PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(sp2): 01–12
https://doi.org/10.14719/pst.5023

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Pepper leaf curl Bangladesh virus (PLCBV) and weed interaction: Consequences for growth, yield and biochemical vicissitudes in chilli (*Capsicum annuum* L.)

Roma[1], Narayan Singh[1,2], Vasudha Maurya[1,2], Vishal Johar[3], Ashutosh Sharma[1], Yogesh Kumar[4], Sapna Thakur[5] & Rahul Kumar[1*]

[1]Faculty of Agricultural Sciences, Dayanand Anglo Vedic University, Jalandhar 144 012, India

[2]Department of Biotechnology, Dayanand Anglo Vedic University, Jalandhar 144 012, India

[3]Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara 144 411, India

[4]Department of Botany, Central University of Jammu, Jammu and Kashmir 181 143, India

[5]Department of Genetics, Plant Breeding and Biotechnology, Eternal University, Baru Sahib, Himachal 173 101, India

*Email: rahulihbt@gmail.com

OPEN ACCESS

ARTICLE HISTORY
Received: 12 September 2024
Accepted: 26 January 2025
Available online
Version 1.0 : 28 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Roma, Narayan S, Vasudha M, Vishal J, Ashutosh S, Yogesh K, Sapna T, Rahul K. Pepper leaf curl Bangladesh virus (PLCBV) and weed interaction: Consequences for growth, yield and biochemical vicissitudes in chilli (*Capsicum annuum* L.) Plant Science Today. 2025; 12(sp2): 01–12. https:/doi.org/10.14719/pst.5023

Abstract

The present study examines the impact of weeds on disease occurrence of Pepper leaf curl Bangladesh virus (PLCBV) and its subsequent impact on growth, yield and biochemical parameters in chilli. The experiment was conducted at the agriculture farm of DAV University, Jalandhar. The field trial revealed a strong correlation between weed presence and leaf curl disease, with the highest disease incidence observed in open plots without weeding. The incidence of PLCBV, with the highest disease incidence (83.3%) was recorded 75 days after transplanting the chilli plants. The study highlighted the role of common weeds like *Physalis* spp., *Solanum* spp., *Solanum* spp. in higher disease incidence. Molecular identification of casual virus PLCBV was executed in chilli and weeds through PCR with degenerate primers. The key growth and yield parameters including plant height, fruit dimensions, number of fruits per plant, number of branches and overall yield were negatively impacted. Biochemical parameters such as photosynthetic pigment content, flavonoids, phenolic content, ascorbic acid, proline, protein content and malondialdehyde (MDA) were also analyzed, along with the enzymatic activities of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX). Results indicated that infected plants showed reduced plant height, fruit length, fruit diameter, yield and content of chlorophyll a, chlorophyll b, total chlorophyll, lycopene, carotenoid, β-carotene and other beneficial compounds like flavonoids, phenolic content, ascorbic acid and protein compared to healthy plants. Conversely, proline content, MDA and enzymatic activities were higher in infected plants. The current study concludes that weeds and PLCBV exacerbated major negative and detrimental consequences in chilli in terms of biochemical, growth and yield parameters.

Keywords

antioxidant enzymes; disease incidence; healthy plants; infected plants; pepper leaf curl Bangladesh virus; weeds; white fly; yield loss

Introduction

Chilli (*Capsicum annuum* L.) is a vegetable crop, native to Mexico, with a chromosome number 2n=24. It grows in warm, humid conditions between 18 –30°C. Chilli produces berries, which are considered as vegetables (1). This is a

nutrient-rich vegetable, rich in essential vitamins and minerals, including vitamin C, manganese, potassium, vitamin K1, vitamin A and vitamin B6. China, Turkey, Indonesia, Mexico and Spain are the top five countries in the world that produce chillies. In India, over 70 tonnes of chilli are produced (2). The top five states producing chillies in India are Karnataka, Madhya Pradesh, Bihar and Andhra Pradesh (2). Production of chilli in Punjab is 17.63 million tonnes (3).

Weeds can significantly affect the production of any field crop by consuming resources for reproductive growth, which may lead to reduced flower and fruit yields by competing for resources such as water, nutrients and sunlight, in addition to causing insufficient sunlight, water stress and nutrient deficiencies (4). Crop growth decreases with weed growth and weeds can inhibit the growth of crop shoots and roots, resulting in smaller plants and reduced crop productivity. Unregulated weed growth acting as alternate host or reservoir, can also attract diseases and pests, increasing the risk of disease outbreaks and insect infestations (5). Changes within an ecosystem can significantly alter cropping patterns and geographic distributions, leading to the emergence of new weed species and an increase in insect vector activity (6). Among the vectors, the whitefly (*Bemisia tabaci*) stands out as a common pest affecting agricultural crops in tropical, subtropical and temperate regions worldwide (7). This highly invasive species poses severe economic challenges to agroecosystems, not only through direct feeding, which causes physiological damage to plants, but also by transmitting plant viruses. These viruses belong to several families, including *Begomovirus, Crinivirus, Carlavirus, Torradovirus* and *Ipomovirus* (8, 9). A *Begomovirus*, Pepper Leaf Curl Bangladesh Virus (PLCBV) causing leaf curl disease in chilli had been characterized in our previous study (9). These viruses can cause symptoms such as leaf curling, stunted growth, reduced yields and mosaic patterns on leaves, leading to lower crop productivity and market value (9).

Biochemical stress occurs due to chemical imbalances, affecting plant growth and increasing vulnerability to other stresses (10). Excess reactive oxygen species (ROS) from oxidative stress damage proteins and lipids, however, plants may mitigate this through antioxidant enzymes like peroxidases, catalase and superoxide dismutase (11). Biochemical studies provide details about physiological responses and offer perspective on the relationship between plants and the pathogen. Chlorophyll in healthy leaves supports optimal growth, while decreased activity can affect photosynthesis and disease resistance (12). Infected leaves may have a lower content of ascorbic acid, phenolic compounds and higher activity of antioxidant enzymes like catalase and superoxide dismutase. These enzymes are essential for reducing reactive oxygen species (ROS) and reducing oxidative harm to essential elements (13). The impact of weeds and their consequences on viral infection and subsequent biochemical, growth and yield parameters need to study to understand as well as mitigate their proliferation in addition to quality and yield loss.

Materials and Methods

The present study on the effect of weeds on leaf curl incidence, biochemical parameters and yield in chilli (Var. Pusa Sadabahar) was conducted during the Kharif season (March to September) 2023 at the experimental farm of Faculty of Agricultural Science, DAV University Jalandhar, Punjab, India.

Location, preparation of experimental field and seedling transplantation

The experiment was conducted at the Faculty of Agricultural Science, DAV University, Jalandhar, during the 2023-2024 period and 238 m above sea level. First, a power tiller was used to cultivate the land, which was then exposed to sunlight for a week. After this, the field was harrowed, ploughed and cross-ploughed multiple times, followed by laddering to achieve a fine tilth suitable for optimal chilli seedling growth. Weeds and stubbles were removed to ensure a desirable soil condition. On March 24, 2023, these seedlings were transplanted into the experimental plots, with a spacing of 60 cm between rows and 45 cm between plants. Light irrigation was provided immediately after transplantation using a water cane.

Intercultural operations

To enhance the growth and development of the plants, the following intercultural methods were used throughout the experiment.

Gap filling, weeding and irrigation

Healthy seedlings from the same stock that had been sown earlier planted in the border area were used to fill in the gaps left by damaged, wilted, or dead seedlings in the field. To maintain the crop without weeds, improve soil aeration and break the soil crust, weeding was done whenever it was needed. It also helps in conservation of soil moisture. Four subsequent weeding were done manually at 15, 30, 45, 60 and 75 days after transplantation (DAT) to keep the plots free from weeds. Throughout the growth season, required irrigations were provided as needed. The experimental plot was semi-flooded with irrigation just after transplantation. Depending on the plants' needs and the soil's moisture level, the crop was watered as needed. Plots for the study of weeds impact on disease incidence were left abandoned without weeding to collect data throughout the crop cycle.

Observation recorded

Assessment of growth and yield parameters

Plant height, fruit diameter, fruit length with stalk and without stalk: Ten healthy and ten infected plants were randomly selected from each plot. At harvest, their heights were measured and the average was calculated. Additionally, ten fruits were collected from each plot, counted and measured for fruit diameter and length (with and without stalks), with averages calculated.

Number of fruits and branches per plant, fresh weight and dry weight: Ten healthy and ten infected plants were randomly selected from each plot. Branches were counted for each plant and the average was calculated. Fruits from ten randomly selected plants were also counted. The fresh

weight of ten fruits per plant was measured using a digital balance and the average was calculated. Additionally, the dry weight of fruits from each plant was determined at harvest using an oven and weighing machine to calculate the mean dry fruit weight.

