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Research Article

Comparative Analysis of Chlorophyll Pigments and Total Protein in Healthy and Virus-Infected Crop Leaves

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ABSTRACT

Plant viral infections significantly alter physiological and biochemical processes, particularly affecting photosynthetic pigments and protein metabolism in crop plants. The present study aims to evaluate the impact of viral infection on chlorophyll pigments and total soluble protein content in healthy and infected crop leaves. Healthy and virus-infected leaves of *Solanum lycopersicum* (tomato), *Capsicum annuum* (chilli), *Lagenaria siceraria* (bottle gourd), and *Luffa acutangula* (ridge gourd) were collected for analysis. Chlorophyll pigments were extracted using 80% acetone and quantified spectrophotometrically. Chlorophyll-a and chlorophyll-b contents were calculated using absorbance values measured at 663 nm and 645 nm following Arnon's formula. The pigment contents of infected leaves were compared with healthy leaves to determine the biochemical impact of viral infection. The study revealed a reduction in chlorophyll content in infected leaves compared to healthy leaves. In tomato plants, chlorophyll-a decreased from 19.27 to 12.93 and chlorophyll-b from 25.60 to 23.31. In chilli, chlorophyll-a reduced from 23.36 to 11.67 and chlorophyll-b from 32.01 to 19.43. Bottle gourd showed a reduction of chlorophyll-a from 15.00 to 12.58 and chlorophyll-b from 27.76 to 21.66, while ridge gourd showed a decrease from 15.66 to 14.06 and 29.50 to 26.19 respectively. Protein estimation also showed variation between healthy and infected plants. Tomato protein OD decreased from 0.33 to 0.056 and chilli from 0.671 to 0.146, whereas bottle gourd increased from 1.071 to 1.278 and ridge gourd from 1.158 to 1.503, indicating possible stress-induced protein responses. The study demonstrates that viral infection significantly alters chlorophyll pigments and protein metabolism in crop plants. Reduction in chlorophyll content indicates impairment of the photosynthetic system, while variation in protein levels suggests activation of plant stress and defense responses. These findings highlight the potential of biochemical markers such as chlorophyll pigments and total soluble proteins for early detection and monitoring of viral stress in crop plants.

1. Introduction

Crop plants play a crucial role in sustainable development and global food security. Among them, members of the Solanaceae family and cucurbit crops are economically, commercially, and nutritionally important (Taiz et al., 2015). However, these crops are highly susceptible to various plant diseases, particularly viral infections, which have become a major threat to agricultural productivity (Agrios, 2005).

Plant diseases account for a major global crop losses each year, with viral infections contributing significantly to this reduction in yield (Agrios, 2005). Viral pathogens not only affect the physical appearance of plants but also alter their internal biochemical processes (Frontiers, 2024). These changes include disruption of photosynthesis and metabolism, leading to reduced chlorophyll content and protein synthesis, ultimately affecting plant growth and productivity (Taiz et al., 2015).

Therefore, understanding the biochemical impact of viral infections is essential for effective crop management. The present study aims to estimate and compare the chlorophyll and protein content in healthy and virus-infected plants in order to evaluate the effect of viral infection on plant metabolism.

2. Materials and Methods

2.1. Sample collection:

Leaf samples of both healthy and virus-infected plants were collected from the Agricultural Research Center, Karimnagar during the previous cropping season. The selected crop plants include (Figure-1) (a)*Solanum lycopersicum* (tomato), (b)*Capsicum annuum* (chilli), (c) *Lagenaria siceraria* (bottle gourd), and (d) *Luffa acutangula* (ridge gourd). These plants were chosen based on their agricultural importance and susceptibility to viral infections.

Figure-1. Selected crop plants

(a)*Solanum lycopersicum* (tomato), (b)*Capsicum annuum* (chilli), (c) *Lagenaria siceraria* (bottle gourd), (d) *Luffa acutangula* (ridge gourd).

a. 

b. 

c. 

d. 

2.1. Chlorophyll estimation:

Chlorophyll content in both healthy and virus-infected leaf samples was estimated using the standard acetone extraction method (Arnon, 1949).

Fresh leaf samples (1 g each) of healthy and infected plants were accurately weighed and taken for analysis. The samples were thoroughly washed with distilled water to remove dust and impurities. The cleaned leaf tissues were then homogenized using a mortar and pestle in the presence of 80% acetone to ensure efficient extraction of chlorophyll pigments. The homogenized mixture was transferred into centrifuge tubes and centrifuged at appropriate speed (approximately 3000–5000 rpm) for 5 minutes. After centrifugation, the supernatant containing the extracted pigments was carefully collected into clean test tubes. The remaining pellet was re-extracted by adding 5 ml of 80% acetone, followed by thorough mixing to ensure complete pigment extraction. The mixture was again centrifuged to separate the residual plant debris from the pigment-containing solution. The supernatants obtained from both extractions were pooled together to ensure maximum recovery of chlorophyll pigments. The final volume of the extract was adjusted by adding 80% acetone (up to 9 ml if required) to maintain consistency between samples.

The absorbance of the chlorophyll extract was measured using a spectrophotometer at wavelengths of 663 nm and 645 nm against 80% acetone as a blank.

Chlorophyll-a, chlorophyll-b, and total chlorophyll contents were calculated using Arnon's equations (Arnon, 1949).:

Chlorophyll-a = 12.7 (od value at 663nm) – 2.69 (OD value at 645nm)
Chlorophyll-b = 22.9 (od value at 645nm) – 4.68 (OD valu at 663nm)

2.2. Protein estimation :

The total soluble protein content in healthy and virus-infected leaf samples was estimated using the standard colorimetric method based on the Biuret reaction followed by Folin–Ciocalteu reagent (Lowry et al., 1951).

