

Study on the Susceptibility of Three Chili Pepper Varieties to Chili Leaf Curl Virus under Greenhouse Conditions

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Abstract

The objective of this study was to evaluate the effect of chili leaf curl virus (ChiLCV) on selected growth and yield traits of chili pepper cultivars grown under plastic house conditions at the College of Agricultural Engineering Sciences, University of Baghdad. The evaluation was conducted 55-85 days after planting. The research included three chili pepper varieties (Barbarian, Singer, and Siadah) to assess their susceptibility and the virus's impact on the percentage and severity of infection, plant height, number of branches, fresh and dry weight of the vegetative and root systems, fruit weight and number, yield per plant, chlorophyll content, and peroxidase enzyme levels. The Barbarian, Singer, and Siadah varieties of chili pepper showed varying resistance to the virus, with significant differences. The cultivar Barbarian was the most tolerant, having the lowest infection rates (4.75 and 8.45%) as severity (1.69 and 3.97%) after 55 and 85 days of emergence respectively. Singer followed, with infection rates of 10.50% and 15.23% and severity of 5.22% and 9.45%, while Siadah showed the highest susceptibility, with infection rates of 12.18% and 18.17% and severity of 7.09% and 11.39%, respectively. Barbarian also significantly outperformed the other cultivars in plant growth, reaching 84.17 cm in height with 33.88 branches per plant at 55 days, and 109.69 cm with 39.15 branches per plant at 85 days. Singer did not differ significantly from Barbarian in plant height at both stages and in branch number at 55 days, but it had fewer branches at 85 days (35.05 branches per plant). Regarding biomass, Barbarian recorded the highest fresh weights for shoots and roots (455.06 g and 57.498 g per plant) and dry weights (160.26 g and 34.813 g per plant, respectively). However, these did not differ significantly from Singer and Siadah. Barbarian also produced significantly higher chlorophyll levels, with 50.07 SPAD at 55 days and 43.13 SPAD at 85 days, compared with lower values in Singer (45.99 and 39.62 SPAD) and Siadah (43.91 and 36.20 SPAD). The Barbarian cultivar also provided the highest peroxidase enzyme level, which reached 138.34 and 98.40 after 55 and 85 days, respectively. In contrast, the Singer cultivar gave enzyme levels of 93.93 and 80.05 after 55 and 85 days, respectively, which differed significantly from the Siadah cultivar, which gave the lowest rates of 86.23 and 73.43 after 55 and 85 days, respectively. The extraction kits provided by Geneaid Company enabled the obtaining of high-purity DNA from infected plant leaves, which can be used for its amplification by Polymerase Chain Reaction (PCR) technology to obtain accurate and effective results. Virus infection was confirmed using Polymerase Chain Reaction (PCR) technique with specific primers (ChiLCVR/ChiLCVF). The PCR results confirmed the amplification of the DNA of the ChiLCV isolate in the selected pepper plants, with a product size of 600 base pairs as expected.

Keywords: Chili leaf curl virus (ChiLCV), *Capsicum annuum*, Cultivar resistance, PCR, Peroxidase activity

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Introduction

Chili pepper (*Capsicum* spp.) is an important economic crop cultivated worldwide in warm and temperate regions for multiple purposes. Globally, chili ranks third after tomato and potato, and it belongs to the family Solanaceae (1). In Iraq, chili pepper production during the summer season reached 306 tons/donum from a cultivated area of 659 donums, whereas in the winter season production was 70 tons/donum from a cultivated area of 130 donums. Yields are generally higher under covered cultivation or plastic house systems (2). On a national scale, chili pepper is grown on approximately 300,000 hectares (2).

The global market value of dried chili pepper was estimated at USD 1.61 billion in 2024 and is projected to reach USD 2.16 billion by 2029, with a compound annual growth rate (CAGR) of 6.11% during 2024–2029. The Asia-Pacific region dominates the global market, with India being the largest producer, consumer, and exporter of dried chili peppers. Major importers of Indian chili include China, the United States, Thailand, Sri Lanka, and Indonesia. Over the past five decades, global chili production has steadily increased (3).

Despite its economic importance, chili pepper is highly susceptible to many pathogens, particularly viral diseases, which cause severe yield losses. Globally, 65 viruses have been reported to infect chili, including members of the genus Begomovirus, which

cause chili leaf curl disease (ChiLCV). This virus is considered one of the most destructive due to its high infection rates and associated yield reductions. The virus can be diagnosed by symptoms of curling, shrinking and reduced leaf area along with stunting of the entire plant and is transmitted by the whitefly *Bemisia tabaci* by continuous transmission (4). Chili leaf curl virus (ChiLCV) caused by Geminivirus transmitted by the whitefly-borne (*Bemisia tabaci*), specifically Pepper leaf curl virus (ChiLCV), has been reported from India, USA, Nigeria and several other countries such as Pakistan, Bangladesh and Indonesia (5, 6). Chili leaf curl virus (ChiLCV) is mainly caused by Chili leaf curl virus (ChiLCV) (Family: Geminiviridae, Genus: Begomovirus). The genome of the virus is a 2.7 kbp circular single-stranded DNA molecule associated with alpha and beta satellites of 1.3 and 1.4 kbp, respectively. The virus genome is packaged in distinctive icosahedral particles about 18–30 nm in size (7). Salari et al. (8) reported that ChiLCV infection begins at an early stage of plant growth, with leaves curling towards the midrib and becoming distorted, leading to stunted plants and wilting of flower buds before reaching full size, in addition to poor pollen development (9).

Materials and Methods

The experiment was conducted in a plastic house at the experimental fields of the Plant Protection Department, College of Agricultural Engineering Sciences, under natural conditions during the spring and summer seasons of 2023–2024. The plastic house had an area of 160 m². The soil was prepared by plowing to a depth of 30–40 cm, leveling, and installing a drip irrigation system. Chili pepper cultivars (Barbarian, Singer, and Saidah) were transplanted as saplings previously raised in polystyrene trays obtained from Al-Reef Al-Khadraa nursery in Al-Yusufiyah. These are among the most widely cultivated and locally demanded chili varieties in Iraqi provinces. The seedlings were transplanted into the field on March 17, 2024, according to a Randomized Complete Block Design (RCBD) within the greenhouse, which measures 8 m in width and 20 m in length. The distance between plants was 40 cm, with six replicates per treatment, and a spacing of 1.5 m between rows. The total number of plants per experimental unit was eight. Routine crop management operations were carried out regularly until the end of the growing season, with daily monitoring of plants to record the development of symptoms and calculate the viral infection incidence and severity.