Yield per plant (g), per plot (g) and tons per hectare (t/ha): Fruits reaching marketable size were picked and weighed and the average yield per plant and per plot was calculated from healthy, infected plants.

Identification of Pepper Leaf Curl Bangladesh Virus symptoms by visual and molecular analysis

Identifying PLCBV infection was carried out by visual observation of typical symptoms like leaf curling, vein clearing and stunted growth as the primary method, with the incidence determined by the number and percentage of infected plants. The virus was identified through molecular analysis as described in the previous study (9).

DNA isolation and molecular conformation of leaf curl virus

Total DNA was extracted from collected healthy and infected leaf samples using modified CTAB (14). Genomic DNA was amplified using *Begomovirus*-specific degenerate primers (AV494 and AC1048) (15).

Number of infected plants per plot

The number of infected plants per plot was observed by counting the infected plants in the field at 30, 60 and 75 DAT.

Disease incidence

A field examination was conducted to observe leaf curl disease incidence in chilli plants, collecting data at 30, 60 and 75 DAT under open field conditions and calculating the incidence (16).

$$\text{Disease incidence} = \frac{\text{Number of infected plants per plot}}{\text{Number of (diseased + healthy) plants per plot}} \times 100$$

Yield reduction (%)

The percentage reduction in yield based on fruit was calculated using the following formula (17).

$$\text{Yield reduction (\%)} = \frac{\text{Yield of healthy plants-Yield of infected plants}}{\text{Yield of healthy plants}} \times 100$$

Biochemical parameters

***Photosynthetic pigments*:** The study quantified photosynthetic leaf pigments chlorophyll-a and chlorophyll-b, total chlorophyll, carotenoid, lycopene and β carotene using spectrophotometric approach based on Lichtenthaler's method (18). Fresh leaves were dried in ethanol for seven days and absorbance was determined at various wavelengths. The following formulae were used to determine the photosynthetic pigments:

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Chlorophyll a = 12.7 (A663) - 2.29 (A645)

Chlorophyll b = 22.9 (A645) - 4.68 (A663)

Carotenoids = (7.6 × A480 - 1.49 × A510 × V) / (1000 × W)

Lycopene = $(0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453})$

Beta-carotene = $(0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453})$

Free proline content

A spectrophotometer was used to measure the absorbance at 520 nm and a proline standard was used to calculate the proline content. The free proline content was determined by the Bates method (19).

Total phenolic content

The study analyzed the total amount of phenols in the plant extract using Singleton's method (20), using Gallic acid as a standard and measuring absorbance at 650nm after 1 h of incubation.

Total flavonoid content

The study used Ardekani's method (21) to determine the total flavonoid content of a plant extract by mixing sodium nitrate, aluminium chloride and sodium hydroxide in a darkroom and evaluating the flavonoid content using catechin per 1g of plant material.

Ascorbic acid content

By mixing plant extract with 50% TCA, activated charcoal and double-distilled water, Roe and Kuether's method (22) was used for calculating the quantity of ascorbic acid. Filtration was done and 2,4 dinitrophenyl hydrazine was added. Ascorbic acid was used as the standard when measuring the absorbance at 520 nm.

Protein extraction and quantification

By crushing leaf tissues into a fine powder and mixing them with phosphate buffer, Bradford's method (23) was used to calculate the amount of protein. the purpose of measuring protein levels and enzyme activity, the supernatant was collected. Bovine serum albumin was employed as a standard to assess the quantity of protein in the extracted sample, which was using for the method.

Malondialdehyde content

The Cakmak and Horst method (24) was used to analyze lipid peroxidation in plant material, which was homogenized, diluted with 0.5% TBA and incubated at 90°C for 25 min.

Superoxide dismutase

The study estimated SOD activity using Beyer and Fridovich's method method (25), with a control reaction without enzyme extract. A reaction mixture was prepared, exposed to fluorescent lamps and incubated for 2 min. By using the inhibition of NBT, the specific activity of SOD was determined and represented in units per minute per milligram of protein.

Catalase

Using Aebi's method (25), the rate of H_2O_2 degradation was studied in order to determine the activity of CAT. A reaction mixture and a stock solution without of enzyme extract were developed. The enzyme activity was determined by measuring the quantity of H_2O_2 that dissolved in one minute for every gram.

Ascorbate peroxidase

The APX activity will be determined using Nakano and Asada's method (26), using a reaction mixture of sodium phosphate buffer, EDTA, ascorbic acid, H_2O_2 and protein and determining oxidized ascorbate.

Glutathione peroxidase

The enzyme GPX, which produces guaiacol per minute per gram of fresh tissue, was measured using the Fernández-Gracía method (27). The activity was determined using an extinction value of 26.6 mM cm based on absorbance at 436 nm.

Statistical analysis

In statistical analysis, data was collected. Three replicates of each sample were used. At a 5% level of significance, one way analysis (ANOVA) was done in T-test to check significance difference in healthy and infected plants by (Student- Newman- Keuls) method at 5% level of significance ($p \leq 0.05$).

Results

Prevalent open field weeds of Jalandhar region of Punjab are enlisted in Table 1. The list is dominated by Solanaceae and Poaceae families.

Identification of chilli leaf curl symptoms by visual and molecular analysis

Leaf samples of the infected chilli plants had typi-cal symptoms of leaf curl infection, showing curling of leaves, yellowing of leaves and reduced leaf size (Fig. 1). Genomic DNA was amplified using begomovirus-specific degenerate primers. In the PCR amplification, the expected ~ 570 bp bands were obtained from infected chilli leaf samples as well as all the three weed leaf samples (Fig. 2). This leaf curl virus has been characterized in previous study (28).

Disease incidence

During the field experiment, chilli plots were affected by leaf curl disease, which was followed by stunted plant growth. Observations from March to September 2023 revealed that the highest disease incidence (83.3%) occurred at 75 DAT in chilli plants grown in the field without weeding (Fig. 3a). In contrast, plants grown under net house conditions showed no signs of the disease (Fig. 3c), while those grown in the open field with weeding exhibited a much lower disease incidence (12.3%) at 75 DAT (Fig. 3b). Fig. 4 shows the overall disease incidence at 30, 45, 60 and 75 DAT in fields without weeding, with weeding and under net house conditions.

Table 1. Prevalent weeds observed in open field

S.No.	Common Name	Botanical Name	Family
1.	Ballon cherry	*Physalis* spp.	Solanaceae
2.	Carrot grass	*Parthenium* spp.	Asteraceae
3.	Green nightshade	*Solanum* spp.	Solanaceae
4.	Tiger's foot	*Lpomoea* spp.	Convolvulaceae
5.	False amaranth	*Digera* spp.	Amaranthaceae
6.	American nightshade	*Solanum* spp.	Solanaceae
7.	Common lambsqarters	*Chenopodium* spp.	Chenopodiaceae
8.	Sheeps sorrel	*Rumex* spp.	Polygonaceae
9.	Crow foot grass	*Dactyloctenium* spp.	Poaceae
10.	Annual meadow grass	*Poa* spp.	Poaceae
11.	Nutgrass	*Cyperus* spp.	Cyperaceae
12.	Congress grass	*Parthenium* spp.	Asteraceae
13.	Purple crabgrass	*Digitaria* spp.	Poaceae
14.	Liverseed grass	*Urochloa* spp.	Poaceae
15.	Crabgrass	*Digitaria* spp.	Poaceae
16.	Doob ghaas	*Cynodon* spp.	Poaceae
17.	Pig weed	*Amaranthus* spp.	Amaranthacea
18.	Little hogweed	*Portulaca* spp.	Portulacaceae
19.	Barnyard grass	*Echinochloa* spp.	Poaceae
20.	Horse purslane	*Trianthema* spp.	Aizoaceae

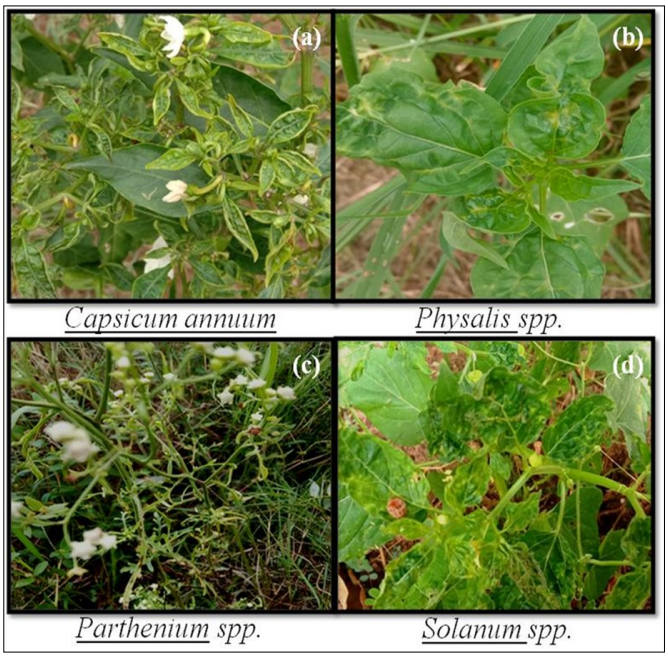

Fig. 1. Visual severe symptoms in chilli and weed plants infected with the leaf curl virus. (a) Curling of *Capsicum annuum* (chilli) leaves due to leaf curl virus infection, (b) Curling of *Physalis spp.* (Balloon cherry) weeds caused by leaf curl virus infection, (c) Curling of *Parthenium spp.* (Carrot grass) weeds due to infection by the leaf curl virus, (d) Curling of *Solanum spp.* (Green nightshade) weeds as a result of leaf curl virus infection.

