

Original Article

Molecular characterization and incidence of new tospovirus: Soybean Vein Necrosis Virus (SVNV) in Egypt

Caracterização molecular e incidência de novo tospovírus: Vírus da Necrose da Veia da Soja (SVNV) no Egito

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Abstract

Field survey study was conducted season (2017). Soybeans and weeds were weekly sampled randomly. Thrips adults were identified and counted. Detection of the virus isolate and the natural incidence was determined using: Mechanical transmission, host range, DAS-ELISA, RT-PCR. The natural incidence thrips individuals was detected depending on the SVNV% in thrips individuals and weeds hosts.

Ten thrips species were associated with soybean plants in the field. The most abundant species was *T. tabaci*, average 256.5 average no. of individuals, followed by *F. occidentalis* (142.5 average no. of individuals), then *N. variabilis* (86.6/ average no. of individuals). Fourteen thrips species occurred on 5 legumes field crops and 41 weed plant species within soybean field. The highest average number 40.6 of individuals were recorded on *Ammi majus*. While the lowest one 3.3 average no. of individuals were on *Urtica urens*. Only 21 diagnostic plant species were susceptible to infection with SVNV. *G. max* and *Vigna radiate*, were the highest percentage of infection 80% followed by *V. unguiculata* & *N. benthamiana*, 75%. Egyptian isolate of Soybean vein necrosis virus (SVNV) in this study showed a high degree of similarity and it is closely related to TSWV from Egypt (DQ479968) and TCSV from USA (KY820965) with nucleotide sequence identity of 78%. Four thrips species transmitted SVNV (*F. fusca* 4.0%, *F. schultzei* 4.3%, *F. tritici* 3.3% and *N. variabilis* 68.0% transmission). Both *C. phaseoli* and *M. sjostedti* can acquire the virus but unable to transmit it. The following species; *T. tabaci*, *F. occidentalis*, *S. dorsalis* and *T. palmi* cannot acquire or transmit SVNV. The incidence of SVNV in the field started by the end of July then increased gradually from 12.7 to 71.3% by the end of the season. In conclusion, few thrips individuals invaded soybean crops are enough to transmit high rate of SVNV within the crop. Furthermore, several vector species are also abundant on weeds, which are the major sources of soybean viruses transmitted to the crops. This information might be important for control and reduce the incidence of SVNV infection.

Keywords: Thysanoptera, thrips, symptoms, virus-transmission, epidemiology.

Resumo

O estudo de pesquisa de campo foi realizado na temporada (2017). A soja e as ervas daninhas foram amostradas semanalmente de forma aleatória. Trips adultos foram identificados e contados. A detecção do vírus isolado e a incidência natural foram determinadas usando transmissão mecânica, gama de hospedeiros, DAS-ELISA, RT-PCR. A incidência natural de trips em indivíduos foi detectada dependendo da % de SVNV em trips e hospedeiros infestantes. Dez espécies de trips foram associadas a plantas de soja no campo. A espécie mais abundante foi *T. tabaci*, com média de 256,5 número médio de indivíduos, seguida por *F. occidentalis* (142,5) e *N. variabilis* (86,6 / número médio de indivíduos). Catorze espécies de trips ocorreram em 5 culturas de leguminosas e 41 espécies de plantas daninhas dentro de campos de soja. O maior número médio de 40,6 indivíduos foi registrado em *Ammi majus*. Enquanto o mais baixo, 3,3 número médio de indivíduos, foi no *Urtica urens*. Apenas 21 espécies de plantas diagnosticadas foram suscetíveis à infecção com SVNV. *G. max* e *Vigna radiate* foram os maiores percentuais de infecção, 80%, seguidos por *V. unguiculata* e *N. benthamiana*, 75%. O isolado egípcio neste estudo mostrou um alto grau de similaridade e está intimamente relacionado ao TSWV do Egito (DQ479968) e ao TCSV dos EUA (KY820965), com identidade de sequência de nucleotídeos de 78%. Quatro espécies de trips transmitiram SVNV (*F. fusca* 4,0%, *F. schultzei* 4,3%, *F. tritici* 3,3% e *N. variabilis* 68,0% de transmissão). Tanto *C. phaseoli* quanto *M. sjostedti* podem adquirir o vírus, mas não podem transmiti-lo. As seguintes espécies, *T. tabaci*, *F. occidentalis*, *S. dorsalis* e *T. palmi* não podem adquirir ou transmitir SVNV. A incidência de SVNV no campo, iniciada no final de julho, aumentou gradativamente de 12,7 para 71,3% no final da temporada. Em conclusão, poucos indivíduos de trips invadiram a cultura da soja e são suficientes para transmitir alta taxa de SVNV dentro da cultura. Além disso, várias espécies de vetores também abundam em ervas daninhas, que são as principais fontes dos vírus da soja transmitidos às lavouras. Essas informações podem ser importantes para controlar e reduzir a incidência de infecção por SVNV.

Palavras-chave: Thysanoptera, trips, sintomas, transmissão de vírus, epidemiologia.

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1. Introduction

Glycine max L. (soybean) considered very important leguminous crop In Egypt, FAS Cairo forecasts that Egypt's soybean area, as well as its production in marketing year 2017 & 2018, estimate of 9,000 hectares (HA) and 25,000 metric tons (MALR) and ARC (2019). Several factors affecting soybean crops productivity such as, fertilization, sowing date, insects and virus infection Hassan et al. (2002) and Soliman et al. (2007). Soybean pests are major biotic constraints limiting soybean production and quality. Crop losses to animal pests, diseases and weeds in soybeans average 26–29% globally (Heinrichs, 2018). Different insect species attack soybean crops around the world such as; Cotton leaf worm, *Spodoptera littoralis* (Boisd) Massoud et al. (2014), Aphid, *Aphis gossypii* (Glov) and *Thrips tabaci* L., cause yield losses from 20 to 50%, Alakhder et al. (2015). 6000 species of thrips insects are known around the world, (Mound and Morris, 2007). There, the 41 thrips species in the genus *Frankliniella* and the 4 of the genus *Thrips* comprise the biggest number of thrips, causing damage to different plant species while feeding and/or through the transmission of viruses (Monteiro et al., 2001).

Thrips transmit plant viruses such as Tospovirus, Ilarvirus, Carmovirus, Sobemovirus and Machlomovirus genera, Jones (2005). Thrips are the most important insect attacked soybean, due to the direct feeding injury and indirect damage caused by transmission of Tospoviruses, Persley et al. (2010), in Puerto Rico. Viteri et al. (2010). Also cause damage to carrot in Alto Paranaíba, Silva et al. (2019).

Tospoviruses, such as, Tomato spotted wilt virus (TSWV). It has a host range exceeding 1000 species in more than 100 plant families Parrella et al. (2003) and Pappu et al. (2009). Other virus such as, Impatiens necrotic spot virus (INSV) causes a major problem for the ornamental plants Daughtrey et al. (1997). and Iris yellow spot virus (IYSV) that infect a large number of economically important crops, Gent et al. (2006); Elnagar et al. (2006) and Abd El-Wahab et al. (2011).

Soybean vein necrosis virus (SVNV) is a *Tospovirus* that rapidly became a widespread and on soybean was first described in 2008 by Zhou et al. (2011), Zhou (2012), (Han et al., 2013) and Sikora et al. (2018). Also, reported in Wisconsin and Iowa by Smith et al. (2013) and in Oklahoma fields, by Ali and Abdalla (2013) while, report in Alabama by Conner et al. (2013).

In Egypt SVNV was first recorded by Abd El-Wahab and El-Shazly (2017), at Giza region. While recorded in Ohio soybean fields by Escalante et al. (2018). Recently, SVNV it is the most widespread soybean viruses in North America, Irizarry et al. (2018). SVNV symptoms showing, vein clearing along the main veins, which became chlorotic and necrotic later, are confirmed by immunology tests Zhou et al. (2011); Zhou and Tzanetakis (2013, 2019).

SVNV is like other viruses in this genus in that its genome consists of a large negative-sense RNA component (L) and two smaller ambience RNA components (M and S) that encode proteins in both the positive and negative-sense (Khatabi et al., 2012; Zhou and Tzanetakis, 2019). Tospovirus species within this genus are typically split between two distinct genetic codes called the 'New World' viruses and the 'Old World' viruses Zhou et al. (2011).

At least 12-15 thrips species were reported to transmit from 13-20 Tospoviruses in nature, Mound (2005), Riley et al. (2011), Zhou and Tzanetakis (2013). Thrips prefer to select major hosts from families including; Asteraceae, Cucurbitaceae, Leguminaceae and Solanaceae, Whitfield et al. (2005) and Pappu et al. (2009).

Soybean thrips, *Neohydatothrips variabilis* (Beach) have been identified as the main vector of SVNV Zhou and Tzanetakis (2013), Abd El-Wahab and El-Shazly (2017). Both alternative weed hosts and thrips vector are the most sources of virus epidemiology, Groves et al. (2002) and Okazaki et al. (2011).

