Fayoum Journal of Agricultural Research and Development
ISSN:1110- 7790
On Line ISSN:2805-2528

Survey of viruses infecting Solanaceous plants and characterization of *Tomato brown rugose fruit virus* (ToBRFV) infecting pepper in Egypt

Khalifa, M.A.A[1,3].; A.M. El- Shazly[1]; M.A.S. El- Kady[2] and A.M. Al Naggar[1]

[1]Agric. Botany Dept., Fac. of Agric. Al- Azhar Univ, Naser City, Cairo 11884, Egypt.
[2]Virus and Phytoplasma Res. Dept. Plant Pathology Res. Inst. Agric. Res. Centre (ARC), Giza, Egypt.
[3]United Eastern Company for Development Egyptian Stockholding Co.

ABSTRACT:

This study investigated the incidence and occurrence of virus-like symptoms in solanaceous plants, peppers (*Capsicum* spp.), potatoes (*Solanum tuberosum*) and tomatoes (*Solanum lycopersicum*) in Egypt. In this study, a total of 640 samples collected from different Governorates in Egypt. 640 samples were tested using DAS-ELISA, with 86.87% of samples showing positive reactions. It was also, revealed that *Potato virus Y* (PVY) was the most prevalent virus (16.41%), followed by *Potato leaf roll virus* (PLRV) (11.87%), *Alfalfa mosaic virus* (AMV) and *Pepper mild mottle virus* (PepMMoV) both (10.94%), However, *Tomato spotted wilt virus* (TSWV) was detected at low frequency (4.22%). Some samples were found coinfected with two or three different viruses (i. e. PVY+ ToMV, TMV+ PepMMoV+ TYLCV). Results demonstrated that potato was the most infected plans (37.65%) followed by tomato (27.97%) and then by pepper (21.25%). Potato was the most infected plant (37.65%), followed by tomato (27.97%), and then pepper (21.25%). Collected pepper leaf and fruit with negative results in all tested antisera were selected for identification the virus isolates which suggestive to be *Tomato brown rugose fruit virus* (ToBRFV) on the basis of symptomatology, host range, modes of transmission and particle morphology. RT-PCR was used to confirm the identity of the virus isolate. Among twenty-five plants species and cultivars, the virus isolate reacted positively with fourteen plants species. The virus was transmitted mechanically and by seed 1:2.5%. Examination of leaf tissue extracts of infected pepper plants by electron microscope revealed rod–shaped particles of 261-348 nm in length, in the range of the *Tobamovirus* group. Concerning varietal susceptibility, Helsinki and Mycelia pepper cultivars were highly susceptible (70%) followed by California Wonder (50%) and Vita Z 461 (40%). On the other hand, Vita Z Dragon cultivar was the lowest susceptible one (30%). RT-PCR analysis indicated that the virus isolate is ToBRFV.

KAY WORDS: *Solanaceae*; ToBRFV; Host rang; Electron microscope; RT- PCR.

Received:5 /11/ 2023 - Accepted: 2 /1./ 2023 - Published:18/2/2024

1- INTRODUCTION:

The family *Solanaceae* comprises approximately 98 genera and about 3000 species, these are distributed around the world in temperate to subtropical regions, the *Solanaceae* are also the third most important plant- taxonomy economically and the most valuable in crops species in terms of agricultural utility, as it includes the peppers (*Capsicum* spp.), potatoes (*Solanum tuberosum*) and tomatoes (*S. lycopersicum*). *Solanaceae* vegetable crops are important source of vitamin C, A, E, thiamine, niacin, pyridoxin, folacin, minerals and dietary fibers which play a significant role in human nutrition and helps to cope with mal nutrition **(FAO,2022& Devi and Nagar, 2017).**

The estimated area of cultivated Solanaceous crops in Egypt reached about 1.062.411 feddans which represent 57% of the vegetable crops grown in Egypt, amounting to 1.867.875 feddans, Pepper crop one of the main vegetable crops for local market and exportation in Egypt, and it had considered a major greenhouse yield cultivated during different seasons to meet increasing demand in Egypt. The cultivation area reached up to 92.327 feddans which yielded about 870.143 tons (Agriculture Economic Report, 2021-2022, Min. of Agric., Egypt).

Plant viruses are minute parasitic organisms which infect cells, altering their chemistry and causing a wide range of symptoms inducing discoloration, distortion and loss of vigor and yield. Most plants viruses are transmitted by vector that feeds on the plant or they are interduce during cultural operations (e.g. graffitiing, pruning, insects, pollen and seed) (**Rodríguez-Verástegui *et al.*, 2022).**

On the other hands, Solanaceous plant are affected by a wide range of viruses and virus-like disease. Some of these, *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Pepper mild mottle virus* (PepMMoV), *Pepper mottle virus* (PepMoV), *Potato leaf roll virus* (PLRV), *Potato virus X* (PVX), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Tomato spotted wilt virus* (TSWV) and *Tomato yellow leaf curl virus* (TYLCV), but there are also many viruses that are not reported here which may cause damage should they arrive (**Hančinský *et al.*, 2020).**

Viral diseases in field-grown pepper plants are the major concern for the pepper growers and the processing industry. Pepper growing areas have been affected by several viruses including *Cucumber mosaic virus* (CMV), *Pepper mottle virus* (PepMoV), *Pepper veinal mottle virus*(PVMV), *Pepper mild mottle virus*(PMMoV), *Tobacco etch virus* (TEV), *Tomato spotted wilt virus* (TSWV), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY) **(Rodriguez-Alvarado *et al.*, 2002).**

Based on the results it can be concluded that, mixed infection types were found in the samples with two or more viruses. **(Kukhaleishvili *et al.,* 2018; Li *et al.,* 2021 and Chan *et al.*, 2022).**

Tomato brown rugose fruit virus (ToBRFV) in the genus *Tobamovirus* is a newly virus identified infected tomato and pepper, has made the epidemic in wide spread areas. ToBRFV was first detected in the Occupied territories in 2014. Since then, the virus has been detected in many more countries around the world such as, Europe, North America, Asia, Turkey etc. ToBRFV spreading widely within greenhouses grown tomato as well as in open field **(Salem *et al.*, 2016)**.

The present study aimed to:

1- Survey of the most relevant viruses infecting Solanaceous plants.

2- Isolate and identify ToBRFV in the infected Pepper plants that gave negative results with all the antisera used in the survey by traditional methods and by RT-PCR (molecular biology technique).

2.MATERIAL AND METHODS:

1- Survey of the most prevalent viruses naturally infecting Solanaceous plants.

1-1- Disease incidence and frequency of virus(es):

Six hundred and forty samples of naturally infected Solanaceous plants (potato, tomato and pepper), exhibiting virus-like symptoms were collected from different fields of Fayoum, Bani-Sweif, Beheira and Giza Governorates, during 2016 to 2017 growing season. The samples were sealed and labelled separately in small bags (one sample/bag) and stored in laboratory at 4^{o}c prior serological analysis.

1-2- Serological detection:

Leaf samples were tested by DAS-ELISA technique according to the method described by **(Hampton *et al.*, 1992)** using the available antisera specific to AMV, CMV, PepMoV, PepMMoV, PVY, PVX, PLRV, TMV, ToMV, TYLCV and TSWV, at concentration of 1:1000. These antisera were provided by Serological lab., Virus and Phytoplasma Research Department, Plant Pathology Res. Institute, ARC. The test was done in polystyrine plates. for recognizing and determining the disease incidence as well as the frequency of naturally infected viruses collected from different Governorates in Egypt.

2- Isolation and identification of the virus isolate:

2-1- Isolation and propagation of the virus isolate:

Collected pepper leaf and fruit samples which gave negative results in all the tested antisera and revealed the characteristic symptoms suggestive to be *Tomato brown rugose fruit virus*(ToBRFV) used for isolation and identification of the virus isolate. The symptoms consisted of mottle or mosaic, discoloration of young leaves ,vein clearing, chlorotic or yellowing and necrotic spots on the leaves (fig.1) , zonatic spots and distortion of the fruits (fig.2), death of growing tips and cessation of growth on one side of the plant. Mechanical inoculation was carried out in the greenhouse to inoculate the healthy tested plants (*Chenopodium quinoa., Ch. amaranticolor*, *Lycopersicon esculentum* cv lugein and *Capsicum annuum* cv .California Wonder) as described by **Noordam (1973)**. The virus isolate was purified biologically by three passages of single- lesion isolation **(Kuhn,1964)** using *C. quinoa* as a local lesion host plant. Finally a single local lesion was propagated in the systemic host (*N. tabacum* cv White Burley) which was used as a source of the virus isolate.

