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Virus diseases limiting greenhouses and open field production of cucurbits in Antalya province

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ABSTRACT

The Mediterranean region in Türkiye is noted for the production of cucurbits and other vegetables. As such, the aim of this study was to sample symptomatic cucurbits crops from open fields and greenhouses where there is active cultivation of these crops. Young shoot, leaves, and fruits exhibiting virus-like symptoms (yellowing, mosaic, necrotic) were collected. Out of 968 plant samples collected and tested through RT-PCR and PCR, 949 were discovered to be infected with several viruses. The identified virus diseases included ZYMV, WMV, PRSV, SqMV, CGMV, CYSDV, BPYV, CABYV, ToLCNDV and CMV. In terms of hosts exhibiting a high incidence of virus infections, cucumber (363 samples), squash (277 samples), melon (201 samples), and watermelon (108 samples) emerged as the top four hosts. Additionally, viruses with notable high incidences in the collected samples, as recorded through molecular testing in decreasing order included ZYMV at 28.1%, CYSDV at 15.5%, and WMV at 14.4%. Also, 90% of samples collected from open fields had single or multiple infection. In contrast, 26.4% of samples from greenhouses exhibited mosaic symptoms and 74.6% showed yellowing symptoms. Notably, MNSV and BPYV, were detected in these samples. The samples also exhibiting mixed infections predominantly displayed mosaic symptoms, including mixed infections such as ZYMV with WMV, ZYMV with CMV, CMV with WMV, and CMV with PRSV. In contrast, samples obtained from open fields showed a higher prevalence of yellowing symptoms, such as ToLCNDV with CMV, ToLCNDV with ZYMV, ZYMV with CMV with CYSDV, and ZYMV with CMV with CVYV.

1. Introduction

The family Cucurbitaceae, referred to as the gourd family within the order Cucurbitales, predominantly inhabits tropical and subtropical regions. Cucurbitaceae consist of approximately 975 species distributed across roughly 98 genera of flowering plants (Xu and Chang 2017). Commercially cultivated members of the Cucurbitaceae family in Türkiye include pumpkin (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), cantaloupe (*Cucumis melo*), cucumber (Cucumis sativus), Armenian cucumber (*Cucumis melo* var. *flexuosus*), butternut squash (*Cucurbita moschata*), and ornamental gourd (*Lagenaria siceraria*).

Pumpkin, an annual vegetable, is cultivated in all parts of the world, however, its production is more dominant in tropical and subtropical regions (Whitaker and Bemis 1975). Fruits of various varieties exhibit diverse shapes, sizes, and colors. The global pumpkin production is estimated at 2.8 million tons over an area of 1.5 million hectares with China leading production with 7.4 million tons, followed by Ukraine with 1.3 million tons, Russia with 1.2 million tons, and the USA with 1.1 million tons. Türkiye's significant economic income earned from pumpkin cultivation amounts to 771.651 tons (FAO 2021).

Watermelon, an herbaceous annual plant within the Cucurbitaceae family, is believed to have been initially cultivated in Africa (Decoteau 2000). Its distribution and cultivation in Anatolia and Europe are estimated to have occurred in the late 14th to early 15th centuries (Kütevin and Türkeş 1987). This crop is reportedly the most extensively cultivated among several cucurbit worldwide, with a total production volume of 101.6 million tons over an area of 3million hectares. China dominates global production with 60.9 million tons, while Türkiye ranks second with 3.5 million tons (FAO 2021).

Melon, a traditional crop of Asian origin, has been cultivated across a wide geographic range since around 2000 BCE (Robinson and Walters 1997). According to FAO data, melon is cultivated across 1.1million hectares globally, yielding a total production of 28.6 million tons. Unsurprisingly, China leads its production with 14 million tons, followed by Türkiye with 1.9 million tons (FAO 2021).

Cucumber, an annual herbaceous plant, is reportedly one of the oldest cultivated vegetables, dating back to 3000 years ago (Yawalkar 1985). It ranks second only to watermelon in terms of global production quantity, with a total of 93.5 million tons produced across 2.2 million hectares. China, Türkiye, Russia, Ukraine, and Mexico are the top cucumber producing countries in the world (FAO 2021). However, yield of these crops are severely threatened by biotic stresses including, bacteria, viruses, fungi, insects, nematodes and other pathogens.