In Egypt little knowledge is yet, available about thrips species associated with soybean as reported by Abd El-Wahab (2016). Preliminary study was done on the isolation of SVNV, Abd El-Wahab and El-Shazly (2017) but no molecular biology, epidemiology and natural incidence of SVNV studies were done, therefore: **The main objectives are:**

- 1- Study the abundance of thrips species and the natural incidence of SVNV in soybean field.
- 2- Study the Molecular characterization of SVNV for the first time in Egypt.
- 3- Throw the light on the reservoir hosts for both thrips and SVNV in field. Such information is important to avoid damage to soybean crop.

2. Materials and Methods

2.1. Field study

Field survey was conducted at the Experimental farm, Giza Governorate. The area received all the recommended normal agricultural practices except insecticides. Thrips were sampled weekly from (Early May, until September 2017) using direct count to determine the species of thrips and count, Irwin and Yeargan (1980). Only thrips adults were collected weekly from 25 soybean plants sampled randomly as well as five random weed plants from each weed species. each sample was shacked over a white plate and number of each thrips species was counted using a pocket lens of 10X magnification and recorded then prepared according to Palmer et al. (1992); Mound and Kibby (1998) and identified under stereo-microscope (ZEISS).

In order to determine SVNV natural incidence, soybean plants showing, vein clearing and necrotic symptoms were weekly collected randomly from the field. Infected plants were sampled according to a zigzag-shaped pattern, with 2-3m of distance between samples. Each sample consist of 10 plants was placed in a separate plastic bag and stored at 4°C until testing by (DAS-ELISA).

2.2. Laboratory studies:

2.2.1. Maintenance of thrips cultures virus-free

A virus-free thrips culture was established as collected adults of different thrips species were separately placed on food plants. *Thrips tabaci*, *Thrips palmi* populations

were reared on cucumber while, *Neohydatothrips variabilis* were established on healthy soybean plants, *F. occidentalis*, *F. schultzei*, *F. fusca*, *C. phasolii* and *M. sjostedti* were reared on pods of French beans (*Phaseolus vulgaris*) in jars covered with thrips -proof -nets. Rearing was incubated at 25 + 2°C and 90 + 2% relative humidity with a photoperiod of 12 h. Adults were allowed to lay eggs on the pods for 3 days and were then removed, Murai and Loomans (2001), first instars were used for the virus acquisition as described by BIRTHIA et al. (2013), then the emerged adults were used in the different transmission tests.

2.3. Virus detection studies

2.3.1. Mechanical transmission and host range

Test plants were grown in the greenhouse for mechanical inoculation and host range studies. The mechanical transmission tests were made by homogenized samples of SVNV-infected plants in distilled water or 0.01 M sodium phosphate buffer, pH 7.0, containing, 0.1% sodium sulfite Zhou and Tzanetakis (2013). The sap was used to inoculate different test plants, dusted with Carborandum 600 mesh. 20 test plants from each species belonging to 10 families, inoculated mechanically. Test plants were kept under greenhouse conditions and observed for symptom expression. Leaf samples were also tested for virus presence using DAS-ELISA test according to Clark and Adams (1977).

2.4. Detection and amplification of SVNV- NP gene using Reverse Transcription -Polymerase Chain Reaction (RT-PCR)

2.4.1. Extraction of total RNA from plant tissue

RNA isolation from Soybean leaves infected with SVNV was carried out using RNase Mini Kit according to manufacturer's instructions (QIAGEN, Germany). The result RNA was dissolved in diethylpyro-carbonate-treated water. To remove any DNA residue, the extracted RNA was incubated with DNase for one hour at 37 °C.

2.4.2. Quantitating the RNA

RNA samples was quantified using Nano Drop spectrophotometer at 260, 280, 230 nm by using 1µl RNA sample to determine quality and quantity of RNA.

2.4.3. cDNA synthesis for the extracted RNA

Reverse transcription reaction was performed in reaction volume 20µl. The reaction mixture contains 2.5µl of 10x buffer with MgCl₂, 2.5µl of dNTPs (10mM), 1µl SVNV-NP reverse primer (10 pmol/µl), 3µl RNA (30ng) and 0.2µl reverse transcriptase enzyme (Biolabs, USA) and 10.8µl sterile water. RT-PCR amplification was performed in a thermal cycler (Eppendorf, Germany) programmed incubation at 42 °C for 2 h and inactivation at 65 °C for 20 min and the cDNA was then stored at -20 °C until used.

2.4.4. Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The primer sequences for SVNV-NP (forward 5'-ACTTGTGCAAGCTTATGGT-3' and reverse 5'-GAAATGATTCCAATCTGTTC-3'), according to Zhou and Tzanetakis (2019) were used to amplify of nucleoprotein (NP) gene of (SVNV). PCR reaction mixture was carried out in total volume 50µl contains 10µl of 10x mixed buffer, 1µl of SVNV-NP primer (10 pmol/µl for forward/reverse), 3µl cDNA and 0.2µl (5 units/µl) Taq DNA polymerase (Bioline, Germany) and final volume up to sterile water. PCR amplification was performed in a thermal cycler (Eppendorf, Germany) programmed for one cycle at 95°C for 3 min, then 30 cycles as follows: 30 sec at 95°C for denaturation, 45 sec at 52°C for annealing and one minute at 72°C for elongation. Reaction was then incubated at 72°C for 5 min for final elongation. A 7µl of the PCR products were separated on agarose gel electrophoresis using 2% (w/v) agarose in 0.5x TBE buffer. Electrophoresis was performed at 80 Volt with 0.5x TBE buffer as running buffer and then the gel was stained in 0.5µg/cm³ (w/v) ethidium bromide solution. The length of each band was estimated using DNA marker. Finally, the gel was photographed by using gel documentation system.

2.4.5. Sequencing and phylogenetic tree for NP gene of SVNV

PCR products were purified by using PCR clean up column kit (Maxim biotech INC, USA). DNA sequence for NP gene was performed by Sigma Company. The sequence was submitted using NCBI-BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to confirm identify the SVNV-NP gene. DNA sequence was alignment compared with other *Tospovirus* available in the Gen Bank database. The sequences were used for comparison using MEGA 4, Tamura et al. (2007) and phylogeny was tested with bootstrap method. The phylogenetic tree was analyzed and generated based on Neighbor joining statistic method.

2.4.6. Thrips transmission test

First instars (24 h-old) of different thrips species were separately allowed an acquisition access period (AAP) of 24 h. to as described by Inoue and Sakurai (2006). The newly emerged adults were transferred in groups (10-15 adults) of each species to (10 test plant) and allowed 24h. Inoculation access period (IAP), then thrips was killed using Malathion 0.01%. Test plants were kept under greenhouse conditions and observed for symptoms appearance. The rate of virus transmission was confirmed by DAS-ELISA. A group of virus free adult thrips were tested as control.

2.4.7. Detection of SVNV in thrips individuals

DAS-ELISA was also applied for testing the presence of SVNV in adult thrips collected in soybean fields Cho et al. (1987). Individual thrips was ground in 500 µL microtubes, 50 µL of the extraction buffer. In this procedure, healthy and SVNV-infected leaves of *N. benthamiana* plants were used as negative and positive controls, respectively. One hour

after adding the substrate, the absorbance value (A405 nm) was measured by DAS-ELISA, Clark and Adams (1977).

2.4.8. Determination of natural incidence of viruleferous thrips

Thrips species associated with soybean and weeds were collected and separated in the laboratory to different species. Individuals of each species were released on healthy soybean seedling for 24h. (IAP) to test their ability to transmit SVNV.

2.4.9. Alternative hosts as a reservoir of SVNV

Different thrips species naturally collected from different weed species associated within soybean field. The collected insects were tested for the presence of SVNV transmitted from weed reservoirs.

3. Results

The survey study revealed, 10 thrips species recorded on soybean plants *Thrips tabaci* (Lindenm), *Frankliniella occidentalis* (Pergande), *Frankliniella tritici* (Fitch), *Frankliniella schultzei* (Trybom), *Frankliniella fusca* (Hinds), *Neohydatothrips variabilis* (Beach), *Caliothrips phaseoli* (Hood), *Scirtothrips dorsalis* (Hood), *Thrips palmi* (Karny), and *Megalurothrips sjostedti* (Trybom).

T. tabaci was the most abundant thrips which averaged 256.5 individuals, followed by *F. occidentalis* 142.5, then *N. variabilis* (86.6/individual). The other species included *F. fusca*, *F. tritici*, *C. phaseoli*, *S. dorsalis* and *T. palmi* were

recorded in low numbers, while the lowest was *M. sjostedti* (13.5/individual) Figure 1.

T. tabaci reached first peak (33.4 average no. /plant) in mid-May and the second was in mid-July (30.1 individual/plant). Also, *F. occidentalis* appeared in late May and increased to reach peak number (21.3 average no./plant) in mid-July while the second peak (16.3 individual/plant) was late in the season Figure 2. Both of *N. variabilis* and *F. tritici* reach the max. average no. (16.3&13.1 individual/plant) late in the season, Figure 2.