2-2- Identification of the virus isolate:

2-2-1- Host range and symptomatology:

A total of 25 species representing six families were used for a host reaction study. Ten plants for each plant species and cultivars were mechanically inoculated. An equal number of healthy seedlings of each species were also used as controls. Inoculated plants were maintained in the greenhouse. Symptoms were monitored for 3-4 weeks post inoculation. All plants species analyzed in at least two independent inoculation experiments with the virus isolate. Symptomless plants were checked for latent infection by back inoculation onto the test plant (*Ch. quinoa*).

2-2-2- Modes of transmission:

2-2-2-1- Mechanical transmission:

Seedling from each of healthy test plants and other hosts used for host range were mechanically inoculated as mentioned before with sap of diseased leaves. Inoculated seedlings were kept under observation in the greenhouse at about ±26°c for at least 30 days, periodically sprayed with insecticides (Imidacloprid 35% using a concentration 35ml/100L water) to prevent contamination through insect transmission.

The percentage of transmission was collected as: $\frac{\text{No.of infected plants} \times 100}{\text{No.of inoculated ones}}$.

2-2-2-2- Seed transmission:

Certified pepper seeds of three cultivars (California Wonder, Vita Z Dragon and Helsinki) tested were supplied by Field Crop Res., Institute, ARC. Seeds were sown in 30 cm-diameter clay pots containing sterilized soil and kept in an insect-proof greenhouse. Half of the resulted seedlings were mechanically inoculated as described above with the virus isolate. The remainder of the seedlings were kept without inoculations to serve as control. Virus inoculated plants showing typical symptoms of the virus isolate as well as healthy ones were kept till maturity. Subsamples composed of 200 seeds from each cultivar harvested from neither infected nor healthy plants separately were sown in pots (5seed/pot) and kept in insect-proof greenhouse. Emerged seedlings were observed for symptoms development and percentage of seed transmission was determined.

2-3- Morphology of virus particles:

Quick–dip preparation method of **Brands (1957),** called cut-squeeze method described sub-sequently by **Bos (1970)** was used for examination of the virus particles by electron microscope.

A leaf of a pepper seedling infected with the virus isolate with distinct symptoms was intensively chopped and squeezed between clean glass-slides, if necessary, with drops of distilled water, and the resultant sap diluted approximately 1:40 with 2% uranyl acetate (negative stains).

A drop of slightly greenish solution was placed on a carbon-reinforced, Formvar-coated copper grid. One min. later, the excess liquid was removed by touching the grid-edge with a piece of filter paper. The grids were then viewed in Electron Microscope Unit, Faculty of Agriculture, Cairo University (JEOL JEM-1400) at magnification of 20,000.

2-4- Varietal susceptibility:

Response of some pepper cultivars to artificial infection with the virus isolate was carried out in the greenhouse. Ten, young potted seedlings of five tested California Wonder (CW), Vita Z 461 (VZ461), Vita Z Dragon (VZD),Helsinki (He) and Mycelia (M) pepper cultivars were inoculated mechanically with expressed sap from infected pepper leaves (as mentioned before). An equal number of healthy seedlings of the same cultivars and age were left without inoculation to serve as controls. Inoculations were conducted in the greenhouse at average temperatures of ±28°C and were observed for symptoms appearance. About 3-4 weeks after inoculation symptoms and percentage of transmission were recorded .Symptomless plants were checked for the presence of the virus by back inoculation onto the test plant (*Ch. quinoa*).

2-5- Molecular detection and characterization of ToBRFV:

Pepper samples showed symptoms of ToBRFV were examined by RT-PCR using different primers:

1- **General primers:** Total RNAs were extracted from leaves of symptomatic and asymptomatic pepper plants, using an BISOLATE II RNA Plant Kit^ (Bioline, UK). Total RNA served as a template for cDNA synthesis using Reverse Transcriptase cDNA kit (Tetro™ cDNA Synthesis Kit, Bioline, UK). The cDNA syntheses reaction were don in a 4µL 5X RT Buffer, 1µL volume, containing 10 mM of dNTPs, 2µL of R-4718 Primer, 1µL of One Script® Plus RTase, 2µL of Nuclease-free H_2O and 10µL Template RNA.

PCR for detecting the main viruses infecting pepper was performed using the primers listed in Table (2) from 1 to 6. The PCR reaction were done in a 25µL volume, containing 0.4 mM of dNTPs, 0.2µM of each primer, one unit of Taq DNA polymerase, 2.5µL of 10X PCR buffer, 0.75 mM $MgCl_2$, and 2.5µL of cDNA. PCR parameters were 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 58 °C and 1 min at 72 °C, and a final extension step for 7 min at 72 °C. **(Salem *et al.,* 2019).**

2- **Specific primers:** The designed primers were obtained by two different methods:

A. To design the pair of specific oligonucleotides for ToBRFV the whole genome of the most representative species of the *Tobamovirus* genus reported by the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) was considered, including *Tobacco mosaic virus* (TMV, *Virgaviridae*) (NC_001367.1, FR878069.1, HE818443.1 and V01408.1), *Tomato mosaic virus* (ToMV, *Virgaviridae*) (NC_002692.1, KY967227.1, MF002490.1 and AF332868.1), *Tomato mottle mosaic virus* (ToMMV, *Virgaviridae*) (NC_022230.1, FX898034.1, KR824950.1 and KF477193.1); the genomes of two ToBRFV isolates (*Virgaviridae*) were also considered an isolate from Occupied Palestinian Territories TBRFV-IL (KX619418.1), and an isolate from Jordan (KT383474.1). A global alignment of the genomes was carried out using the BioEdit program version 7.0.5.3 **(Hall, 1999)** and the Clustal W algorithm in order to create a matrix and identify variable regions between them. The selected region was used to obtain the oligonucleotides sequences, which were then analyzed in silico using the Oligo Analyzer server version 3.1 (https://www.idtdna.com/calc/Analyzer/Home/Instructions) to predict their physical and chemical properties (Annex 1). The oligonucleotides were synthesized by Integrated DNA Technologies, Inc. (IDT). according to **Rodriguez- Mendosa *et al.* (2019)**

B. Complete genome of ToBRFV isolate 22006291-H (GenBank: MW284987.1 with 6390 bp linear RNA using www.primer3.ut.ee as described by the program manual.

Viral RNA extraction was performed using Accuprep Viral RNA Extraction kit (Bioneer, Daejeon, Korea). The PCRBIO cDNA synthesis kit (PCR Biosystems, London, UK) was employed and the obtained cDNA was subjected to PCR amplification (Plant Pathology Lab, Faculty Agriculture, Fayoum University). The primer sets used for identification of ToBRFV were listed in (Table 2). The primer sets used for whole-genome sequencing were 7–12 (Table 1). Obtained amplicons were run on agarose gels for viral detection and confirmation.

Table 1. Primer pairs served for *Tomato brown rugose fruit virus* (ToBRFV) detection and whole-genome sequencing:

Primer name		Primer sequence	Exp. Size	Reference
1	ToBAMO S1	5′ GGGAATCAGTTTCAAACRCA-3′	560 bp	Menzel *et*
2	ToBAMO AS1	5′ GGGGGGATTCGAACCYCT- 3′		*al.* 2019
3	ToBRFV F1	5′ GTATTTTTGTTTTACAACATATACCAAC-3‘	1300 bp	Salem *et al.*
4	ToBRFV R1	5′ AGTGCGAATGTGATTTAAAACTGTGAA-3′		2019
5	ToBRFV F7	5′ GAAGAAGTCCCGATGTCTGTAAGGCTT-3′	697 bp	Salem *et al.*
6	ToBRFV R7	5′ ATGCAGGTGCAGAGGACCATTGTAAAC-3′		2019
7	ToBRFV F1	5′ ACATCACTACCAAGGACGCA-3′	247 bp	P1
8	ToBRFV R1	5′ GCAAGCCTTACAGACATCGG-3′		
9	ToBRFV F2	5′ TGGTCAGCTTGCAGATTTTG-3′	250 bp	P2
10	ToBRFV R2	5′ AGCTGGCGTCTTCCTTGTAA-3′		
11	ToBRFV F3	5′ GTGCTCAAGGAGTCGGACAA-3′	361 bp	P3
12	ToBRFV R3	5′ TAGCCGTTGCCATGTGGAAT-3′		

3- Results and Discussion:

1- Survey of the most prevalent viruses infecting the solanaceous plants

1-1- Disease incidence and frequency of virus(es):

Six hundred and forty samples of naturally infected plants showing virus-like symptoms consisting of mosaic or mottling, yellowing, ring spot, necrosis, leaf rolling, leaf deformation and leaf curling in leaves in addition to infected fruits of three different solanaceous plants (pepper, potato and tomato) were collected from different fields of Bani-Sweif, Beheira, Fayoum and Giza Governorates, Egypt during 2016/2017 (Figs 1,2,3 and 4).

