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Tomato chlorosis virus AND *Tomato yellow leaf curl virus* CAUSING MIXED INFECTION IN PROTECTED EGGPLANT (*Solanum melongena*) CROPS IN TURKEY

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ABSTRACT

Eggplant (*Solanum melongena*) is an important source of food for people. Eggplant cultivations, located in the Mediterranean region province of Antalya, corresponds to 20% of the total eggplant production in Turkey. In Antalya province, yellowing type symptoms were observed in eggplant areas and it was found that these symptoms were not caused by fungal or bacterial cause. The yellowing symptoms characteristic for *Solanaceae* family, such as tomato and pepper, are also observed in the same family of eggplant. Total nucleic acids were obtained from infected eggplants were tested by RT-PCR/PCR with specific primers of TYLCV (*Tomato yellow leaf curl virus*) and ToCV (*Tomato chlorosis virus*). Their PCR amplicons were directly sequenced, and phylogenetic analyses were performed. Additionally, TYLCV infected plants were determined in the strain level. This study is the first record of *Tomato chlorosis virus* and *Tomato yellow leaf curl virus* causing infection in eggplant in Antalya province and characterized by yellowing type symptom in infected plant.

Key words: eggplant, survey, virus, yellowing type symptoms

INTRODUCTION

Eggplant (*Solanum melongena*), known as aubergine or brinjal, is an economically important vegetable in many countries. Dash et al. [2019] is known in India in ancient times and the first record in Europe was made in the 15th century. Eggplant (*Solanum melongena*) is called eggplant because the first known varieties had egg-shaped fruits [Sao and Metha 2010]. The most important producer countries are China (32 mln t), India (12.5 mln t), Egypt (1.2 mln t), Turkey (0.85 mln t) and Iran (0.67 mln t). Eggplant in Asia and the Mediterranean is among the five most important plant products (tomato, pepper, potato, tobacco, eggplant) [FAO 2016]. According to TUIK data, in 2018, 836.284 t of eggplant was produced in Turkey. Mediterranean region in the area of 69,191 with a maximum of 431.506 t of eggplant production. 190.125 t of eggplant were produced in 23.559 daa of production areas in Antalya province, which is located in the Mediterranean region. Antalya province is in the 20% share of the eggplant production in Turkey [TUIK 2018].

In the Mediterranean region of our country, the most common undergrowth vegetables are tomato, pepper, cucumber and eggplant. There are many biotic and abiotic factors in the production of eggplants, causing cultivations damages in different periods. Biotic factors that may cause yellowing and wilt type symptom infections in eggplant include *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp. *michiganensis* bacterial species; *Fusarium oxysporum* f. sp. *melongenae* and *Verticillium* spp. fungus species. In addition to fungal and bacterial diseases,

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there are also various types of virus diseases affecting eggplants such as *Tospovirus*, *Geminivirus* and *Crinivirus* [https://www.seminis-us.com/resources/disease-guides/pepper-eggplant/].

Eggplant is in the same family as tomatoes, so it can also be affected by biotic factors that cause significant damage to tomatoes. *Tomato chlorosis virus* (ToCV) and *Tomato yellow leaf curl virus* (TYLCV) which are carried by whiteflies and frequently encountered in tomato cultivation also gain importance in eggplant cultivation.

Whitefly population in Turkey's Mediterranean province of Antalya, where climate is favorable for this kind, is very high. Kil et al. [2016] reported that *Tomato yellow leaf curl Virus* (TYLCV) belonging to the *Begomovirus* genus of the family *Geminiviridae* and a single-stranded circular DNA genome infected tomato plants in Korea, while Green et al. [2003] reported infections in eggplant in Thailand and Vietnam. In April 2013, eggplants exhibiting yellow mosaic symptoms were detected in Laos, Vietnam. The PCR results found that plants were infected with a *Begomovirus*. They reported that this virus associated with eggplant yellow mosaic disease is an isolate of TYLCV and it was transmitted by whiteflies. Plants belonging to *Solanum* family were experimentally infected with TYLCV, and showed symptoms like chlorotic leaf edges, upward leaf curling, yellow mosaic, stunting, leaf mottling, and reduced leaf size symptoms that were identical to those observed in the field. There are different strains of this virus such as *Tomato yellow leaf curl Sicilya* (TYLCSV-Sic), *Tomato yellow leaf curl virus*-Mild (TYLCV-Mld) ve Sardunya (TYLCV-Sa), *Tomato yellow leaf curl virus*-Moracco (TYLCV-Mo), *Tomato yellow leaf curl virus*-Mild (TYLCV-Mld), *Tomato yellow leaf curl Malaga virus* (TYLCMalV), *Tomato yellow leaf curl Axarquia virus* (TYLCAxV), *Tomato yellow leaf curl China* (TYLCV-Ch), *Tomato yellow leaf curl Thailand* (TYLCV-Th.) [Anfoka et al. 2009].

