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Interactive Effects of Abiotic Stress and Bacterial Treatments on the Severity of Viral Infection in Greenhouse-Grown Cucumbers: A Case Study from Erbil, Iraq and Syria, Raqqa

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ABSTRACT: Viral diseases and climate change are the most significant challenges facing cucumber cultivation in greenhouses. This study aims to study the impact of Pseudomonas fluorescens, Bacillus subtilis, water, and heat stress on viral infection cultivars in three commercial cucumber cultivars (Master, CODE11, and AYOUBI) in Erbil and Raqqa, within greenhouses conditions. In Erbil, the efficacy rate was 100% during W1,W2 and W3. Moreover, the reduced rate for Bacillus was (100 to 16.32)% from W1 to W7 respectively. The Master cultivar showed a high response to both types of bacteria compared to the other cultivars. In Raqqa, the effectiveness of Pseudomonas bacteria ranged from 81.52% in W1 to 15.91% in W7, while the effectiveness of Bacillus ranged from 36.95% to 6.57% from W1 to W7. Meanwhile, the CODE11 cultivar showed a greater response to both types of bacteria, with a rate of 52.21% for Pseudomonas and 0.94% for Bacillus.

The effect of water and heat stress was clear in W5 in Erbil and in W4 in Raqqa. In Erbil, the percentage increase in infection severity in W5 was 25.92% due to water stress and 15.22% due to heat stress. In Raqqa, the infection rate increased in the Master cultivar more than in the other cultivars, which the infection percentage was 16% of water stress. Furthermore, the highest effect of the time was noticed in W 4, which the percentage of infection severity in W4 was 21.00% due to water stress and 9.58% due to heat stress.

INTRODUCTION:

Cucumber (Cucumis sativus L.) is a cucurbitaceous vegetable that is highly sought after in the world (Pradeepkumara et al., 2025). Cucumber production is significantly impacted by both biotic and abiotic variables; the prevalence of pests and diseases, which are brought on by a range of environmental conditions, act as significant constraints, resulting in yield losses ranging from 35 to 60% (Jain et al., 2024, Khadka et al., 2025).In natural settings, over 90 viruses have been reported to have infected cucurbits. Their agronomic impact and prevalence are drastically different, and about ten virus species are responsible for most global issues (Desbiez, 2020). Cucumber Mosaic Virus (CMV) is one of the many viruses that infect cucumbers. It is present in most countries worldwide, and has the widest host range (Palukaitis et al., 1992), according to plant virologists affiliated with Molecular Plant Pathology, CMV is ranked fourth among the ten most common plant viruses in terms of on economic and scientific importance (Scholthof et al., 2011). CMV is common on greenhouse-grown cucumbers in Iraq (Adhab and Ali, 2021) and on cucurbits in Syria, where it coexists with watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) (Chikh-Ali et al., 2019, Kassem et al., 2005). Zucchini yellow mosaic virus (ZYMV), an economically significant viral infection that causes severe mosaic symptoms and ugliness in cucurbits, poses a danger to cucurbit crops globally (Ahsan et al., 2023, Topkaya and ErtunÇ, 2022). The watermelon mosaic virus (WMV), which has been identified in Iraq, Iran, and Turkey, also

spreads to cucurbits and results in production losses (Karabulut and Koklu, 2025, Alinizi et al., 2024). Cucurbit crops are being severely harmed by the Squash Mosaic Virus (SqMV). Symptoms have been documented in Turkey, Syria (Kassem et al., 2005, YEŞİL1, 2019). The production of cucurbits suffers significant financial losses due to the Papaya Ringspot Virus watermelon strain (PRSV-W) (Huang et al., 2019).

Abiotic stresses such as severe drought or waterlogging, temperature extremes, salinity or alkalinity, and abrupt rainfall patterns are made worse. These factors ultimately have a cultivar of effects on plants (Shahzad et al., 2021). The biggest environmental barrier to greenhouse productivity is high temperatures. Cucumbers produced in greenhouses are among the most heat-sensitive crops. (Fanourakis et al., 2025). Climate change is expected to alter many significant viral and bacterial epidemic components in different ways (Jones and Barbetti, 2012). The dynamics of plant-virus interactions are drastically changed as temperatures rise and weather patterns change (Yin et al., 2024). High temperatures can reduce the transmission of cucumber mosaic virus by decreasing the efficiency of the Myzus persicae (Sulzer) aphid in transmitting the virus, although its survival is not affected by high temperatures (Balagalla et al., 2025). Likewise, the experiments showed that whereas CMV and water deficit together were more detrimental than treat alone (Jaisval et al., 2023).