Description of chili pepper cultivars used in the study

- 1.Saidah: Origin – Switzerland. Purity: 99.9%, Germination: 98%, Package: 500 seeds. A hot pepper cultivar characterized by vigorous growth and very good resistance to powdery mildew. It has high productivity, producing dark green fruits about 0.15 m long with a shoulder length of 0.02 m. The recommended plant density is 20,000 plants/ha. Produced by Syngenta and imported by Nada Al-Awrad Company, Iraq.
- 2.Singer: Origin – USA. Purity: 90%, Germination: 99%. A hot pepper cultivar with dark green, thick-walled fruits and high yield potential. Produced by US Agriseeds and imported by Ashjar Al-Bustan Company.
- 3.Barbarian: Origin – India. Purity: 99%, Germination: 90%. A high-yielding hot pepper cultivar producing dark green fruits that turn red at maturity. Fruit size is 15 × 18 cm with an average weight of 70–80 g. Produced by United Genetics and imported by Ard Al-Zira'a Company.

Calculation of infection rate

The infection rate was calculated according to the following formula: (10)

Table (1). Scale used to assess the severity of chili leaf curl virus (ChiLCV) infection.

Severity grade	Symptoms and infection percentage
0	0% Healthy, no infection, immune to infection
1	0–4% Transparent spotting on upper leaves (slight leaf distortion)
2	4–25% Leaf curling, few yellow spots, and leaf swelling
3	25–50% Leaf curling, yellowing, and vein swelling
4	50–75% Leaf curling, growth inhibition, and blister formation on nodes
5	75–100% Leaves curled, small and deformed; stunted plant growth; appearance of small flowers; absence of fruits or presence of small, deformed, and twisted fruits

The severity of the infection was calculated according to the following scale used by (Sharma et al., 2018):

The studied parameters included plant height, number of branches per plant, chlorophyll content, peroxidase enzyme activity, yield in terms of number of fruits per plant, fruit weight per treatment, fresh and dry weight of the vegetative mass, and fresh and dry weight of the root system after 85 days of planting.

Statistical analysis of the experiment:

The experiment was carried out according to a randomized complete block design (RCBD). The experiment included three

treatments with six replicates per treatment, with eight plants per experimental unit. The number of experimental plants was $3 \times 6 \times 8 = 144$. Data were analyzed using Microsoft Excel, and treatment means were compared using the Least Significant Difference (LSD) test at a 0.05 probability level (11).

Diagnosis using polymerase chain reaction (PCR) technology

The PCR test was performed using the Cat.NO.25026 kit; The Maxime PCR premix kit (I-Taq), supplied by the Korean company Bioneer, performed the polymerase chain reaction (PCR) using specific primers for the ChiLCV virus:

CATATTCGCCAGACACATTAG ChiLCV-F-
CGTGCCATTCCTCAAGAC ChiLCV-R-

The nucleic acid of the ChiLCV virus samples was amplified using the following PCR steps and conditions: initial denaturation of the DNA for 5 minutes at 94°C, followed by 35 cycles consisting of final denaturation for 30 seconds at 94°C, primer annealing for 45 seconds at 53°C, and initial elongation of the amplified DNA product for 1 minute at 72°C, and finally, the reaction was terminated with a final elongation step at 72°C.

Electrophoresis Using Agarose Gel:

A 2% agarose gel was prepared by melting in a microwave at 100°C for 15 minutes using TBE1XL, then allowing it to cool to 50°C. Next, 5 microliters of 10% ethidium bromide dye was mixed into the agarose gel solution. The solution was poured into a specialized agarose casting mold equipped with a comb to create wells in the gel. After being left to solidify for 15 minutes at room temperature, the comb was gently removed once the agarose gel had completely solidified. The mold, now with the wells, was placed back in the electrophoresis apparatus, and TBE1X buffer solution was added. Finally, 10 microliters of PCR product were loaded into one set of wells, while 5 microliters of a 100 bp ladder were loaded into the other. Electrophoresis was carried out at 135 Volts and 80 mA for 30 minutes. The PCR products were visualized using an EZ-Capture MG imaging device from ATTO (Japan).

Chlorophyll Measurement:

Chlorophyll The content of chlorophyll was measured using the SPAD-502 Plus Chlorophyll Meter. Three readings were taken from three leaves per plant, and the average of these readings was calculated for each experimental unit, following the methodology described by Kamarianakis, and Panagiotakis (12).

Estimation of peroxidase activity in chili pepper plants

Fresh leaves were collected from chili plants and immediately transported to the laboratory of the Agricultural Research Directorate in Abu Ghraib in an ice-cooled container. The leaves were thoroughly washed with water, blotted dry using filter paper, and 1 g of tissue was weighed and manually cut into small pieces. The leaf tissue was homogenized in a porcelain mortar with 2 mL of sodium phosphate buffer (Na_2PO_4) at 0.01 M and pH 6.5. The homogenate was centrifuged at 6000 rpm for 2 minutes at 4 °C. 100 microliters of the supernatant extract were taken and mixed with 1.5 ml of Progal solution at a concentration of 0.05 M, then a 100 microliters of 1% hydrogen peroxide was added.

The reaction mixture was immediately transferred to a spectrophotometer, and absorbance was recorded at 420 nm over four to six consecutive readings, taken at 15-second intervals.

100 microliters of the supernatant extract were taken and mixed with 1.5 ml of progall solution at a concentration of 0.05 M, followed by the addition of 100 microliters of 1% hydrogen peroxide. Enzyme activity was calculated using the following formula:

$$(\text{Enzyme units/ml}) = ((3 \times \Delta A)) / (0.001 \times \Delta T))$$

Where:

- ΔA = Change in absorbance

- ΔT = Time interval for the change in absorbance

One unit of enzyme is defined as the amount of enzyme that causes an increase in absorbance of 0.01 units per minute at a wavelength of 420 nm. Peroxidase activity was determined according to the method described by Hammerschmidt et al. (13).