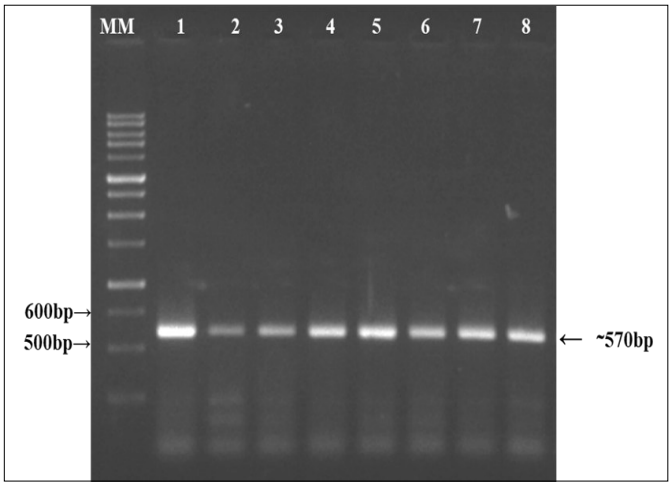

Fig. 2. Gel image displays PCR amplifications of PLCBV coat protein gene using the degenerate primer pair AV494 and AC1048. MM represents the 1000 bp marker (Genei, India). Lanes 1 and 5 show amplification from 2 and 6 represent infected *Physalis spp.* (balloon cherry) weed; Lanes 3 and 7 from infected *Parthenium spp.* (carrot grass) weed; and Lanes 4 and 8 represent infected *Solanum spp.* (green nightshade) weed.

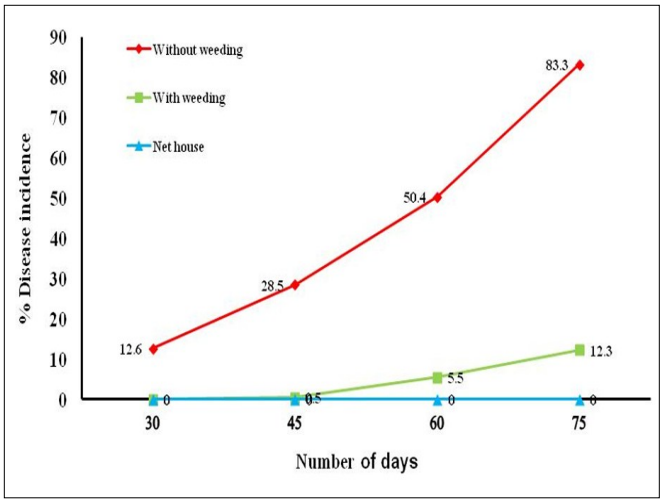

Fig. 4. Comparative effect of weeds on PLCBV disease incidence in Chilli growing under net house (Blue), open field with weeding (green) and open field without weeding (red) plots at 30 DAT, 45 DAT, 60 DAT and 75 DAT.

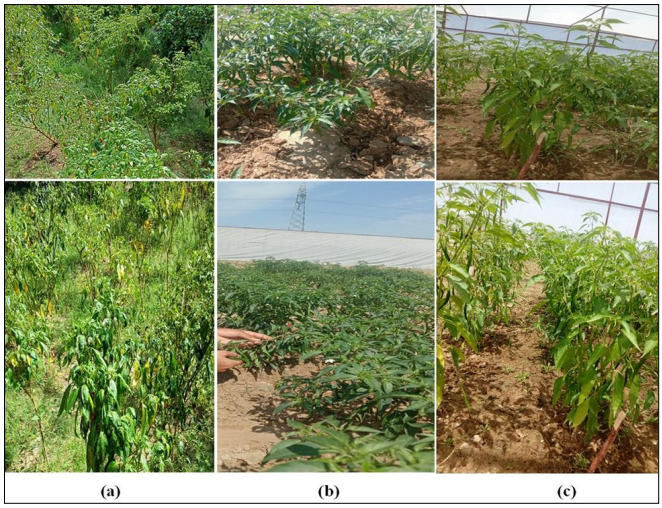

Fig. 3. Plants grown in an open field without weeding show severe leaf curl symptoms (a). In contrast, those grown with weeding exhibit fewer leaf curl symptoms (b). Plants grown under net house conditions display no disease symptoms (c).

Growth parameters

***Plant height and fruit diameter*:** That plant height at 30 DAT (Fig. 5a), 60 DAT (Fig. 5b), 75 DAT (Fig. 5c) and fruit diameter (Fig. 5d) differed in healthy and infected chilli plants. The plant height of healthy plants was taller than infected chilli plants and the fruit diameter of healthy plants was also higher than that of infected chilli plants.

Fruit length with and without stalk per plant and Numbers of fruit per plant

The fruit length with and without stalk and the number of fruits per plant differed in healthy and infected chilli plants. Healthy chilli plants had the longest fruit length with or without stalk than infected chilli plants (Fig. 6a & 6b). Healthy chilli plants had the highest numbers of fruit per plant than that of infected chilli plants (Fig. 6c).

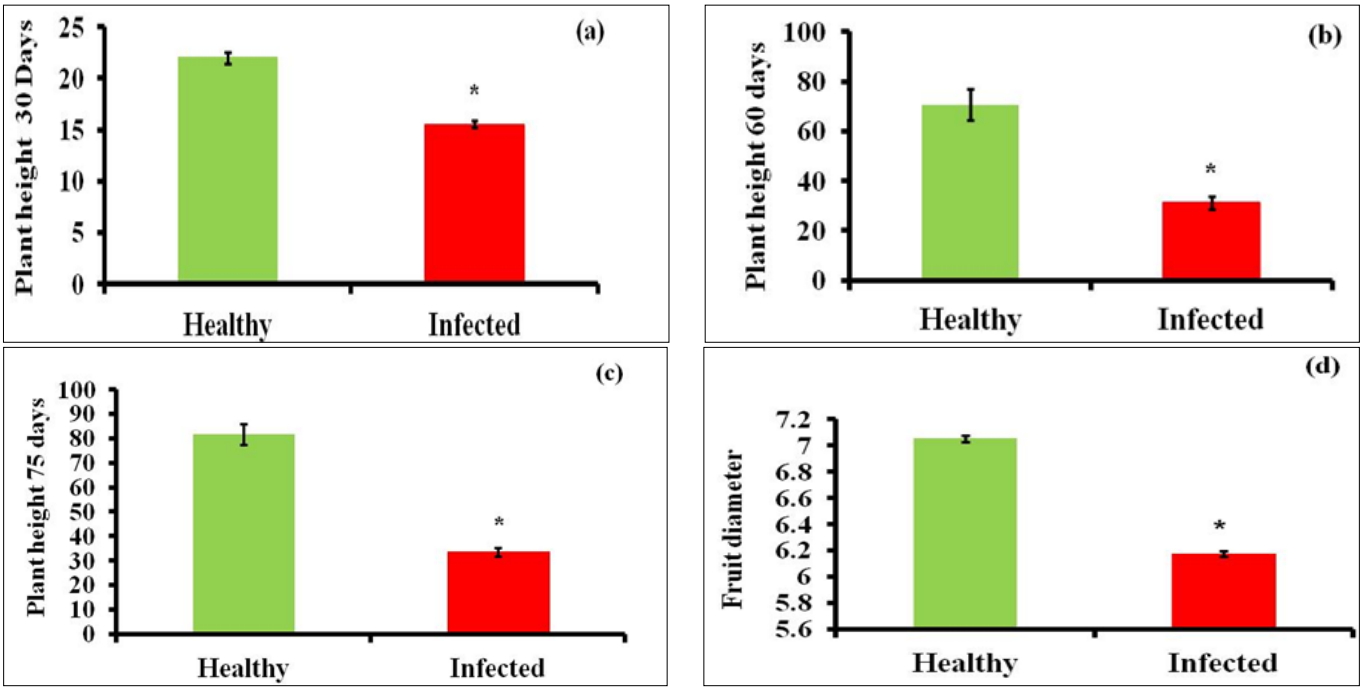

Fig. 5. Effect of weeds on (a) plant height 30 days, (b) plant height 60 days, (c) plant height 75 days and (d) fruit diameter in healthy and infected chilli plants. (*) indicates a significant difference between healthy and infected chilli plants at the level ($p \leq 0.05$).

