



Determination of Zucchini Yellow Mosaic Virus and Watermelon Mosaic Virus Infections in Cucurbit Production Areas of Çanakkale Province from Türkiye

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ABSTRACT

Viral diseases are among the most significant challenges in protecting plants of the *Cucurbitaceae* family, with viruses from the Potyvirus genus, such as zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV), causing up to 100% yield losses under favorable conditions. Despite the importance of these viruses, there have been no previous studies investigating potyvirus diseases in *Cucurbitaceae* production areas in Çanakkale province. Consequently, the status of these diseases in the region remains unknown. This study aims to address this gap by analyzing the presence of potyviruses in *Cucurbitaceae* production areas in Çanakkale. In the 2021 production year, a total of 137 samples exhibiting virus and virus-like symptoms were collected from various *Cucurbitaceae* production sites in Çanakkale province and its districts. The samples were tested using RT-PCR with primer pairs specific to WMV and ZYMV. From the infected samples, seven isolates were selected for further analysis, and the coat protein (CP) genes were amplified and sequenced. The results revealed that WMV was detected as a single infection in 78 samples, ZYMV in one sample, and mixed infections of ZYMV+WMV were found in 39 samples, indicating that WMV is notably prevalent in Çanakkale. Bioinformatics analyses demonstrated that the Turkish WMV and ZYMV isolates share more than 90% similarity with other isolates in both the local samples and the GenBank database. Phylogenetic analysis further revealed that Turkish WMV and ZYMV isolates are closely related to each other. This is the first study to reveal the presence and phylogenetic relationships of ZYMV and WMV in *cucurbitaceous* plants in Çanakkale province of Türkiye.

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Introduction

Viruses that negatively impact plant growth and fruit production pose a significant threat to yields in *Cucurbitaceae* cultivation. *Cucurbitaceae* species are known to host over 90 viruses, with approximately 15 belonging to the Potyvirus genus within the *Potyviridae* family. Among the most widespread potyviruses affecting cucurbit production globally are watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) (Sharma, 2023).

Potyvirus which comprises the largest number of species among plant-infecting viruses, is responsible for substantial economic losses in cultivated plants worldwide. The genus includes 167 pathogenic species that infect a broad range of many plants. Symptoms caused by potyviruses in plants changeable based on the virulence, species, and strain of the virus. Common symptoms include mosaic or streak patterns on leaves, chlorosis, leaf deformation, stunting, and even plant death (Revers and García, 2015; Abd El-Aziz, 2020).

Potyviruses are primarily transmitted by aphids in a non-persistent manner with over 200 vector aphids. Some

potyviruses are also known to be seed-transmitted (Nigam et al., 2019). Potyviruses are characterized by their filamentous, non-enveloped, helical symmetry, measuring 680–900 nm and 11–13 nm in length and in width, respectively. They possess a single-stranded, positive-sense RNA genome, ranging from 9.300 to 10.800 nucleotides (Inoue-Nagata et al., 2022).

Numerous studies on cucurbit viruses in Türkiye have been reported that these infections are widespread and significantly diminish the market value of affected crops (Özer et al., 2012; Kamberoğlu et al., 2016; Kamberoğlu and Keçe, 2016; Güller and Usta, 2019; Topkaya et al., 2019; Güller and Usta, 2020; Yeşil, 2021; Karanfil, 2022; Karanfil et al., 2023; Güller et al., 2024a). Similar studies conducted in various provinces of the Marmara region have indicated that potyviruses are prevalent in *Cucurbitaceae*, causing severe mosaic symptoms on leaves and fruit deformations such as blistering or warts, which render the fruits unmarketable (Köklü and Yılmaz, 2006; Kaya and Erkan, 2011; Karakurt, 2015; Altınay, 2017; Karabulut, 2020; Sarı, 2023; Güller et al., 2024b). Despite their

economic impact, no comprehensive study has been conducted on the diagnosis and molecular characterization of cucurbit viruses in Çanakkale province. Therefore, this study aims to identify and molecularly characterize the economically important potyviruses, ZYMV and WMV, in cucurbit-growing regions across Çanakkale and its districts.