Viruses which cause yield and quality losses in cultivated plant species are notably distinct from other plant pathogens. The absence of chemical control measures for viruses and the insufficient implementation of existing controls by growers contribute to more devastating symptoms and greater yield losses compared to those caused by other plant pathogens.

Cucurbits serve as hosts to various viral pathogens, resulting in economic losses due to disease. Nearly 60 viral pathogens have been reported on cucurbits with the notable ones being Zucchini yellow mosaic virus (ZYMV), Cucumber mosaic virus (CMV), Watermelon mosaic virus (WMV), Papaya ringspot virus (PRSV), Melon necrotic spot virus (MNSW), Cucurbit chlorotic yellows virus (CCYV), Beet pseudo-yellows virus (BPYV), Squash mosaic virus (SqMV), Cucurbit yellow stunting disorder virus (CYSDV), and Cucumber vein yellowing virus (CVYV) (Lecoq and Desbiez 2012).

The presence of more than 60 viruses, with varying modes of transmission (seed-borne and insect vectors, mechanical) often leads to confusion with symptoms that are due to environmental stresses. In order to mitigate this confusion and assess the status of viral diseases in cucurbits in both open field and greenhouse settings, surveys were conducted over a span of two years in the Antalya province. This study aimed to elucidate the current situation in both open field and greenhouse environments, as well as to identify any new potential harmful viral diseases. Based on the symptoms exhibited by viral diseases, the study proceeded to categorize them into three classes, as shown in Table 1a.

As observed in Table 1a viruses transmitted by whiteflies are characterized by yellowing symptoms whereas those transmitted by seeds and aphids, or leafhoppers, typically result in mosaic-like symptoms, and the others, transmitted by fungal pathogens, are majorly distinguished by necrotic lesions on infected plants. For this purpose, samples in surveys were examined in three broad symptom groups: yellowing, mosaic, and necrotic.

2. Materials and Methods

2.1. Sample collection

Survey studies were conducted by visiting open fields and greenhouses where cucurbit cultivation took place from the easternmost (Gazipaşa, Alanya, Serik Aksu) to the westernmost (Demre, Kumluca Finike, Elmalı) parts of Antalya province. Cucurbit samples collected during these studies were observed based on symptoms (yellowing, mosaic, and necrotic) and collected using a symptom-driven sampling method. Plant samples showing symptoms typical of viral infections were obtained from young shoot leaves and fruits.

The collected plant samples were packaged in plastic bags and placed in a cold chain and then taken to the laboratory with each sample bearing the information of location of collection, date of collection, as well as the species or variety of the plant. They were then stored in a deep freezer at -20°C.

2.2. Nucleic acid extraction, PCR (RT-PCR) and gel electrophoresis

Total nucleic acid (TNA) isolation was carried out on all collected leaf samples. The CTAB method as previously reported by Doyle and Doyle (1990) was slightly modified and used for isolation. Isolated nucleic acids were adjusted to a concentration of 80-100 ng using the nanodrop spectrophotometer.

For RNA-based viruses, RT-PCR protocols were applied using the One-step RT-PCR kit purchased from Thermo Scientific. In addition, specific primer pairs listed in Table 1b were used for the virus identification of viral pathogens. In the study, only Tomato yellow leaf curl virus (TYLCV) was identified as a DNA-based pathogen among the viral pathogens. To detect the presence of TYLCV, PCR studies were conducted using Dream Taq Green Buffer Master mix and its protocol from Thermo Scientific with specific primer pairs listed in Table 1b.

The total volume of the study was prepared to be 25 µl. For the detection of each virus, 1 µl of TNA containing an average of 80-100 ng RNA/DNA was used. After, PCR products were run on 1.5% agarose gel for an hour. The gel was stained with a solution containing ethidium bromide at a concentration of 0.5 µg ml^{-1}, and resulting bands were visualized under UV light.