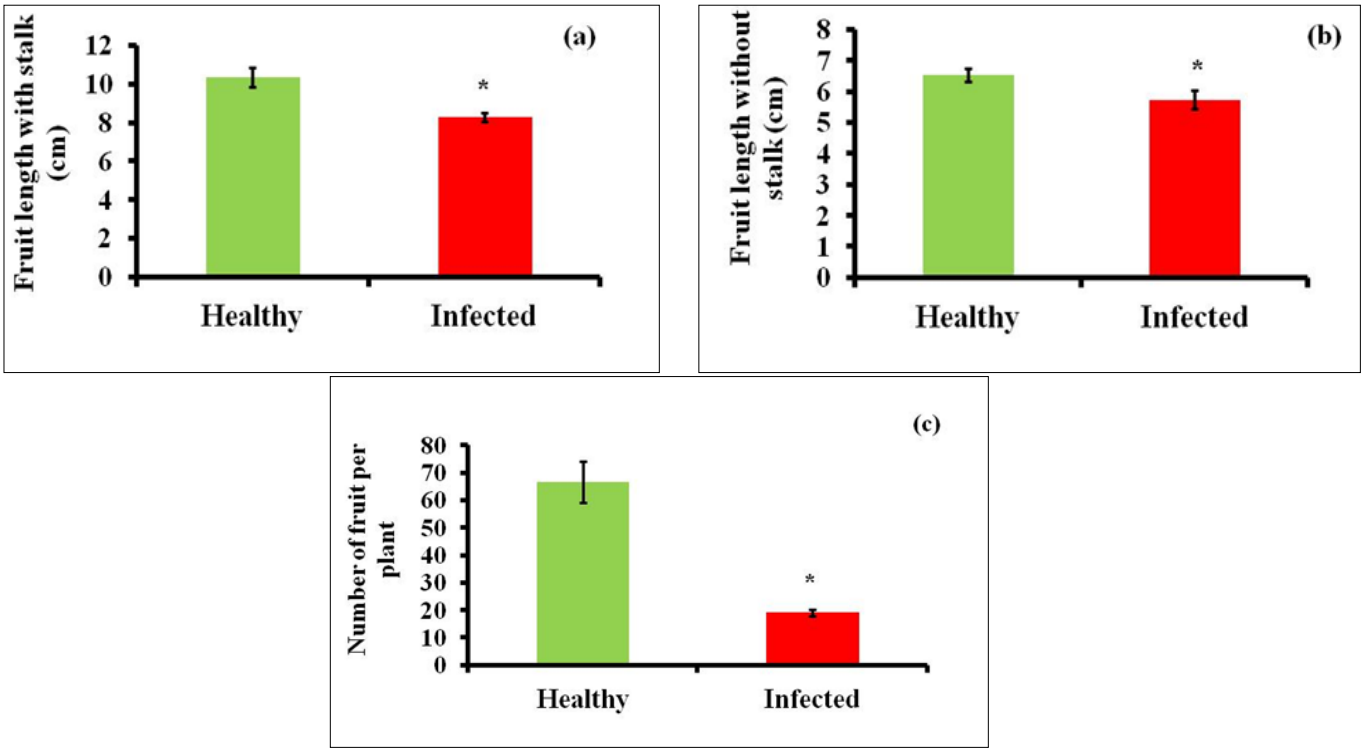

Fig. 6. Effect of weeds on (a) fruit length with stalk, (b) fruit length without stalk and (c) number of fruits per plant in healthy and infected chilli plants. (*) indicates a significant difference between healthy and infected chilli plants at the level (p≤0.05).

Number of branches

The number of branches at 30 DAT (Fig. 7a), 60 DAT (Fig. 7b) and 75 DAT (Fig. 7c) differed in healthy and infected chilli plants. Healthy chilli plants had more branches than the infected chilli plants.

Yield parameters

Fresh weight, dry weight of fruit and yield per plant **:** In the present study, it was observed that fresh weight of fruit, dry fruit weight, yield per plant and yield per plot differed between healthy and infected chilli plants. Healthy chilli plants had higher fresh and dry fruit weights than infected chilli plants (Fig. 8a & 8b). The yield per plant was higher in healthy chilli plants than in infected chilli plants (Fig. 8c).

Yield per plot and tons per hectare

The highest fresh yield per plot (12.62 kg) was observed with proper weeding in the net house plots (Fig. 9). However, the open field plots with weeding had a yield of 11.64 kg/plot, which was higher than the open field without weeding (4.81 kg/plot) and hence, the lowest yield per plot was recorded in the open field without weeding. The maximum fresh yield (21.03 t/ha) was achieved in the net house with weeding,

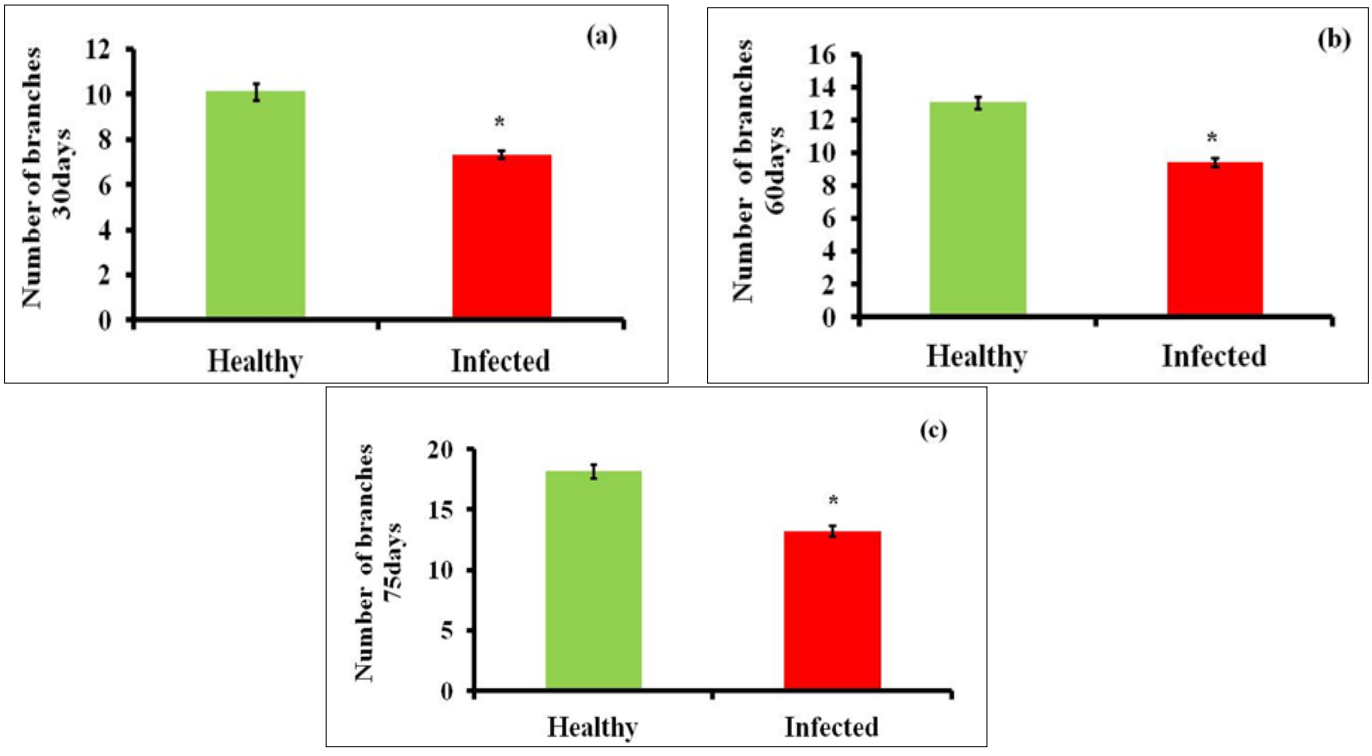

Fig. 7. Effect of weeds on (a) number of branches 30 days, (b) number of branches 60 days and (c) number of branches 75 days in healthy and infected chilli plants. (*) indicates a significant difference between healthy and infected chilli plants at the level (p≤0.05).