Materials and Methods

Sampling

Field studies for sample collection were conducted during the summer and autumn seasons of 2021. The sampling covered various locations in Çanakkale province and its districts, including open fields, greenhouses, and home gardens. Samples were collected from several *Cucurbitaceae* species, including pumpkin (*Cucurbita pepo*), squash (*Cucurbita* spp.), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), melon (*C. melo*), bottle gourd (*Lagenaria siceraria*), and snake melon (*C. melo* var. *flexuosus*). During the sampling, plants with symptoms such as yellow mosaic patterns, leaf deformation, blistering, excessive growth or stunting, fruit deformation, and discoloration; indicative of viral and virus-like infections were specifically targeted. In fields where multiple plants appeared infected, a maximum of three samples were collected to represent the area adequately. The samplings were carried to the plant virology laboratory in ice bag to ensure their integrity. As a result of the field studies, samples were collected from eight districts of Çanakkale province.

Testing

To determine the infection of WMV and ZYMV in the samplings from Çanakkale province and its districts, the RT-PCR method was employed. Initially, total RNA was isolated using the CTAB (cetyltrimethyl-ammonium bromide) procedure (Li et al., 2008). The isolated RNA samples were then subjected to denaturation, reverse transcription (RT), and PCR (amplification) steps sequentially. For the RT-PCR tests, WMV-specific primer pairs from Ali et al. (2012) and ZYMV-specific primers from Khanal et al. (2021) were used (Table 1). The RT-PCR test conditions were established according to the protocols of the respective primer references (Ali et al., 2012; Khanal et al., 2021).

Molecular Characterization Studies

The molecular characterization of WMV and ZYMV isolates were conducted based on their coat protein (CP) gene regions. For molecular characterization, a total of seven isolates were selected from those identified as infected with WMV and ZYMV, considering different districts and host species (Table 2). The selected isolates of CP gene were amplified using PCR, and the resulting PCR products were addressed to sequencing. The nucleotide sequencing of the WMV and ZYMV isolates was carried out by a commercial service provider (BMLabosis, Ankara). Amino acid sequences of the ZYMV and WMV isolates were derived from the obtained sequence data. These data were subsequently used to perform multiple sequence alignments and phylogenetic analyses, comparing the similarities of the selected isolates with each other and with other global isolates.

Sequence Analysis and Phylogenetic Studies

The nucleotide and amino acid sequences obtained from WMV and ZYMV isolates in Çanakkale were aligned using Clustal W in the CLC Main Workbench V.20 software. Comparisons were performed between the nucleotide and amino acid sequences of the isolates themselves and with other global isolates (Table 3 and 4) to assess similarity percentages. The similarities between the selected isolates and other global isolates were also analyzed using Clustal W in the CLC software.

To further determine the percentage similarity of WMV and ZYMV isolates, the Sequence Demarcation Tool Version 1.2 (SDTv1.2) was used to create a similarity matrix. This matrix was color-coded according to the percentage similarity values obtained (Muhire et al., 2014).

Phylogenetic analyses of the WMV and ZYMV isolates were conducted using CP gene sequences at the nucleotide level, utilizing the multiple sequence alignment files generated by Clustal W. Phylogenetic relationships between global isolates and the WMV and ZYMV isolates were examined using the neighbor-joining method to construct phylogenetic trees. A 1000-replicate bootstrap analysis was performed to statistically validate the accuracy of the constructed phylogenetic trees. ZYMV was used as an outgroup to determine the phylogenetic relationships of WMV isolates and ZYMV was used as an outgroup to determine the phylogenetic relationships of WMV isolates.

Table 1. Primer pairs specific to coat protein genes of zucchini yellow mosaic virus and watermelon mosaic virus.

Primer Direction	Primer Sequence	Amplificon Length	Reference
Forward	GAACAAGGAGACACTGTGAT	902	Khanal et al., 2021
Reverse	GCAGCGAAACAATAACCTAG		
Forward	AACACACAACCAAGTGAATT	979	Ali et al., 2012
Reverse	TAACGACCCGAAATGCTAACT		

Table 2. Information for watermelon mosaic virus and zucchini yellow mosaic virus isolates used in molecular characterization studies

Species	Isolate Code	Districts	Host
WMV	WVM10	Çan	Watermelon
	WMV47	Eceabat	Melon
	WMV78	Bozcaada	Pumpkin
	WMV88	Merkez	Bottle gourd
ZYMV	ZYMV9	Çan	Melon
	ZYMV10	Çan	Watermelon
	ZYMV29	Gelibolu	Melon