Fourteen thrips species were associated with 41 weed plant species within soybean field, the highest species number of different thrips 7 species were recorded on both *B. vulgaris* and *M. intertexta* followed by 6 different species of thrips were occurred on, *A. majus*, *Amaranthus sylvestrus*, *Cynanchum acutum*, *Cynodon dactylon*, *Malva parviflora*, *Ipomoea tricolor*, *Setaria verticillata*, *Spinacia oleracea*, and *Trifolium alexandrinum* as show in Figures 3 and 4.

The most common thrips species associated with different weeds was *T. tabaci* (216.9 general average), followed by *F. occidentalis*, *F. tritici* and *N. variabilis* by an average 111, 62 & 41.8 individual respectively . While the lowest species was *S. dorsalis* 6.6 individual, Figure 5. *A. majus* harbored the highest average number of different thrips (40.6 individual/plant), while the lowest average number 3.3 individual occurred on *Urtica urens*, Figure 6.

3.1. Soybean vein necrosis virus SVNV symptoms

Soybean field layout, c111, Giza Egypt (Figure 7A-L) infected plants showing SVNV symptoms that often begin as chlorotic (light green to yellow) blotchy patches near

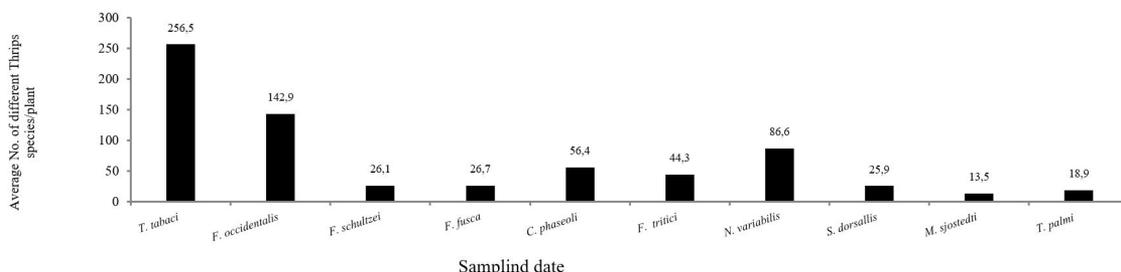


Figure 1. Different thrips species associated with soybean plants Giza111 in the field season 2017, Giza region Egypt.

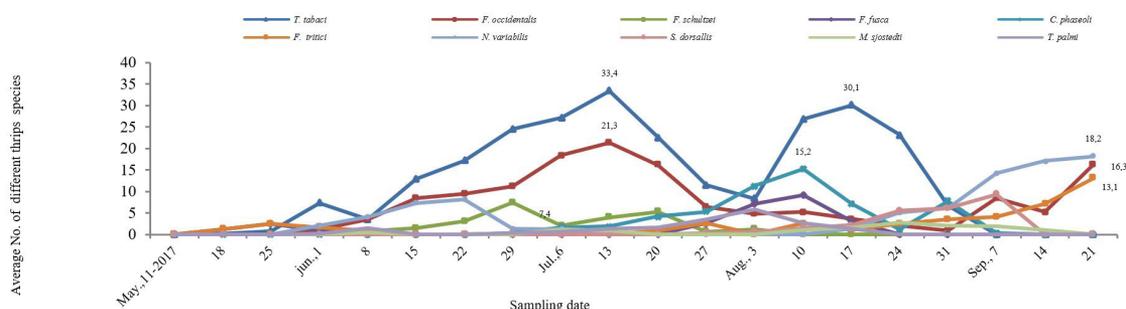


Figure 2. Weekly average No. of different thrips species associated with soybean plants in the field season 2017, Giza region, Egypt.

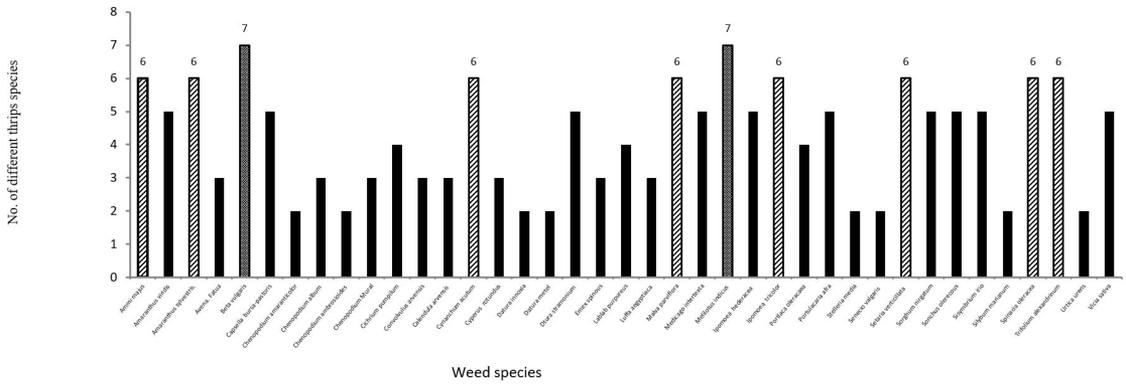


Figure 3. Number of different thrips species recorded on different weeds associated with soybean plants in the field, Giza, Egypt.

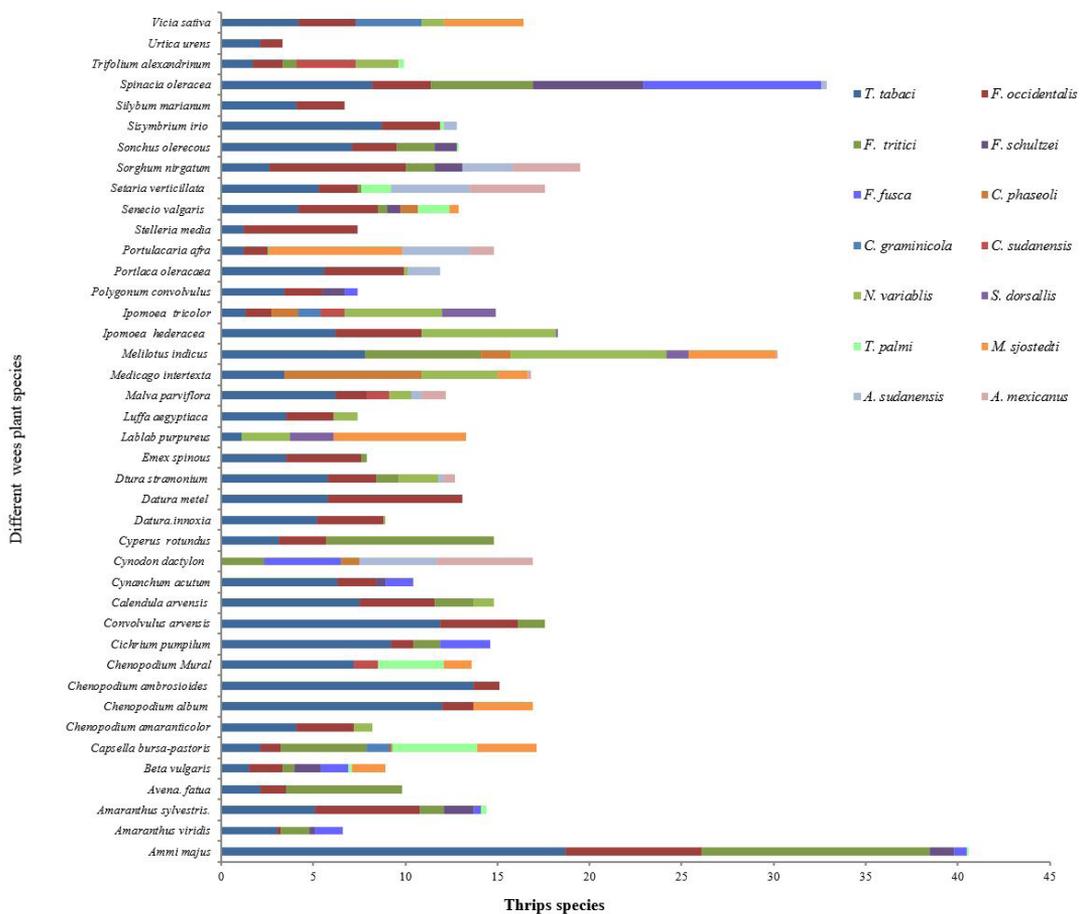


Figure 4. Average No. of different thrips species recorded on the weed plants associated with soybean plants season 2017 in the field, Giza region.

the main veins started near the main leaf veins. Natural symptoms showed reddish-brown areas with a browning of the veins. These areas may have a scaly or scabby appearance. These symptoms detected using RT-PCR as SVNV. The detected isolate was used in the study of the virus host range, transmission testes mechanically and by thrips species.