Therapeutically managing plant viruses is difficult due to their intracellular nature. Therefore, management strategies for diseases caused by plant viruses must include risk reduction and preventative measures (Tatineni and Hein, 2023). In order to biologically control plant diseases, microbial biological control agents (MBCAs) are administered to crops and work through a cultivar of mechanisms (Kohl et al., 2019). Utilizing bacterial biocontrol agents, especially plant growth-promoting rhizobacteria (PGPR), has emerged as a practical, sustainable, and effective crop protection strategy (Sokra et al., 2026, Essalimi et al., 2022). PGPR has effectively activated plant systemic resistance mechanisms in a range of crops, lessening the effects of viral infections including Cucumber Mosaic Virus (CMV) and Tobacco Mosaic Virus (TMV) (Khalid et al., 2025). When plant growth-promoting bacteria (PGPB) like Pseudomonas indica, Bacillus paramycoides, Bacillus thuringiensis, Bacillus mycoides, Paenibacillus glucanolyticus, and Niallia circulans are applied foliar or in the soil, squash plants can significantly lessen the severity of WMV and increase plant growth (Bashandy et al., 2025). Through systemic acquired resistance (SAR) and induced systemic resistance (ISR), several bacterial isolates, such as Streptomyces variabilis, Rhizobium sophorae, and Bacillus subtilis subsp. subtilis, have proven effective in reducing the incidence and severity of Cucumber Mosaic Virus (CMV) infection in a cultivar of crops, including cucumbers, faba beans, and squash in greenhouses and open fields (Elsharkawy et al., 2022, Abdelkhalek et al., 2025, Osama et al., 2025).

MATERIALS AND METHODS

Experiment implementation areas

Two different locations from two countries were selected:

Iraqi Kurdistan Region: Erbil - Pir Dawood, inside greenhouse (28.5 X 9)meter.

Syria: Raqqa – Al-Mansoura, Hinda village, inside greenhouse (52 X 9)meter.

Cucumber cultivars

The field survey conducted prior to the experiment's implementation phase, three commercially cultivated cucumber selected in Erbil, used by farmers, varied in their susceptibility to viral infections and were grown in different seasons. (Table 1).

Table 1. Characteristics of Cucumber Varieties Used in the Experiment

Characteristics / Varieties	Variety Code	Source	Prod. Date	Purity	Germination
Top Green (Master) F1	V1	Jordan	10/2024	99%	98%
CODE 11	V2	Spain	11/2024	99%	95%
AYOUBI VERDE F1	V3	Netherlands	7/2023	99%	95%

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Planting Date and Method

The seeds were planted directly in the greenhouse. The greenhouse was divided into five rows, with one meter between rows, each row 60 cm wide, and 30 cm between plants. Three rows were planted (one cultivar per row) according to the following schedule:

Erbil: August 11 to November 11, 2025

Raqqa: October 4, 2025, to January 4, 2026

Mechanical Infection

Cucumber plant leaves were collected from greenhouses exhibiting mosaic virus, leaf distortion, and yellowing in the experimental areas (Erbil and Raqqa).

Infected cucumber plant leaves were crushed in a solution containing 0.01 M phosphatase buffer at pH 7.3 on the day of inoculation (1 gram of leaves per 4 mmol of buffer). Thereafter, the mixture was filtered through two layers of gauze to produce a filtrate that was used as the viral inoculum. Carborandome sparingly applied to the upper surface of the examined cucumber plant leaves (three leaves per plant), rubbed off, and then sprayed with sterile distilled water to get rid of any leftover powder. When the plants reached the 4-6 leaf stage, the prepared viral inoculum was then applied by dipping a finger into it and gently wiping the upper surface of the leaves (Al-Ani and Adhab, 2013).

Cover Cucumber Plants with Polyethylene Plastic – Cover Drip irrigation Pipes with Plastics Strips:

In both experiments, the treatment plants were covered with polyethylene plastic sheeting to raise the heat inside the treatment plant chamber. Every week, the chamber's temperature was recorded and contrasted with the greenhouse's heat. During the experiment, there was a 4-6°C temperature differential, with the chamber being 4-6°C warmer than the greenhouse (treatment plants covered with polyethylene after 4 days of artificially infecting).

To investigate how drought impacts the severity of viral infection in cucumber, drip emitters in both tests covered with plastic rubber bands. To keep water from getting to the treatment plants, which were routinely watered every three days following infection, these bands were placed every three days. Throughout the experiment, the plants were occasionally deprived of water while being watered, and the rubber bands were taken off for the subsequent watering.

After intentionally infecting cucumber plants, both treatments were administered on the same day that viral symptoms infected (after 4 days of artificially infecting).



Fig. 1 Covering cucumber plants with polyethylene nylon in a temperature stress experiment (A: Erbil, B Raqqa).

Bacterial Preparations

For both irrigation and spraying, two commercially available, ready-to-use bacterial preparations were combined directly with water at a rate of 3 g/liter of water. 35 ml of irrigation was applied to each plant four days prior to infection. This was followed by two spray applications, one week after infection and two weeks after infection. The two commercial preparations are:

1 -Bsciland (Bacillus subtilis): Microcapsule granule, active ingredient and contents is Bacillus subtilis 100 million CFU/g. Source: China, Imported company: Green Gold Land .