3. Results

In the survey conducted in Antalya province, a total of 968 plant specimens were collected, with 949 testing positive for various viral infections through molecular analyses (Table 2a). Molecular testing identified 11 distinct viral pathogens, including ZYMV, WMV, PRSV, SqMV, CGMV, CVYV, CYSDV, BPYV, CABYV, ToLCNDV, and CMV (Figure 1). Separate studies in Korea and Europe identified seven viruses - WMV, ZYMV, PRSV, CFMMV, CGMMV, KGMMV, and ZGMMV in cucurbit crops (Lee et al. 1981; Kim et al. 1995; Lee et al. 1990; Ko et al. 2007; Ryu et al. 2000; Rhee et al. 2010; Kwon et al. 2014).

Host-specific virus infections were investigated, revealing cucumber as the crop with the highest number of positive samples (363). Second to cucumber was pumpkin which exhibited 277 positive samples for the examined viruses, while melon had 201 samples, and watermelon displayed the lowest infection rate with 108 testing positive for the viruses.

Upon analysis of the data presented in Tables 2a and 2b it becomes evident that ZYMV predominates among the viral pathogens tested. Specifically, out of 267 leaf and fruit samples, ZYMV was detected in 28.1% of samples collected. This finding is coherent with the report by Kamberoglu et al. (2016), where ZYMV was determined as a prevalent virus in the Çukurova region (Adana and Mersin). Secondly, 147 samples tested positive for CYSDV (15.5%). Following closely, in third place, 137 samples showed presence of WMV (14.4%). Research conducted in China has consistently reported the widespread prevalence of WMV across nearly all regions of cucurbit production (Zhang et al. 2007; Tian et al. 2016; Wang et al. 2017; Zhao et al. 2022).

Upon examination, based on the cultivation environment, it was observed that out of 502 samples collected from open fields, 452 were found to be infected with mosaic-type symptomatic viruses (90.03%). Among the samples exhibiting yellowing symptoms in open fields (Figure 2a, b) (Figure 3), 50 samples showed a 10% infection rate. Among the samples collected from greenhouses, 118 (26.4%) out of the total (447) were discovered to be infected with mosaic-causing viruses (Figure 4) (Figure 5a and b). Moreover, 329 samples collected from greenhouses exhibited yellowing symptoms, with a 74.6% infection rate.

Upon examination of symptom types as delineated in Tables 2a and 2b, it becomes evident that among mosaic-type viral pathogens Zucchini yellow mosaic virus (ZYMV) prevail as the most common. Specifically, ZYMV was predominantly detected in open-field pumpkin species, with 89 positive samples, followed by 65 positive samples in cucumbers, 35 positive samples in melons, and 27 positive samples in watermelons.

No viral pathogens showing necrotic symptoms were observed. From all of the samples collected 24 randomly selected samples were subjected to RT-PCR using specific primers for MNSV, all of which tested negative. In Turkey, MNSV was first detected in cucumber species in 2010 by Fidan et al. (2010). A subsequent study by, Koç et al. (2014) confirmed the presence of the agent in pumpkins, indicating an expanded inter-species host range. Kwak et al. (2015) reported the detection of four different strains of MNSV in watermelon plants in different regions of Korea.

Among viral pathogens showing yellowing symptoms, CYSDV was the most predominant. 58 of the samples which were collected from covered cucumber fields were confirmed positive for CYSDV (Figure 2b). This was followed by 41 positive samples collected from covered melon fields and 24 positive samples in covered pumpkin fields. Additionally, Brown et al. (2007) reported that economic losses attributed to CYSDV could be as high as 80% in highly susceptible melon varieties (Arslan et al. 2020a)

ToLCNDV was detected in greenhouse production as follows :31 melon samples, 24 cucumber samples 18 pumpkin samples and 4 watermelon samples, In open field production ToLCNDV was detected in13 cucumber samples, 9 melon samples, 6 pumpkin and 3 watermelon samples. (Figure 3). Panno et al. (2016) identified ToLCNDV in pumpkin species cultivated in the open fields of Sicily, Italy, while Juárez et al. (2014) were the first to report ToLCNDV detection in pumpkin plants.