Table 3. Information for watermelon mosaic virus isolates retrieved from genbank

Accession Number	Host	Origin	Isolate Code
MG952635.1	<i>Cucumis melo</i>	Türkiye	Alakoy 2
MG952634.1	<i>Cucumis melo</i>	Türkiye	Alakoy 1
LC434453.1	<i>Panax ginseng</i>	South Korea	AS
LC434452.1	<i>Panax ginseng</i>	South Korea	SJ
MN814408.1	<i>Bromus sp.</i>	Spain	E1P_87
MN814378.1	<i>Cucumis melo</i>	Spain	M3V_6
KC447295.1	<i>Citrullus lanatus</i>	Saudi Arabia	WMV-SA
KF021299.1	<i>Cucurbita pepo</i>	Türkiye	W26
KF021300.1	<i>Cucurbita pepo</i>	Türkiye	W59
KF021298.1	<i>Cucumis sativus</i>	Türkiye	W2
MZ130405.1	<i>Cucumis melo</i>	Türkiye	Igdir 7
GQ421158.1	<i>Cucurbita pepo</i>	İran	Meşhed
MN966673.1	<i>Cucurbita pepo</i>	Egypt	WMV-Egy1
MH992141.1	<i>Cucurbita moschata</i>	Poland	D2
MT437295.1	<i>Cucumis melo</i>	Türkiye	Bingol W4
MT413451.1	<i>Cucumis melo</i>	Türkiye	Bingol W2
MZ055421.1	<i>Citrullus lanatus</i>	Türkiye	Igdir 6
MW962978.1	<i>Cucurbita pepo</i>	Türkiye	Diyarbakir D3
AY464948.1	-	China	WMV-HLJ
MG021273.1	<i>Citrullus lanatus</i>	USA	KY-1
MG021301.1	<i>Citrullus lanatus</i>	USA	TX-20
MG021250.1	<i>Citrullus lanatus</i>	USA	OK-4
AB001994.1	<i>Habenaria radiata</i>	Japan	Habenaria
MG021268.1	<i>Cucurbita pepo</i>	USA	MS-3
L22907.1	<i>Vanilla fragrans</i>	Australia	Tonga

Table 4. Information on other zucchini yellow mosaic virus isolates retrieved from genbank

Accession Number	Host	Origin	Isolate Code
KP872543.1	<i>Cucurbita pepo</i>	Türkiye	ER6-8
KP872575.1	<i>Cucurbita pepo</i>	Türkiye	G3
KP872574.1	<i>Cucurbita pepo</i>	Türkiye	G2
KP872573.1	<i>Cucurbita pepo</i>	Türkiye	G1
KP872550.1	<i>Cucurbita pepo</i>	Türkiye	AS6
KP872581.1	<i>Cucumis melo</i>	Türkiye	S5
KP872578.1	<i>Cucurbita pepo</i>	Türkiye	E-7
KP872576.1	<i>Cucurbita pepo</i>	Türkiye	K3
KP872546.1	<i>Cucurbita pepo</i>	Türkiye	KAR12-4
KP872541.1	<i>Cucurbita pepo</i>	Türkiye	AKS5-7
KP872572.1	<i>Cucurbita pepo</i>	Türkiye	AYS7
MK689858.1	<i>Cucurbita pepo</i>	Türkiye	ZYMV- Bingol
JF317296.1	<i>Cucumis sativus</i>	Türkiye	ZYMV-Adana
KP872577.1	<i>Cucurbita pepo</i>	Türkiye	K17
KP872571.1	<i>Cucumis sativus</i>	Türkiye	D14
AJ420019.1	-	Germany	Berlin 1
AJ420015.1	-	Austria	Austria 10
AJ420017.1	-	Austria	Austria 12
KP872561.1	<i>Cucurbita pepo</i>	Türkiye	BE26
AJ251527.1	<i>Cucumis sativus</i>	Hungary	10
AJ420018.1	-	Slovenia	Slovenia 1
AJ459956.1	-	Hungary	H272-8
KP872565.1	<i>Cucurbita moschata</i>	Türkiye	BRD4
JF317297.1	<i>Cucumis melo</i>	Türkiye	ZYMV-Ahlat
JF795797.1	<i>Mukia maderaspatana</i>	Austria	Cvn-13
KP872580.1	<i>Cucurbita pepo</i>	Türkiye	Y4
KP872579.1	<i>Cucurbita pepo</i>	Türkiye	Y23

Results

The distribution of these samples by district is presented in Figure 1. As shown in Figure 1, 41 samples were collected from the Çanakkale Central district, 32 from Eceabat, 24 from Çan, 18 from Gelibolu, 9 from Bozcaada, 7 from Lapseki, 4 from Biga, and 2 from Bayramiç (Figure 1).