Plant virus diseases cause very important damages in the *Solanaceae* family, but there are no detailed studies of this problem in eggplant cultivations in Antalya province. The main aim of this study is to determine the presence virus diseases in Antalya, where eggplant production is most common in the Mediterranean region, regardless of the degree of damage in eggplant production areas. For this purpose, in 2016–2018 vegetation periods, survey studies were done in the areas where eggplant cultivation was performed and virus diseases were tried to be determined.

MATHERIALS AND METHODS

Field observation and sample collection

Field samples of commercial eggplant crops have been collected from 2016 to 2018, surveys were carried out in Alanya, Aksu, Konyaaltı, Kumluca, Manavgat and Serik districts of Antalya province (Tab. 3). Observations were made in the eggplant fields in these districts and the yellowing symptom sample was collected (Fig. 1). During symptomatological observations, eggplant plant samples that did not show virus symptoms but were suspected of being infected be collected. Total of 96 samples including exhibiting mild chlorosis, yellowing symptom and abnormal leaves were collected from plants (Tab. 1).

Molecular studies

Total RNA was extracted from 96 samples showing symptoms of virus infection, using GeneJet RNA Purification Kit (Termo Fisher Scientific), according to manufacturer's instructions. Total DNA was extracted using GeneJet Genomic DNA Purification Kit (Termo Fisher Scientific). Total RNA was used as template for polymerase chain reaction with reverse transcription (RT-PCR) with primers for ToCV and TICV (Tab. 1); total DNA extracts were used to check presence of *Tomato yellow leaf curl virus* (TYLCV), *Tomato yellow leaf curl Sardinia virus* (TYLCSV), *Tomato yellow leaf curl virus*-Israel (TYLCV-Is), *Tomato yellow leaf curl virus*-Mild (TYLCV-Mld), *Tomato yellow leaf curl virus*-Sicily (TYLCV-Sic) – Table 2 – common viruses in Antalya province. The genetic material was checked on a spectrophotometer for quality and quantity determination and optimized for using as a template in PCR & RT-PCR.

RT-PCR was performed for ToCV and TICV using Verso 1-Step RT-PCR Kit (Thermo Scientific) under the following conditions: 50°C for 15 min and 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 60°C for 45 s and 72°C for 30 s, and a final extension at 72°C for 7 min. The same genetic material was tested by the PCR using DreamTaq Green PCR Mas-

Fig. 1. Eggplant plants that showing yellowing type symptoms

Table 1. Primer sequences of *Tomato chlorosis virus* (ToCV), *Tomato infectious chlorosis virus* (TICV) used in RT-PCR and *Tomato yellow leaf curl virus* (TYLCV) used in PCR studies

Viruses' names	The sequence of primers	Amplicon size	Source
Tomato chlorosis virus (ToCV)	F: ATCGGTGAAACCCCGATGAC R: CCGGAACCCAAAGTCACAGT	574bp	Sulley 2016
Tomato infectious chlorosis virus (TICV)	F: TCAGTGCGTACGTTAATGGG R: CACAGTATACAGCAGCGGCA	500bp	Vaira 2012
Tomato yellow leaf curl virus (TYLCV)	F: ATACTTGGACACCTAATGGCTATTTG R: TGCCTTGGACARTGGGGRCAGCAG	543bp	Fidan et al. 2019

Table 2. Primer sequences representing genome of *Tomato yellow leaf curl virus* (TYLCV) used in PCR studies [Fidan et al. 2019]