Table 1(a). Modes of Transmission and Symptom Types Induced by Viral Pathogens

Type of symptoms	Plant virus	Mode of transmission
Yellowing and chlorosis	Cucumber vein-yellowing virus (CVYV)	Silverleaf whitefly *(Bemisia tabaci)*
	Cucurbit yellow stunting disorder virus (CYSDV)	Silverleaf whitefly *(Bemisia tabaci)*
	Beet pseudo-yellows virus (BPYV)	Greenhouse whitefly *(Trialeurodes vaporariorum)*
	Cucurbit aphid-borne yellows virus (CABYV)	Several aphid species
	Tomato Leaf Curl New Delhi virus (ToLCNDV)	Silverleaf whitefly *(Bemisia tabaci)*
	Cucurbit chlorotic yellows virus (CCYV)	Silverleaf whitefly *(Bemisia tabaci)*
Necrosis	Melon necrotic spot virus (MNSV)	*Olpidium bornovanus* (Fungi)
Mosaic	Zucchini yellow mosaic virus (ZYMV)	Aphis spp.
	Watermelon mosaic virus (WMV)	Aphis spp.
	Papaya ringspot virus (PRSV)	Aphis spp.
	Squash mosaic virus (SqMV)	Striped cucumber beetle (*Acalymma spp.)* Spotted cucumber beetle (*Diabrotica spp.)*
	Cucumber mosaic virus (CMV)	Aphis spp.
	Cucumber green mottle mosaic virus (CGMMV)	Mechanical, Seed

Table 1(b). The list of PCR primer pairs used for virus diseases in this study

Virus diseases	Primer Pairs	Annealing temperature (°C)	Amplicon size (bp)	References
CVYV	CVYVF: AGCTAGCGCGTATGGGGTGAC CVYVR: GCGCCGCAAGTGCAAATAAAT	55	450	Papayiannis et al. 2005
CCYV	CCYVF: TCCCGGTGCCAACTGAGACA CCYVR: TACGCGCGGCAGAGGAATTT	55	375	Sarıkaya et. al. 2023
CYSDV	CYSDVF: AGTGACATGCCTAACTGTTACTT CYSDVR: ATAGCTGCTGCAGATGGTTC	54	364	Papayiannis et al. 2005
BPYV	BPYVF: TCGAAAGTCCAACAAGACGT BPYVR: CTGATGGTGCGCGAGTG	55	251	Papayiannis et al. 2005
CABYV	CABYVF: GAATACGGTCGCGGCTAGAAATC CABYVR: CTATTTCGGGTTCTGGACCTGGC	62	600	Kassem et al. 2007
TOLCNDV	To-A1F: GGGTTGTGAAGGCCCTTGTAAGGTGC To-A1R: AGTACAGGCCATATACAACATTAATGC	55	504	Fidan et al. 2023a
MNSV	MNSVF: CTCCATAAGCGCCAAGCAACC MNSVR: AGCGGGGGAAAACAGAAGAA	50	485	Koç et al. 2014
ZYMV	ZYMVF: TCACCACACATGGAGTTTC ZYMVR: ATGCAACCTTGTTGAGCA	61	550	Zhao et al. 2015
WMV	WMVF: GGCTTCTGAGCAAAGATG WMVR: CCCAYCAACTGTYGGAAG	53	408	Desbiez et al. 2009
PRSV	PRSVF: ATCACAATGATTACGCGCTGCG PRSVR: CTCTCATTCTAAGAGGCTCGAATAG	59	1200	Usharani at al. 2013
SqMV	SqMVF: TTACAGACTTGGCTCTAGTG SqMVR: AAATAACAGCATCTGGCATAT	61	550	Zhao et al. 2015
CMV	CMVF: TAACCTCCCAGTTCTCACCGT CMVR: CCATCACCTTAGCTTCCATGT	52	513	Fidan et al. 2023b
CGMMV	CGMMVF:CTAATTATTCTGTCGTGGCTGCGGATGC CGMMVF: CTTGCAGAATTACTGCCCATA	56	976	Tian and Posis 2014

Table 2(a). Viral pathogens identified through PCR/RT-PCR tests conducted on collected cucurbit samples