When the sampled cucurbit plants were analyzed for species and potyvirus presence, 45 samples were collected from squash, of which 40 showed single or mixed infections with watermelon mosaic virus (WMV), and 14 were infected with zucchini yellow mosaic virus (ZYMV). Additionally, 5 samples tested negative for virus presence. In pumpkin, 35 samples were collected, with 19 showing single WMV infections and 11 infected with both viruses, while 5 samples were negative for any virus. For melon and watermelon plants, 23 and 19 samples were collected, respectively; 15 melon samples were infected with WMV, 5 with mixed infections, and 12 watermelon samples were infected with WMV, with 3 showing mixed infections. Additionally, 3 melon and 4 watermelon samples were negative for virus presence. Seven cucumber samples were collected, with 4 infected with WMV and 1 with ZYMV, but no mixed infections were detected. Due to limited cultivation, 4 samples were collected from both Armenian cucumber and bottle gourd. One WMV infection was detected in each species, and 3 samples from each exhibited mixed infection. Of all collected samples, single infections were determined 78 and 1 plants infected with WMV and ZYMV, respectively. Furthermore, mixed infections were determined in 39 plants. Potyvirus presence was not detected in 19 samples (Table 5).

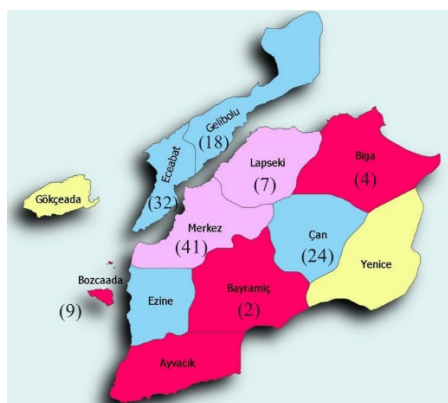


Figure 1. Districts of Çanakkale where field studies were conducted (The number of samples collected is given in parentheses).



Figure 2. Mosaic symptoms in melon (A) and gourd (B) plants mixed infected with watermelon mosaic virus and zucchini yellow mosaic virus

Table 5. Number of infected and collected samples based on hosts as a result of field and virus identification studies

Host	Number of WMV		Number of ZYMV		Number of Samples	
	Infected/Collected	Samples	Infected/Collected	Samples	WMV+ZYMV Infected/Collected	Samples
Melon	15	23	0	23	5	23
Watermelon	12	19	0	19	3	19
Squash	26	45	0	45	14	45
Pumpkin	19	35	0	35	11	35
Snake melon	1	4	0	4	3	4
Cucumber	4	7	1	7	0	7
Bottle gourd	1	4	0	4	3	4
Total	78	137	1	137	39	137

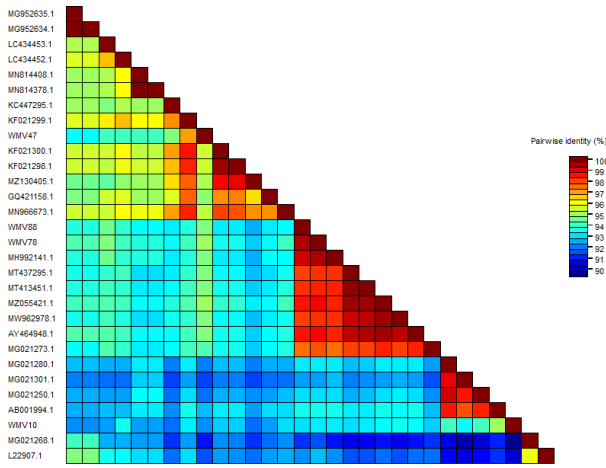


Figure 3. Similarity rates of coat protein nucleotide sequences between Çanakkale and global watermelon mosaic virus isolates