Primers' names	The sequence of primers
TYLCV-Is F	ATACTTGGACACCTAATGGCTATTTGG
TYLCV-Is R	TGCCTTGGACA(A/G)TGGGG(A/G)CAGCAG
TYLCV-Mld F	AAGTGGGTCCCACATATTGCAAGAC
TYLCV-Mld R	ATTGACCAAGATTTTTACACTTATCCC
TYLCV-Sic F	TGGAAAGTACCCCATTCAAGAACATC
TYLCV-Sic R	ACGTAGGTCTTGACATCTGTTGAGCTC
TYLCV-Sa F	ATATTGATGGTTTTTTCAAAACTTAGAAG
TYLCV-Sa R	TTTTATTTGTTGGTGTTTGTAGTTGAAG

terMix under following conditions 95°C for 3 min, followed by 35 cycles 94°C for 30 s, 52°C for 45 s and 72°C for 30 s, and a final extension at 72°C for 7 min. The resulting amplified products were checked on a 1.5% agarose gel for 60 min for 70 V. After the run was completed, the gel was treated with ethidium bromide and observed under UV (Integrated Biometra Gel Imager, Goettingen/Germany) and directly sequenced at Sentebiolab company. For sequencing of samples infected with the virus, another 40 µl PCR product was prepared and directly sequenced by the Sentebiolab (Company of Synthetic Biology, Turkey). Sequence alignment were carried out using Chromas (Technelsium DNA Sequencing Software Australia) software. After the checked of peaks and obtained the clear, sequences of these PCR products were obtained, and their results were compared with isolates in the NCBI database. The phylogenetic analysis of the isolates selected from NCBI according to the Construct/ Test Neighbor-Joining Tree method was carried out using the Mega 7 program.

RESULTS

In the survey studies, plants showing viral symptoms such as yellowing, chlorosis, deformity, mottling and curling were observed. It has been indicated that whitefly populations are common in greenhouses where yellowing symptoms were observed in eggplants. TYLCV, which is a serious problem in tomatoes and causes yellowing symptoms, has been observed to be noticeably yellowing and slight curling even though tomato plants do not cause serious curl in leaves and curling and becoming smaller in green areas. In ToCV symptoms, no curl occurred but growth retardation, shortening between the nodules and stunting were observed with yellowing. These results suggest that the cause of yellowing symptoms may be related to viral diseases, not fungal diseases.

The molecular analysis performed with DNA extract from 96 plant samples and 31 of them were yielded the expected 574 bp DNA fragment for ToCV; 44 of which were infected with TYLCV and showed presence of the expected 543 bp DNA (Fig. 2). 17 of the plants were detected as infected at the same time with both viruses. According to the results, 21 samples were found to be negative form both examined viruses.

RT-PCR and PCR studies showed that 23 of the simptomatic plants were not infected with any of viruses. It was thought that these plants may be infected with *Tomato infectious chlorosis* which is closely related to *Tomato chlorosis virus*. ToCV (574 bp) and TICV (500bp) are difficult to distinguish symptomatologically and their symptoms are similar to those of nutrient deficiencies. *Bemicia tabaci*, the vector of ToCV, is a common whitefly species in Antalya province, and on the contrary, the *Trialeurodes vaporarium* whitefly, the vector of TICV, can never be related to this species. As a result of molecular studies, 17 different samples were positively identified with both viruses in the study subject. A positive sample of 2 different viruses, which were infected with ToCV and TYLCV, were directly sequenced for identification of partial sequences. The resulting sequences were BLAST analyzed in the NCBI database and their

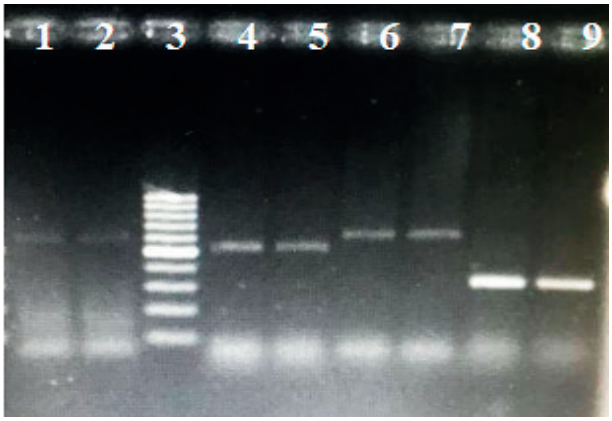

Fig. 2. 1–2: ToCV positive samples (574bp); 3: 100bp DNA Ladder; 4–5: TYLCV positive samples (543bp); 6–7: TYLCV-Is positive samples (634bp); 8–9 TYLCV-Mld positive samples infected plants (316bp)

similarities with other isolates recorded from different parts of the world were compared. Sequence analysis showed that the isolate TRAntToCVEgp is originated from Mediterranean region (Fig. 3b). When BLAST analysis was performed in NCBI database, our isolate TRAntToCVEgp (MK248741.1) showed 97% similarity with the isolate HG380084.1, Greece – *Lactuca sativa* isolate was closely related. ToCV-positive isolate which Genbank accession is MK248741.1 isolates

showed the highest similarity to isolates that reported in Greece (97%), Israel (96%), Portugal (96%) and Spain (95%) – Table 4. Also, TRAntToCVEgp located on the same branch with one of the Greek and Israeli isolates. The result indicates that the TRAntToCVEgp isolate identified in Antalya, Turkey most probably originates from Greece.