Symptom types	Plant virus	Cucumber		Pumpkin		Melon		Watermelon		Total
		F*	GH*	F*	GH*	F*	GH*	F*	GH*	
Mosaic	ZYMV	65	12	89	27	35	8	27	4	267
	CMV	42	13	11	12	4	5	6	4	97
	WMV	43	4	27	13	21	-	25	4	137
	PRSV	23	4	14	12	-	-	12	-	65
	SqMV	1	-	3	-	-	-	-	-	4
	CGMV	-	-	-	-	-	-	4	-	4
Necrosis	MNSV	-	-	-	-	-	-	-	-	0
Yellowing	CVYV	3	42	-	6	-	13	-	4	68
	CCYV	-	-	-	8	-	-	-	-	-
	CYSDV	4	58	3	24	6	41	3	8	147
	BPYV	-	-	-	-	-	-	-	-	0
	CABYV	-	12	-	4	-	28	-	-	44
	ToLCNDV	13	24	6	18	9	31	3	4	108
	TOTAL	194	169	153	124	75	126	80	28	949

*F: Open Field * GH: Greenhouse

Table 2(b). Percentages of Viral Pathogens Observed in Cucurbit Species

Plant virus	Cucumber (363)	Pumpkin (277)	Melon (201)	Watermelon (108)	Total	%
CVYV	45	6	13	4	68	7.1
CYSDV	62	27	47	11	147	15.5
BPYV	0	0	0	0	0	0
CABYV	12	4	28	0	44	4.6
ToLCNDV	37	24	40	7	108	11.4
CCYV	0	8	0	0	8	0.84
MNSV	0	0	0	0	0	0
ZYMV	77	116	43	31	267	28.1
WMV	47	40	21	29	137	14.4
PRSV	27	26	0	12	65	6.8
SqMV	1	3	0	0	4	0.42
CMV	55	23	9	10	97	10.22
CGMMV	0	0	0	4	4	0.42

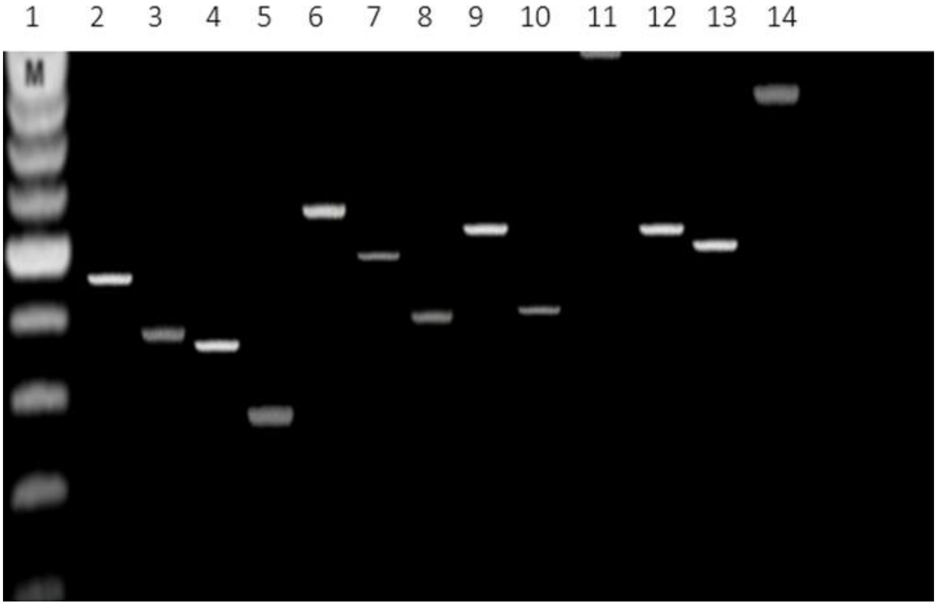

Figure 1. Agarose gel electrophoresis results of PCR and RT-PCR, 1) 100bp DNA marker; Lane 2) MNSV: 485 bp; Lane 3) CCYV: 375 bp; Lane 4) CYSDV: 364 bp; Lane 5) BPYV: 251 bp; Lane 6) CABYV: 600 bp; Lane 7) ToLCNDV: 504 bp; Lane 8) WMV: 408 bp; Lane 9) ZYMV: 550 bp; Lane 10) CVYV: 450 bp; Lane 11) PRSV: 1200 bp; Lane 12) SqMV: 550 bp; Lane 13) CMV: 513 bp; Lane 14) CGMMV: 976 bp.