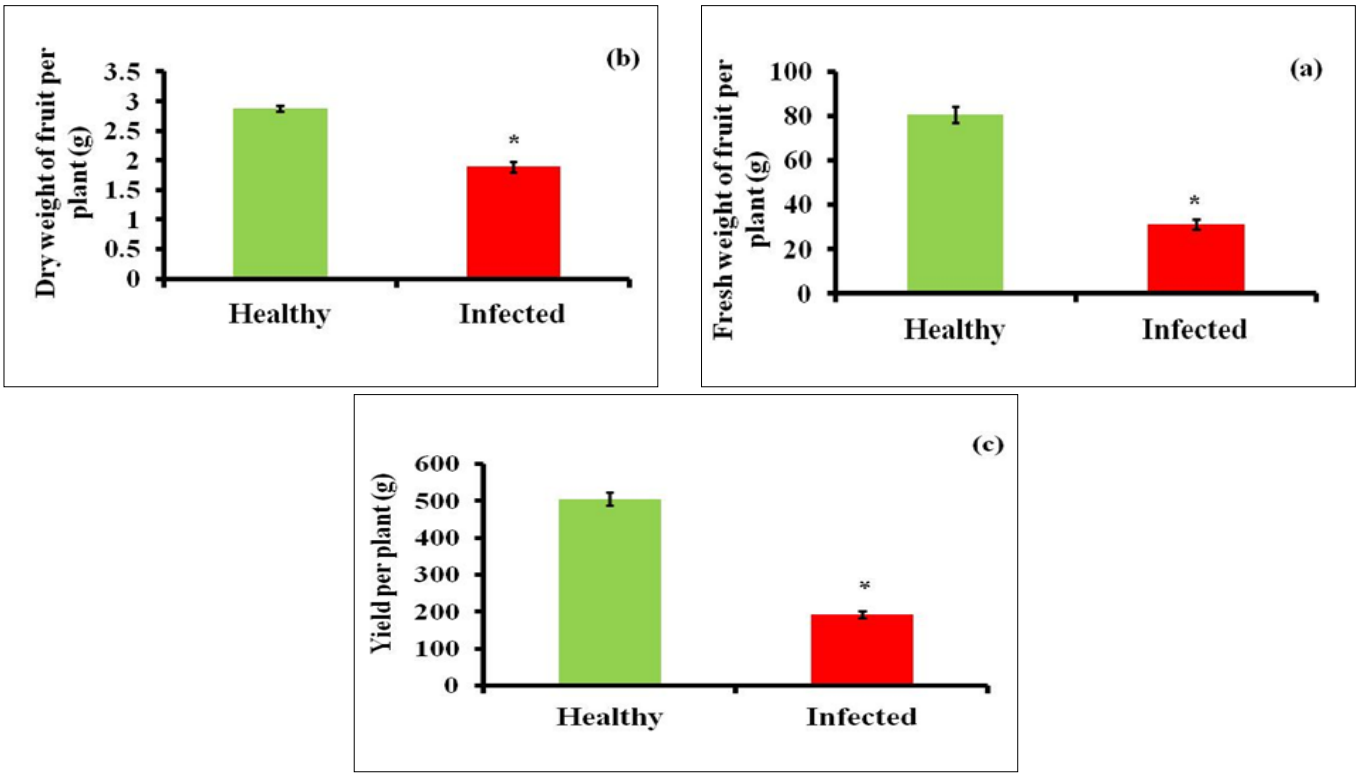

Fig. 8. Effect of weeds on (a) fresh weight of fruit per plant, (b) dry weight of fruit per plant and (c) yield per plant in healthy and infected chilli plants. (*) indicates a significant difference between healthy and infected chilli plants at the level (p≤0.05).

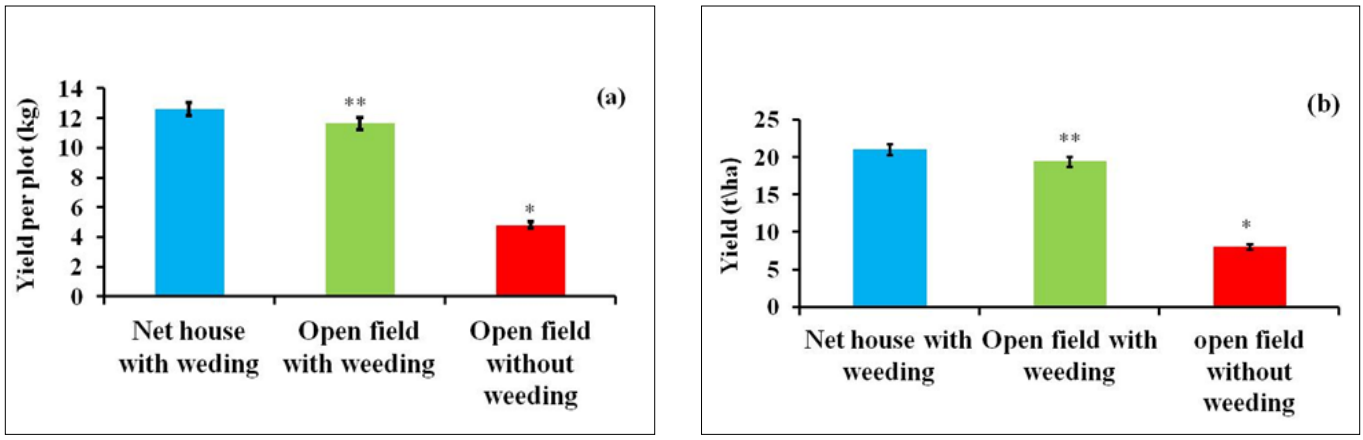

Fig. 9. Comparison of yield in net house with weeding (blue), open field with weeding (green) and open field without weeding (red) (kg per plot (a) and tons per hectare (b). (* and **) indicates a significant difference between the plots in net house with weeding and open field without weeding at the level (p≤0.05).

while the open field with weeding showed a slightly lower fruit yield (19.41 t/ha) as compared to the net house. The minimum fruit yield (8.03 t/ha) was recorded in the open field without weeding.

Yield reduction

In present study 61.8% reduction in yield per plot and yield per hectare was observed in chilli plots infested with predominant weeds as compared to plots that were properly weeded and kept clean in net house.

Photosynthetic pigments

The photosynthetic pigments differed in healthy and infected chilli plants. Healthy chilli plants had higher chlorophyll a (Fig. 10a), chlorophyll b (Fig. 10b), total chlorophyll (Fig. 10c), lycopene (Fig. 10d), carotenoid (Fig. 10e) and β-carotene content (Fig. 10f) than infected chilli plants.

Total flavonoid and phenolic content

The flavonoid content and phenolic content differed between healthy and infected chilli plants. Healthy chilli plants had higher flavonoid (Fig. 11a) and phenolic content (Fig. 11b) than infected chilli plants.

Ascorbic acid, protein content, proline content and MDA content

The ascorbic acid, protein content, proline content and MDA differed in healthy and infected chilli plants. Healthy plants had higher ascorbic acid content (Fig. 12a) and protein content (Fig. 12b) than infected chilli plants. Whereas, healthy plants had lower proline content (Fig. 12c) and MDA content (Fig. 12d) than the infected chilli plants.

Enzymatic changes (SOD, CAT, APX, GPX)

The enzymatic parameters differed in healthy and infected chilli plants. SOD (Fig. 13a), CAT (Fig. 13b), APX (Fig. 13c) and GPX of healthy plants (Fig. 13d) were lower than infected chilli plants.

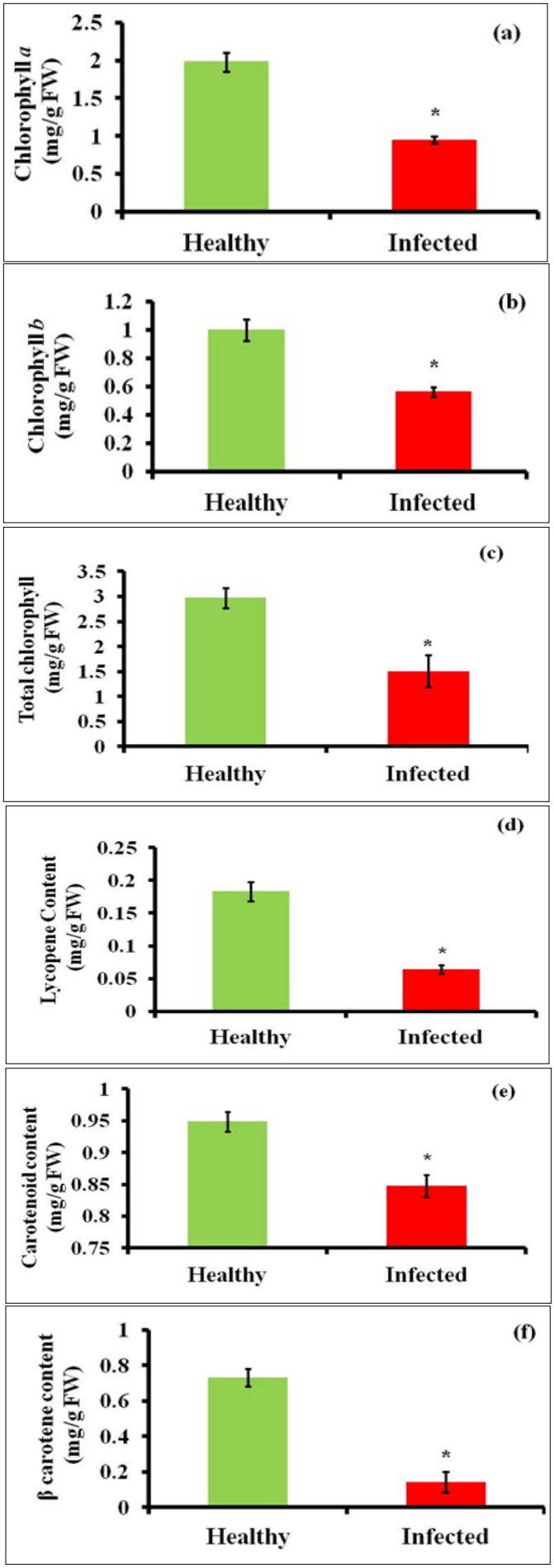

Fig. 10. Comparison of healthy and PLCBV-infected plants in terms of (a) chlorophyll a, (b) chlorophyll b, (c) total chlorophyll, (d) lycopene content, (e) carotenoid content and (f) β-carotene content. (*) indicates a significant difference between healthy and infected chilli plants at the level (p≤0.05).