Confirmation of ChiLCV Presence in Naturally Infected Cultivars via Polymerase Chain Reaction (PCR):

Samples were collected from chili pepper plants exhibiting symptoms ranging from mild to severe leaf curling and twisting, to verify natural infection with ChiLCV. Viral DNA was extracted from all infected leaves and subjected to (PCR) amplification using the universal primers ChiLCVF/ChiLCVR to confirm the presence of the virus.

The PCR results showed amplification of a fragment approximately 600 base pairs in length, representing a region of the viral genome. The outcome was separated using a 2% Agarose gel and dyed with Et. Br., confirming the presence of the virus in some

randomly selected samples from naturally infected plants (Figure 2).



Figure (2): Electrophoresis of ChiLCV on agarose gel showing a molecular weight of 600 bp.



Figure (3): Development and variation of disease symptoms in chili pepper cultivars infected with ChiLCV.

Evaluation of chili pepper cultivars for tolerance to ChiLCV infection under natural conditions

Analysis of variance revealed significant differences among chili pepper cultivars in their response to ChiLCV infection, both in terms of infection rate and severity. Statistical results also revealed significant differences of infection in the hot pepper cultivars. The difference in cultivar response may be due to a number of factors such as Bemisia tabaci population dynamics as the vector, environmental conditions in the agro-ecosystem and genetic differences associated with resistance or susceptibility to ChiLCV.

The study further demonstrated the presence of two distinct cultivar groups: one more susceptible and the other relatively more resistant, based on infection rates and severity levels. Clear differences in response to viral infection were observed among the cultivars Barbarian, Singer, and Saidah. Barbarian showed significant superiority over Singer and Saidah, recording the lowest infection rates of 4.75% and 8.45% and severity of 1.69% and 3.97% after 55 and 85 days, respectively. Singer followed with infection rates of 10.50% and 15.23% and severity of 5.22% and 9.45% at 55 and 85 days, while Saidah was the most susceptible, exhibiting the highest infection rates of 12.18% and 18.17% and severity of 7.09% and 11.39% at 55 and 85 days, respectively (Table 1).

Table (1) Incidence and severity of ChiLCV infection in chili pepper cultivars.

Cultivar	Incidence after 55 days (%)	Severity after 55 days (%)	Reaction	Incidence after 85 days (%)	Severity after 85 days (%)	Reaction
Saidah	12.18	7.09	R	18.17	11.39	R
Barbarian	4.75	1.69	HR	8.45	3.97	HR
Singer	10.50	5.22	R	15.23	9.45	R
LSD (0.5%)	2.4852	4.268		6.51	4.9673	

These results are consistent with Devi et al. (14) regarding the presence of active defense mechanisms in ChiLCV-resistant cultivars, as virus accumulation in resistant cultivars was significantly slower compared to susceptible cultivars. In this study, ChiLCV infection led to distinct morphological changes in the leaves, stems, flowers, and fruits of chili plants. Initially, leaves appeared smooth, medium-sized, oval, and narrow, gradually changing in color from light green to dark green. Early infection caused noticeable disturbances in plant growth and development.

Typical ChiLCV symptoms were evident on chili plants, beginning with curling of young leaves, followed by upward or downward curling of older leaves, accompanied by stunted growth in most cases. Additional symptoms included leaf margin curling, reduced leaf size, blistering and swelling between veins, and thickened tissues. Infected plants acquired a bushy appearance due to stunting, with older leaves becoming leathery and brittle. Young shoots often developed in clusters, bearing oval-shaped leaves curled upwards or downwards, accompanied by flower drop, malformed fruits, and bud necrosis. Some buds showed twisted, abnormal growth (Figure 4-1). These symptoms are consistent with earlier descriptions by Vasudeva and Samraj (15), Muniyappa (16), Muniyappa and Veeresh (17), Kumar et al. (18), Singh et al. (19), and Jones (21).

The results also showed that ChiLCV weakens cell walls in susceptible cultivars, facilitating viral entry, as these cultivars lack resistance-related immune responses that restrict viral spread. In contrast, resistant cultivars contain effective resistance genes that enhance defense responses (22). Similar studies demonstrated that ChiLCV disrupts plant metabolism, particularly in susceptible cultivars, affecting water and nutrient transport and thereby increasing infection rates while weakening antiviral immune responses. This disruption accelerates viral spread in sensitive cultivars, whereas resistant cultivars have greater capacity to induce early defense responses, including the enhanced production of antioxidant proteins (23, 24, 25).

Moreover, ChiLCV infection was shown to enhance the production of reactive oxygen species (ROS), causing oxidative stress, cellular injury and resulted in more severe infection in susceptible varieties. Resistant varieties, however, could limit the increase of ROS levels more effectively through a simultaneously-established antioxidant defense system and was therefore beneficial in reducing viral damages (26, 27).

In addition, other researches revealed that in certain chili cultivars resistance to the ChiLCV was due to genetic modification and changed the distinct proteins prevent the entry of virus inside plant cells. Resistant genotypes frequently have higher doses of ChiLCV-inducible particles, particularly resistance (R) genes that activate plant defense systems by early immune responses that interfere or hinder viral multiplication (4; 28; 29). This was associated with an increased production of phenolic compounds and antioxidants by resistant genotypes like flavonoids and tannins which counteract the negative effect caused by virus via reduction of oxidative stress in plant cells (30, 31). Susceptible cultivars, however, suffered metabolic and tissue repair damage due to ChiLCV infection, which led to higher infection rates and weakened immune responses in those cultivar compared with resistant ones, which maintained higher levels of antioxidant defense enzymes restricting viral spread (32, 33).

Another study's results indicated that the virus affects sugar levels, increases free radical production, and causes oxidative stress, leading to cellular damage and increased infection severity in susceptible varieties. Resistant varieties, however, contain higher sugar levels and demonstrate a greater ability to restrict free radicals due to strong defense systems, thereby reducing the impact of infection (26, 27, 34). These findings also align with Devi et al. (14), who noted active defense mechanisms in ChiLCV-resistant varieties, with significantly slower virus accumulation compared to susceptible varieties.