2- Pseudoland (Pseudomonas fluorescens): Microcapsule granule, active ingredient and contents is Pseudomonas fluorescens 100 million CFU/g. Source: China, Imported company: Green Gold Land

Greenhouse Evaluation of Biological Control and Abiotic Stress Treatments in Cucumber Plants

The experiment included 6 treatments with 4 replicates per cultivar (3 cultivars) at each experimental site (Erbil, Raqqa):

1 -Negative control, coded VT1

2 -Positive control, coded VT2.

3- Virus-inoculated plants treated with Bacillus subtilis, coded VT3

4- Virus-inoculated plants treated with Pseudomonas fluorescens, coded VT4

5 -Virus-infected plants under heat stress, coded VT5

6 -Virus-infected plants under drought Stress, coded VT6

All treatments (excluding the control VT1) are infected artificially with viruses.

Viral Diseases Severity Scale

The six severity levels of the cucumber mosaic virus on leaves, as depicted in Fig. 2 (McKinney, 1923, Al -Fahd et al., 2020), were utilized. The following formula was used to get the arithmetic mean of the severity levels on the leaves after the number of infected leaves was tallied based on the severity level for each replicate:

Average severity for each replicate = Number of leaves (sum of severity levels 1-5) / Total number of infected leaves per plant

Average severity for the treatment = Sum of the average severity level for the replicates / Number of replicates

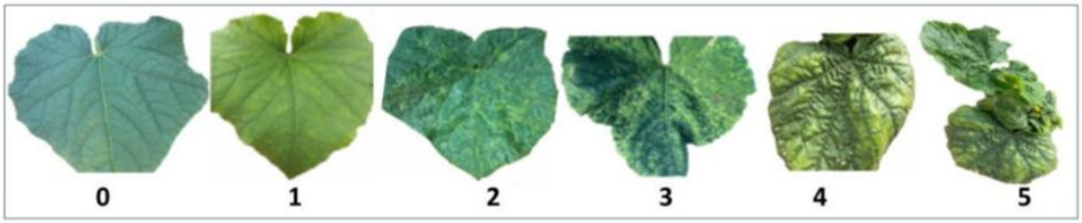


Fig.2 Disease scale used for assessing severity of cucumber infection with CMV. 1= Healthy, 2- Mild mosaic, 2= Severe mosaic on the leave, 3= Severe mosaic and leaf rolling, 4= Yellowing and severe mosaic and severe leaf rolling, 5= Severe mosaic and leaf rolling with malformation and reduction in leaf blade area.

Identification of Viral Species Molecularly

RNA Extraction, cDNA Synthesized and cDNA Amplification: RNA of viruses' sample was extracted via spin column chromatography by using AddPrep. Total RNA Extraction Kit, Product Code: 10119 (Add Bio, Korea). The single strand RNA extracted was converted to double strand complementary DNA by using AddScript cDNA Synthesis Kit Add Bio with Product code 22701. PCR amplification for partial genes was done in 50 µl of reaction mixture containing 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), 10 picomoles (pmol) of forward primer and 10 pmol of reverse primer of the ZYMV, WMV, and CMV viruses (Table 2), DNase-free water, and template DNA (Table 2) by Bioresearch PTC-200 Gradient thermocycler. The total volume of PCR amplification reagent was 50 µl, including master mix 25 µl, forward primer 3 µl, reverse primer 3 µl, DNase-free water 14 µl, and cDNA template 5 µl. The temperature profile included step one, an initial denaturation at 95°C for 5 min, followed by step two, 35 cycles of a denaturation at 95°C for 35 sec., a primer annealing at 60°C for 40 sec., an extension at 72°C for 1 min., and final step is an extra extension at 72°C for 10 min. An intercalating dye of Ethidium bromide is added to1.5% melted agarose gel in 1X TBE buffer after 30minutes inside of electric

field of electrophoresis in 80-volt electric field; the location of bands determined by examining the gel under UV trans-illuminator.

Table 2 The Primers are Used for Identifying Viruses

No.	Virus name	Forward primer	Amplicon size	Source
1	ZYMV	GCTCCATACATAGCTGAGAC	850	(Chinnaraja et al., 2015)
		AACGGAGTCTAATCTCGAGC		
2	WMV	AACTCGCTGCATCCGGAAAA	1100	(Sharifi et al., 2008)
		CGCAAATGCTAACTGTGACC		
4	CMV	TTGAGTCGAGTCATGGACAAATC	678	(Lin et al., 2004)
		AACACGGAATCAGACTGGGAG		

Sequencing DNA: The PCR products were purified using the Promega Wizard® SV Gel and PCR clean up system manufactured by Promega Corporation, USA, following the manufacturer's protocol. DNA sequencing was performed to determine the nucleotide sequence of the viral cap protein region. All PCR products of all samples were applied to the ABI Prism Terminator Sequencing Kit (Applied Biosystem) at the Microgene Center in Korea.

Sequence alignment and submission gene to GenBank: Sequenced data were checked for quality by BioEdit v.7.0.5 software. Homology, insertions, deletions, stop codons, and frameshifts were checked using NCBI BLAST. The website was compared, and the laboratory or query sequence was aligned with other biological sequences to find more similarity with other targets. BankIt, a WWW-based submission tool with wizards to guide the submission process, was used. The GenBank database was intended for new sequences of data that were determined and annotated by the submitter. All sequences were uploaded to GenBank.