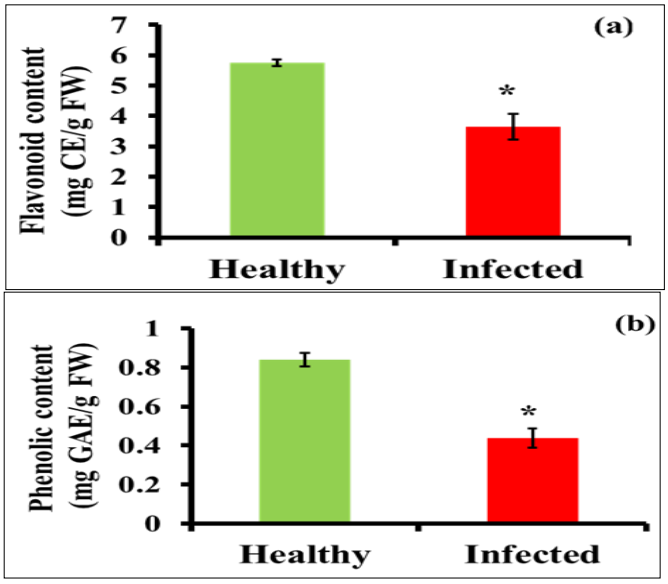

Fig. 11. Comparison of healthy and PLCBV-infected plants in terms of (a) Flavonoid content and (b) Phenolic content in healthy and infected chilli plants. (*) indicates a significant difference between healthy and infected plants at the level (p≤0.05).

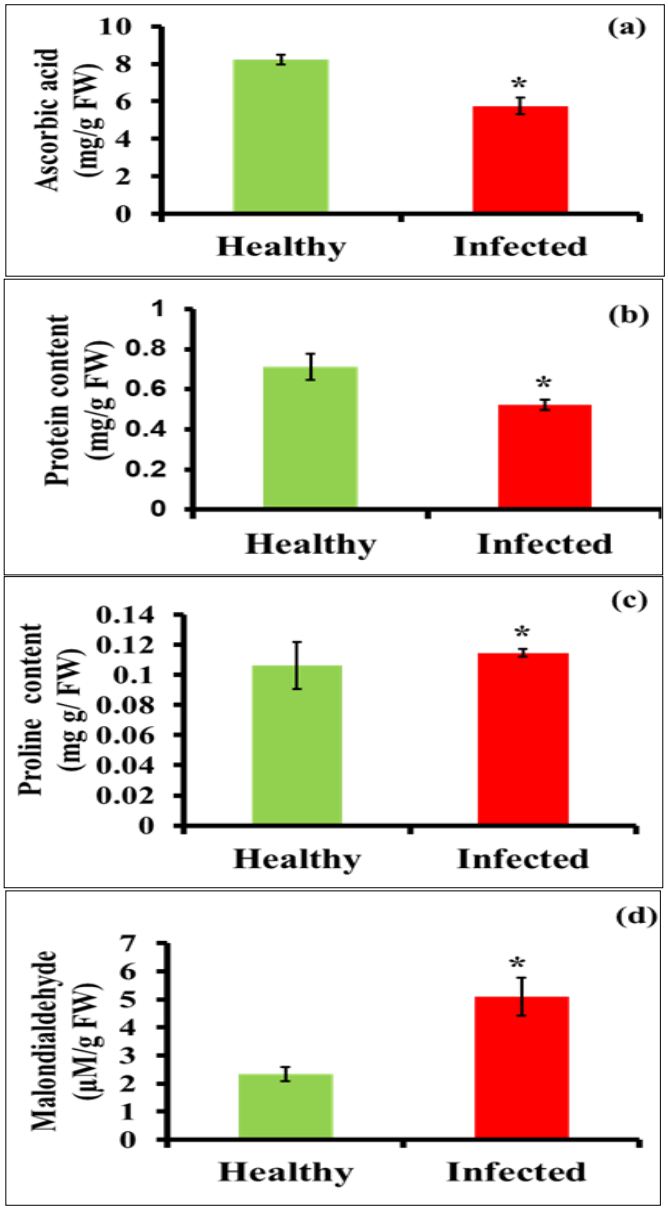

Fig. 12. Comparison of healthy and PLCBV-infected plants in terms of (a) ascorbic acid, (b) protein content, (c) proline content and (d) malondialdehyde in healthy and infected chilli plants. (*) indicates a significant difference between healthy and infected chilli plants at the level (p≤0.05).

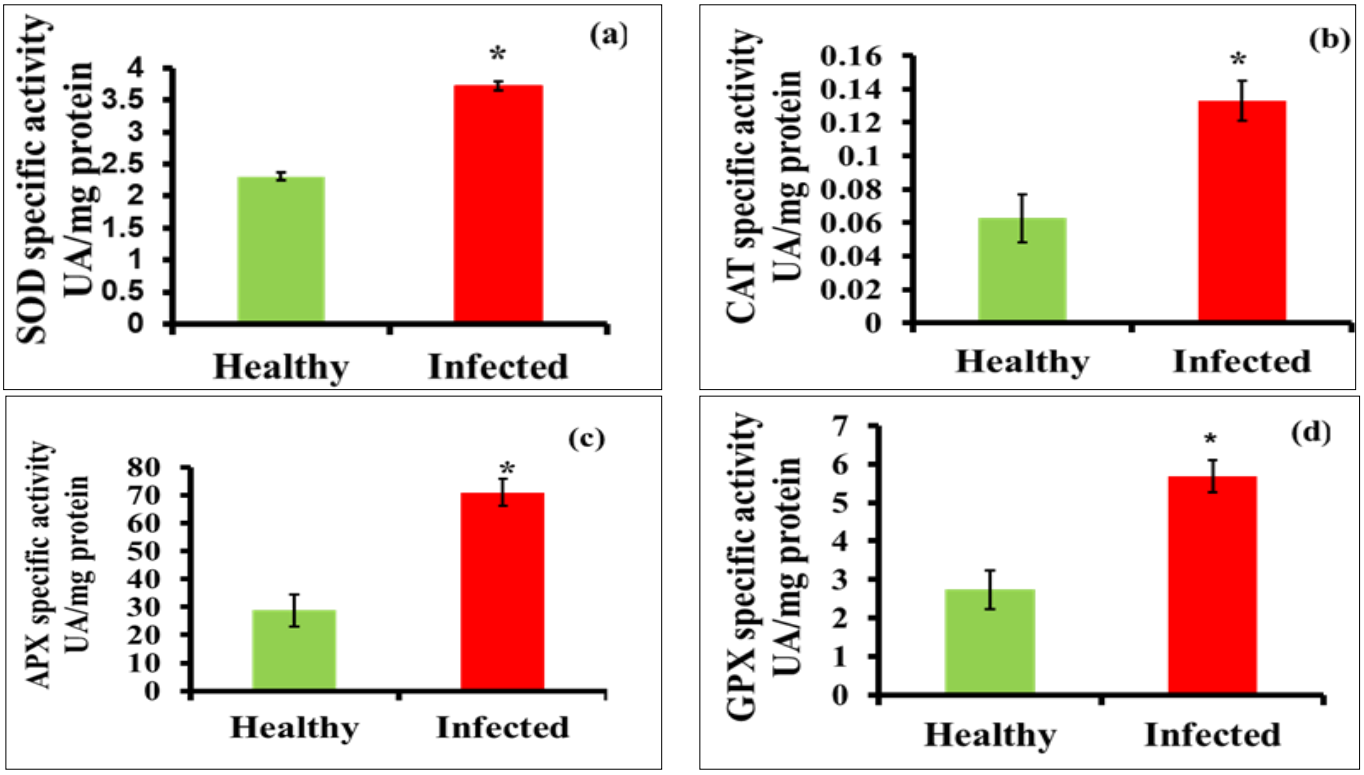

Fig. 13. Comparison of healthy and PLCBV-infected plants in terms of (a) Superoxide dismutase, (b) Catalase, (c) Ascorbate peroxidase and (d) Guaiacol peroxidase in healthy and infected chilli plants. (*) indicates a significant difference between healthy and infected chilli plants at the level ($p \leq 0.05$).