1-2- Serological detection:

All of the collected samples were tested using direct ELISA, using the available antisera specific for AMV, CMV, PepMoV, PepMMoV, PVY, PVX, *PLRV,* TMV, ToMV, TYLCV and TSWV, at concentration of 1:1000.

Symptomatic and asymptomatic plants were screened by ELISA test for the presence of several different pathogenic viruses potentially present in the area. Results in Table (2) revealed that out of 640 samples tested 556 (86.87%) gave positive reaction against virus infection, while 84 (13.12%) tested were negative by using the available antisera. It was founded that PVY was the most prevalent virus (16.41%), followed by PLRV (11.87%), AMV and PepMMoV both (10.94%), CMV (9.53%), TYLCV (8.75%), PepMoV (7.19%), ToMV (6.87%), PVX (6.72%), TMV (6.56%) and TSWV (4.22%).

Some samples were coinfected with two or three different viruses (i. e. PVY+ ToMV, TMV+ PepMMoV+ TYLCV).

Results demonstrated that potato was the most infected plans (37.65%) followed by tomato (27.97%) and then by pepper (21.25%).

All samples which gave negative results in ELISA tests were tested and characteristic symptoms of ToBRFV were selected for virus identification.

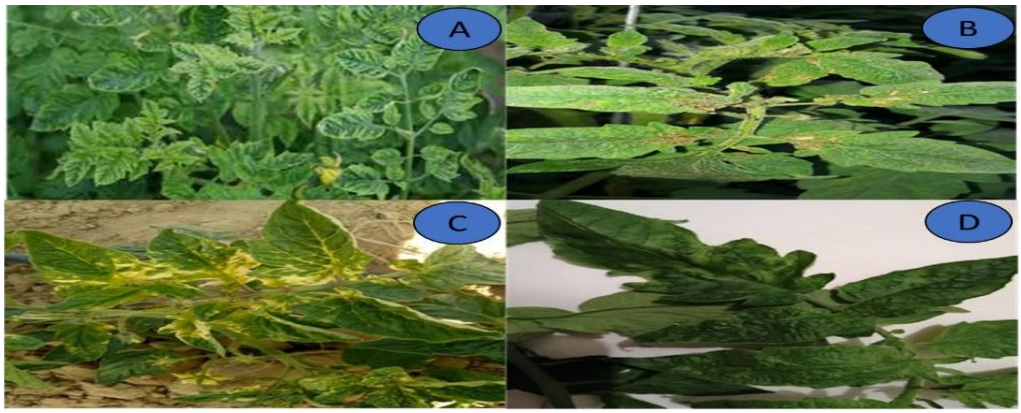

Fig. 1. Naturally infected pepper plants showing: A- blister and leaf deformation, B-mosaic or mottling and leaf curling C- necrosis and D- chlorotic spots.

Fig. 2. Naturally infected pepper fruits showing: A and E healthy fruit B, C, F, G and D, H discoloration, zonatic spots and distortion of the pepper fruit.

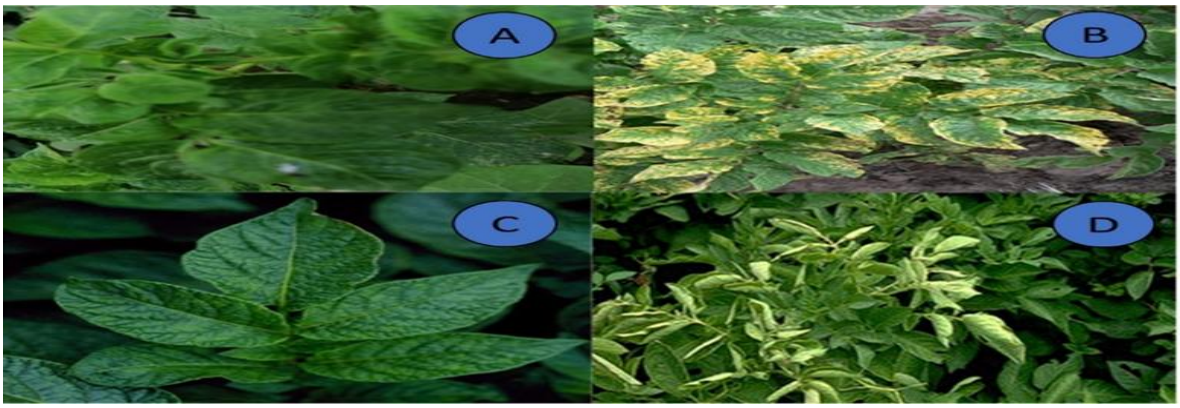

Fig. 3. Naturally infected potato plants showing A- vein banding, B- yellowing, C- systemic mottling or mosaic and D- leaf curling

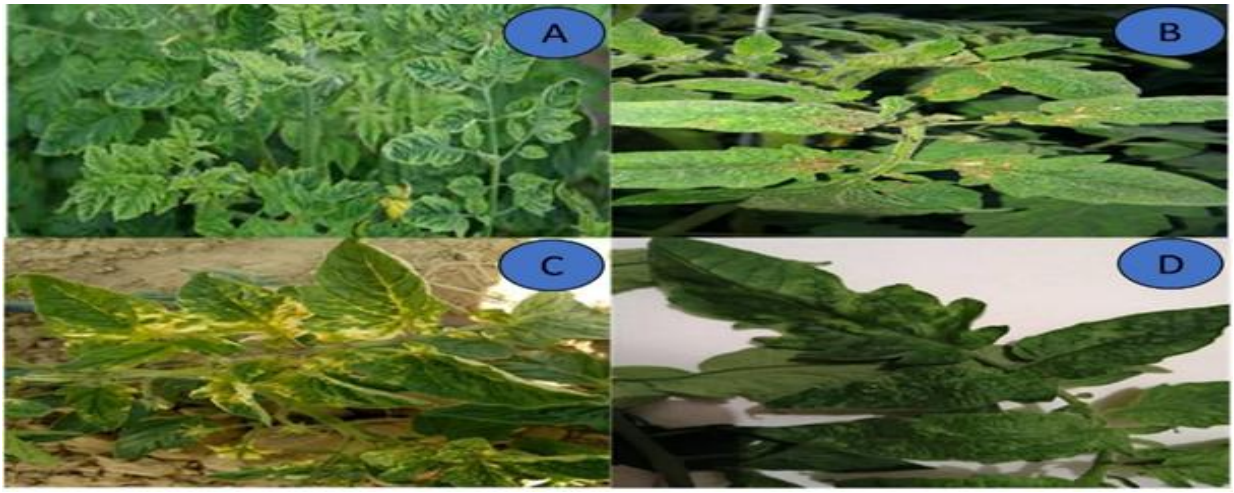

Fig. 4. Naturally infected tomato plants showing A- green Yellowing, vein banding and leaf curling B-Top necrosis, C-Yellow mosaic and D- blister and mottle.

Table 2. Prevalence of virus infections revealed by visual inspection and ELISA tests in three different solanaceous plants (potato, tomato, and pepper) collected from different regions in Egypt during 2016/2017:

Governorat	Crops	N. of tested samples			Virus detected by DAS-ELISA (A405nm)										
		ollecte	Positive	egativ	AMV	CMV	epMo	epMMo	PVY	PVX	PLRV	TMV	ToMV	YLCV	SWV
ani-Swe	Pepper	30	24	6	3	3	4	6	6	1	2	3	0	0	2
	Potato	58	58	0	7	5	2	5	17	6	16	0	6	0	0
	Tomato	52	41	11	5	6	3	5	3	3	1	8	3	14	6
	Total	140	123	17	15	14	9	16	26	10	19	11	9	14	8
Beheira	Pepper	46	37	9	5	3	6	9	7	2	1	5	3	0	2
	Potato	65	63	2	8	7	3	6	16	6	15	0	8	0	0
	Tomato	56	44	12	7	6	3	5	5	3	2	6	2	14	4
	Total	167	144	23	20	16	12	20	28	11	18	11	13	14	6
Fayoum	Pepper	46	37	9	4	4	7	8	6	2	3	3	3	0	2
	Potato	56	54	2	9	4	2	2	13	6	15	0	6	0	0
	Tomato	58	47	11	5	6	3	5	6	2	0	6	2	13	7
	Total	160	138	22	18	14	12	15	25	10	18	9	11	13	9
Giza	Pepper	48	38	10	4	4	7	11	6	3	1	4	2	0	1
	Potato	66	66	0	9	8	3	3	15	8	20	0	6	0	0
	Tomato	59	47	12	4	5	3	5	5	1	0	7	3	15	7
	Total	173	151	22	17	17	13	19	26	12	21	11	11	15	8
Total number		640	556	84	70	61	46	70	105	43	76	42	44	56	31
ncidence (%)			86.87%	13.12%	10.94%	9.53%	7.19%	10.94%	16.41%	6.72%	11.87%	6.56%	6.87%	8.75%	4.22%

2- Isolation and identification of the virus isolate

2.1. Isolation and propagation of the virus isolate:

After isolation and biological purification of the virus isolate that suggestive to be *Tomato brown rogues fruit virus* (ToBRFV), it was propagated in *N. tabacum* cv. White Burley which was used as a source of the virus isolate and was then identified as so on the basis of host range, modes of transmission and particle morphology. Because no antiserum specific to ToBRFV was available, we used RT-PCR to confirm the identity of the virus isolate:

2.2: Identification of the virus isolate:

2.2.1: Host range and symptomatology:

Reactions of twenty-five plants species and cultivars belonging to six different families to virus infection are summarized in Table (3) and Figs. (5:12).