3.2. Hosts range and diagnostic hosts

Out of 43 plant species belonging to 11 plant family listed in (Table 1). mechanically inoculated, only 21 plant species were susceptible to infection with SVNV, *G. max* and *V. radiate*, gave highest percentage 80% followed by 75% for both *V. unguilata* & *N. benthamiana*, then *N. tabacum* 46% and *N. rastics* 15% (Table 1).

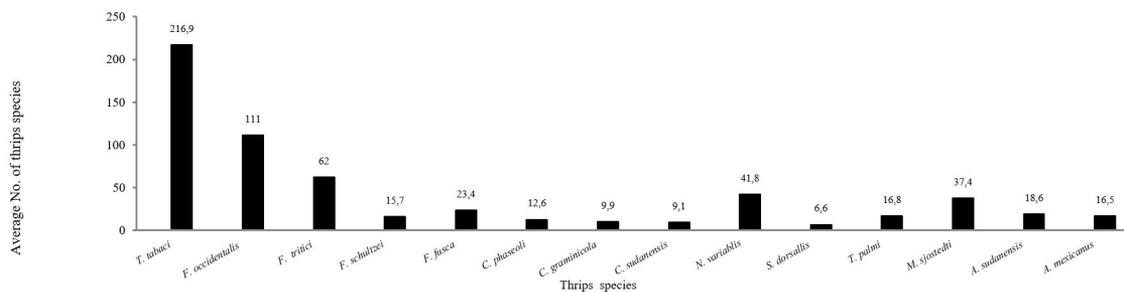


Figure 5. Different thrips species occurred on weed plants associated with soybean plant cv G. 111 in the field, season 2017, Giza, Egypt.

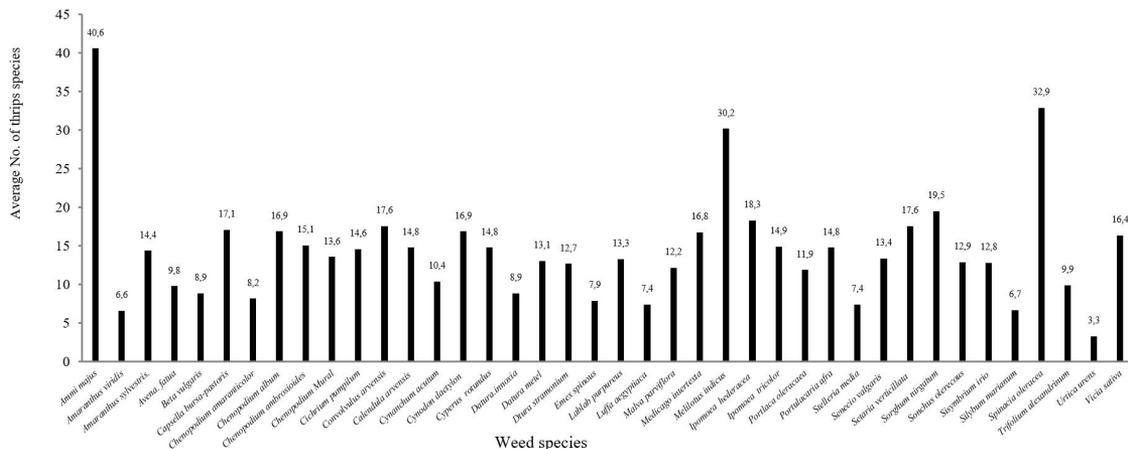


Figure 6. Different thrips species occurred on weed plants associated with soybean plant cv G. 111 in the field, season 2017, Giza, Egypt.

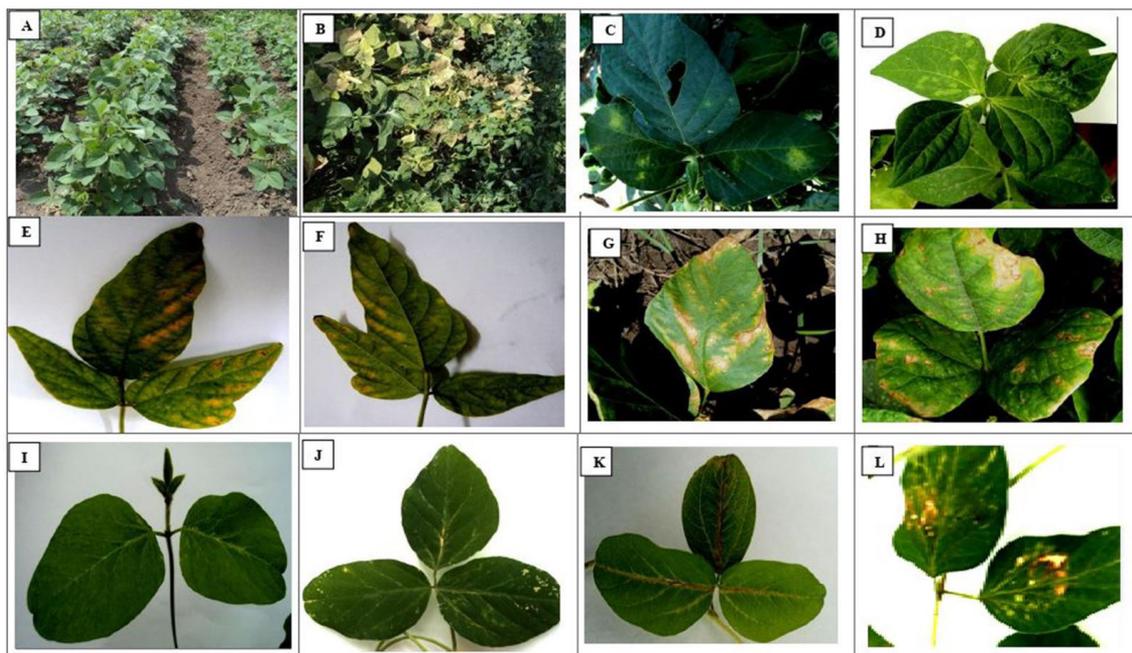


Figure 7. Soybean vein necrosis symptoms (SVNV). A: Layout of soybean field, c111,Giza Egypt. B: Nnaturally symptoms of SVNV. C: Early SVNV symptoms include yellowing tissue around leaf veins. D: Small light-green to yellow patches develop near main leaf veins. E & F: The upside and underside of soybean leaf shows vein discoloration. G & H: The symptoms were appeared as chlorotic spots on leaves which turned necrotic. I :Vein clearing in early stages of mechanical inoculation , J: Vein clearing and discoloration of veins, yellowing ,chlorosis along the veins of the leaf, K:Vein clearing and yellowing , chlorosis along the veins , L: light green patches a mottled and green areas associated with veins, red-brown and leaf tissue begins to die.

Table 1. Sap (mechanical) transmission of the Soybean vien necrosis virus to different tested hosts.

Scientific Name	Common Name	Symptoms	No. I / No. T	%	DAS- ELISA
1-Amaranthaceae					
<i>Amaranthus viridis</i>	Green pigweed	-	0/20	0	-
<i>Beta vulgaris</i>	Wild beet	-	0/20	0	-
<i>Gomphrena globosa</i>	globe amaranth	-	0/20	0	-
2-Apiaceae					
<i>Ammi majus</i>	bishop's weed	-	0/20	0	-
2-Asteraceae					
<i>Cichrium pumilum</i>	Chickory	-	0/20	0	-
<i>Helianthus annuus</i>	Sunflower	-	0/20	0	-
<i>Sonchus oleraceus</i>	Sowtistle		0/20	0	-
4-Apocynaceae					
<i>Cynanchum acutum</i>	Climbing vine swallowworts	Local lesion	2/20	10	+
5-Convolvulaceae					
<i>Convolvulus arvensis</i>	Field bindweed	Symptomless	1/20	5	+
<i>Ipomoea hederacea</i>	ivy-leaved morning glory	Symptomless	2/20	10	+
<i>Ipomoea tricolor</i>	Morning Glory, Granny vine	Symptomless	1/20	5	+
6-Cucurbitaceae					
<i>Cucumis melo</i>	Cantaloupe	Necrotic lesions	1/20	0	-
<i>Cucumis sativus</i>	Cucumber	-	0/20	5	+
<i>Cucurbita pepo</i>	pumpkin and squash	Necrotic lesions	1/20	5	+
<i>Cucurbita argyrosperma</i>	Pumpkins	Symptomless	2/20	10	+
<i>Luffa aegyptiaca</i>	Sponge gourd	Symptomless	1/20	5	+
7-Leguminaceae					
<i>Archis hypogeal</i>	Groundnut	-	0/20	0	-
<i>Cajanus cajan</i>	pigeon pea	Local lesion	1/18	5.6	+
<i>Cicer arietinum</i>	Chick pea	-	0/20	0	-
<i>Glycine max</i>	Soybean	Necrotic lesion	16/20	80	+
<i>Lablab purpureus</i>	lablab bean, Egyptian kidney bean	-	1/20	5	+
<i>Lens culinaris</i>	Lentil	-	0/20	0	-
<i>Lupinus luteus</i>	Yellow Lupin	Necrotic lesions	1/21	4.7	+
<i>Medicago sativa</i>	Alfalfa	Local lesions	1/20	5	+
<i>Phaseolus vulgaris</i>	common bean	-	0/20	0	-
<i>Pisum sativum</i>	Pea	-	0/20	0	-
<i>Trifolium alexandrinum</i>	Egyptian clover	Necrotic lesions	1/15	6.7	+
<i>Vigna radiate</i>	Mung bean	Necrotic lesions	16/20	80	+
<i>Vigna unguilata</i>	Cowpea	Necrotic lesions	15/20	75	+
<i>Vicia faba</i>	Broad bean	Necrotic lesions	1/20	5	+
8-Malvaceae					
<i>Gossypium herbaceum</i>	Levant cotton	-	0/20	0	-
<i>Malva parviflora</i>	cheeseweed mallow, Egyptian mallow	-	0/20	0	-
9-Portulacaceae					

(+) Positive; (-) Negative.