The effect of ChiLCV on some vegetative growth indicators:

The results showed a clear effect of ChiLCV infection 55-85 days after planting on plant heights and branch counts. Variations were observed in the heights and number of branches of chili pepper plants and all tested varieties after 55-85 days. The Barbarian variety produced the highest rates of plant height and branch count, 84.17 cm/plant and 33.88 branches/plant, respectively, after 55 days, and 109.69 cm/plant and 39.15 branches/plant, respectively, after 85 days. It was followed by the Singer variety, which did not differ significantly from the Barbarian variety in plant heights after 55 and 85 days and branch count after 55 days. However, they differed significantly in the number of branches after 85 days, reaching 35.05 branches/plant. It was followed by the Saidah variety, which showed a significant difference, with plant heights reaching 109.69 cm/plant and 39.15 branches/plant. 71.85 and 82.93 cm/plant and number of branches, 25.48 and 31.66 branches/plant, respectively, after 55 and 85 days (Table 2 and Figure 4).

These findings agree with Das et al. (33), who reported that ChiLCV infection substantially reduced plant height and branch number. The results are also consistent with previous studies showing that infected plants develop smaller leaves and shorter branches, giving them a dense, stunted appearance (35). The reduction in growth is linked to the virus's effect on nutrient and water uptake, which diminishes primary metabolic processes, leading to weakened plant growth and reduced height. ChiLCV also limits photosynthetic energy production, reduces assimilate availability for branch formation, and impairs nutrient transport within the plant, negatively

affecting vegetative growth and branching (32, 36).

Chaubey and Mishra (34) and Roy et al. (2023) estimated the effect of several factors on density of insect vector and transmission rate of virus ChiLCV to infected host plant since these vectors feed with sap of host plants, during feeding period, the virus penetrates into the plant tissues resulting in infection and plant growth rate is reduced. Mohamed (38) and Kone et al. (39) explained that dense plant coverage and narrow spacing provide suitable sites for whitefly egg-laying, while delayed planting leads to an intensification of whitefly numbers and an increase in ChiLCV virus incidence, as overcrowding of plants in narrow areas results in reduced light access and ventilation due to dense tree canopies, thereby decreasing photosynthesis and vegetative growth, and reducing plant height and branch numbers (38; Das 40).

Table (2) Plant height, number of branches, and fresh and dry weights of shoots and roots of chili pepper cultivars.

Cultivar	Plant height after 55 days (cm)	Number of branches after 55 days (branches/plant)	Plant height after 85 days (cm)	Number of branches after 85 days (branches/plant)	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots (g)
Saidah	71.85	25.49	82.93	31.66	376.30	149.77	51.197	24.625
Barbarian	84.17	33.88	109.69	39.15	455.06	160.26	57.498	34.813
Singer	76.78	29.01	91.03	35.05	396.88	153.83	48.420	28.02
LSD 0.05	3.3353	3.5343	4.9093	1.9069	115.52	22.196	8.809	5.766



Figure 4: Plant height, number of branches, and fresh weight of different chili pepper cultivars.

In similar studies, it was found that whitefly-transmitted viruses affect cytokinin hormone, which is one of the main regulatory molecules in vital plant interactions and plays a role in reshaping primary and secondary metabolism, associated with the plant's defense mechanisms against biotic and abiotic stresses (22, 41).

These results align with previous findings reported by Ghafoor et al. (42), which confirmed that the ChiLCV virus led to a reduction in the fresh and dry weights of the vegetative and root systems, possibly due to a decrease in nutrients such as zinc sulfates, copper sulfates, manganese sulfates, and uric acid, which play roles in numerous processes that can alter the plant's response to viral

infection.

Kumar et al. (26) and Kushwaha et al. (32) reported that under ChiLCV infecting conditions, toxic metabolites including free amino acids and phenolics accumulate in the infected tissues. These compounds lead to oxidative stress and decreased capacity for water, nutrients uptake and transport by the plant, as well as damage to vascular tissue resulting in inhibition of growth and decrease in shoot and root mass.

Additionally, ChiLCV directly affects amino acid-related enzymes, affecting the photosynthesis rate, reduces energy production and chloroplast integrity which in turn declines nutrient synthesis (32, 34).

Estimation of peroxidase activity

The cultivars under study exhibited clear differences in peroxidase enzyme activity. Barbarian recorded the highest levels, with 138.34 and 98.40 units at 55 and 85 days, respectively. Singer followed with 93.93 and 80.05 units at 55 and 85 days, while Saidah recorded the lowest levels, with 86.23 and 73.43 units at 55 and 85 days, respectively. These differences were statistically significant (Table 3).

Table (3) Peroxidase enzyme activity against ChiLCV infection in chili pepper cultivars.

Cultivar	Peroxidase enzyme after 55 days	Peroxidase enzyme after 85 days
Saidah	86.23	73.43
Barbarian	138.34	98.40
Singer	93.93	80.05
LSD (0.5%)	7.0655	13.318

The results are consistent with previous studies, which reported that ChiLCV infection leads to the accumulation of toxic compounds such as lipid peroxides in plant tissues, causing oxidative damage to cell membranes, tissue necrosis, reduced calcium levels, and buildup of amino acids inside plant cells. This breaks down protein syntheses that are crucially needed for defense mechanism, resulting in diminished performances of a number of important enzymes (43, 44).

Peroxidase is implicated in cellular functions and promotes host defense against pathogen by polymerizing cell wall components to glycoproteins for reinforcement of the wall upon infection (45). And It also participates in plant physiological activities from seed germination to leaf aging and can be localized in the cell wall or cytoplasm (46).

Studies have also shown that the peroxidase enzyme is the main enzyme in the biosynthesis of lignin and suberin deposition, and the synthesis of phytoalexins (47, 48). This enzyme oxidizes phenolic compounds when exposed to pathogens, converting these compounds into quinone compounds (Quinones), and it contributes to transforming them into semi-quinone materials in plant cell walls at penetration sites, which contributes to the appearance of infection symptoms.