Statistical Analysis

Analysis of variance (ANOVA) was performed on the gathered data. Duncan's Multiple Range Test was used to compare mean differences at a significance level of 0.05 ($P < 0.05$). The statistical program GenStat (version 19.1) was used for all statistical studies (Duncan, 1975).

RESULTS AND DISCUSSION

1. Evaluation of biocontrol on the viral infection

1.1 The effect of bacteria on virus infection with the time:

Erbil:

As shown in table 3, the mean viral infection severity interaction between time and treatments was statistically significant p value(\(P <0.001\)).The role of Pseudomonas fluorescens and Bacillus subtilis in decreasing virus infection (Table 3) showed that depending on the time, the virus infection increased with time and reached the top in W 7. The highest mean virus infection was 2.94 which was recorded in W7 in the plant infected by virus only followed by plant treated with Bacillus, Pseudomonas and control (no treated) 2.46, 2.32 and 1.76 respectively, while the lowest infection was recorded in W 1.

The effect of Bacillus and Pseudomonas on virus infection (Table 3), the result showed that Pseudomonas more effective than Bacillus in reducing virus infection where the mean of virus infection decreased to 0.8 when plant treated with Pseudomonas compared with Bacillus 1.15, while in positive control (virus infection) and negative control (no treated plant) were 2.07 and 0.35 respectively. While the infection on negative control appeared later than other treatments, with the infection appearing in the W5 with mean severity 0.08, moreover, the infection severity increasing over time.

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As result, this effectiveness of biocontrol refers to the role of these factors to promote plants to products which induced resistance to combating viruses and preventing their spread inside plans cells.

Table 3 Interactive Effects of Bacteria on the Temporal Development of Viral Disease Severity in Cucumber Plants under Greenhouse Conditions in Erbil.

Treatments / Duration	Negative control	Positive control	Bacillus subtilis	Pseudomonas fluorescens
W1	0 k	0.58 h	0 k	0 k
W2	0 k	1.56 f	0.17 jk	0 k
W3	0 k	2.05 d	0.44 hi	0 k
W4	0 k	2.3 c	1.22 g	0.35 ij
W5	0.08 k	2.43 c	1.9 de	1.13 g
W6	0.63 h	2.66 b	1.89 de	1.8 e
W7	1.76 e	2.94 a	2.46 bc	2.32 c
Mean	0.35	2.07	1.15	0.8
P- value	<0.001			

Means with different letters within the same column (or row) differ significantly from each other according to Duncan's multiple tests at a probability level of ($P \leq 0.05$).

Raqqa:

As shown in table 4, the mean viral infection severity interaction between time and treatments was statistically significant p value(\(P <0.001\)). In Raqqa experiment, the effect of Pseudomonas fluorescens and Bacillus subtilis in decreasing virus infection (Table 4) showed that depending on the time, the most decreasing rate was in W1 and W2 in Pseudomonas and bacillus which means virus infection in W2 was 0.29 and 0.53 respectively, while the mean was 1.62 in infected control. From W 3, the effective of both bacteria decreased to W7. Virus infection increased with time and reached the top in week 7. The highest mean virus infection was 2.12 which was recorded in W7 in the plant infected by virus only followed by plant treated with Bacillus, Pseudomonas and control (no treated) 2.7, 2.43 and 2.31 respectively.

The result showed (Table 4) that Pseudomonas are more effective than Bacillus in reducing virus infection where the means of virus infection decreased to 1.34 when plant treated with Pseudomonas compared with Bacillus 1.68, while in positive control (virus infection) and negative control (no treated plant) were 2.12 and 0.91 respectively. While the infection on negative control appeared later than other treatments, with the infection appearing in the W4 with mean severity 0.7.

Overall, the effect of both bacteria in reducing the virus infection was more in Erbil experiment than Syria experiment as result showed (Table 3, Table 4), may be due to the difference in weather conditions, the nature of the region, and the conditions of the experiment, especially since one experiment was carried out at the end of summer and the other experiment was carried out in autumn and the beginning of winter.

Table 4 Interactive Effects of Bacteria on the Temporal Development of Viral Disease Severity in Cucumber Plants under Greenhouse Conditions in Raqqa.

Treatments / Duration	Negative control	Positive control	Bacillus subtilis	Pseudomonas fluorescens
W1	0 oq	0.92 k	0.58 lm	0.17 no
W2	0 oq	1.62 i	0.53 m	0.29 n
W3	12:00 PM	1.91 g	1.44 j	1.02 k
W4	0.7 l	2.19 f	1.68 hi	1.46 j
W5	1.43 j	2.56 cd	2.3 ef	1.8 gh
W6	1.94 g	2.73 ab	2.55 cd	2.25 f

W7	2.31 ef	2.89 a	2.7 bc	2.43 de
Mean	0.91	2.12	1.68	1.34
P- value	<0.001			

Means with different letters within the same column (or row) differ significantly from each other according to Duncan's multiple tests at a probability level of ($P \leq 0.05$).