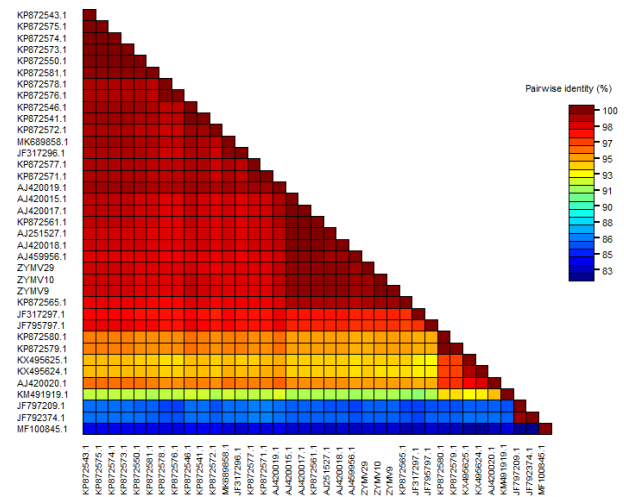


Figure 4. Similarity rates of coat protein nucleotide sequences between Çanakkale and global zucchini yellow mosaic virus isolates

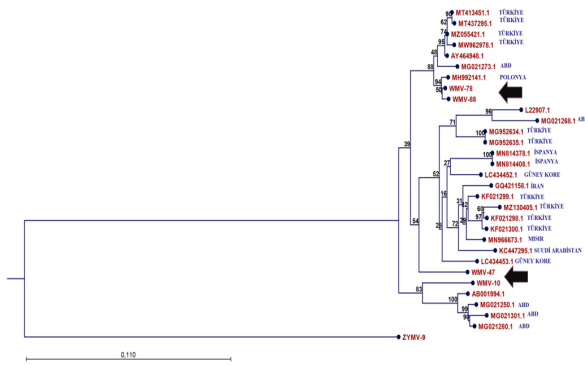


Figure 5. Phylogenetic family tree of Çanakkale watermelon mosaic virus isolates based on nucleotide sequences

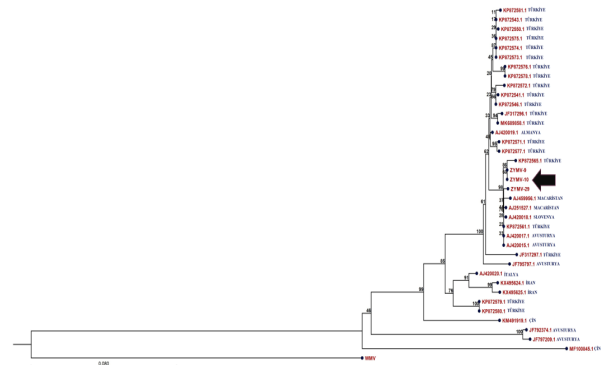


Figure 6. Phylogenetic family tree of Çanakkale zucchini yellow mosaic virus isolates based on nucleotide sequences

The phylogenetic tree of ZYMV isolates is shown in Figure 6. The tree comprised four main groups. Phylogenetic analyses indicated that, unlike the Çanakkale WMV78, WMV88, WMV47, and WMV10 isolates, the ZYMV9, ZYMV10, and ZYMV29 isolates were clustered in the same main group, with ZYMV9 and ZYMV10 being closely related. Notably, the ZYMV29 isolate showed a close resemblance to isolates from Hungary, Slovenia, Turkey, and Austria.

Discussion

The study investigated the prevalence of two major potyviruses affecting cucurbit crops, revealing that WMV is significantly more prevalent than ZYMV. In Türkiye, previous studies have documented the widespread occurrence of both viruses in cucurbits. For instance, Köklü and Yılmaz (2006) reported ZYMV infection rates of 45.5% in watermelon and 40.3% in melon, while WMV infection rates were 34.3% in watermelon and 31.2% in melon out of 502 samples collected from melon and watermelon fields in the Thrace region. Similarly, a study conducted in Tokat by Korkmaz et al. (2018) found that 12% of the 571 leaf samples from pumpkin plants were infected with ZYMV, while 37% were infected with

WMV. These findings indicate that both viruses are prevalent and have high infection rates in regions neighboring our study area, such as Thrace and Central Anatolia, consistent with the results of our research.

