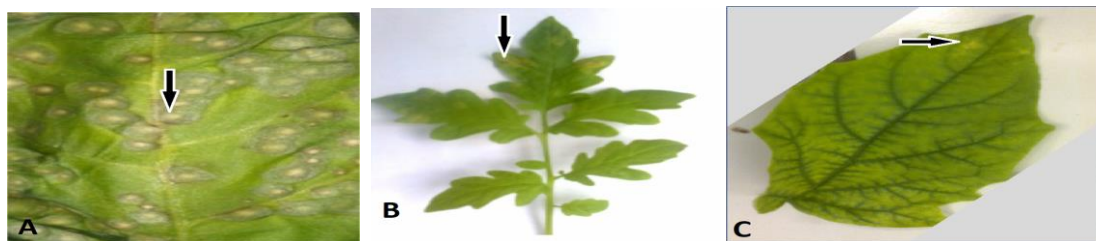


Fig. 6. Necrotic local lesions on a) *D. stramonium* (Jimson weed), mechanically infected with TSWV; b) systemically infected leaves on tomato; c) *N. benthamiana* (tobacco), showed chlorotic and necrotic ring spots in line patterns.

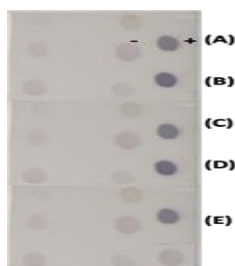


Fig. 7. Dot blot test reaction of five plant species infected with TSWV against different tospovirus antisera (A) Positive control (infected with TSWV), (B) Cucumber, (C) Squash, (D) Tomato and (E) *N. benthamiana*

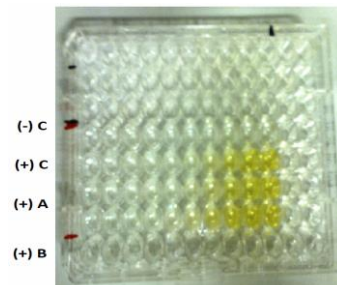


Fig.9. DAS-ELISA test results using TSWV antisera, (-) C= negative Control (Cucumber); (+) C= positive control; A= infected cucumber plant; B= infected squash plant.

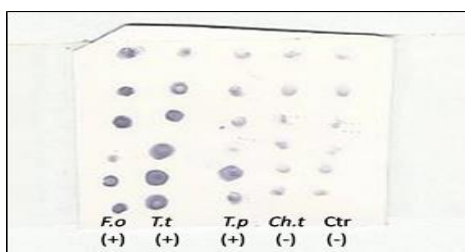


Fig.8. Dot blot immunoassay test reaction of four thrips species were tested against TSWV antisera asserted that the tested thrips species were the vector of TSWV. (+F.o.=*F. occidentalis*), (+T.t.=*T. tabaci*), (+T.p.=*T. palmi*), (- Ch.t.=*Ch. texanus*) and (- control= *T. tabaci*).



Fig. 10. IC-PCR amplification of N gene from TSWV infected cucumber, TSWV infected plants and three thrips species (*T. tabaci*, *T. palmi*, *F. occidentalis*). 1: infected cucumber, 2: *T.t.*, 3: *Ch.t.*, 4: *F.o.* and 5: *T.p.* and healthy *N.b.* was used as negative control (-C). 1Kb ladder was used. The PCR product represent the expected bands of 500 bp.

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الطرق المختلفة لاكتشاف وتشخيص إصابة بعض نباتات العائلة القرعية لفيروس الذبول المتبقي في الطماطم ونقله الرئيسي لأنواع التريسي في الحقل والصوب الزراعية في مصر

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سجلت الدراسة لأول مرة في مصر وجود فيروس الذبول المتبقي في الطماطم على القرعيات والتي تم تأكيدها باستخدام عدة اختبارات سيرولوجية مثل المقاييس المناعية المرتبطة بالإنزيم و التفاعل المناعي والبلمرة المتسلسل واختبار الطبع. لتثبت النتائج قدرة 3 أنواع فقط من حشرات التريسي وهم تريسي البصل وتريسي الأزهار الغربي وتريسي القرعيات (النخيل) على نقل فيروس الذبول المتبقي في الطماطم لكل من الكوسة والخيار والطماطم والدخان والجموفرينا في حين ان تريسي الحشائش ليس له القدرة على نقل الفيروس. وقد كان هناك تفاعل ايجابي ناتج عن النقل الميكانيكي لفيروس الذبول المتبقي في الطماطم لتعطي اعراض جهازية علي اوراق كلا من نباتات الخيار والكوسة والدخان والداتورا البلدي والطماطم بنسب معدلات نقل كانت ٤٦,٥ ٪، ٤٠ ٪، ٤٦,٥ ٪، ٤٦,٥ ٪ و ٨٦,٥ ٪ على التوالي. لتثبت الدراسة أن ثلاث أنواع نباتية أظهرت الاعراض الجهازية كعوائل كاشفة لفيروس الذبول المتبقي في الطماطم مطابقة لاعراض الإصابة الحقلية بالفيروس بعد ١٥-٢٠ يوم من بداية تلقيح النباتات بالفيروس. وبالكشف عن وجود الفيروس باستخدام اختبار الاليزا ELISA على نباتات الخيار والكوسة والطماطم والدخان المرباه في الصوبة سواء بالنقل الميكانيكي اوبالحقن بحشرات التريسي اظهرت النتائج ان جميع النباتات المختبرة أظهرت تفاعل موجب مقارنة بالنباتات السليمة. كما تم الحصول على حزم من المادة الوراثية ذات وزن جزيئي مكافئ للوزن الجزيئي للمادة الوراثية RNA الخاصة بفيروس الذبول المتبقي في الطماطم من خلال التفاعل المناعي - البلمرة المتسلسل Immunocapture-PCR.