1.2 Bacterial treatments and varietal response:

Erbil:

As fig. 3 shows, Master cultivar was the most responsive to Pseudomonas fluorescens and Bacillus subtilis in reducing the virus infection, which means virus infection was 0.63 and 0.81 respectively, while in positive control and negative control were 1.99 and 0.44 respectively. However, the lowest responsive as fig 3 shows, was AYOUBI cultivar to both bacterium types.

The effect of Pseudomonas was more effectiveness in reducing virus infection than Bacillus in all cultivars studied.

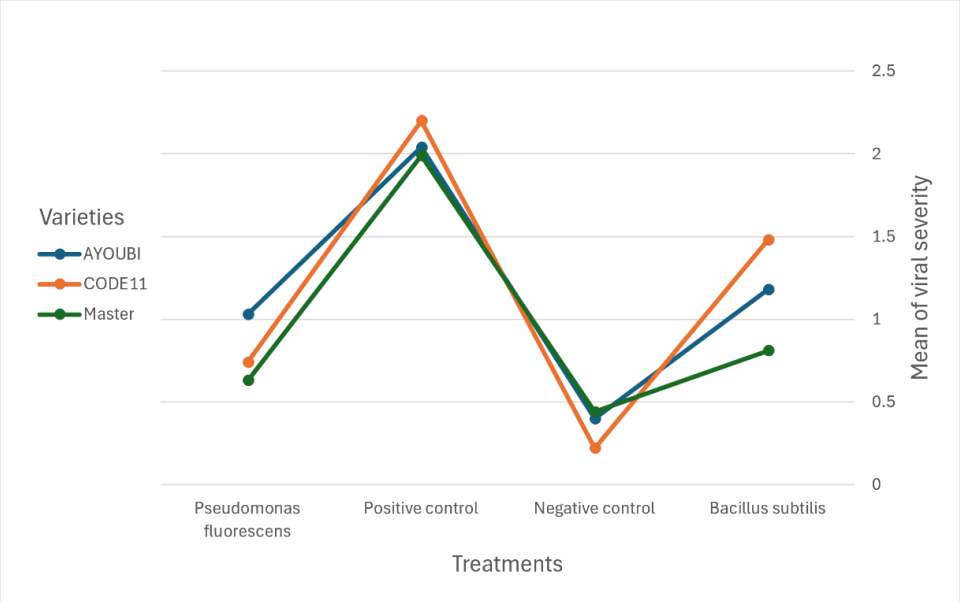


Fig. 3 Combined effect of cucumber cultivars and treatments on mean viral infection severity over the experimental period in Erbil.

Raqqa:

In Raqqa experiment, CODE11 cultivar was the most responsive to Pseudomonas fluorescens and Bacillus subtilis in reducing the virus infection, which means virus infection was 1.04 and 1.6 respectively, while in positive control and negative control were 2.19 and 0.72 respectively. However, the lowest responsive as fig 4 shows, was Master cultivar to both bacterium types .

The effect of Pseudomonas was more effectiveness in reducing virus infection than Bacillus in all cultivars studied.

In general, as fig 3 and fig 4 shows, the effect of both bacterium types was more active in all cultivars in Erbil experiment compared with Syria experiment.

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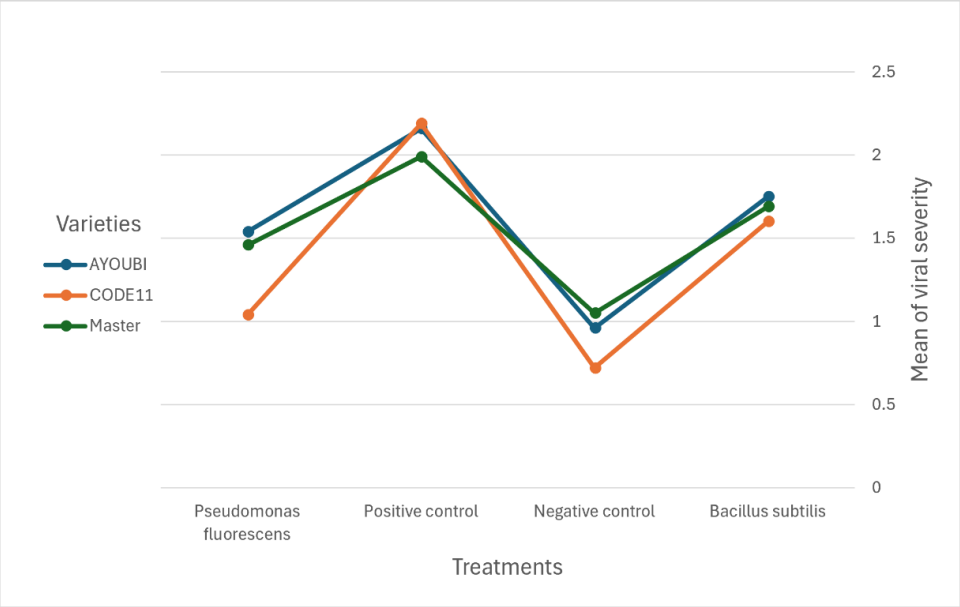


Fig. 4 Combined effect of cucumber cultivars and treatments on mean viral infection severity over the experimental period in Raqqa.