Infection was confirmed by back inoculation to *Ch. quinoa* as an assay host plant. Symptoms appeared on host plants might be grouped into four categories:

A-Plants reacted only with local symptoms: *Ch. Amaranticolor, Ch. quinoa*, *Datura stramonium* and *N. benthamiana* developed yellow chlorotic local lesions, 4-6 days after inoculation by the virus isolate (Figs.5 and 6).

B-Plants reacted only with systemic symptoms: Systemic mosaic, mottling or mosaic, chlorosis or yellowing, blistering and leaf deformation were developed, on *Ocimum basilicum* and *physalis floridana,* 12-15 days after inoculation (Figs. 7 and 8).

C-Plants reacted with local followed by systemic symptoms: These are: *C. annuum* L. cv. California Wonder**,** *C. annuum L cv. VitaZ dragon*, *C. annuum* L cv. VitaZ 461, *Solanum lycopersicum* cv. Lugein, *S. nigrum, Nicotiana tabacum* cv White Burley, *N. benthamiana, N. rustica*, *N. glutinosa* and *N. debneyi***.** (Figs. 9,10,11 and 12)

D-No symptoms were observed on any of the following inoculated species: *Gomphrena globosa, Cucumis sativus* cv. Balady, *Cucurbita pepo* L. cv Eskandarani*, Cucumis melo*, *Mentha longfolia* L, *Petunia hybrida, Solanum melongena* L.*, Solanum tubrosum* cv. *Sponta* and *S. tubrosum* cv. *Nicola*. No infection was detected by back inoculation on the local lesion host plant.

Table 3. Reaction of different hosts to infection with the virus isolate.

Host plant	Local symptoms	Systemic symptoms
1- Family: Amaranthaceae		
Gomphrena globosa L.	0	0
2- Family: Chenopodiaceae		
Chenopodium. amaranticolor Caste & Reyn	YCLL	0
Ch. quinoa Wild	YCLL	0
3- Family: Cucurbtaceae		
Cucumis sativus L. cv. Balady	0	0
Cucurbita pepo L. cv. Eskandarani	0	0
Cucumis melo L.	0	0
4- Family: Lamiaceae		
Ocimum basilicum L	0	M, LD
Mentha longfolia L	0	0
5-Family: Solanaceae		
Capsicum annuum L. cv. California Wonder.	NLL, Y	VB, M, Y, LD, PC
Capsicum annuum L cv. VitaZ dragon	NLL, Y	VB, M, Y, LD, PC
Capsicum annuum L cv. VitaZ 461	NLL, Y	VB, M, Y, LD, PC
Solanum lycopersicum cv. Lugein Mill.	NLL, Y	M, B, LD
Nicotiana tabacum L.cv White Burley	NLL	SM, B
N. benthamiana L	NLL	0
N. rustica L.	NLL	MM
N. glutinosa L.	NLL	MM
N. debneyi Domin	YNLL	SM
D. stramonium L.	YNLL	0
Physalis floridana Rydb.	0	M, B, LD
Solanum nigrum L. (Black nightshade)	MM	M, B, LD
Petunia hybrida L.	0	0
Solanum melongena L. (Eggplant)	0	0
Solanum tubrosum cv. *sponta* (potato)	0	0
Solanum tubrosum cv. *Nicola* (potato)	0	0
6- Family: Apocynaceae		
Catharanthus vinca L.	ZS	0

0: no symptoms **YCLL**: yellow chlorotic local lesion **YNLL**: yellow necrotic local lesion **NLL**: necrotic local lesion **MM**: mild mottle or mosaic **M**: mottle or mosaic **Y**: yellow **L D**: leaf deformation **NLL**: necrotic local lesion **VB**: vein band **PC**: plant collapsed **SM**: severe mosaic **B**: blister **ZS: zonatic spot**

Fig. 5 A- Healthy control, B-Yellow chlorotic local lesions produced were developed on inoculated leaves of *ch. amaranticolor* by the virus isolate.

Fig. 6 A- Healthy control, B-Yellow chlorotic local lesions developed on inoculated leaves of *ch. quinoa* produced by the virus isolate.

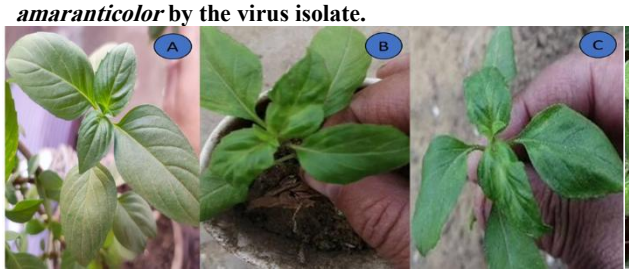

Fig. 7 A- Healthy control, B and C- Systemic mosaic, mottle, blister and leaf and leaf deformation were developed on *Ocimum basilicum* by the virus isolate.

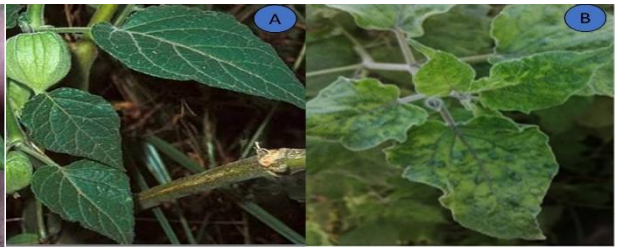

Fig. 8 A- Healthy control, B and C- Systemic mosaic, mottle, blister and leaf and leaf deformation were developed on *Physalis floridana* by the virus isolate.

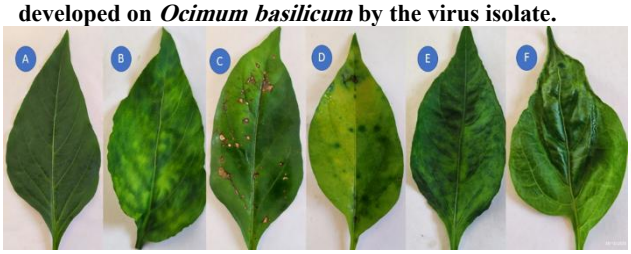

Fig. 9. A- healthy control, B- vein band, C-Necrotic local lesions, D-yellow, E- mottle or mosaic and F- leaf deformation were developed on *Capsicum* spp. du to virus infection.

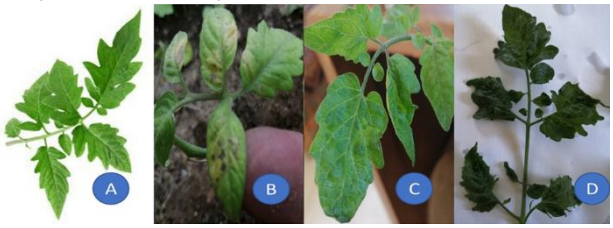

Fig 10 A- healthy control, B- Necrotic local lesions, C-mottle or mosaic and D-blister and leaf deformation were developed on *Solanum lycopersicum* after inoculation by the virus isolate.

Fig 11 A- healthy control, B, C- Necrotic local lesions, sever mosaic and blister were developed on *N. tabacum* L. cv White Burley produced by the virus isolated

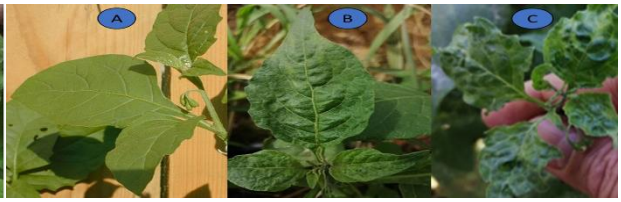

Fig 12 A- healthy control, B -mottle or mosaic and C- blister and leaf deformation were developed on *Solanum nigrum* after inoculation by the virus isolate.