Total Plant Chlorophyll Content (SPAD):

The results showed clear differences between chili pepper cultivars in chlorophyll content. Barbarian outperformed the other cultivars, with SPAD values reaching 50.07 after 55 days and 43.13 after 85 days. This significantly differed from Singer and Siadah, which had lower SPAD values of 45.99 and 43.91, respectively, after 55 days and 39.62 and 36.20, respectively, after 85 days (Table 4).

Table (4) Effect of ChiLCV after 55–85 days on chlorophyll content in chili pepper cultivars.

Cultivar	chlorophyll content after 55 days	chlorophyll content after 85 days
Saidah	43.91	36.20
Barbarian	50.07	43.13
Singer	45.99	39.62
LSD (0.5%)	3.6779	4.18

These results are consistent with the findings of researchers that the ChiLCV virus leads to a significant decrease in chlorophyll content in the leaves of susceptible plants compared to plants resistant to the virus (ChiLCV) as a result of the disruption of the chloroplast membrane system during infection, which affects the efficiency of photosynthesis in plants (34, 49, 50, 51). Higher

accumulation of phenolic compounds, tannins, and chlorophyll was observed in resistant varieties, with lower amounts in susceptible varieties of infected plants, through participation in metabolic interactions associated with resistance (34, 52, 53). Kushwah et al. (32) and Sran et al. (54) observed that ChiLCV infection greatly reduces photosynthesis and vegetative efficiency of the plant, which resulted in a reduction of chlorophyll content in leaves and more specifically affecting efficient transportation of nutrients within the plant as well as degraded vegetative growth in infected plants. Furthermore, the photosynthetic ability of the plant is lowered by inhibition of chlorophyll biosynthesis through inactivation of specific enzymes involved in its formation as well as the production of toxic substances accumulating in plant cells, that interfere with absorption of important nutrients such as nitrogen and magnesium (both essential for chlorophyll synthesis) (23, 24, 55). In addition, degradation of chloroplast structure and disruption in basic photosynthetic processes was noticed during ChiLCV infection. The virus causes accumulation of water within the cellular compartments of leaf, which is responsible for decrease in the potentiality of producing chlorophyll (26, 34). Furthermore, the virus leads to a disruption in the system of vitamin and mineral delivery within the plant, and its ability to take up major elements such as magnesium and iron that are vital for chlorophyll synthesis (43, 56).

Effect of ChiLCV on yield and productivity traits of chili pepper cultivars

In light of the results, differences were observed among the varieties in rates of fruit number formed, fruit weight, and plant yield after 85 days from planting (Figure). Barbarian recorded the highest averages, reaching 29.80 g/plant, 3.153 g/fruit, and 0.445 g/plant, respectively. These values differed significantly from Singer and Saidah, which showed lower averages of 23.55 g/plant, 2.356 g/fruit, and 0.2972 g/plant for Singer, and 19.86 g/plant, 2.325 g/fruit, and 0.2755 g/plant for Saidah, respectively (Table 5).

Table (5): Effect of ChiLCV on fruit weight, number of fruits, and yield per plant in chili pepper cultivars.

Cultivar	Fruit weight (g/plant)	Number of fruits (g/fruit)	Yield per plant (g/plant)
Saidah	2.325	19.86	0.2755
Barbarian	3.153	29.80	0.4450
Singer	2.356	23.55	0.2972
LSD (0.5%)	0.5575	6.0317	0.0779

Table 5 illustrates the effect of ChiLCV infection on fruit weight, fruit number, and yield per plant in chili pepper cultivars.

The results confirmed that ChiLCV exerts a strong negative effect on the tested cultivars. Susceptible plants exhibited early reductions in fruit number, fruit weight, and overall yield compared with resistant cultivars. These findings align with previous studies reporting that ChiLCV infection significantly reduces fruit productivity and quality. Das et al. (40) found that infection reduced fruit yield, fruit number, fruit weight, and fruit length in chili plants, with early-infected plants failing to set fruit. During the flowering stage, infected flowers dried, abscised, or produced deformed fruits. Variations among cultivars in fruit number were attributed to genetic differences in resistance and to the viral impact, which reduces flower initiation, inhibits fruit set, and increases flower drop (57).

Rao et al. (58) and Singh et al. (20) reported similar reductions in fruit number and fruit size in sensitive cultivars. Thakur et al. (4) also observed that heavily infected plants with high ChiLCV incidence remained stunted and failed to bear fruit. The reduction in fruit yield may also be linked to increased levels of capsaicinoids in diseased fruits, which showed nearly a fourfold increase in dihydrocapsaicin and a threefold increase in capsaicin and nordihydrocapsaicin content. Higher levels of proteins, total phenolics, antioxidants, and free radical activity were also reported in infected fruits (59).

Singh et al. (20) and Reddy et al. (60) reported that infection with ChiLCV results in a decrease in fruit number because of a reduction in length and setting of the fruits, and it shortens flowers and its diameter. This may be due to the lack of significant heteroblasty metabolism involved in fruit yield, capsaicin, oleoresin and ascorbic acid (61, 62, 63, 64).

ChiLCV infection resulted in a significantly reduced fruit ratio and weight of various cultivars (65; Abbas and Al-Abadi, 2017). The reduction of productivities in infected lines is attributable to the impact of the virus on many basic plant metabolic processes involved in maintaining plant life cycle, such as a lowered rate of synthesis of chlorophyll, regulation by hormones affecting growth (e.g., gibberellin), and absorption by plants from growing medium mineral elements such as magnesium and calcium adversely influencing flowering, number of flowers per plant, fruit setting and overall yield (22)

Differences in the cultivar tested, the viral isolate used, transmission method of virus and virus accumulation in plant tissue under different environmental conditions (i.e., temperature) may cause discrepancies between our study and others (4; 7; 37).

Conclusions

This study showed that the chili leaf curl virus (ChiLCV) greatly reduced growth and yield of chili pepper cultivars, with great differences in susceptibility difference among them. Barbarian was highly resistant, presenting reduced incidence and severity of infection, better vegetative growth, higher fruit yield, and greater physiological performance than the other cultivars. According to these results, the use of resistant cultivars such as Barbarian is recommended as an effective strategy for minimizing yield losses caused by ChiLCV in chili pepper. Furthermore, since molecular diagnostic methods such as PCR are used, it is a sensitive method for tracking ChiLCV dispersal and serving the screening of new resistant cultivars.