Table 1. Continued...

Scientific Name	Common Name	Symptoms	No. I / No. T	%	DAS-ELISA
<i>Portulaca oleraceae</i>	Common purslane	–	0/20	0	–
<i>Portulaca afra</i>	elephant plant, small leaf jade	–	0/20	0	–
10-Polygonaceae					
<i>Polygonum convolvulus</i>	Wild buckwheat	Necrotic lesions	2/20	10	+
11-Solanaceae					
<i>Capsicum annum</i>	Peppers	–	0/20	0	–
<i>Datura innoxia</i>	moonflowers, jimsonweed	–	0/20	0	–
<i>Datura stramonium</i>	Jamestown weed, datura	–	0/20	0	–
<i>Lycopersicon esculantum</i>	Tomato	–	0/20	0	–
<i>Nicotiana benthamiana</i>	Tobacco	Systemic	15/20	75	+
<i>Nicotiana glutinosa</i>	Tobacco	Local lesion	9/20	45	+
<i>Nicotiana tabacum</i>	Tobacco	Local lesion	1/20	15	+
<i>Petunia hybrid</i>	Petunia	–	0/20	0	–

(+) Positive; (-) Negative.

The 6 tested plant species, *C. arvensis*, *I. hederacea*, *I. tricolor*, *C. moschata*, *L. aegyptiaca* and *L. purpureus* gave from 4% to 12% infection although all of them were symptomless.

C. pepo, showing yellow necrotic lesions on the margins, then spread on leaf veins (Figure 8A). *C. melo*, shows systemic necrotic lesions (Figure 8B). Symptoms of SVNV infection on *C. acutum*, showing, chlorotic and necrotic lesions (Figure 8C). While *N. benthamiana*, showing local lesion and systemic infection (Figure 8D). necrotic lesions were observed on *P. Sativum*, (Figure 8E). SVNV lesions have yellow margins on *L. luteus* that spread on leaf veins. (Figure 8F). Faba bean showing chlorotic and necrotic lesions (Figure 8J). Local lesions caused by SVNV on *mung bean* (Figure 8H).

In the current study, the viral RNA was extracted from soybean leaves infected with SVNV and we found that the RT-PCR using the NP gene was a highly sensitive method for detecting SVNV. RT-PCR reactions were used at different dilution of cDNA and cycles number. The expected band ~348 bp sized of nucleoprotein (NP) is illustrated in Figure 9.

The phylogenetic tree observed for sequence of NP gene from SVNV-DA1 isolate from Egypt is closely related with other *Tospovirus* available in the Gen Bank database. Furthermore, the NP gene of SVNV-DA1 Egyptian isolate in this study showed a high degree of similarity and it is closely related to *Tomato spotted wilt virus* from Egypt (DQ479968) and *Tomato chlorotic spot virus* from USA (KY820965) with nucleotide sequence identity of 78% (Figure 10).

3.3. Incidence of viruliferous thrips

Fourteen different thrips species presented in (Table 2) Figure 11 were collected from 41 weeds and 5 legume field crops. Most of these species invaded soybean and weed plants, Seven weeks earlier to the appearance of virus

syndromes. The viral symptoms gradually increased to reach its maxim. in late Sep., while thrips appeared from mid-May in relatively high numbers, then decreased in early Jul., then increased again from Late Jul., to mid Sep., (Figure 11). However small numbers were encountered during Aug./Sep.

3.3.1. Detection, identification, sequencing and phylogenetic tree of NP gene of SVNV

Different thrips species field collected listed in (Table. 2) were tested for their role in of the spread of SVNV. Within the soybean crop, weeds and neighbor crops. Weed hosts are the possible reservoirs of virus from which thrips vectors transmit it to the new crops. *T. tabaci* *F. occidentalis* and *F. tritici* collected from 40, 36& 11 host weed plants did not transmit SVNV by feeding and gave a negative reaction using DAS- ELISA test, therefore they may not play a role as a vector of SVNV. *F. schultzei* collected from 10 host and weed plants, (*Trifolium alexandrinum*, *Vigna unguiculata* and *Lablab purpureus*), were able to transmit SVNV by 7.8% 10% and 5% respectively.

F. fusca collected from 13 host and weed plants, (*Glycine max*, *Polygonum convolvulus* *Ipomoea hederacea* and *Trifolium alexandrinum*), were able to transmit SVNV by 11.7% 10% 9% & 8.3%, respectively.

N. variabilis collected from *Cynanchum acutum* and *Ipomoea hederacea* transmitted SVNV by rate 20% and 12.5%, respectively and those collected from *Cucurbita moschata*, *Luffa aegyptiaca*, *Trifolium alexandrinum*, and *Medicago intertexta* transmit the SVNV by 10%. The high rate 70%, 53% & 20%, of transmission using *N. variabilis* individuals were from *Vigna unguiculata* *G. max* and *Vigna radiate* respectively. While the lower rate of SVNV transmission 5%. was by *N. variabilis* Collected from *Lablab purpureus*

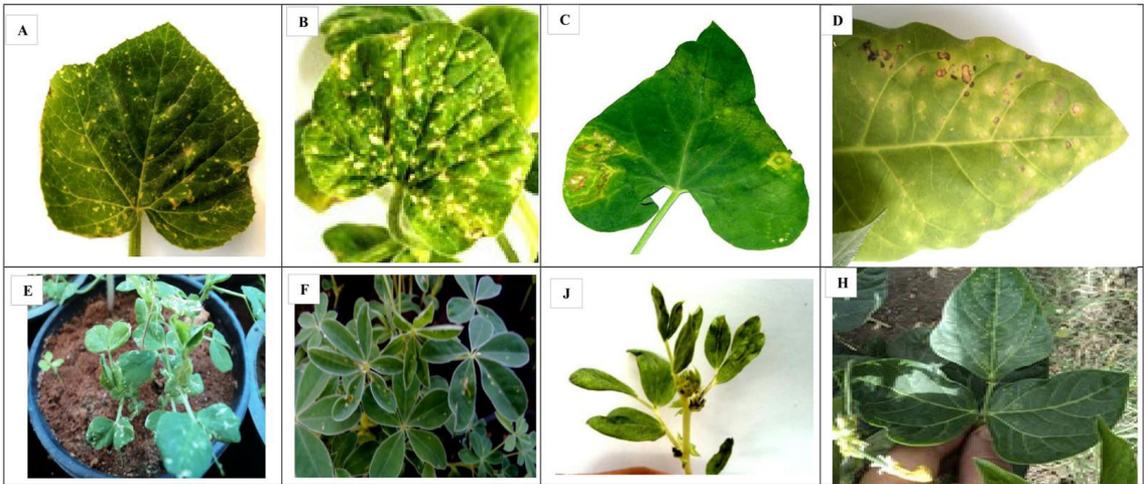


Figure 8. SVNV symptoms: A: *Cucurbita pepo*, pumpkin, squash, have yellow necrotic lesions on the margins that spread on leaf veins. B: kamtalop (*Cucumis melo*) showing systemic necrotic lesions. C: *C. acutum*, the wild buckwheat leaf showing chlorotic and necrotic lesions. D: *Nicotiana benthamiana*, showing Local lesion and Systemic infection. E: Pea *Pisum sativum* necrotic lesions. F: SVNV lesions have yellow margins on *Lupinus luteus* that spread on leaf veins. J: Faba bean showing chlorotic and necrotic lesions. H: Local lesions caused by SVNV on mung bean.