2.2.3: Morphology of virus particles:

Examination of clarified pepper- leaf extracts preparations infected by the virus isolate, using quick-dip method negatively by an electron microscope at Magnification of 20000), revealed rod–shaped particles of 261-348 nm in length and 15-19 nm wide, in the range of the *Tobamovirus* group (Fig. 13).

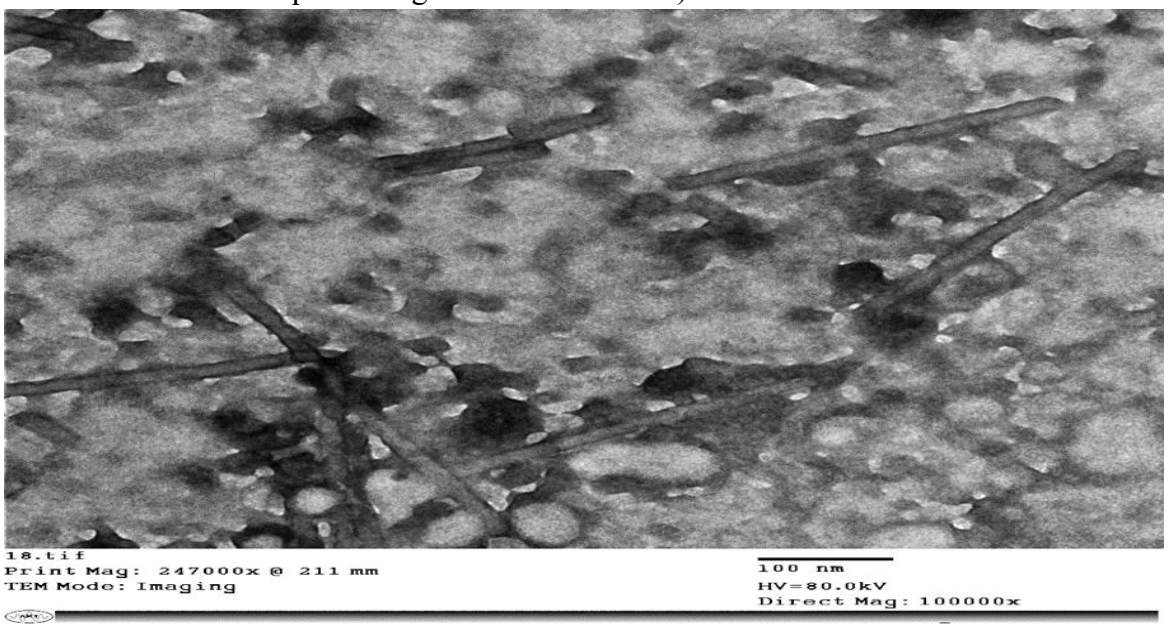

Fig. 13. Electron micrograph of quick- dip preparation method ToBRFV rod-shaped particles ranged from 261-348nm in length and 15-19nm wide.

2.2.2: Modes of transmission:

2.2.2.1: Mechanical transmission:

This has been proven in all inoculation experiments during this study

2.2.2.2: Seed transmission:

Results demonstrated in Table (4) revealed that, the percentage of seed transmission of the virus was varied according to the pepper cultivars tested. It was ranged from 1 to 2.5 %.

Table 4. The percentage of seed transmission on pepper cultivar:

Cultivar tested	No. infected seeds / No. tested seeds	% of infection
California Wonder	3/200	1.5
Vita Z Dragon	2/200	1
Helsinki	5/200	2.5

2.3: Varietal susceptibility:

As indicated in Table (5), Helsinki and Mycelia pepper cultivars were highly susceptible (70%) followed by California Wonder (50%) and Vita Z 461 (40%). On the other hand, Vita Z Dragon cultivar was the lowest susceptible one (30%).

Table 5. Response of five pepper cultivars to artificial infection with ToBRFV.

Cultivar	Infection%
California Wonder	50
Vita Z 461	40
Vita Z Dragon	30
Helsinki	70
Mycelia	70

2.4: Molecular characterization:

In this study, ToBRFV isolate was further identified by RT- PCR amplification. Three negative DAS-ELISA samples with viral symptoms (S1, S2 and S3) were detected using previously tested primers. General primer for Tobamoviruses designed by **Menzel et al. (2019)** and other two specific ones for detecting RNA-dependent RNA polymerase (RdRp) and CP of ToBRFV designed by **Salem et al. (2019**). The amplified fragments of 560bp, 1300bp, 697bp, respectively were detected from RNA infected extracts after agarose electrophoresis of RT- PCR products (Fig. 14).

Tested samples were amplified using the designed three specific primers **(P1, P2 and P3)** amplifying part of coat protein gene for ToBRFV**.** The amplified fragments of 247bp, 250pb and 361bp, respectively were detected from RNA infected extracts after agarose electrophoresis of RT- PCR products (Fig.15). Whereas, no such fragments were amplified from RNA extracted from the comparable healthy plants or other plants with negative DAS-ELISA results.

Other runs of agarose gel were conducted comparing RT-PCR products of each designed primers (P1, P2, and P3) separately with the three confirmed positive ToBRFV samples (S1, S2 and S3) by **Salem et al. (2019** and three Tobamovirus (TMV, ToMV and ToMMoV) as negative control. The three primers were successfully amplified the ToBRFV from the tested samples. The P3 primer reacted as a specific primer for ToBRFV with amplifications of around 361bp (Fig. 16) whereas, other two designed primers reacted as general primers (P1and P2) for Tobamoviruses with amplifications of around 247bp and 250bp, respectively gives positive reaction with all samples and Tobamoviruses (Fig. 17 and Fig. 18, respectively).

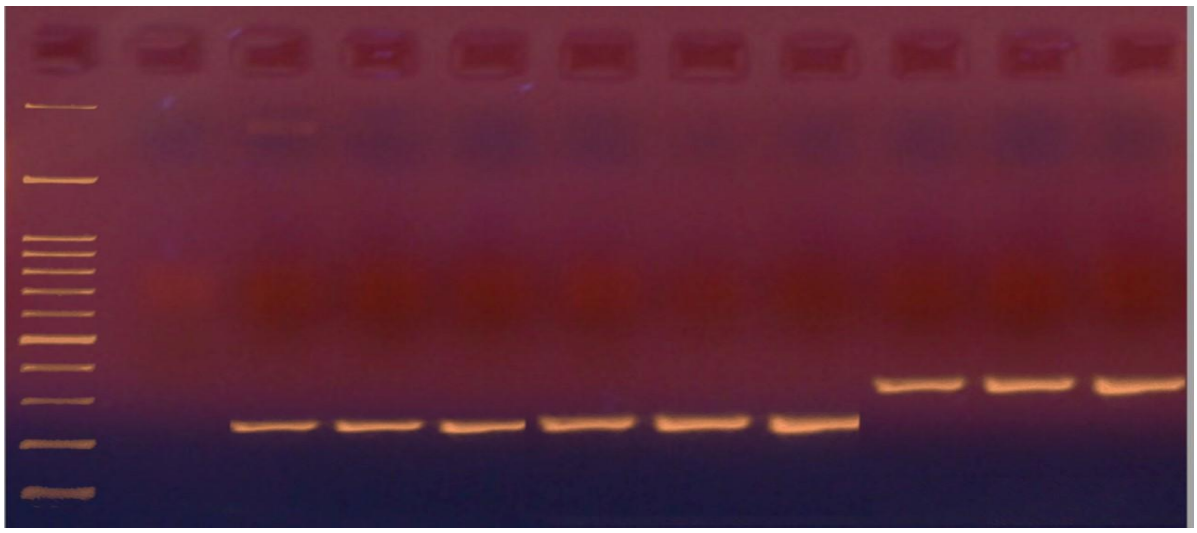

Fig. 14. Agarose gel electrophoresies of three RT-PCR amplifications for negatively DAS-ELISA with viral symptoms using Menzel *et al.* (2019) (Mz) and Salem *et al.* (2019) (Sm_1 and Sm_2) primers infected by ToBRFV; *M:* Fast Gene 100 bp DNA Marker (100pb), control (healthy sample), *1, 2, 3;* S1, S2, S3 samples tested with Mz primer (amplicon of 560 pb), *4, 5, 6;* samples tested with Sm_1 primer (amplicon of 1300 pb), *7, 8, 9;* samples tested with Sm_2 primer (amplicon of 697 pb), *N:* negative control (healthy sample).