References

1. Ahmed W, Imran M, Yaseen M, ul Haq T, Jamshaid MU, Rukh S, Ikram RM, Ali M, Ali A, Maqbool M, Arif M. 2020. Role of salicylic acid in regulating ethylene and physiological characteristics for alleviating salinity stress on germination, growth and yield of sweet pepper. *PeerJ*. 2020 8: e8475. doi:10.7717/peerj.8475.
2. Central Statistical Organization. Agricultural production statistics of vegetable crops – Pepper in Iraq. Ministry of Planning. 2023.
3. Food and Agriculture Organization of the United Nations. World food and agriculture – statistical yearbook. FAO. 2020.
4. Thakur H, Jindal SK, Sharma A, Dhaliwal MS. Chilli leaf curl virus disease: A serious threat for chilli cultivation. *Journal of Plant Diseases and Protection*. 2018. 125:239–249. doi:10.1007/s41348-018-0146-8.
5. Mishra MD, Raychaudhuri SP, Ashrafi J. Virus causing leaf curl of chili (*Capsicum annum* L.). *Indian Journal of Microbiology*. 1963. 3:73–76.
6. Mishra M, Verma RK, Pandey V, Srivastava A, Sharma P, Gaur R, Ali A. Role of diversity and recombination in the emergence of chili leaf curl virus. *Pathogens*. 2022. 11(5):529.
7. Shingote PR, Wasule DL, Parma VS, Holkar SK, Karkute SG, Parlwar ND, Senanayake DMJB. An overview of chili leaf curl disease: Molecular mechanisms, impact, challenges, and disease management strategies in the Indian subcontinent. *Frontiers in Microbiology*. 2022. 13:899512. doi:10.3389/fmicb.2022.899512.
8. Salari K, Heydarnejad J, Massumi H. Genome characterization of chili leaf curl virus, the associated alphasatellite and betasatellite, and demonstration of pathogenesis of the virus in south-eastern Iran. *Iranian Journal of Plant Pathology*. 2023. 59(1):156–166. doi:10.22034/ijpp.2023.2010123.425.
9. Kumar, RV; Singh, AK; Singh, AK; Yadav, T; Basu, S; Kushwaha, N; Chattopadhyay, B and Chakraborty, S, Complexity of Begomovirus and Betasatellite populations associated with Chilli leaf curl disease in India. *Journal of General Virology*, 2015. 96(10);3143-3158.doi: 10.1099/jgv.0.000254.
10. Sharma A, Jindal SK, Thakur H. Phenotypic classes of leaf curl virus disease severity for nursery screening in chilli pepper. *Plant Disease Research*. 2018. 33(1):99–103.
11. Al-Rawi KM, Khalaf-Allah AAM. Design and analysis of agricultural experiments. University of Mosul, Ministry of Higher Education and Scientific Research. 2000.
12. Kamarianakis, Z. and Panagiotakis, S., Design and Implementation of a low-cost chlorophyll content meter. *Sensors*, 2023. 23(5), p.2699.
13. Hammerschmidt R, Nuckles EM, Kuc J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology*. 1982. 20(1):73–82.
14. Devi OP, Sharma SK, Sanatombi K, Devi KS, Pathaw N, Roy SS, Chanu NT, Sanabam R, Devi HC, Singh AR, Baranwal VK. A simplified multiplex PCR assay for simultaneous detection of six viruses infecting diverse chilli species in India and its application in field diagnosis. *Pathogens*. 2022. 12(1):6.
15. Vasudeva RS, Sam Raj J. A leaf-curl disease of tomato. *Phytopathology*. 1948. 38:364–369.
16. Muniyappa V. Whiteflies—Vectors of plant pathogens. Academic Press. 1980.
17. Muniyappa V, Veeresh GK.. Plant virus diseases transmitted by whiteflies in Karnataka. *Proceedings: Animal Sciences*. 1984. 93:397–406.
18. Kumar S, et al. A new monopartite Begomovirus species, Chilli leaf curl Vellanad virus, and associated Betasatellites infecting Chilli In the Vellanad region of Kerala, India. *New Dis Rep*, 2012. 25(20). DOI 2044-0588doi: 10.5197/12044-0588.2012.025.020.
19. Singh-Pant P, Pant P, Mukherjee SK, Mazumdar-Leighton S. 2012. Spatial and temporal diversity of begomoviral complexes in papayas with leaf curl disease. *Archives of Virology*. 2012. 157:1217–1232.
20. Singh AP, Singh S, Pal M, Singh R, Singh RS, Kumari R. Screening and identification of chilli leaf curl virus resistance genotypes in chilli. *The Pharma Innovation Journal*. 2021. 10(2):531–533.
21. Jones I. Research methods for sports studies. Routledge. 2022.
22. Al-Tamimi MQH, Kazem JA, Hameed KB, Aqeel N, Al-Amousi BH. Genetic variation of some isolates of tomato leaf curl virus and its effect on the content of tomato plants (*Solanum lycopersicum* L.) of some mineral elements. *Series of Agricultural Research Institute Conferences: Soil and Environmental Sciences*. 2022. 106(1):12.
23. Bhattacharyya D, Gnanasekaran P, Kumar RK, Kushwaha NK, Sharma VK, Yusuf MA, Chakraborty S. A geminivirus