In the study, potyvirus infection was not detected in some samples showing typical viral symptoms, which is probably due to other viruses affecting cucurbits. Karanfil and Korkmaz (2021) identified the presence of Cucumber mosaic virus (CMV) in 10 out of 72 cucurbit samples from the Balıkesir, Çanakkale and Bursa provinces. Numerous studies in Turkey have also identified other viruses in cucurbits besides WMV and ZYMV, such as CMV, papaya ringspot virus (PRSV), squash mosaic virus (SqMV), and melon necrotic spot virus (MNSV) (Köklü and Yılmaz, 2006; Korkmaz et al., 2018). These studies highlight the diversity of viruses, beyond potyviruses, that cause infections in cucurbits in Türkiye.

The infection rates of WMV and ZYMV in Çanakkale vary across districts and crop types. These variations may be influenced by factors such as the transmission capacity and population density of virus-carrying vectors, differences in sample types and varieties, cultivation techniques, and environmental factors.

In terms of sequence similarity, Gara et al. (1997) reported that the WMV-Habenaria isolate (AB001994.1)

from Japan showed sequence similarities ranging from 78% to 96% with other isolates in GenBank. The nucleotide sequence similarity between the WMV isolates from Çanakkale and those from Japan ranged from 95% to 96%. In Iran, Sharifi et al. (2008) analyzed the amino acid sequence similarity of the coat protein gene region of 14 WMV isolates, finding a similarity range from 97.4% to 100%. When compared to two Iranian isolates in the GenBank, the similarity rates ranged from 91.6% to 93.4%. These findings indicate a high degree of similarity in the amino acids encoded by the coat protein gene region of WMV isolates. In Türkiye, Yeşil (2013) reported sequence similarity rates of 99% based on CP gene sequence analyses of positive samples among 652 collected in 2009 and 2010. Studies conducted globally and in Türkiye consistently show that WMV isolates coat protein genes exhibit over 90% similarity, aligning with the results of our study.

Vučurović et al. (2012) conducted a study in Serbia in 2011, where they found that 6 out of 26 watermelon samples were infected with ZYMV. They sequenced one of these isolates using RT-PCR, finding nucleotide similarity rates between the Serbian isolate and other global isolates ranging from 93.7% to 99.9%. In a study conducted in Türkiye, Topkaya et al. (2019) identified the presence of WMV, ZYMV, CMV, PRSV, and CGMMV viruses in the Ankara and Antalya provinces between 2009 and 2014. They sequenced the CP nucleotide sequences of 45 ZYMV isolates and compared them with global isolates from GenBank, finding similarity rates ranging from 96% to 99%. The similarity between isolate BE26 (accession number KP872561, Ankara) and the Çanakkale isolates (ZYMV9, ZYMV10, and ZYMV29) was 100%, demonstrating high similarity between isolates from the same geographical regions.

Nematollahi et al. (2021) collected 305 samples showing virus symptoms from watermelon fields in northern Iran, finding that 80 samples were infected with WMV. Their sequence and phylogenetic analyses of the CP and P1 regions identified three main groups (CL, EM, and G2) formed by WMV isolates, each further subdivided into several branches. Our study also identified three main clusters of WMV-infected samples based on CP gene phylogenetic analysis, demonstrating parallel results between the two studies.

The phylogenetic relationships of ZYMV isolates have been extensively studied in Türkiye, consistently showing that Turkish isolates are closely related (Kamberoğlu et al., 2016; Yeşil, 2021). These findings align with our study, confirming the consistency and mutual support among various studies conducted in Turkey.

Conclusion

This study provides insights into the infection rates of WMV and ZYMV species in Çanakkale province. Additionally, the molecular characterization of seven isolates based on the CP gene region was conducted. Future research should include sequencing other genes beyond the CP gene or even the entire viral genome to generate comprehensive genomic information on isolates specific to Türkiye, contributing valuable data to the scientific community.

Declarations

Author Contribution Statement

M.S. conducted the experiments; A.K. contributed to the writing of the manuscript and the analyses; S.K. designed the study, contributed to the writing of the manuscript and the analyses. All authors have made significant contributions to the final manuscript and have approved its content.

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Conflict of Interest

The authors declare no conflict of interest.

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