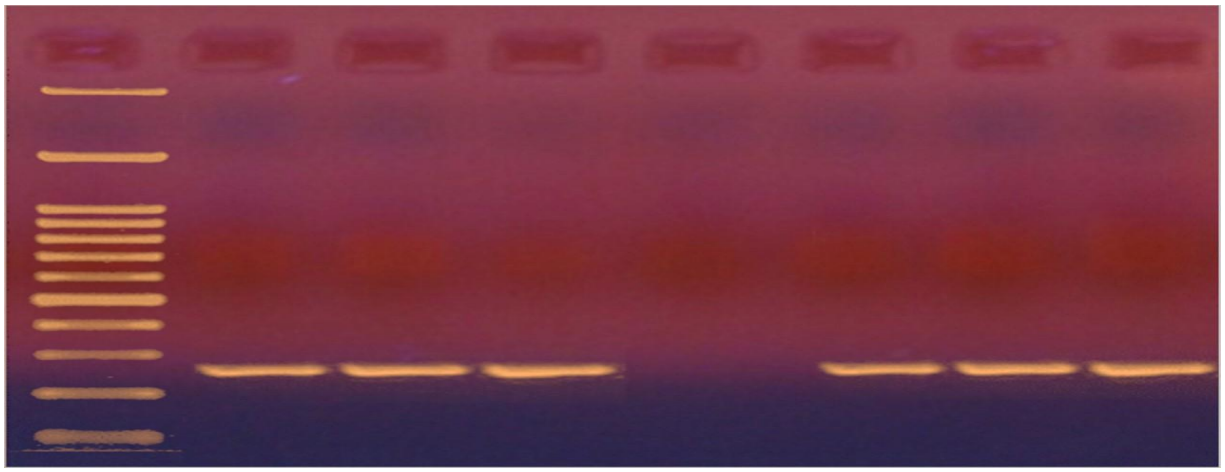

Fig. 15. Agarose gel electrophoresies of three RT-PCR amplifications for negatively DAS-ELISA with viral symptoms using P1, P2 and P3 designed primers infected by ToBRFV; *M:* Fast Gene 100 bp DNA Marker (100pb), N: negative control (healthy sample), *1, 2, 3;* S1, S2, S3 samples tested with P1 primer (amplicon of 247pb), *4, 5, 6;* samples tested with P2 primer (amplicon of 250pb), *7, 8, 9;* samples tested with P3 primer (amplicon of 361pb).

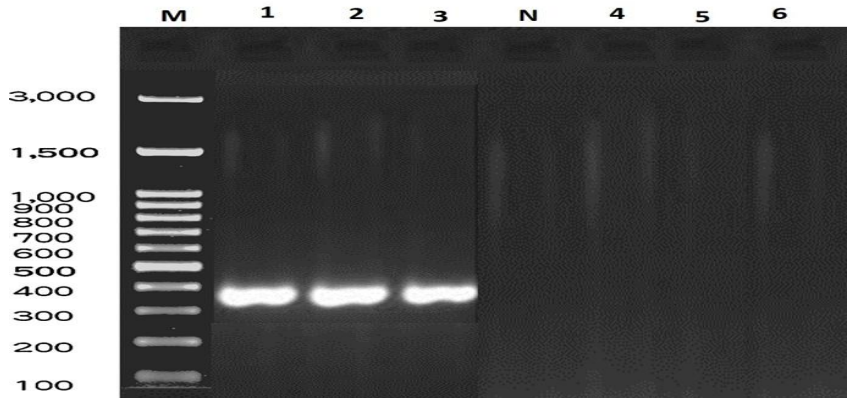

Fig. (16): Agarose gel electrophoresies of RT-PCR amplification for negatively DAS-ELISA with viral symptoms using P3 designe primer infected by ToBRFV; M: 100pb DNA ladder, *1, 2, 3;* S1, S2, S3 samples tested with P1 primer (amplicon of 361 pb), N negative control (healthy sample) and *4, 5, 6;* three Tobamoviruses (TMV, ToMV and ToMMoV) amplified.

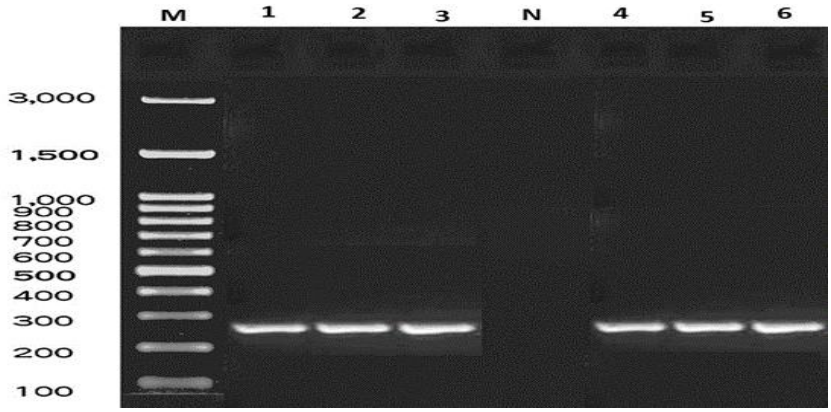

Fig.17. Agarose gel electrophoresies of RT-PCR amplification for negatively DAS-ELISA with viral symptoms using P1 designed primer infected by ToBRFV; *M:* 100pb DNA ladder, *1, 2, 3;* three Tobamoviruses (TMV, ToMV and ToMMoV) amplified *N:* negative control (healthy sample), *4, 5, 6;* S1, S2, S3 samples tested with P1 primer (amplicon of 247 pb).

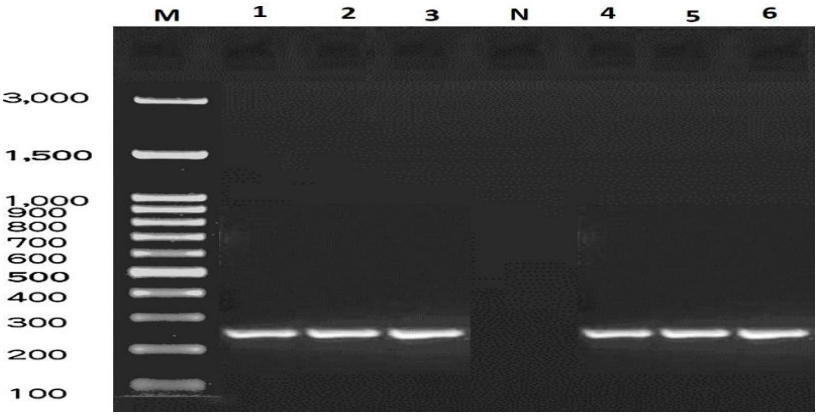

Fig.18. Agarose gel electrophoresies of RT-PCR amplification for negatively DAS-ELISA with viral symptoms using P3 designed primer infected by ToBRFV; *M:* 100pb DNA ladder, *1, 2, 3;* three Tobamoviruses (TMV, ToMV and ToMMoV) amplified, N: negative control (healthy sample),*4, 5, 6;* S1, S2, S3 samples tested with P1 primer (amplicon of 250 pb).

In this study, the survey conducted in potato, tomato and pepper fields in different Governorates in Egypt during 2016/2017 growing season.

A total of eleven viruses were identified serologically in collected samples. there was no clear association between symptoms observed in the field and detected viruses, maybe because of coinfection of plants with several viruses or other biotic and abiotic stresses. This may also be due to the presence of other undetected viruses that were not covered by the antisera used. The use of ELISA technique greatly facilitated the identification of viruses and provided more accurate and consistent results than did symptomatology and host range **(Mathews, 2002)**. Analysis of the collected samples by ELISA using the available antisera revealed that out of 640 samples collected from potato, tomato and pepper tested 86.87% gave positive reaction, while 13.12% recorded negative results. It was founded that PVY was the most prevalent virus (16.41%), followed by PLRV (11.87%), AMV and PepMMoV both (10.94%), CMV (9.53%), TYLCV (8.75%), PepMoV (7.19%), ToMV (6.87%), PVX (6.72%), TMV (6.56). However, TSWV was detected at low frequency (4.22%). Because serological cross-reactivity occurs between TMV and other tobamoviruses like ToMV, the precise to which the virus detected by DAS- ELISA belongs remains to be determined **(Bolou Bi et al., 2018).** It was found that some samples were coinfected with two or three different viruses (i. e. PVY+ ToMV, TMV+ PepMMoV+ TYLCV). These results were in agreement with that reported by **Luria et al. (2017)** and **Jamous et al. (2022).** This study indicated that regular survey of virus diseases was very important in management of viral diseases. We considered that more investigations are needed to identify new viruses such as ToBRFV. So that specific virus resistance can be integrated in potato, tomato and pepper breeding programs.