- betasatellite damages the structural and functional integrity of chloroplasts leading to symptom formation and inhibition of photosynthesis. *Journal of Experimental Botany*. 2015. 66(19):5881–5895.
24. Bhattacharyya D, Chakraborty S. Chloroplast: The Trojan horse in plant–virus interaction. *Molecular Plant Pathology*. 2018. 19(2):504–518.
 25. Kushwaha, N.K.; Mansi, Sahu, P.P.; Prasad, M. and Chakraborty, S., Chilli leaf curl virus infection downregulates the expression of the genes encoding chloroplast proteins and stress-related proteins. *Physiology and Molecular Biology of Plants*, 2019. 25;:1185-1196.<https://doi.org/10.1007/s12298-019-00693-1>
 26. Kumar, S; Raj, R; Raj, SK; Agrawal, L; Chauhan, PS and Srivastava, A, Study of biochemical and histopathological changes induced In the sweet pepper (*Capsicum annuum* L.) in response to Chilli leaf curl virus infection. *Physiological and Molecular Plant Pathology*, 2018. 104;:95-102.
 27. Taufik, M; Firihi, MZ; Gusnawaty, HS; Variani, VI; Hasan, A; Botek, M; Tihuraa, EF and Wulansari, TYI, The changes of chili leaf structure by Geminivirus infection. *Journal Hama dan Penyakit Tumbuhan Tropika*, 2024. 24(1);:109-119.<https://doi.org/10.23960/jhptt.124109-119>.
 28. Mishra, M.; Verma, R.K.; Pandey, V.; Srivastava, A.; Sharma, P.; Gaur, R. and Ali, A., Role of diversity and recombination if the emergence of Chilli leaf curl virus. *Pathogens*, 2022. 11(5), p.529.
 29. Mangal, M; Srivastava, A; Mandal, B; Solanki, V; Mirajkar, SJ; Shashank, PR; Kalia, P; Rana, JC and Sharma, VK, Exploring Host Resistance against Chilli Leaf Curl Disease in a Tolerant Chilli Genotype. *Plants*, 2024. 13(12), p.1647<https://doi.org/10.3390/plants13121647>.
 30. Mondal, C. K., Acharyya, O., & Hazra, P. Biochemical basis of plant defense for leaf curl virus of chili (*Capsicum annuum* L.). In *Proceedings of the XV EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant*. 2019; (pp. 315–322).
 31. Ramazani, M A, Ayazpour, K, Niazmand, A R, & Najafipour, G . Detection of begomoviruses of Solanaceae crops in southern Iran. *Indian Phytopathology*, 2022 ; 75(4), 1137–1142. <https://doi.org/10.1007/s42360-022-00545-1>
 32. Kushwaha, N.K.; Mansi, Sahu, P.P.; Prasad, M. and Chakraborty, S., Chilli leaf curl virus infection downregulates the expression of the genes encoding chloroplast proteins and stress-related proteins. *Physiology and Molecular Biology of Plants*, 2019; 25;:1185-1196.<https://doi.org/10.1007/s12298-019-00693-1>
 33. Das S, Rahman M, Dash PK, Kamal MM. Suppression of chili leaf curl virus (ChiLCV) incidence in chili (*Capsicum annuum* L.) across Bangladesh via manipulated planting date and spacing. *Journal of Plant Diseases and Protection*. 2021; 128:535–548. doi:10.1007/s41348-020-00397-9.
 34. Chaubey AN, Mishra RS. Survey of chili leaf curl complex disease in eastern part of Uttar Pradesh. *Biomedical Journal of Scientific & Technical Research*. 2017. 1(7). doi:10.26717/BISTR.2017.01.000589.
 35. Senanayake, D M J B, Varma, A, & Mandal, B . Virus–vector relationships, host range, detection and sequence comparison of chilli leaf curl virus associated with an epidemic of leaf curl disease of chilli in Jodhpur, India. *Journal of Phytopathology*, 2012 ; 160(3), 146–155. <https://doi.org/10.1111/j.1439-0434.2011.01876.x>
 36. Zeeshan N, Kudada N. Eco-friendly management of chilli leaf curl disease complex through plant products. *Journal of Pharmacognosy and Phytochemistry*. 2019; 8(1):1045–1049.
 37. Roy, B., Venu, E., Kumar, S., Dubey, S., Lakshman, D., Mandal, B., & Sinha, P. Leaf curl epidemic risk in chilli as a consequence of vector migration rate and contact rate dynamics: A critical guide to management. *Viruses*, 2023; 15(4), 854. <https://doi.org/10.3390/v15040854>
 38. Mohamed MA. Impact of planting dates, spaces and varieties on infestation of cucumber plants with whitefly, *Bemisia tabaci* (Genn.). *The Journal of Basic & Applied Zoology*. 2012; 65(1):17–20. doi:10.1016/j.jobaz.2012.01.003.
 39. Koné N, Asare-Bediako E, Silué S, Koné D, Koita O, Menzel W, Winter S. Influence of planting date on incidence and severity of viral disease on cucurbits under field conditions. *Annals of Agricultural Sciences*. 2017; 62(1):99–105.
 40. Das, S., Rahman, M., Dash, P. K., & Kamal, M. M. Suppression of chili leaf curl virus (ChiLCV) incidence in chili (*Capsicum annuum* L.) across Bangladesh via manipulated planting date and spacing. *Journal of Plant Diseases and Protection*, 2021; 128, 535–548. <https://doi.org/10.1007/s41348-020-00397-9>
 41. Giron D, Glevarec G, Erb M, Dicke M, Pieterse CMJ. Cytokinins as key regulators in plant–microbe–insect interactions: Connecting plant growth and defence. *Functional Ecology*. 2013; 27(3):599–609. doi:10.1111/1365-2435.12042.
 42. Ghafoor A, Ali S, Zeshan MA, Ghani MU, Mahmood R, Azmat S, Khan AA. Management of chili leaf curl disease (ChiLCD) through resistant germplasm and nutrients in relation to environmental factors. *Abasyn Journal of Life Sciences*. 2022; 5(1):134–146.
 43. Ali A, Zeshan MA, Iftikhar Y, Abid M, Ehsan SF, Ghani MU, Khan AA. Role of plant extracts and salicylic acid for the management of chili veinal mottle virus disease. *Pakistan Journal of Phytopathology*. 2020; 32(2):147–157.
 44. Nair, M., Radhika, N. S., Johnson, J. M., Sajeesh, P. K., Kurian, S., Binitha, N. K., & Abinaya, B. Role of antioxidants in the management of chili leaf curl virus in chili using beneficial fungal root endophyte *Piriformospora indica*. *International Journal of Plant & Soil Science*, 2024 ; 36(8), 241–254. <https://doi.org/10.9734/ijps/2024/v3684853>
 45. Wang Q, Luo C, Wang R. Insecticide resistance and its management in two invasive cryptic species of *Bemisia tabaci* in