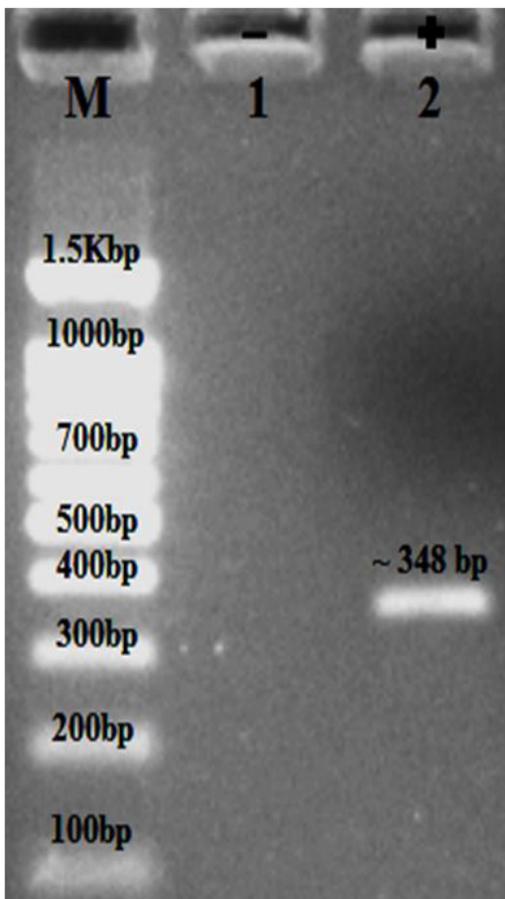


Figure 9. 2% agarose gel electrophoresis for RT-PCR detection of Soybean vein necrosis virus (SVNV) using primers SVNV-NP. M, DNA ladder; lane1, negative control (-); lane 2, SVNV-infected sample (+).

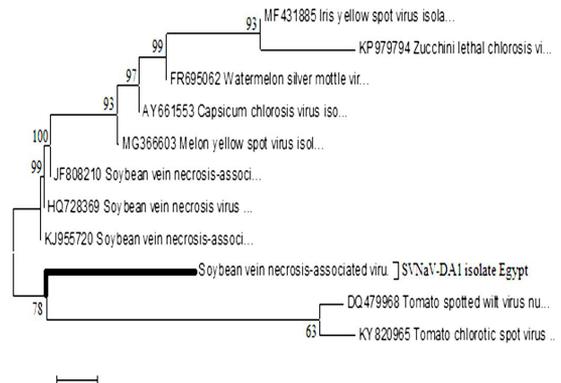


Figure 10. Unrooted phylogenetic tree generated from nucleotide sequences of NP gene for SVNV was alignment compared with other *Tospovirus* available in the GenBank database. The dendrogram was constructed by the Neighborjoining method of bootstrapped 1,000 times. Bootstrap scores are shown at major nodes.

The present results indicated that, *C. acutum*, *I. hederacea*, *C. moschata*, *L. aegyptiaca*, *G. max*, *L. purpureus*, *T. alexandrinum*, *M. intertexta*, *V. unguiculata* and *V. radiata* are hosts for *F. schultzei*, *F. fusca* and *N. variabilis* in the field and a source of SVNV. However, *T. tabaci*, *F. occidentalis*, *F. tritici*, *S. dorsalis*, *T. palmi*, *M. sjostedti*, *A. sudanensis* and *A. mexicanus* may not play a role as vectors of SVNV.

3.4. Transmission efficiency of different thrips species

Out of 10 tested thrips species only the following four species; *F. tritici*, *F. schultzei*, *F. fusca*, and *N. variabilis* are able to acquire and transmit SVNV at an infection rate of 2.0%, 4.3%, 4.0% & 68.0% respectively from the virus source soy bean seedling under greenhouse conditions (Table 3).

Table 2. Natural Incidence of viruliferous thrips carrying (SVNV) among collected individuals from soybean and associated wild plants in the field (Giza, 2017).

Host plant Scientific Name	Common Name	Thrips species	*No.I/**No.T	***%
Amaranthaceae				
1- <i>Amaranthus viridis</i>	Green pigweed	<i>T.tabaci</i> & <i>F. occidentalis</i>	0/20	0
2- <i>Amaranthus sylvestris</i> .	Pigweed	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F. tritici</i> , <i>F. schultzei</i> , <i>T. palmi</i> & <i>F. Fusca</i>	0/60	0
3- <i>Beta vulgaris</i>	Wild beet	<i>T. tabaci</i> , <i>F. occidentalis</i>	0/20	0
4- <i>Spinacia oleracea</i>	Spinach	<i>F. occidentalis</i> & <i>T.tabaci</i>	0/20	0
Apiaceae				
5- <i>Ammi majus</i>	Bishop's weed	<i>T. tabaci</i> & <i>F. occidentalis</i>	0/20	0
Apocynaceae				
6- <i>Cynanchum acutum</i>	Climbing vine swallowworts	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F. tritici</i> , <i>F. schultzei</i> <i>N. variabilis</i>	0/40 2/10	0 20
7- <i>Calendula arvensis</i>	Field marigold	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F. tritici</i> & <i>N. variabilis</i>	0/40	0
8- <i>Cichrium pumilum</i>	Chickory	<i>T. tabaci</i> , <i>F. occidentalis</i> & <i>F. fosca</i>	0/30	0
9- <i>Senecio vulgaris</i>	Groundsel, old-man-in-the-Spring	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F. tritici</i> , <i>F. schultzei</i> , <i>F. fusca</i> , <i>C. phaseoli</i> , <i>T. palmi</i> & <i>M. sjostedti</i>	0/80	0
10- <i>Silybum marianum</i>	Milk thistle,	<i>T. tabaci</i> & <i>F. occidentalis</i>	0/20	0
11- <i>Sonchus oleraceus</i> L.	Sowtistle	<i>F. occidentalis</i>	0/9	0
Brassicaceae				
12- <i>Capsella bursa-pastoris</i>	Shepherd's purse	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F. tritici</i> , <i>C. graminicola</i> , <i>C. sudanensis</i> , <i>N. variabilis</i> , <i>T. palmi</i> & <i>M. sjostedti</i>	0/80	0
13- <i>Sisymbrium irio</i>	London rocket	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>T. palmi</i> , <i>M. sjostedti</i> & <i>A. sudanensis</i>	0/50	0
14- <i>Stellaria media</i>	Common chickweed	<i>T. tabaci</i> , <i>F. occidentalis</i>	0/20	0
Chenopodiaceae				
15- <i>Chenopodium album</i>	Goosefoot .pigweed. lambquarters	<i>F. occidentalis</i> , <i>T. tabaci</i> & <i>T. palmi</i>	0/30	0
16- <i>Chenopodium amaranticolor</i>	Purple Goosefoot / Giant Lambquarters	<i>F. occidentalis</i>	0/10	0
17- <i>Chenopodium ambrosioides</i>	Wormseed, Jesuit's tea, Mexican-tea	<i>T.tabaci</i> & <i>F. occidentalis</i>	0/20	0
18- <i>Chenopodium murale</i>	Nettle-leaved Goosefoot	<i>T. tabaci</i>	0/10	0
Convolvulaceae				
20 - <i>Convolvulus arvensis</i>	Field bindweed	<i>T. tabaci</i> , <i>F. occidentalis</i> & <i>F.fusca</i>	0/30	0
21- <i>Ipomoea hederacea</i> Jacq	Ivy-leaved morning glory	<i>T. tabaci</i> , <i>F. occidentalis</i> <i>N. variabilis</i> <i>F.fusca</i>	0/20 2/15 1/11	0 12.5 9
22- <i>Ipomoea tricolor</i>	Morning Glory, Granny vine	<i>F. occidentalis</i>	0/10	0
Cucurbitaceae				
23- <i>Cucurbita moschata</i>	Pumpkins	<i>N. variabilis</i>	1/10	10

*I/T = No. of Infected plants. **No. of tested ones at least 10 individuals from each species were tested. ***% = Percentage of infection.

Table 2. Continued...