Figure 2. (a) The yellowing type symptoms of CVYV observed in cucumber plants and melon plants, (b)The symptoms of CYSDV observed in cucumber plants (A, B) and melon plants, as well as the symptoms of CABYV observed in melon plants (C).

Figure 3. Symptoms of ToLCNDV observed in cucumber plants (A) and pumpkin plants (B, C).

Figure 4. Symptoms of PRSV observed in pumpkin fruit (A) and symptoms of WMV observed in watermelon fruit (B, C).

The least recorded viral pathogens were Squash Mosaic Virus (SqMV) and Cucumber Green Mottle Mosaic virus (CGMMV) (Figure 5b), with infection rates of 0.42%. No detections of Beet pseudo-yellows virus (BPYV) and Melon necrotic spot virus (MNSV) were recorded. According to Bi et al. (2019), the rapid spread of CGMMV in watermelon cultivation has led to massive yield reductions and substantial economic losses in regions such as Israel (Reingold et al. 2013) and Australia (Tesoriero et al. 2016). Noteworthy, CGMMV was exclusively identified as a viral pathogen in watermelon in this study.

CCYV, a newly reported Crinivirus with limited geographical distribution, has been reported only in Japan (Gyoutoku et al. 2009), Taiwan (Huang et al. 2010), and China (Gu et al. 2011; Kavalappara et al. 2021). In this study, CCYV was detected in pumpkin plants at a rate of only 0.84%.

PRSV was found in cucumber, pumpkin, and watermelon but not in melons. CMV, ToLCNDV, CYSDV, and ZYMV were observed in all species examined (cucumber, melon, watermelon, and pumpkin) and in both greenhouse and open field environments. A decade ago, the presence of WMV, ZYMV, CMV, CABYV, and PRSV agents in cucumber, melon, watermelon, and pumpkin plants grown intensively in Diyarbakır and Mardin provinces was determined by Kızmaz et al. (2016). In Kastamonu province and its surroundings, where cucurbits are grown, the presence of WMV, CMV, ZYMV, and PRSV was confirmed, while SqMV infection was not reported in the study (Topkaya 2020).

In total, the top five pathogens observed in cucurbits, in order of frequency, were ZYMV (28.1%), CYSDV (15.5%), WMV (14.4%), ToLCNDV (11.4%), and CMV (10.22%) (Figure 6). China accounts for approximately 64.02% of cucumber production, making it the largest producer of cucurbits (FAO 2021). Among the most significant RNA viruses affecting the cucurbit group in China are Tobacco mosaic virus (TMV), ZYMV, WMV, CGMMV, and CMV (Liu et al. 2019; Desbiez et al. 2009 Zhao et al. 2022).

Figure 5. (a) Symptoms of ZYMV observed on watermelon plant (A), cucumber plant (B), pumpkin plant (C), and cucumber fruit (D). (b) Symptoms of CGMMV observed on watermelon leaves (A, B) and watermelon fruit (C).

Figure 6. Symptoms of CMV (1), PRSV (2), WMV (3), and ZYMV (4) observed in squash plants.

Mixed infections have been identified in both greenhouse and open field cultivation. These are typically observed as mixed infections of viruses causing mosaic symptoms, while in some cases, they appear as mixed infections of viruses causing both mosaic and yellowing symptoms. When mixed infections are examined according to the cultivation environment, mosaic-type symptoms ZYMV+WMV with 18 positive samples, ZYMV+CMV with 21 positive samples, CMV+WMV with 14 positive samples, CMV+PRSV with 5 positive samples were more commonly observed in greenhouse cultivation conditions, whereas yellowing-type symptoms ToLCNDV+CMV with 9 positive samples, ToLCNDV+ZYMV with 3 positive samples, ZYMV+CMV+CYSDV with 3 positive samples, ZYMV+CMV+CVYV with 4 positive samples, were more prevalent in open fields, as confirmed in this study.