- China. *International Journal of Molecular Sciences*. 2023; 24(7):6048.
46. Iqbal, M. J., Zia-Ur-Rehman, M., Ilyas, M., Hameed, U., Herrmann, H. W., Chingandu, N., Manzoor, M. T., Haider, M. S., & Brown, J. K.. Sentinel plot surveillance of cotton leaf curl disease in Pakistan: A case study at the cultivated cotton–wild host plant interface. *Virus Research*, 2023; 333, 199144.
 47. Asif, M., Haider, M. S., & Akhter, A. Impact of biochar on Fusarium wilt of cotton and the dynamics of soil microbial community. *Sustainability*, 2023; 15(17), 12936.
 48. Singh, K., Kumar, M., Rawat, K., Ranebennur, H., Meena, V. S., Shekhawat, N., Sharma, M., Chawla, M. P., Jadon, K. S., Choudhary, M., & Rao, G. P. Characterization of ‘Candidatus *Phytoplasma asteris*’ associated with witches’ broom disease of fenugreek and preliminary germplasm screening for disease resistance. *Phytopathogenic Mollicutes*, 2023 ; 13(2), 163–176.
 49. Chia, T. F., & He, J.. Photosynthetic capacity in *Oncidium* (Orchidaceae) plants after virus eradication. *Environmental and Experimental Botany*, 1999; 42(1), 11–16.
 50. Funayama, S., Hikosaka, K., & Yahara, T. Effects of virus infection and growth irradiance on fitness components and photosynthetic properties of *Eupatorium makinoi* (Compositae). *American Journal of Botany*, 1997 ; 84(6), 823–829.
 51. Guo, D. P., Guo, Y. P., Zhao, J. P., Liu, H., Peng, Y., Wang, Q. M., Chen, J. S., & Rao, G. Z.. Photosynthetic rate and chlorophyll fluorescence in leaves of stem mustard (*Brassica juncea* var. *tsatsai*) after turnip mosaic virus infection. *Plant Science*, 2005; 168(1), 57–63.
 52. Mahjabeen, Akhtar, K.P.; Sarwar, N.; Saleem, M.Y.; Asghar, M.; Iqbal, Q. and Jamil, F.F., Effect of cucumber mosaic virus Infection on morphology, yield and phenolic contents of tomato. *Archives of Phytopathology and Plant Protection*, 2012; 45(7);766-782.
 53. Singh, A., Jaiswal, R. K., Maurya, S., & Singh, U. P. Analysis of phenolic and indole acetic acids in *Meloidogyne graminicola* infected rice plants (*Oryza sativa* L.). *International Journal of Advanced Research*, 2013; 1, 71–76.
 54. Sran, T. S., Jindal, S. K., Sharma, A., & Chawla, N. Genetics of novel leaf curl disease-resistant pepper genotypes and antioxidative profile analysis of their progenies. *Scientia Horticulturae*, 2023; 308, 111563. <https://doi.org/10.1016/j.scienta.2022.111563>
 55. Hamida, R., & Suhara, C. Pengaruh infeksi Cucumber mosaic virus (CMV) terhadap morfologi, anatomi, dan kadar klorofil daun tembakau cerutu. *Buletin Tanaman Tembakau, Serat & Minyak Industri*, 2013; 5(1), 11–19.
 56. Ganefianti, D. W., Hidayat, S. H., & Syukur, M. Susceptible phase of chili pepper due to tomato yellow leaf curl Begomovirus infection. *International Journal of Advanced Science, Engineering and Information Technology*, 2017; 7(2), 594–601.
 57. El-DougDoug, K. A., Gomaa, H. H. A., & El-Maaty, S. A. The impact of interference between tomato yellow plants. *Journal of Applied Sciences Research*, 2006; 2(12), 1151–1155.
 58. Rao, A. M., Prasad, G., & Susmitha, B. The leaf curling in *Capsicum* species: A review. *Mysore Journal of Agricultural Sciences*, 2020; 54(2). <https://www.researchgate.net/profile/Balaraju>
 59. Khan, M. S., Raj, S. K., & Singh, R.. First report of Tomato leaf curl New Delhi virus infecting chilli in India. *Plant Pathology*, 2006; 55(2), 289. <https://doi.org/10.1111/j.1365-3059.2006.01324.x>
 60. Reddy, M. G., Baranwal, V. K., Sagar, D., & Rao, G. P. Molecular characterization of chickpea chlorotic dwarf virus and peanut witches’ broom phytoplasma associated with chickpea stunt disease and identification of new host crops and leafhopper vectors in India. *3 Biotech*, 2021; 11(3), 112.
 61. Bhutia, N. D., Seth, T., Shende, V. D., Dutta, S., & Chattopadhyay, A. Estimation of heterosis, dominance effect and genetic control of fresh fruit yield, quality and leaf curl disease severity traits of chili pepper (*Capsicum annuum* L.). *Scientia Horticulturae*, 2015; 182, 47–55.
 62. Kumar, R.A.K.E.S.H.; Kumar, V.I.N.A.I.; Kadiri, S. and Palicherla, S.R., Epidemiology and diagnosis of Chilli leaf curl virus in central India, a major Chilli growing region. *Indian Phytopathol*, 2016; 69(4s):.61-64.
 63. Krishnan, N., Kumari, S., Krishnan, S., Dubey, V., Singh, A. K., & Kumar, R. First report of tomato leaf curl Joydebpur virus infecting chilli (*Capsicum annuum*) in Andaman and Nicobar Islands. *Plant Disease*, 2019; 103(11), 2974. <https://doi.org/10.1094/PDIS-03-19-0451-PDN>
 64. Sreenivas, M., Bhattacharjee, T., Sharangi, A. B., Maurya, P. K., Banerjee, S., Chatterjee, S., Maji, A., Mandal, A. K., Chakraborty, I., & Chattopadhyay, A.. Breeding chili pepper for simultaneous improvement in dry fruit yield, fruit quality and leaf curl virus disease tolerance. *International Journal of Vegetable Science*, 2020; 26(5), 457–486. <https://doi.org/10.1080/19315260.2019.1648351>
 65. Kareem, M. H. Immunological and molecular diagnosis of tomato yellow leaf curl virus and classification of tomato cultivars for infection (Master’s thesis). College of Agriculture, Al-Muthanna University. (2016).