TYLCV-positive isolate Genbank accession is MK238543.1 showed 95% similarity with the isolate DQ423476.1 (Fig. 3a). In this study, only two of the strains of the *Tomato yellow leaf curl virus* have been identified in our region; TYLCV-Mild (19/44) and TYLCV-IL (25/44). Analysis of TYLCV strains showed that the world bases isolates clustered from 97 to 92% similarity with the Mediterranean countries (Tab. 5). Sequences of isolates with high similarity to our isolate have been subjected to phylogenetic tree analysis in the Mega 7 program.

The product size of the TYLCV-Mld isolate was 316 bp and the TYLCV-Is isolate was 634 bp and when the phylogenetic tree formed after BLAST analysis in NCBI database was examined, our TRAntTYLCVIs isolate was located in the same branch as the tomato isolate recorded in Turkey with GenBank accession number KM096585.1 and our TRAntTYLCVMld isolate was located in the same branch as pepper isolate recorded from Jordan with GenBank accession number FJ376387.1.

This result shows that the source of infection comes from a common background and these countries affect each other with the exchange of plant material. When the countries and isolates in the phylogenetic tree are examined, it becomes clear that there are countries that have interactions with each other in global trade. Not only was ToCV the same as our border neighbor country Greece (Fig. 3b), but also TYLCV was similar to Israel (Fig. 3a), where we had a vegetative exchange. Considering the fact that TYLCV and ToCV can be transmitted by whitefly which are able to distribute either migrate or wind ways to neighbor countries, infections of plants can be caused by vector transmission.

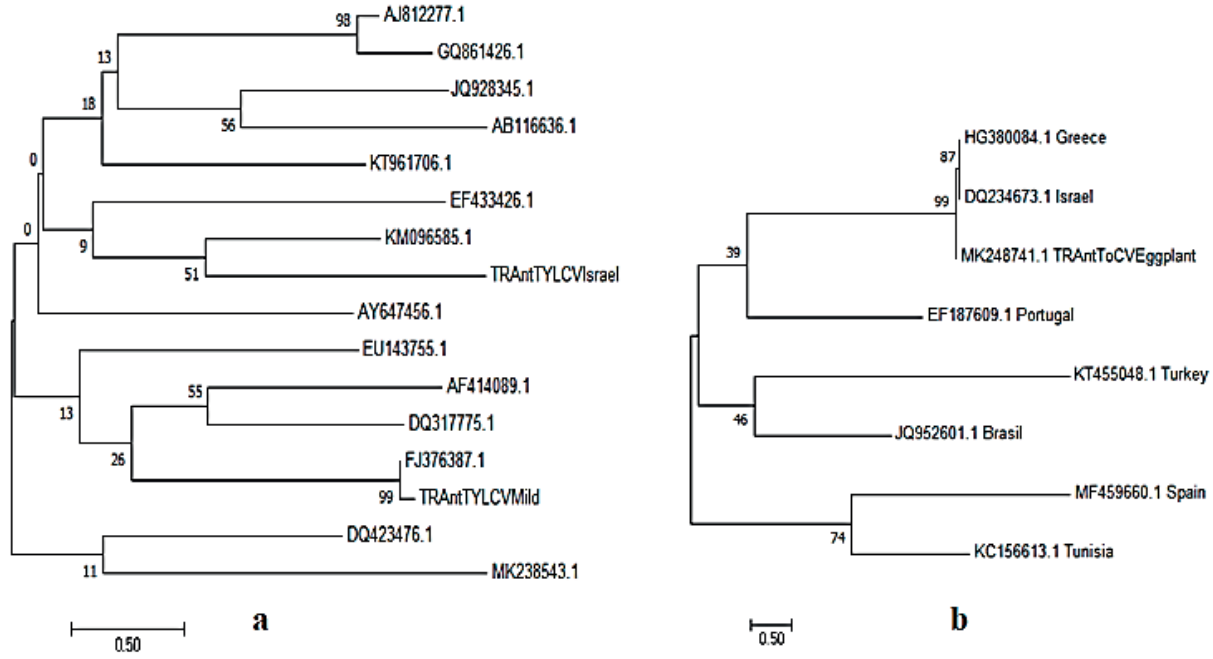

Fig. 3a: Phylogenetic analysis showing the relationship between TRAntTYLCVEgp (MK238543.1); **3b**: TRAntToCVEgp (MK248741.1) isolates with sequences from word bases