Discussion

The results in present study are consistent with a number of earlier reports, which have shown that chilli plants exhibiting symptoms of leaf curl have been found to harbor mixed infections with various begomoviruses (8, 28). These viruses belong to the family Geminiviridae, genus *Begomovirus* and are transmitted primarily by the vector whitefly *Bemisia tabaci* (6). Leaf curl viruses affecting chilli plants are classified within the genus *Begomovirus*, which comprises a large group of plant viruses characterized by their circular, single-stranded DNA genomes (29). Chilli being the Solanaceae crop may directly attract the pathogens and pests from other Solanaceae crops or plants including weeds. These weeds can act as alternate hosts or reservoirs for the spread of the vector transmitted diseases/ pathogens especially in the same family (30). Growth parameters are essential to assess plant health and yield estimation (31). In comparison to healthy plants, the present investigations showed that infected chilli plants had typical symptoms like leaf curling, reduced height, lower fruit diameter, fewer branches and fewer and shorter fruits. These findings are consistent with earlier studies in other crops like tomato (32) and sunflower (33). Reduction in disease incidence, providing and maximum utilization of resources and maintaining food security all depend on an understanding of the yield of both healthy and diseased plants (34).

The present study on yield parameters showed that infected chilli plants had lower dry weight, yields per plant and lower fresh weight. Further, the negative effect of weeds was evident in yield reduction resulting in lower yield per plot and per hectare. Similarly, chilli yellow leaf curl virus (35) and tomato yellow leaf curl virus (36) affected chilli and tomato crops which showed comparable results. Reduced yield correlates with disease incidence, which peaked at 83.3% at 75 DAT which was lower at 12.6% at 30 DAT. These findings are consistent with a study by Senanayake et al. (37), which reported a high occurrence of leaf curl in chilli plants during the flowering period in Rajasthan and in the Sultanpur and Faizabad districts of Uttar Pradesh in 2014 and 2015 (38).

The present study results indicate that healthy plants have a greater amount of chl *a*, chl *b*, total chl, β-carotene, carotenoids and lycopene than diseased plants. These pigments are reduced by viral infections in leaf curl disease, which affects the structure and function of chloroplasts (39). In the case of tomato yellow leaf curl virus in tomatoes (34) similar results were observed. Healthy plants have higher flavonoid and phenolic content compared to diseased plants, which are important for resisting pathogens (40). Lower phenolic content was also seen in diseased chilli with leaf curl disease (41). Ascorbic acid is a plant antioxidant that determines reactive oxygen species (ROS) and protects cells from oxidative damage (42). According to the present study, the vitamin C levels of virus-infected chilli plants as compared to healthy plants were considerably lower. Ascorbic acid content is also observed to decrease in response to viral infections, including pepper mild mottle virus (43). In the current investigation, the protein content of healthy plants is higher than that of diseased plants. A similar trend was observed in lucerne crops infected with *Candidatus Phytoplasma australasia* (44). Proline is essential for a plant's adaptability, growth control and tolerance to environmental problems (45). As per the findings of the current research, plants with disease symptoms had a significantly greater proline concentration than healthy plants. Similar results were found in tomato leaf curl New Delhi virus (46). According to a previous study,

MDA in plants is a key indicator of oxidative stress and lipid peroxidation (47). As compared to healthy plants, diseased leaves had greater MDA levels. Similar results were found in lucerne crops infected with *Candidatus Phytoplasma australiense* (44). CAT and SOD are essential for detoxifying ROS and protecting plants from oxidative damage (48). In the present study, healthy plants had low CAT and SOD activity than infected plants. These results match with other research and findings in chilli (49). APX and GPX activity is essential to reducing oxidative stress (50). Healthy plants had lower APX and GPX activity than in infected plants in the present study. The present study revealed the effect of weeds on subsequent spread of PLCBV followed by loss in yield and quality as suggested by the cascading biochemical changes. However, in future, all the PLCBVs can be characterized through their whole genome sequencing. In addition to that, based on the cascading mechanism, the mitigation strategies can also be designed to check the spread and effect of PLCBVs and other *Begomoviruses*.

Conclusion

On the basis of the current study, we conclude that weeds exacerbated major negative and detrimental consequences in chilli in terms of biochemical, growth and yield parameters. Severe symptoms, including leaf curling, leaf deficits, curled fruit and stunted plants, were caused by PLCBV. It negatively impacted yield metrics (dry and fresh fruit weights, yield per plant, yield per plot and yield per hectare) as well as growth characteristics (plant height, fruit diameter, fruit length and number of fruits per plant). Additionally, PLCBV lowered biochemical parameters such as protein content, phenolic content, ascorbic acid, lycopene, flavonoids, carotenoids, β-carotene, total chlorophyll and chlorophyll a and b. Plants that are not infected by PLCBV exhibited increased MDA activity and proline levels.

Acknowledgements

The authors are thankful to the DAV University management for providing financial and basic infrastructure support at DAV University, Jalandhar, through project no. DAVU/REGR/2023/278(a). In addition, the corresponding author acknowledges the financial grant received from the Council of Scientific and Industrial Research–Human Resource Development Group (CSIR-HRDG), New Delhi, via project no. 38(1477)/19/EMR-II.

Authors' contributions

R carried out field and biochemical work. YK carried out molecular work studies. NS and VM participated in the execution and statistical analysis. AS and VJ assisted in disease incidence analysis. VJ, ST and RK drafted the manuscript. Rahul conceived the idea of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors declare that they have no competing interests.

Ethical issues: None

References

1. Bosland PW, Votava EJ, editors. Peppers: vegetable and spice capsicums. Cabi; 2012. https://doi.org/10.1079/9781845938253.0000
2. Seth T, Lyngdoh YA, Chattopadhyay A, Sharangi AB, Mishra GP. Export of onion, garlic and chilli: Three essential spices in daily kitchen. In: Indian spices. Cham: Springer International Publishing; 2018. p. 359–78. https://doi.org/10.1007/978-3-319-75016-3_13
3. Anonymous. Area and production, National Horticulture Board Statistics: Area and production; 2022.
4. Adenubi OO, Sanni KO. Weed interference and fruit yield of chilli Pepper (*Capsicum annum)* as influenced by plant density. Open J Plant Sci. 2020;5(1):030–32. https://doi.org/10.17352/ojps.000020
5. Daramola OS, Adigun JA, Adeyemi OR. Efficacy and economics of integrated weed management in chilli pepper (*Capsicum frutescens* L.). J Crop Improv. 2021;35(1):38–50. https://doi.org/10.1080/15427528.2020.1795770
6. Mangang NL, Devi KS, Singh R, Saha S, Gupta N, Sharma SK. Plant virus diseases dynamics under modified environments and their impacts on host virus-vector landscape. In: Climate change impacts on soil-plant-atmosphere continuum. Singapore: Springer Nature Singapore; 2024. 2:485–506. https://doi.org/10.1007/978-981-99-7935-6_18
7. Li H, Wang J, Peng Y, Guo C, Qu W, Yang N, et al. Invasion genomics uncover complex introduction patterns of the globally invasive whitefly, *Bemisia tabaci* MED. Divers Distrib. 2023;29 (9):1172–89. https://doi.org/10.1111/ddi.13751
8. Idrees MA, Abbas A, Saddam B, Bashir MH, Naveed H, Khan AK, et al. A comprehensive review: persistence, circulative transmission of begomovirus by whitefly vectors. Int J Trop Insect Sci. 2024;44:405–17. https://doi.org/10.1007/s42690-024-01170-3
9. Sharma J, Lager P, Kumar R, Kumar Y. First report and genome characterization of pepper leaf curl Bangladesh virus infecting chilli in India. Plant Cell Biotechnol Mol Biol. 2022;23(39-40):75–88. https://doi.org/10.56557/pcbmb/2022/v23i39-408075
10. Borges CV, Orsi RO, Maraschin M, Lima GP. Oxidative stress in plants and the biochemical response mechanisms. In: Plant stress mitigators: Types, techniques and functions; Academic Press: Cambridge, MA, USA; 2023. p. 455–68. https://doi.org/10.1016/B978-0-323-89871-3.00022-7
11. Meitha K, Pramesti Y, Suhandono S. Reactive oxygen species and antioxidants in postharvest vegetables and fruits. Int J Food Sci. 2020;2020(1):8817778. https://doi.org/10.1155/2020/8817778
12. Chaubey AN, Mishra RS. Alternations in biochemical components in Chilli (*Capsicum annuum* L.) plants infected with chilli leaf curl viral disease. J Exp Agric Int. 2021;43(11):146–52. https://doi.org/10.9734/jeai/2021/v43i1130766
13. Cho SY, Kim HW, Lee MK, Kim HJ, Kim JB, Choe JS, et al. Antioxidant and anti-inflammatory activities in relation to the flavonoids composition of pepper (*Capsicum annuum* L.). Antioxidants. 2020;9(10):986. https://doi.org/10.3390/antiox9100986
14. Zhao L, Ding Q, Zeng J, Wang FR, Zhang J, Fan SJ, He XQ. An improved CTAB–ammonium acetate method for total RNA isolation from cotton. Phytochem Anal. 2012;23(6):647–50. https://doi.org/10.1002/pca.2368

15. Wyatt SD, Brown JK. Detection of subgroup III gemini virus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathology. 1996;86(12):1288–93. https://doi.org/10.1094/Phyto-86-1288

16. Mayee CD, Datar VV. Phytopathometry. Technical Buletien-1 (Special Buletein-3), Marathwada Agric Univ Parbhani, India. 1986;218.