Fig 5 A: Symptoms of viral infection on cucumber plants of the Master cultivar in the Raqqa experiment. B: Symptoms of infection on control plants of the AYOUBI cultivar in Erbil.

2. Influence of climate factors on viral infection

2.1 The effect of climate factors on virus infection with the time

Erbil:

As shown in table 5, the mean viral infection severity interaction between time and treatments was statistically significant p value(\(P <0.001\)). Depending on the time, climate factors have an impact on the spread of viruses (Table 5). Throughout the experiment, water stress had varying effects; the most effect was in W1, when the mean virus infection was 0.83, compared to 0.58 and 0 in the positive and negative controls, respectively. The impact of drought diminished in W 2 and W 3, while the effect started from W 4. However, the effect decreased and the virus infection decreased from W 6, while mean virus infection in W 5 was 3.06 compared to positive and negative control 2.08 and 2.43 respectively.

The role of heat stress started in W3 and increased depending on the time. The effect was moderate in increasing virus infection (Table 5). The most effect was in W 6, which means virus infection was 3.1, while in positive and negative controls were 2.66 and 0.63 respectively.

In general, the effect of drought stress was more than the heat stress in increasing the rate of virus infection.

Table 5 Interactive Effects of Heat and Water Stress on the Temporal Development of Viral Disease Severity in Cucumber Plants under Greenhouse Conditions in Erbil.

Treatments / Duration	Negative control	Positive control	Heat Stress	Water Stress
W1	0 n	0.58 m	0.58 m	0.83 l
W2	0 n	1.56 k	1.56 k	1.5 k
W3	0 n	2.05 i	2.18 hi	1.84 j
W4	0 n	2.3 gh	2.48 f	2.81 de
W5	0.08 n	2.43 fg	2.8 de	3.06 bc
W6	0.63 m	2.66 e	3.1 bc	3.16 ab
W7	1.76 j	2.94 cd	3.29 a	3.32 a
Mean	0.35	2.07	1.28	2.36
P- value	<0.001			

Means with different letters within the same column (or row) differ significantly from each other according to Duncan's multiple tests at a probability level of ($P \leq 0.05$).

Raqqa:

As shown in table 6, the mean viral infection severity interaction between the time and treatments was statistically significant p value(\(P <0.001\)). Water stress had varying effects; the effect started in increasing virus infection from W1, while mean virus infection decreased in W2 as the lowest effect, the mean virus infection in W 1 was 1.07 and in W 2 was 1.63, compared with positive control in W1 and W 2 which was 0.92 and 1.62 respectively. The impact of drought decreased in W 2, while the effect increased again from W 3 to W 4, moreover decreased from W5 to W 7. The most role of drought stress in increasing the virus infection was in W 4, which mean virus infection was 2.65, while in positive and negative controls were 2.19 and 0.7 respectively.

The temporal dynamics of the heat stress effect were very similar to the dynamics of the drought stress effect, as the effect began in the W 1, decreased in the W 2, then its role in increasing the severity of the infection returned in W3 and W 4, and it decreased again from the W 5 to W 7. The most effect of heat stress (table 5) showed in W 1, while mean virus infection was 1.04 compared with positive and negative control, which were 0.92 and 0 respectively.

In general, the effect of drought stress was more than the heat stress in increasing the rate of virus infection.

Table 6 Interactive Effects of Heat and Water Stress on the Temporal Development of Viral Disease Severity in Cucumber Plants under Greenhouse Conditions in Raqqa.

Treatments / Duration	Negative control	Positive control	Heat Stress	Water Stress
W1	12:00 PM	0.92 n	1.04 m	1.07 m
W2	12:00 PM	1.62 k	1.59 k	1.63 k
W3	12:00 PM	1.91 j	2.08 i	2.23 h
W4	0.7 o	2.19 hi	2.4 g	2.65 ef
W5	1.43 l	2.56 f	2.71 e	2.99 abc
W6	1.94 j	2.73 de	2.86 cd	3.02 ab

W7	2.31 gh	2.89 bc	2.99 abc	3.1 a
Mean	0.91	2.12	2.24	2.38
P- value	<0.001			

Means with different letters within the same column (or row) differ significantly from each other according to Duncan's multiple tests at a probability level of ($P \leq 0.05$).

2.2 Climate factors and varietal response

Erbil:

As fig. 6 shows, Master cultivar was more affected by water and heat stress compared to the CODE11 and AYOUBI cultivars, with the mean infection severity under water stress and heat stress was 2.57 and 2.37 respectively, compared to positive and negative controls 1.99 and 0.44 respectively.