After the survey of the most prevalent viruses infecting tomato, potato and pepper plants, an isolate suggestive to be ToBRFV which did not give any reaction against the antisera used. It was biologically purified and propagated in *N. tabacum* cv. White Burley and identified on the basis of host range, modes of transmission and particle morphology. In addition, its identity was confirmed molecularly in the absence of specific antisera available to it.

Tomato brown rogues fruit virus (ToBRFV) belongs to the genus *Tobamovirus*, a newly identified virus infecting tomato, potato and pepper was isolated from symptomatic plans grown under greenhouse conditions in Jordan in 2015 **(Salem et al., 2016)**. Since then, the virus has been detected in many countries in the world **(Van de Vossenberg et al., 2020).**

Disease symptoms on plants alone may be misleading or inadequate. This particularly so when several viruses cause similar symptoms. Thus, since the early days of plant virology, host range was used as indicator hosts know to give clear, characteristic and consistent symptoms for the virus or viruses being studied, usually under greenhouse conditions **(Matthews, 1993).**

The symptoms produced on inoculated host range by the virus isolate varied from local symptoms on *Ch. amaranticolor*, *Ch. quinoa* and *N. benthamiana* to systemic symptoms such as mosaic, chlorosis or yellowing, blistering and leaf deformation or systemic only on *Osmium basilicum* and *Solanum nigrum* or by local followed by systemic ones such as *C. annuum* L. cv. California Wonder,

C. annuum L cv. Vita Z dragon, *C. annuum* L cv. Vita Z 461, *Solanum lycopersicum* cv. Lugein, *S. nigrum, Nicotiana tabacum* cv White Burley, *N. benthamiana, N. rustica*, *N. glutinosa* and *N. debneyi.* Out of twenty – five plants species and cultivars belonging to six different families, the virus isolate infecting fifteen but did not infect *G. globosa, Cucumis sativus* cv. Balady, *Cucurbita pepo* L cv. Eskandarani, *Cucumis melo*, *Mentha longfolia* L., *Petunia hybrida*, *Solanum melongena* L., *Solanum tubrsum* cv. Sponta and *S. tubrsum* cv. Nicola. These results appear to be in the line with other workers **(Salem et al., 2016, Bally et al., 2018 and Yan et al., 2021)**. Difference in host range and symptom syndrome under Egyptian environmental conditions might due to difference in the expression of genetic interaction between the tested hosts and the virus isolate. These results appear to be in the line with other workers **(Dombrovsky & Smith, 2017; Cambrón-Crisantos et al., 2019 and AHDB, 2019).**

Viruses usually depend for survival on their ability to spread from one susceptible individual plant to another fairly frequently.

Knowledge of the ways in which viruses are transmitted from plant to plant is important for recognizing a particular viral disease and developing satisfactory control measures **(Matthews, 2002).**

Seed transmission provides a very effective means of introducing virus into a crop at an early stage, giving randomized foci of infection throughout the planting. Thus, when other methods of transmission can operate to spread the virus within the growing crop, seed transmission may be of very considerable economic importance. Viruses may persist in seed for long periods so that commercial distribution of a seed- borne virus over long distances may occur **(Matthews, 2002).**

In this work, the virus was found to be transmitted mechanically and by seeds. The percentage of seed transmission of the virus was varied according to the pepper cultivars tested (Vita Z Dragon, 1%, California Wonder 1.5% and Helsinki, 2.5%). It is evident from all available reports the ToBRFV is capable of being mechanically transmitted through infected sap via any means **(Wilstermann and Ziebell, 2019).** The virus was found in the seed coat and the endosperm but does not infected embryo inside seed and instead contaminates the seed coat. So, it can be preserved on the seed for several years **(Dombrovsky and Smith, 2017, Oladokun et al., 2019 and Oepp& Bulletin, 2020).** The external virus can be readily inactivated by certain treatments eliminating all or almost allseed- borne infection**. Davino *et al.* (2020)** and **Salem *et al.* (2022)** reported that the seed transmission rate from ToBRFV- contaminated seeds to their seedlings is low, ranging from 0.08 to 2.8%.

It is generally believed that there are no specific insect vectors that transmit ToBRFV **Oladokun et al. (2019).**

Measurements made on electron micrographs of isolated virus particles, or thin sections of infected cells, offer very convenient estimates of the size of viruses **(Matthews, 2002).** Examination of clarified pepper – leaf extract preparations infected by the virus isolates, using quick-dip analysis and the samples revealed rod-shaped particles of 261- 348 nm in length and 15- 19nm in wide. This type of particles is in the range of the *Tobamovirus*. Our results were in agreement with those obtained by other workers **(Gibbs. 1977, Adams et al., 2017 and Eicheier et al., 2023).**

Reverse transcription – polymerase chain reaction is popular technique for detection and identification of RNA and DNA plant viruses. The procedure is highly sensitive, simple in overcoming many difficult encountered with serological methods. Such as low antigen titer, availability of antibodies **(Webster et al., 2014).**

In this study, RT-PCR, which was performed to detect ToBRFV isolate. Three samples used in RT- PCR were selected based on negative DAS-ELISA samples with viral symptoms (S1, S2 and S3) were detected using previously tested primers, general primer for Tobamoviruses designed by **Menzel et al. (2019)** and other two specific ones for detecting RNA-dependent RNA polymerase (RdRp) and CP (1300 and 679bp, respectively) of ToBRFV designed by **Salem et al. (2019)**. All tissue (leaves or fruits) samples of selected pepper were tested positive for ToBRFV by representing the expected size (1052bp) for the amplification fragments of the Open Reading Frame (ORF) encoding the RNA dependent RNA polymerase **(Luria *et al.*, 2017)**. Samples were tested by reverse transcription-polymerase chain reaction (RT-PCR) for most common Tobamoviruses infecting pepper, including TMV, ToMV and ToMMoV **(Takeuchi et al., 2005)**. In addition, generic primers for detection of Tobamoviruses were also used **(Dovas *et al.*, 2004)**. In RT-PCR amplicons of the expected product size 247, 250 and 361bp using the generic Tobamovirus and specific primer, respectively in all symptomatic plant samples were obtained, but such amplicons which not obtained from healthy plants extracts or water negative controls. The three primers were successfully amplified the ToBRFV from the tested samples. The P3 primer reacted as a specific primer for ToBRFV with amplifications of 361bp. Whereas, other two designed primers reacted as general primers (P1 and P2) for Tobamoviruses with amplification of around 247 and 250bp, respectively gave positive reaction with all samples. Similar results were reported by **Luria et al. (2017)** and **Salem et al. (2019)**.

4- Conclusion and Recommendation

The survey result provided, a base line information on the distribution of some viruses infected three different solanaceous plants (potato, tomato and pepper) in different regions in Egypt. Virus diagnosis showed that PVY was the most prevalent virus, followed by CMV, AMV, PepMoV, PVX, TSWV, PLRV, TMV, TYLCV, TRV and PepMMoV. The viruses existed in combinations of two to three and characterized by symptoms which are not specific to a particular virus. These facts present a good starting point for virus diseases diagnosis in these locations surveyed. These viruses have a wide crop range and are potentially very damaging to these crops. ToBRFV was also characterized by traditional identification methods and by Rt-PCR. It is considered one of new emerging and identified viruses. There is need, therefore, for constant monitoring through regular disease survey to identify new and emerging viruses.

5- Acknowledgment

The authors briefly acknowledge those who helped them in conducting this research specially virus and phytoplasma Res. dept., plant pathology Res. Inst., ARC, Giza, Egypt and fac. of Agric., Fayoum univ. for providing facilities valuable and materials during this course of investigation.

4. REFERENCES:

Adams M. J., Adkins S., Bragard C., Gilmer D., Li D., MacFarlane S. A., Wong S. M., Melcher U., Ratti C., Ryu K. H. 2017. ICTV virus taxonomy profile: Virgaviridae. Journal of General *Virology* 98: 1999–2000**.**

AHDB Horticulture 2019. Tomato Brown Rugose Fruit Virus (ToBRFV). *AHDB Horticulture* pp. 1–2.

Bally J., Jung H., Mortimer C., Naim F., Philips J. G., Hellens R. *et al.* 2018. The rise and rise of *Nicotiana benthamiana*: a plant for all reasons. *Annual Review of Phytopathology* 56: 405– 426.

Bolou Bi B. A., Moury B., Abo K., Sorho F., Cherif M., Girardot G., Kouassi N. P. and Kone D. 2018. Survey of viruses infecting open-field pepper crops in Cote^ d'Ivoire and diversity of *Pepper veinal mottle virus* and *Cucumber mosaic virus*. *Plant Pathology* 67: 1416–1425. https://doi:10.1111/ppa.12849.