Host plant Scientific Name	Common Name	Thrips species	*No.I/**No.T	***%
		<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F.fusca</i> , <i>T.palmi</i> & <i>F. schultzei</i>	0/50	0
24- <i>Luffa aegyptiaca</i>	Sponge gourd	<i>N. variabilis</i>	1/10	10
		<i>T. tabaci</i> , <i>F. occidentalis</i> & <i>M. sjostedti</i>	0/30	0
Cyperaceae				
25- <i>Cyperus rotundus</i>	Purple nut sedge, red nut sedge	<i>T. tabaci</i> , <i>F. occidentalis</i> & <i>F. tritici</i>	0/30	0
Leguminaceae				
26- <i>Archis hypogaeal</i>	Groundnut	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>M. sjostedti</i> , <i>F.fusca</i>	0/40	0
27- <i>Glycine max</i>	Soybean	<i>N. variabilis</i>	8/15	53
		<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>T.palmi</i>	0/20	0
		<i>M. sjostedti</i>	1/15	6.7
		<i>F.fusca</i>	2/17	11.7
		<i>F. schultzei</i>	1/20	5
28- <i>Lablab purpureus</i>	Lablab bean, Egyptian kidney bean	<i>N. variabilis</i>	1/20	5
		<i>F. occidentalis</i> , <i>F.fusca</i> , <i>T.palmi</i> , <i>F. schultzei</i> & <i>T. tabaci</i>	0/50	0
29- <i>Trifolium alexandrinum</i>	Egyptian clover	<i>N. variabilis</i>	1/10	10
		<i>F.fusca</i>	1/12	8.3
		<i>F. schultzei</i>	1/13	7.8
		<i>T. tabaci</i> , <i>T.palmi</i> , <i>M. sjostedti</i> & <i>F. occidentalis</i>	0/40	0
30- <i>Medicago intertexta</i>	Hedgehog medick, Calvary clover	<i>T. tabaci</i> , <i>C. phaseoli</i> & <i>M. sjostedti</i>	0/30	0
		<i>N. variabilis</i>	1/10	10
31- <i>Melilotus indicu</i>	Sweet-clover, sour clover	<i>T. tabaci</i> , <i>F. tritici</i> , <i>C. phaseoli</i> , <i>N. variabilis</i> & <i>S. dorsallis</i>	0/50	0
32- <i>Vicia sativa</i>	Common vetch, garden vetch	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>C. graminicola</i> , <i>N. variabilis</i> , <i>M. sjostedti</i>	0/50	0
33- <i>Vigna unguiculata</i>	Cowpea	<i>N. variabilis</i>	7/10	70
		<i>F. occidentalis</i> , <i>T. tabaci</i> , <i>M. sjostedti</i> , <i>F.fusca</i> , <i>T.palmi</i>	0/50	0
		<i>F. schultzei</i>	1/10	10
34- <i>Vigna radiate</i>	Mung bean	<i>N. variabilis</i>	4/20	20
		<i>F. occidentalis</i> , <i>M. sjostedti</i> & <i>T. tabaci</i>	0/30	0
Malvaceae				
35- <i>Malva parviflora</i>	Cheeseweed mallow, Egyptian mallow	<i>T. tabaci</i> & <i>F. occidentalis</i>	0/20	0
Portulacaceae				
36- <i>Portulaca oleraceae</i>	Common purslane	<i>T. tabaci</i>	0/10	0
37- <i>Portulica afra</i>	Elephant plant, small leaf jade	<i>T. tabaci</i>	0/10	0
Polygonaceae				

*I/T = No. of Infected plants. **No. of tested ones at least 10 individuals from each species were tested. ***% = Percentage of infection.

Table 2. Continued...

Host plant Scientific Name	Common Name	Thrips species	*No.I/**No.T	***%
38- <i>Emex spinous</i>	Devil's thorn or lesser jack	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F. tritici</i> & <i>T. tabaci</i>	0/40	0
<i>Polygonum convolvulus</i>	Bindweed, black or Wild Buckwheat	<i>F. fusca</i> ,	1/10	10
		<i>T. tabaci</i> , <i>F. occidentalis</i> & <i>F. schultzei</i>	0/30	
Poaceae				
39- <i>Avena. Fatua</i>	Common wild oat	<i>T. tabaci</i> , <i>F. occidentalis</i> & <i>F. tritici</i>	0/30	0
40- <i>Cynodon dactylon</i>	Bermuda grass	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F. tritici</i> , <i>F. schultzei</i> & <i>F. Fusca</i>	0/50	0
41- <i>Setaria verticillata</i>	Hooked bristlegrass	<i>T. tabaci</i> , <i>F. occidentalis</i> , <i>F. tritici</i> , <i>T. palmi</i> , <i>A. sudanensis</i> & <i>A. mexicanus</i>	0/60	0
Solanaceae				
42- <i>Datura inoxia</i>	Moonflowers, jimsonweed	<i>T. tabaci</i>	0/10	0
43- <i>Datura metel</i>	Downy thorn-apple, metel	<i>F. occidentalis</i>	0/10	0
44- <i>Datura stramonium</i>	Jamestown weed, Jimsonweed, datura,	<i>T. tabaci</i>	0/10	0
45- <i>Solanum nigrum</i>	Black nightshade	<i>T. tabaci</i> & <i>F. occidentalis</i>	0/20	0
Urticaceae				
46- <i>Urtica urens</i>	Dog nettle or burning nettle	<i>T. tabaci</i> & <i>F. occidentalis</i>	0/20	0

*I/T = No. of Infected plants. **No. of testedones at least 10 individuale from each species were tested. ***%= Percentage of infection.

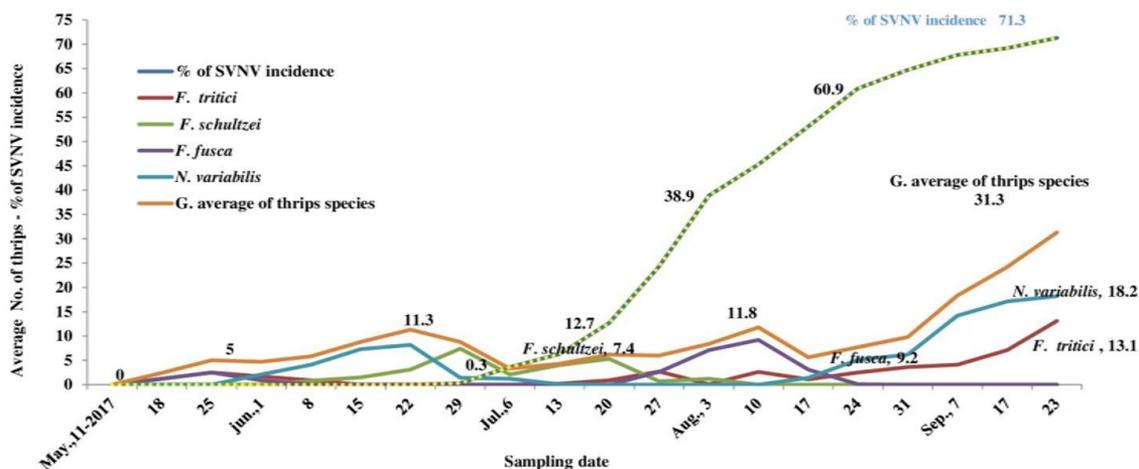


Figure 11. Natural incidence of SVNV in relation to abundance of thrips species associated with soybean plants in the field, Giza region, Egypt.

3.5. Detection of SVNV in thrips individuals naturally collected from soybean field

Results obtained from Table 4 showed that thrips individuals naturally collected from soybean fields gave 23.6% highly positive reaction in *N. variabilis*, while very low reaction with (*F. tritici*, *F. shultize* and *F. fusca*) ranged from 1.6% - 1.9% respectively. These results indicated that,

soybean thrips was the most efficient vector of SVNV. In conclusion all of *F. tritici*, *F. schultzei*, *F. fusca*, and *N. variabilis* are vectors of SVNV.

3.6. Natural incidence of (SVNV)

Weekly observations were made, to determine the level of the natural incidence of SVNV in the field. The rate

Table 3. Efficiency of different thrips species in transmitting soybean vein necrosis virus (SVNV).

Thrips species	I/T*	%	ELISA /OD**
<i>T. tabaci</i>	0/50	0.0	-(0.125-0.177)
<i>F. occidentalis</i>	0/50	0.0	-(0.120-0.165)
<i>F. fusca</i>	1/50	2.0	+(1.181-0.398)
<i>F. schultzei</i>	2/47	4.3	+(1.123-0.746)
<i>F. tritici</i>	2/50	4.0	+(1.130-0.512)
<i>C. phaseoli</i>	0/45	0.0	-(0.434-0.466)
<i>N. variabilis</i>	34/50	68.0	+(0.786-1.634)
<i>S. dorsallis</i>	0/48	0.0	-(0.608-0.129)
<i>M. sjostedti</i>	0/40	0.0	-(0.130-0.471)
<i>T. palmi</i>	0/45	0.0	-(0.056-0.102)

Absorbance values were recorded after 30 min. Mean absorbance value calculated from four wells. *(+) Positive reaction values 0.2 to 0.7. (Hicks and Smitamana, 1993). **(-) Negative reaction Samples with A 405 values less than 0.1 assumed to be free of SVNV.

Table 4. Detection of SVNV in adult thrips collected from three soybean fields in Giza Egypt using ELISA test.

Thrips species	No. / T.	No. / I.	OD	%
<i>T. tabaci</i>	27	0	(0.061) -	0
<i>F. occidentalis</i>	23	0	(0.063)-	0
<i>F. tritici</i>	60	1	(1.473)+	1.6
<i>F. schultzei</i>	58	1	(1.413)+	1.7
<i>F. fusca</i>	53	1	(1.504)+	1.9
<i>T. palmi</i>	45	0	(0.034)-	0
<i>C. phaseoli</i>	30	0	(0.059)-	0
<i>N. variabilis</i>	55	13	(1.761)+	23.6
<i>S. dorsallis</i>	20	0	(0.062)-	0
<i>M. sjostedti</i>	21	0	(0.058)-	0

An average of A405 nm values of the absorbance values obtained for positive and negative controls were 1.466 and 0.042, respectively.

of infection showed 12.7, 38.4, 60.9 to reached 71.3% by the end of the season. Thrips species appeared 7 weeks before symptoms which suggest the role of thrips vectors in virus dispersal Figure 11.

In conclusion, few thrips invading soybean crops are enough to transmit a high rate of virus infection within the crop. Furthermore, several vector species are also abundant on weed plant species, which are the major reservoirs of soybean viruses.