The lowest effect of heat and drought stress was in AYOUBI cultivar, with almost no effect and no significant difference between the treatments and the positive control, as illustrated in fig. 6.

Overall, the effect of drought stress was more than the heat stress in all studied cultivars.

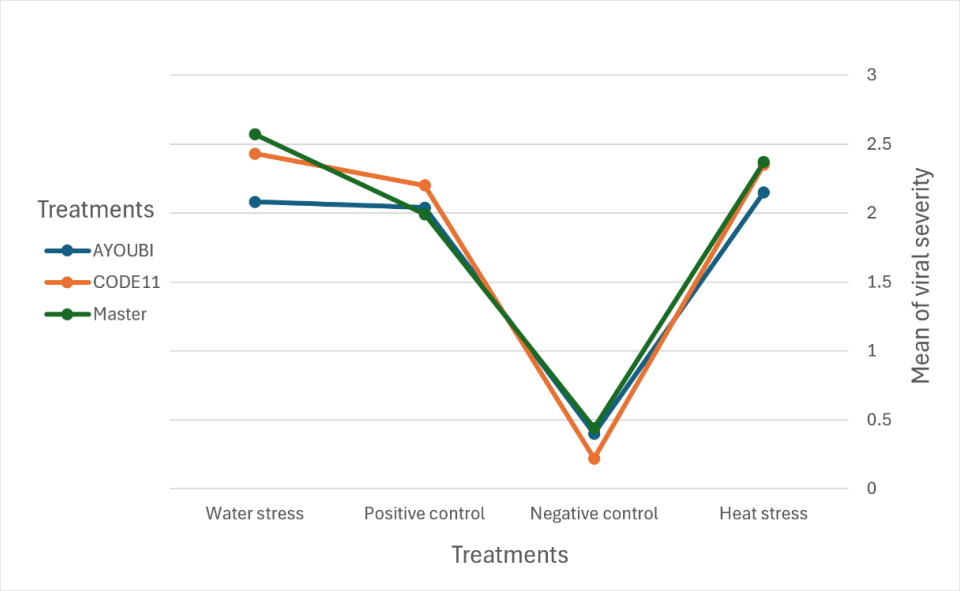


Fig. 6 Combined effect of climate factors and cucumber cultivars on mean viral infection severity over the experimental period in Erbil

Raqqa:

In Raqqa experiment, the most effect of water stress on viral infection severity was in Master and AYOUBI cultivars, while the highest virus infection was in master, where mean virus infection in drought and heat stress was 2.32 and 2.15 respectively, while in positive and negative controls was 2 and 1.05 respectively.

The lowest effect of both treatments was in CODE11 cultivar compared with Master and AYOUBI as fig. 7 shows.

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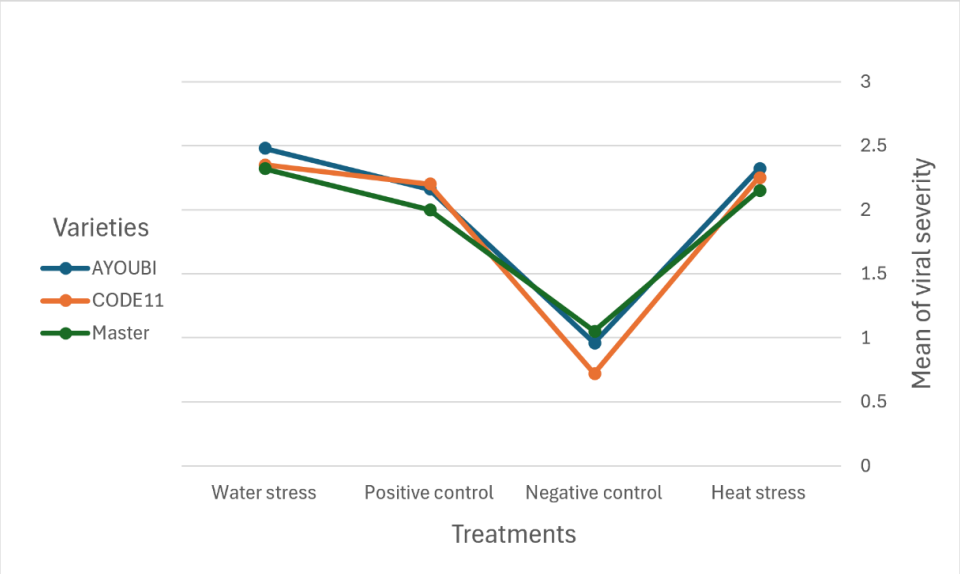


Fig 7 Combined effect of climate factors and cucumber cultivars on mean viral infection severity over the experimental period in Raqqa.



Fig 8 Symptoms of viral infection varied across the different cucumber cultivars used in the experiment.

Molecular Detection and Identification of Viruses

The chromatograms were converted to FASTA format using FinchTV chromatogram viewer software. The DNA sequences in the ABI file were manually edited using BioEdit v.7.0.5. Results of sequence editing were analyzed using BLAST (Basic Local Alignment Search Tool) NCBI to indicate the homology from the closest species (Table 7). A phylogenetic tree was constructed using the maximum likelihood method, calculating using Bootstrap with 1000 times of repetition in the Molecular Evolutionary Genetics Analysis (MEGA) software program (Altschul et al., 1997, Tamura et al., 2004, Tamura et al., 2021).