Table 3. Number of samples collected from surveys and number of samples infected with viruses on the basis of districts

Districts of Antalya	Samples	TYLCV positive plants	ToCV positive plants
Alanya	19	9	7
Aksu	16	3	9
Gazipaşa	11	8	–
Konyaaltı	15	6	3
Kumluca	22	11	8
Serik	13	7	4
Total	96	44	31

Table 4. World-based isolates with the highest similarity selected with the isolation of TRAntToCVEgp (MK248741.1) by nucleotide comparison in the NCBI blast system

No.	GenBank (accesion)	Host	Country	Identity (%)
1	KT455048.1	*Solanum lycopersicum*	Turkey	97
2	HG380084.1	*Lactuca sativa*	Greece	97
3	MF459660.1	*Solanum lycopersicum*	Spain	96
4	DQ234673.1	*Solanum lycopersicum*	Israel	96
5	JQ952601.1	*Solanum lycopersicum*	Portugal	96
6	EF187609.1	*Physalis ixocarpa*	Spain	95
7	KC156613.1	*Solanum elaeagnifolium*	Tunisia	95

Table 5. World-based isolates with the highest similarity selected with the isolation of TRAntTLYCVEgp (MK238543.1) by nucleotide comparison in the NCBI blast system

No.	GenBank (accesion)	Host	Country	Identitiy (%)
1	DQ317775.1	*Solanum lycopersicum*	Spain	99
2	EU143755.1	*Cucumis sativus*	Jordan	99
3	AF414089.1	*Capsicum annuum*	Cuba	98
4	EF433426.1	*Cucumis sativus*	Jordan	98
5	KT961706.1	*Solanum lycopersicum*	Iraq	97
6	KM096585.1	*Solanum lycopersicum*	Turkey	96
7	AJ812277.1	*Solanum lycopersicum*	Turkey	96
8	GQ861427.1	*Solanum lycopersicum*	Jordan	95
9	DQ423476.1	*Solanum lycopersicum*	Egypt	95
10	FJ376387.1	*Capsicum annuum*	Jordan	95
11	AB1166636.1	*Solanum lycopersicum*	Japan	95
12	AY647456.1	*Capsicum annuum*	Jordan	94
13	JQ928345.1	*Solanum lycopersicum*	Iran	92

DISCUSSION

This research showed that a large part of yellowing type symptoms were caused by the viral diseases. Eggplant crops are commonly infected by several viruses such as *Potato virus Y* (PVY), *Tomato spotted wilt virus* (TSWV) and *Eggplant mottled dwarf virus* (EMDV), which can infect eggplant plants, which have also been detected in our country [Colak-Ates et al. 2018, Kamberoğlu 2009, Osman and Baloglu 2018, respectively]. Our results provide evidence that this *Crinivirus* (ToCV), only discovered in diseased tomatoes so far, is spreading to other Mediterranean regions, causing severe symptoms, also in combination with other viruses. The unusual *Tomato chlorosis virus* and *Tomato yellow leaf curl virus* disease on eggplant has not been studied in our region. The symptomatology described is 'unusual', if it is considered that in eggplant plants the most significant effects of ToCV are the color alterations on the leaves (yellowing), while those of TYLCV occur of interveinal yellowing and curling on the leaves. It was found that yellowing type symptoms became more prominent in eggplants with mixed infection with TYLCV and ToCV. Mixed infection eggplants also showed distinguishable symptoms such as yellowing in tomato. Studies should be conducted on the losses caused by these viruses in single infections.

The Mediterranean region has large agricultural distribution areas and supplies of all kinds of commercial products. This showed the importance of TYLCV's natural distribution and occurrence in located areas. Our experiments showed the vector types present in tomato fields with molecular techniques and sanitary conditions for TYLCV were transmitted by whiteflies. Additionally, the presence of vector whiteflies in and out of the greenhouse, the loss of biological and chemical control agents, enhances the disease.