4. Discussions

Ten thrips species were associated with soybean crop and 14 thrips species occurred on weeds at Giza region, Egypt. Similar results were obtained by Irwin and Yeargan (1980), recorded ten species of thrips on soybean crops. *T. tabaci* was the most abundant species found in soybean field, this result goes parallel with Viteri et al. (2010), they reported that Soybean thrips, *N. variabilis* (Beach) was one of the most abundant thrips species found in soybean fields.

Other species that are present include eastern flower thrips, *F. tritici* and tobacco thrips, *F. fusca* (Irwin et al., 1979). While others reported that thrips associated with soybean include *C. impurus*, *C. phaseoli*, *F. insularis*, *F. occidentalis*, *F. schultzei*, *S. dorsalis*, *S. occipitales*, *T. sjostedti*, *T. palmi* and *T. tabaci* Viteri et al. (2010).

On the other hand in Puerto Rico, *T. palmi* was the most common species of thrips that was found on soybean crops, Medina (2003) and Viteri et al. (2010). While *F. fusca*, *F. tritici*, and *N. variabilis* were common species in Brazil (Monteiro, 2001).

On another way the different three species; *C. phaseoli*, *F. gossypiana* and *E. americanus* were recorded attacking soybean crops. Similar result obtained by Abd El-Wahab (2016) found that, *T. tabaci* and *F. occidentalis* are the most abundant species in soybean fields in Egypt, in contrast, Reisig et al. (2012), reported that the most abundant thrips species was *F. fusca*. While, *N. variabilis*, was more common later in the season. Also, *N. variabilis* was the most abundant species in Missouri Irwin et al. (1979). However, Burriss et al. (2002), observed that the

S. variabilis, *F. fusca* and *F. tritici* are the most abundant species in soybean fields in the mid southern of the U.S. On the other hand *C. phaseoli* has been reported as an important pest of soybean in Mexico, United States and in Central and South America, Irwin et al. (1979); Mound and Marullo (1996).

SVNV infection causes similar symptoms on different legume species including cowpea, mung bean, medicago and pea. The symptoms include; chlorotic spots on leaves which turned necrotic, this result agree with that obtained by Sikora et al. (2018). Present results showing systemic infection of SVNV in buckwheat and clear local infection with possible systemic infection on melon were shown, also our result suggested that alternative host crops may harbor SVNV and may be a source of the virus inoculums for soybean crops this goes on line with Irizarry et al. (2018).

21 plant species were susceptible to infection with SVNV, our finding go parallel with that obtained by Zhou and Tzanetakis (2013). Ten plant species that were sources of SVNV. The symptoms on these plant species differed, resulting in asymptomatic response, local lesions, and systemic infection. Cowpea and all three *Nicotiana* species had a substantially greater success rate with mechanical inoculation than the other positive species. This result goes online with Zhou and Tzanetakis (2013). Common bean did not infect with SVNV parallel to that obtained by Oliveira et al. (2012). previous studies showed that out of 25 tested plants, only three were found to be hosts of SVNV Costa and Carvalho (1961); Salazar et al. (1982); Han et al. (2019); Zhou and Tzanetakis (2013); Irizarry (2016).

Similarly, using the same technique (Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Zhou and Tzanetakis (2013) confirmed the presence of SVNV in all SVNV material in contrast with negative controls by SVNV-NP primer set. The sensitivity of the RT-PCR was 4 and 400 pg of RNA after 30 or 20 PCR cycles. These results suggest that SVNV-NP F/R has both universality and sensitivity, providing a useful, efficient tool for virus detection. In another study, Groves et al. (2016), they found that the three plants from the 48 sampled were determined by RT-PCR to be infected with SVNV and the results were confirmed the presence of SVNV in the AG2433 seed lot examined.

In the same case, Dewey et al. (1996); Kormelink et al. (1992) and Pappu et al. (2006), they reported that the NP gene has commonly been used in Tospovirus diversity studies and was chosen to evaluate the SVNV population structure. In addition, Khatabi et al. (2012), found the nucleotide sequences of 37 isolates collected from AR, DE, IL, KS, MD, MS, and TN were determined and deposited in Gen Bank as accession numbers HQ728355-84, HQ728386, and JQ946869-74. Together with 11 isolates from KY and TN (accession numbers JF808207-13, JF8082115, and JQ277450-52), 48 isolates were used to analyze the population structure of the virus. Pairwise comparisons revealed identities of 98 to 100% at the nucleotide level. Moreover, the SVNV was fully sequenced, which revealed several typical and atypical characteristics for the members of the genus *Tospovirus*. All the SVNV segments have the highly conserved *Tospovirus* 50 terminal sequence

(AGAGCA1-6) predicted to be crucial as replication and transcription signals, Sherwood et al. (2000).

The role of weeds in the epidemiology and natural incidence of SVNV in soybean field in Egypt, Giza region showed that, Bindweed, Buckwheat, Ivy- morning glory and Egyptian clover, were harbored different thrips species that able to transmit SVNV and in the fact these plant species are natural hosts of SVNV. Similarly results obtained with another weed (kudzu) a weed species in the Fabaceae as an asymptomatic, systemic host of SVNV it is possible that this plant species may serve as the major reservoir for SVNV Irizarry et al. (2018). Also these weed providing overwintering or early season population growth habitats for viruliferous thrips prior to moving to soybean.

Mechanical transmission for SVNV have a low success rate while thrips transmission has almost a 100% success rate, indicating the importance of thrips as vectors Khatabi et al. (2012); Zhou and Tzanetakis (2013).

N. variabilis was the most efficient vector of SVNV followed by other three species *F. fusca*, *F. schultzei* and *F. tritici* these results goes online with Zhou et al. (2019). SVNV is transmitted also by, tobacco thrips and eastern flower thrips Keough et al. (2016). While in Carolina and Virginia *F. fusca* and *N. variabilis* were the predominant species during the first 5 week after planting, while *F. occidentalis* and *F. tritici* were also collected, Reising et al. (2012).

While several investigations reported that more than ten species in the genera *Thrips*, *Frankliniella* and *Scirtothrips* can transmit over 15 virus species. *F. occidentalis* is one of the most efficient tospovirus vectors transmitting TSWV, GRSV, INSV, TCSV CSNV (Nagata et al., 2004). Similar results with other tospoviruses showed that, *T. tabaci* is the most efficient vector of IYSV in Egypt, Abd El- Wahab, (2004). Some *Tospoviruses* can be transmitted by multiple species of thrips, such as TSWV, which can be transmitted by 7 different species of thrips, Whitfield et al. (2005).

The natural incidence of SVNV symptoms at soybean crops, Giza region, Egypt started Seven weeks after thrips appeared in mid- to late June, goes online with Chitturi et al. (2018); Sikora et al. (2018), they reported that arrival of thrips to soybean field couple of weeks earlier SVNV symptoms appear, also the three species of thrips; *S. variabilis*, *F. tritici* and *F. fusca* migrate each spring from south-central states and even from Mexico. Early in the growing season soybean thrips colonize alfalfa and other broadleaf plant species before they immigrate to soybean fields, where they reproduce throughout the growing season. In contrast, flower thrips colonize a wider variety of plants, including corn and some grasses, before colonizing soybean fields. Hence, thrips could acquire and bring SVNV each spring or could acquire it from local weeds and move it into neighboring soybean fields.

In Egypt SVNV similar to several Tospovirus species; (INSV), (IYSV) and (TSWV), have been reported in Giza region to affected several hosts, El-Shazly et al. (2006). Tospovirus infections are typically the result of primary spread, from nearby alternative hosts into the susceptible crops, Garcia et al. (2000) and Culbreath et al. (2003). Secondary spread is more limited, which is the movement of viruliferous thrips within the same field Garcia et al.

(2000). Alternative hosts, such as weeds, can also contribute to the spread of SVNV.

Tospoviruses and thrips vectors may infect winter weed hosts, which would increase the virus inoculum that could potentially be spread to more important field crops, Chellemi et al. (1994). The relation between the vectors, viruses and hosts are complicated. Weed hosts, play a role in virus incidence in soybean field, similar results of these plant species, it is the plants that can also act as reproductive hosts for thrips vectors that are the most important for virus spread Morsello et al. (2008) and Whitfield et al. (2005).

The present findings show that soybean thrips, currently the main vector of SVNV has the highest abundance later in the growing season compared to other species of thrips, and that increase coincides with the timing of SVNV symptoms found in soybean fields.

Management of SVNV, as with many viral diseases, may include reduction of inoculum sources and monitoring of insect vectors.

5. Conclusion

Understanding the relations between SVNV and thrips vectors in the field, is fundamental in avoiding the damage to soybean by the spread of SVNV and disease.

6. Future Work

In the future, it would be necessary to complete important aspects of SVNV epidemiology that to cover the stats of the virus infection over all Egyptian governorates. Second, is to determine the effect of the virus infection on the yield crop quality and quantity. Then design a map of Tospoviruses affecting soybean and other crops.

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