Table 7 Indicates BLAST Analysis to Identify of Observed Samples

Code	Cultivars	Query Accession Numbers	query cover %	Identity %	Gene bank Acc. No.	Species name
M	Master.EBL	PZ584015	100	99.37	OQ454911	Watermelon mosaic virus
AS	AYOUBI.SYR	PZ584017				
MS	Master. SYR	PZ584014	100	99.16	MK659926	
CS	CODE11.SYR	PZ584016				
AY	AYOUBI.EBL	PZ584008	100	100	PV939586	Cucumber music virus
CV	CODE11.EBL	PZ584007				
AS	AYOUBI.SYR	PZ584009	100	100	PX354906	

MS	Master.SYR	PZ584010				
CS	CODE11.SYR	PZ584011	100	99.81	MW926530	
M	Master.EBL	PZ584006	100	100	OQ136664	Zucchini yellow mosaic virus

Maximum likelihood (ML) was analyzed to find the phylogenetic relationship among the samples of plant viruses, and using MEGA X software, the phylogeny was constructed (Fig. 8) by the MEGA X software using analyzed sequences (marked Isolated) of three types of viruses. A total of eight sequences from different parts of the world available in the NCBI GenBank were used for a proper comparison.

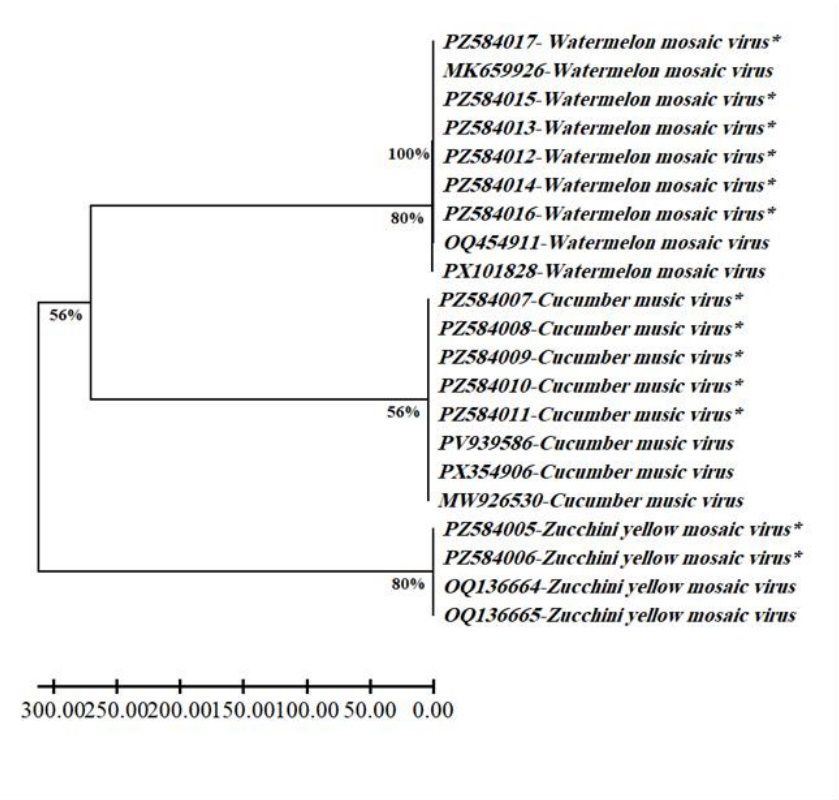


Fig. 8 Phylogenic tree of plant viruses samples from Iraq Kurdistan region (Erbil) and Syria (Raqqa). The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 100 re-samplings. . Partial DNA sequences of concatenated partial coat protein viruses gene were used as input data.

CONCLUSION

In conclusion, the experiment demonstrates that the biotic factor decreased viral infection especially during the treatments and after treatments for about two weeks in both locations.

As some cucumber cultivars showed greater effectiveness and response compared to other cultivars in the experiment, in addition to the increase in effectiveness during and after the application of the bacterial treatments, it is recommended to choose the appropriate cultivars for each growing season.

As result, the finding indicates that the climate factors (water stress and temperature stress) increased the mean severity of viral infection at both locations, with significant differences favoring water stress over temperature stress. These findings highlight the importance of farmers considering climatic conditions and climate change when cultivating greenhouses. This consideration should improve plant health and increase resistance to viral diseases. Applying IPM strategy and selecting suitable cultivars for each growing season are the most significant methods to avoid the harmful effects of viral diseases.

In general, Pseudomonas were more effective than Bacillus, and the effect of water stress was greater than that of temperature stress at a significant level ($P < 0.05$) in both locations. However, the effectiveness of both bacteria and the effect of abiotic factors were greater in the Erbil experiment compared to Raqqa.

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