Mixed viral infections manifest as mosaic+mosaic symptoms (ZYMV+WMV, ZYMV+CMV, CMV+PRSV) and mosaic+yellowing symptoms (ToLCNDV+CMV). Upon examination of triple infections, mosaic+yellowing symptoms (ZYMV+CMV+CVYS, ZYMV+CMV+CYSDV) have beenobserved. In 2020, mixed infections of WMV+ZYMV and WMV+PRSV were found in cucurbit fields in Kastamonu (Topkaya 2020).

4. Discussion

When examining viral diseases that limit cucurbit production based on symptoms, it was observed that they could be categorized into three groups. Among samples displaying symptoms of yellowing, 375 out of 384 were found to be infected with one or more viruses causing yellowing. Similarly, out of 584 samples showing mosaic-like symptoms, 574 were found to be infected with viruses. No samples showing necrotic symptoms were found. However, 24 randomly selected samples displaying necrotic symptoms were tested and found to be negative for viral infection. These results indicate the effectiveness of symptomology in distinguishing virus-infected areas within cucurbit crops.

Observations based on symptoms revealed that whitefly-transmitted viruses predominantly caused symptoms of yellowing. Specifically, only CABYV was identified as a virus transmitted by aphids and causing yellowing. Mosaic-like symptoms were generally attributed to viruses transmitted by aphids, seed, and mechanical means. It was observed that viruses causing yellowing symptoms were prevalent in greenhouses, while those causing mosaic-like symptoms were common in open fields (Arslan et al. 2020b). Factors contributing to the prevalence of viruses causing yellowing symptoms in greenhouses include the conducive environment created by unsuitable climate conditions for protected cultivation, allowing whiteflies to thrive. Additionally, the development of resistance of whiteflies against effective pesticides hinders their population control. The widespread of monoculture through the large cultivation of a single economic crop on a large area of land quickens the adaptability of the virus to a host and thereby contributing to their easy spread.

In open fields, the prevalence of mosaic-like symptoms is attributed to factors such as dense planting, larger cultivation areas compared to protected cultivation, and less frequent monitoring and maintenance, leading to increased mechanical transmission. Furthermore, the inability to conduct timely spraying during periods of heavy rainfall and overcast weather in open fields, unlike in protected cultivation, contributes to the highest incidence of mosaic-like symptoms.

Observations indicated that viruses causing symptoms of yellowing were intense in greenhouses, while mosaic-like symptoms were prevalent in open fields, initially transmitted by aphids and later dispersed mechanically during harvesting. Symptoms observed in cucurbit viruses provide a general idea of which viruses are present. However, leaf symptoms caused by ZYMV, PRSV, and WMV are very similar, making it impossible to distinguish these viruses based solely on leaf symptoms. Therefore, in areas where symptoms are observed, testing must be conducted using DAS-ELISA, RT-PCR, and qRT-PCR techniques to distinguish among the three viruses in cases of mixed infections. This study demonstrated the reliable use of RT-PCR/PCR techniques in viral disease diagnostics.

In areas where viral diseases are prevalent in cucurbits, effective vector control, including the use of biological control agents against vectors, and proper weed management are essential. Moreover, given that ZYMV is the most widespread virus in this study and globally, preference should be given to varieties resistant or tolerant to viruses. Otherwise, efforts should focus on breeding for resistance or employing molecular techniques such as CRISPR-Cas9. With the continuous emergence of new virus species or entirely new viruses, overcoming them economically is becoming increasingly challenging. Viruses possess significant potential to adapt to natural selection pressures due to factors such as large population sizes and the absence of repair mechanisms in their genomes, enabling rapid replication.

In summary, research is needed to understand the distribution of reservoir plants for viruses and their transmission to cultivated plants, the detection and characterization of local isolates from different locations inTürkiye, including both wild and cultivated forms, and the collection of isolates from common host pools at specific times, which may lead to the formation of new sub-isolates capable of breaking resistance in plants. The impact on agricultural ecology and economic losses in agricultural fields due to both mixed infections and the development of new strains through mutation cannot be predicted.

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