17. Mousanezhad S, Alizadeh A, Safaei N. Assessment of yield loss due to rice blast disease in Iran. J Agric Sci Technol. 2010;357–64.

18. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Methods Enzymol. 1987 Jan 1;148:p. 350–82. https://doi.org/10.1016/0076-6879(87)48036-1

19. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39:205–07. https://doi.org/10.1007/BF00018060

20. Vl S. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152–78. https://doi.org/10.1016/S0076-6879(99)99017-1

21. Ardekani MR, Hajimahmoodi M, Oveisi MR, Sadeghi N, Jannat B, Ranjbar AM, et al. Comparative antioxidant activity and total flavonoid content of Persian pomegranate (*Punica granatum* L.) cultivars. Iran J Pharm Res. 2011;10(3):519.

22. Roe JH, Kuether CA. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivavative of dehydroascorbic acid. J Biol Chem. 1943;399–407. https://doi.org/10.1016/S0021-9258(18)72395-8

23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1-2):248–54. https://doi.org/10.1006/abio.1976.9999

24. Cakmak I, Horst WJ. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). Physiol Plant. 1991;83(3):463–68. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

25. Vyas D, Kumar S, Ahuja PS. Tea (*Camellia sinensis*) clones with shorter periods of winter dormancy exhibit lower accumulation of reactive oxygen species. Tree Physiol. 2007;27(9):1253–59. https://doi.org/10.1093/treephys/27.9.1253

26. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 1981;22(5):867–80.

27. Fernández-garcía NI, Carvajal M, Olmos E. Graft union formation in tomato plants: peroxidase and catalase involvement. Ann Bot. 2004;93(1):53–60. https://doi.org/10.1093/aob/mch014

28. Rai VP, Kumar R, Singh SP, Kumar S, Kumar S, Singh M, Rai M. Monogenic recessive resistance to Pepper leaf curl virus in an interspecific cross of Capsicum. Sci Hortic. 2014;172:34–38. https://doi.org/10.1016/j.scienta.2014.03.039

29. Kandhro AG, Khoso FN, Soomro AH, Qazi ST, Soomro WA, Jatoi GH, et al. Spatial distribution of chili leaf curl disease and its correlation with environmental factors in Southern irrigated plain zone of Pakistan. Pure Appl Biol. 2020;9(4):2543–53. https://doi.org/10.19045/bspab.2020.90270

30. Kumar S, Bhowmick MK, Ray P. Weeds as alternate and alternative hosts of crop pests. Ind J Weed Sci. 2021;53(1):14–29. https://doi.org/10.5958/0974-8164.2021.00002.2

31. Langensiepen M, Jansen MA, Wingler A, Demmig-Adams B, Adams III WW, Dodd IC, et al. Linking integrative plant physiology with agronomy to sustain future plant production. Environ Exp Bot. 2020;178:104125. https://doi.org/10.1016/j.envexpbot.2020.104125

32. Khalil RR, Bassiouny FM, El-Dougdoug KA, Abo-Elmaty S, Yousef MS. A dramatic physiological and anatomical changes of tomato plants infecting with tomato yellow leaf curl germinivirus. Int J Agric Sustain. 2014;10:1213–29.

33. Deepa D, Sunkad GS, Govindappa MR, Naik MK, Suresh SR. Estimation of yield loss in sunflower due to new sunflower leaf curl virus disease at different stages of crop growth. Int J Plant Prot. 2015;8(1):138–41. https://doi.org/10.15740/HAS/IJPP/8.1/138-141

34. Nicaise V. Crop immunity against viruses: outcomes and future challenges. Front Plant Sci. 2014;5:660. https://doi.org/10.3389/fpls.2014.00660

35. Ganefianti DW, Hidayat SH, Syukur M. Susceptible phase of chili pepper due to yellow leaf curl Begomovirus infection. Int J Adv Sci Eng Inf Technol. 2017;7(2):594–601. https://doi.org/10.18517/ijaseit.7.2.1872

36. Karem MH, Al-Abedy AN, Kadhim JH. Effect of Tomato yellow leaf curl virus (TYPLCBV) infection of some tomato (*Solanum lycopersicom* L.) genotypes on fruits content of lycopene and some vitamins. In: IOP Conf Ser Earth Environ Sci. IOP Publishing; 2023 Nov 1. 1259(1):p. 012092. https://doi.org/10.1088/1755-1315/1259/1/012092

37. Senanayake DM, 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. J Phytopathol. 2012;160(3):146–55. https://doi.org/10.1111/j.1439-0434.2011.01876.x

38. Chaubey AN, Mishra RS. Survey of chilli leaf curl complex disease in eastern part of Uttar Pradesh. Biomed J Sci Tech Res. 2017;1(7):1941–44. https://doi.org/10.26717/BJSTR.2017.01.000589

39. Saqib M, Shahzad U, Abid Z, Zulfiqar F, Tiwari RK, Lal MK, et al. Melatonin alleviates cadmium phytotoxicity through regulation of growth, photosynthesis and antioxidant potential in two pepper genotypes. Acta Physiol Plant. 2024;46(5):49. https://doi.org/10.1007/s11738-024-03672-6

40. Al-Khayri JM, Rashmi R, Toppo V, Chole PB, Banadka A, Sudheer WN, et al. Plant secondary metabolites: The weapons for biotic stress management. Metabolites. 2023;13(6):716. https://doi.org/10.3390/metabo13060716

41. Chaubey AN, Mishra RS. Leaf curl disease of *Capsicum annuum* and its impact on secondary metabolites. J Pharmacogn Phytochem. 2020;9(2):2247–51.

42. Foyer CH, Kyndt T, Hancock RD. Vitamin C in plants: novel concepts, new perspectives and outstanding issues. Antioxid Redox Signal. 2020;32(7):463–85. https://doi.org/10.1089/ars.2019.7819

43. Dikilitas M, Guldur ME, Deryaoglu A, Ozcan ER. Antioxidant and oxidant levels of pepper (*Capsicum annuum* cv.'Charlee') infected with pepper mild mottle virus. Not Bot Horti Agrobot Cluj Napoca. 2011;39(2):58–63. https://doi.org/10.15835/nbha3925881

44. Ayvacı H, Güldür ME, Dikilitas M. Physiological and biochemical changes in lucerne (*Medicago sativa*) plants infected with 'Candidatus Phytoplasma australasia'-related strain (16SrII-D Subgroup). Plant Pathol J. 2022;38(2):146. https://doi.org/10.5423/PPJ.OA.12.2021.0189

45. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: a review. Plant Signal Behav. 2012;7(11):1456–66. https://doi.org/10.4161/psb.21949

46. Kumar R, Lal MK, Tiwari RK, Chourasia KN, Kumar A, Kumar R, et al. Investigating the interplay between tomato leaf curl New Delhi virus infection, starch metabolism and antioxidant defence system in potato (*Solanum tuberosum* L.). Antioxidants. 2023;12(7):1447. https://doi.org/10.3390/antiox12071447

47. Morales M, Munné-Bosch S. Malondialdehyde: facts and artifacts. Plant Physiol. 2019;180(3):1246–50. https://doi.org/10.1104/pp.19.00405

48. Rao GS, Reddy NR, Surekha C. Induction of plant systemic resistance in Legumes *cajanus cajan, Vigna radiata, Vigna mungo* against plant pathogens *Fusarium oxysporum* and *Alternaria alternata*-a *Trichoderma viride* mediated reprogramming of plant defense mechanism. Int J Recent Sci Res. 2015;6(5):4270–78.

49. Nitiema LW, Sombié PA. Antioxidant responses of three pepper (*Capsicum annuum*) varieties against pepper veinal mottle virus. J Exp Agric Int. 2019;41(2):1–10. https://doi.org/10.9734/jeai/2019/v41i230397

50. Raza A, Sharif Y, Chen K, Wang L, Fu H, Zhuang Y, et al. Genome-wide characterization of ascorbate peroxidase gene family in peanut (*Arachis hypogea* L.) revealed their crucial role in growth and multiple stress tolerance. Front Plant Sci. 2022;13:962182.