Bemisia tabaci (*Hemiptera*: *Aleyrodidae*) biotypes epidemiological presence and feeding behaviors for TYLCV strains on Tomato AgroEcology in Turkey have become well known as an agricultural host reservoir. Such studies will develop new management strategies for the dynamics of disease vectors to protect sanitized and resistant crops, frequently depending on vector and pathogen activation [Fidan et al. 2019].

Whiteflies are insects of the *Aleyrodidae* family that cause direct phloem-fed and indirect damage via virus transmission like *Begomoviruses* [Brown and Czosnek 2002, Jones 2003, Navas-Castillo et al. 2011]. TYLCV transmission by whiteflies is semi-persistent in circulation. The transmission of TYLCV by whiteflies circulates semi-permanently. Turkey's whiteflies and there are several reports on the economic damage [Bayhan et al. 2006, Ulusoy et al. 2012]. Morphological differentiation of whitefly biotypes is difficult. However, the possibility of molecular filtration [Papayiannis et al. 2009]. Most common whitefly biotypes in Turkey are biotype B and Q [Satar and Ulusoy 2016] similar with various reports around the world [Konjević et al. 2018].

The most important factor in spreading virus diseases is vector insects. Vector insects such as aphids, thrips, whiteflies have gained importance in vegetable growing in the Mediterranean region. In our region, the whitefly population is particularly high in areas close to sea level. The presence of emerging virus diseases spread by whiteflies significantly restricts the sustainable production of economically significant crops like tomatoes (*Solanum lycopersicum*), one of the world's largest vegetable crops [Navas-Castillo et al. 2011].

Antalya is an important place for greenhouse and open field production of *Solanaceace* family and the products grown in the region are very important for whitefly. Eggplant is in the same family with the tomato which is main host of whiteflies. Also important in rotation as well as in easy propagation, the high whitefly population with greenhouses and open spaces where polyculture is applied showed obvious yellowing symptoms as in tomato plants. It was observed that the eggplants showing the symptoms for yellowing also exhibited the *Tomato chlorosis virus* and *Tomato yellow leaf curl virus* disease. In coastal areas, where heat and whitefly population are high, ToCV and TYLCV infections have been established whereas in areas where the altitude is high and the temperature is low, this virus infection has not been found.

In summary, this study illustrated the presence of ToCV and TYLCV causing diseases in commercial eggplant crops in Turkey and characterized the symptomatology of the infection disorder caused in this host species. The coming about noticeable yield mis-

fortunes might seriously constrain eggplant generation in regions where expanded predominance happens, which in turn may be decided by changes in vector populaces. This will at that point posture a unused risk to eggplant, a edit of awesome financial significance around the world. A more complex epidemiological circumstance for ToCV and TYLCV are too anticipated in those zones ideal to infection nearness, basically in the event that both whitefly vectors, *B. tabaci* and *T. vaporariorum*, are display tomato, pepper and eggplant crops cover.

The management of TYLCV and ToCV in eggplant is difficult and costly both in development greenhouse and open field production. There are many different approaches for controlling these infections such as evacuating whiteflies and changing development season connected to diminish misfortunes due to TYLCV and ToCV. Since a single approach isn't successful. For this reason, a combination of chemical and biological control techniques for integrated pest management should be employed to reduce the population and migration of the whitefly vector and eliminate inoculum sources of TYLCV and ToCV.

Tomato chlorosis virus and *Tomato yellow leaf curl virus* that occurs diseases on eggplant have not been studied in our region. In this study, the present situation was revealed in the study on the absence of data on the fruit attitude, yield and quality changes of the eggplant infected with the virus disease. In the following studies, *Tomato yellow leaf curl virus* and *Tomato chlorosis virus*, which is one of the most common viruses, the effect on the yield needs to be investigated. Epidemiological studies suggest that *Tomato chlorosis virus* and *Tomato yellow leaf curl virus* diseases are highly related to the tomato isolate in the NCBI database. As a result of the cultivation of tomatoes and eggplants in the same family and often in the Antalya region, it was observed that these viruses reached the areas of tomato cultivation. It was determined that these two viruses carried by whiteflies caused severe yellowing symptoms in eggplants and some viruses showed infection in some plants. Therefore, the results proved that the unusual disorder found in greenhouse eggplants in Turkey can be associated with infections by TYLCV and ToCV, reported for the first time in a mixed infection.

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