Bos, L. 1970. The identification of three new viruses isolated from *Wisteria* and *Pisum* in the Netherlands and the problem of rariation within the potato virus Y group. *Neth. J. Pl. Path*. 76:8-46.

Brandes J. 1957. Eine elektronenmikroskopische Schnellmethodezum Nachweis faden- und stabchenformiger Viren, insbesondere kartoffeldunkelkeimen**.** *Nachr Bl. Dt. Pflschutzdienst, Braunschweig* 9:151-152.

Cambrón-Crisantos J. M., J. Rodríguez-Mendoza J. B. Valencia-Luna S. A. Rangel C. De Jesús García-Ávila and López-Buenfil J. A. 2019. First Report of *Tomato brown rugose fruit virus* (ToBRFV) in Michoacan, Mexico." *Mexican Journal of Phytopathology* 37:185–92.

Chan Y. L., Saidov N., Lee L.M., Kuo F. H., Shih S.L. and Kenyon L. 2022. Survey of Viruses Infecting Tomato, Cucumber and Mung Bean in Tajikistan. Horticulturae 2022, 8, 505. https://doi.org/10.3390/horticulturae8060505.

Davino S., Caruso A. G., Bertacca S., Barone S. and Panno S. 2020. *Tomato brown rugose fruit virus*: seed transmission rate and efficacy of different seed disinfection treatments. *Plants* 9: 1615.

Dive S. and Nagar A. 2017. Nutritional and Medicinal Properties of Solanaceous Vegetables. Division of Vegetable Science, IARI, New Delhi, 110012. http://www.biotechecharticles.com/submit guide.php.

Dombrovsky A. and Smith E. 2017. Seed transmission of tobamoviruses: aspects of global disease distribution. In: Jimenez-Lopez, J.C. (Ed.) *Advances in seed biology*. London, UK: IntechOpen, pp. 233–260.

Dovas C. I., Efthimiou K. and Katis N. I. 2004. Generic detection and differentiation of tobamoviruses by a spot nested RT-PCR-RFLP using dI-containing primers along with homologous dG-containing primers. J Virol Methods 117: 137– 144. https://doi.org/10.1016/j.jviromet.2004.01.004.

Eicheier A., Hejlova M., Orsagova H., Frej Lichova L., Hakalova E., Tomankova K., Linhartova S, Kulich P., Germak V. and Cechova J. 2023. Characterization of *Tomato brown rugose fruit virus* (ToBRFV) detected in Czeh Repablic. Diversity 15: 301. https://doi.org/10.3390d15020301.

Fidan H. 2020. *Tomato brown rugose fruit virus* (ToBRFV): Current situation

and future prospects. ***Mediterr Agric Sci.*** **33(1): 43-49. https://DOI:10.29136/mediterranean.705740.**

FAO 2022. World Food and Agriculture-Statistical Yearbook 2022. Rome, Italy. https://doi.org/10.4060/cc2211en.

Gibbs A. J. 1977. Tobamovirus group. *CM1/AAB Dezcr. Pl. Viruses No.* 184.

Hall T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucl. Acids. Symp. 41: 95–98.

Hampton R. O., Shukla D. D. and Jordan R. L. 1992. Comparative *Potyvirus* host rang Serological and coat protein peptide profiles of white *Lupin mosaic virus. Phytopathology* 82:566-571.

Hančinský R., Mihálik D., Mrkvová M., Candresse T. and Glasa M. 2020. Plant Viruses Infecting *Solanaceae* Family Members in the Cultivated and Wild Environments: A Review. Plants (Basel). 25;9(5):667. https://doi:10.3390/plants9050667.

Jamous R. M, Abu Zaitoun S. Y., Mallah O. B. and Ali-Shtayeh M. S. 2022. Biological and molecular characterization of *Tomato brown rugose fruit virus* (ToBRFV) on tomato plants in the state of Palestine *Res.Plant Dis.*28(2): 98-107.

Kukhaleishvili M., Bulauri E., Megrelishvili I., Shamatava T. and Chipashvili T. 2018. Survey of Potato Viral Infection Using Das-Elisa Method in Georgia. World Academy of Science, Engineering and Technology International Journal of Bioengineering and Life Sciences Vol:12, No:12.

Kuhn C. W. (1964): Separation of cowpea virus mixture. *Phytopathology* 54:739-740.

Li Y., Tan G., Xiao L., Zhou W., Lan P., Chen X., Liu Y., Li R. and Li F. 2021. A Multiyear Survey and Identification of Pepper- and Tomato-Infecting Viruses in Yunnan Province, China. *Front. Microbiol.* 12:623875. https://doi:10.3389/fmicb.2021.623875.

Luria N., Smith E., Reingold V., Bekelman I., Lapidot M., Levin I. and Dombrovsky A. 2017. A new Israeli Tobamovirus isolate infects tomato plants harboring Tm-22 resistance genes. *PloS one* 12(1), e0170429.

Matthews R. E. F. 1993. Diagnosis of Plant Virus Diseases CRC Press *Boca Raton,* Ann Arbor, London, Tokyo374pp.

Matthews R. E. F. 2002. Plant virology, 4th ed. Academic Press, London1001pp.

Menzel W., Knierim D., Winter S., Hamacher J. and Heupel M. 2019. First report of *Tomato brown rugose fruit virus* infecting tomato in Germany. *New Disease Reports* 39: 1.

Noordam D. 1973. Identification of plant viruses. Methods and experiments. Center for Agric. Publishing and documentation, Wageninen, Nether lands 207 pp.

Oepp B. and Bulletin, E. 2020. *Tomato brown rugose fruit virus. EPPO Bulletin 50*(3): 529–534. https://doi.org/10.1111/epp.12711

Oladokun J. O., Halabi M. H., Barua P. and Nath P. D. 2019. Tomato brown rugose fruit disease: current distribution, knowledge, and future prospects. Plant Pathology 68(9): 1579-1586.

Rodriguez -Alvarado G., Fernandes - Pavia S., Creamer R. and Liddell C. 2002. *Pepper mottle virus* disease in Chile pepper in Southern New Mexico. *Plant Disease* 86:603-605.

Rodríguez-Verástegui L. L., Ramírez-Zavaleta C. Y., Capilla-Hernández M. F. and Gregorio-Jorge J. 2022. Viruses Infecting Trees and Herbs That Produce

Edible Fleshy Fruits with a Prominent Value in the Global Market: An Evolutionary Perspective. Plants (Basel). 13;11(2):203. https://doi:10.3390/plants11020203.

Salem N. M., Cao M. J., Odeh S., Turina M., and Tahzima R. 2019. First Report of *Tobacco Mild Green Mosaic* Virus and *Tomato Brown Rugose Fruit Virus* Infecting *Capsicum annuum* in Jordan." *Plant Disease* 104 (2): 601.

Salem N. M., Mansour A., Ciuffo M., Falk B. W. and Turina M. 2016. A new tobamovirus infecting tomato crops in Jordan. Archives of Virology 161: 503–506.

Salem N. M., Sulaiman, A., Samarah, N., Turina, M. and Vallino, M. 2022. Localization and mechanical transmission of tomato brown rugose fruit virus in tomato seeds. *Plant Disease* 106: 275– 281.

Takeuchi S., Hamada H., Toyoda K., Suzuki K., Kiba A., Hikichi Y., *et al.* 2005. Discrimination between tobamoviruses and their pathotypes for L-gene-mediated resistance in green pepper (*Capsicum annuum* L.) by reverse transcription-polymerase chain reaction. Journal of General Plant Pathology. 71(1):60-67. https://doi.org/10.1007/s10327-004-0161-4.

Van de Vossenberg B. T. L. H., Visser M., Bruinsma M., Koenraadt H. M. S., Westenberg M. and Botermans M. 2020. Real-time tracking of *Tomato brown rugose fruit virus* (ToBRFV) outbreaks in the Netherlands using

Webster C. G., E. N. Rosskopf L. Lucas H. C. Mellinger and S. Adkins. 2014. First Report of *Tomato Mottle Mosaic Virus* Infecting Tomato in the United States." *Plant Health Progress* 15 (4): 151–2.

Wilstermann, A. and Ziebell, H. 2019. *Tomato brown rugose fruit virus (ToBRFV). JKI Data Sheets Plant Dis. Diagn.* 1: 1–4.

Yan Z. Y., Ma H. Y., Wang L., Tettey C., Zhao M. S. Geng C. *et al.* 2021. Identification of genetic determinants of tomato brown rugose fruit virus that enable infection of plants harbouring the *Tm- 2*^{2} resistance gene. *Molecular Plant Pathology* 22: 1347– 1357.