Fresh leaf samples (approximately 0.5 g) of both healthy and infected plants were accurately weighed and washed thoroughly with distilled water to remove surface impurities. The samples were then homogenized using a mortar and pestle in a suitable extraction medium to obtain a uniform extract. The homogenized mixture was transferred into centrifuge tubes and centrifuged at 8000 rpm for 5 minutes. After centrifugation, the clear supernatant containing soluble proteins was carefully collected and used for further analysis. To the collected supernatant, alkaline reagent was added, which consisted of sodium carbonate, sodium potassium tartrate, and copper sulphate. This reagent facilitates the formation of a copper-protein complex under alkaline conditions. The mixture was incubated at room temperature for approximately 10 minutes.

Following incubation, Folin–Ciocalteu (FC) reagent was added to the reaction mixture. This resulted in the development of a blue-colored complex due to the reduction of the reagent by the protein–copper complex. The intensity of the blue color is directly proportional to the protein concentration present in the sample (Lowry et al., 1951; Plummer, 2001). The absorbance of the developed color was measured at 660 nm using a spectrophotometer against a suitable blank. Protein concentration in the samples was estimated based on optical density (OD) values obtained from the spectrophotometric readings.

3. Results and Discussion

3.1. Chlorophyll Content Analysis

The chlorophyll content of healthy and virus-infected leaf samples was estimated and compared across selected crop plants. The results clearly indicate a reduction in chlorophyll-a and chlorophyll-b content in infected plants when compared to their healthy counterparts.

In *Solanum lycopersicum* (tomato), chlorophyll-a decreased from 19.27 in healthy leaves to 12.93 in infected leaves, while chlorophyll-b decreased from 25.06 to 23.31. Similarly, in *Capsicum annuum* (chilli), chlorophyll-a showed a significant reduction from 23.36 to 11.69, and chlorophyll-b from 32.01 to 19.43. In *Lagenaria siceraria* (bottle gourd), chlorophyll-a decreased from 15.00 to 12.58 and chlorophyll-b from 27.26 to 21.66. Likewise, *Luffa acutangula* (ridge gourd) showed a reduction in chlorophyll-a from 15.66 to 14.06 and chlorophyll-b from 29.50 to 26.19.

The reduction in chlorophyll content in infected plants may be attributed to the damage caused by viral infection to chloroplast structure and function. Viral pathogens are known to interfere with photosynthetic machinery, leading to decreased pigment synthesis and increased degradation (Agrios, 2005; Taiz et al., 2015). This ultimately affects photosynthetic efficiency and plant growth.

Table 1. calculated chlorophyll values using Arnon formula.

Plants	Chlorophyll-a Healthy leaves	Chlorophyll-a Infected leaves	Chlorophyll-b Healthy leaves	Chlorophyll-b Infected leaves
Tomato	19.27	12.93	25.06	23.31
Chilli	23.36	11.69	32.01	19.43
Bottle gourd	15.00	12.58	27.26	21.66
Ridge gourd	15.66	14.06	29.50	26.19

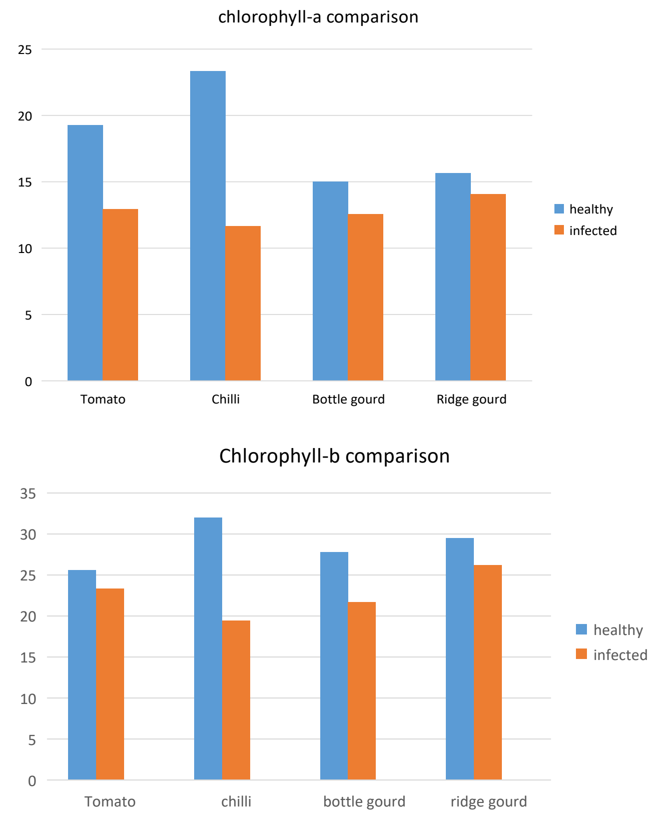

Figure-1. chlorophyll content in infected plants compared to healthy ones.

Figure-1 shows a decrease in chlorophyll content in infected plants compared to healthy ones. This reduction indicates damage to chloroplasts and decreased to chloroplasts and decreased photosynthetic efficiency due to viral infection.

3.1. Protein Content Analysis:

The protein estimation results revealed variations in total soluble protein content between healthy and virus-infected plants. In tomato, protein content showed a marked decrease from 0.33 to 0.056, indicating a significant reduction in protein synthesis due to viral infection. Similarly, chilli plants exhibited a decrease from 0.671 to 0.146.

In contrast, bottle gourd and ridge gourd showed an increase in protein content in infected samples. Bottle gourd protein levels increased from 1.071 to 1.278, while ridge gourd showed an increase from 1.158 to 1.503.

The decrease in protein content observed in tomato and chilli may be due to viral interference with cellular metabolism, leading to reduced synthesis of structural and enzymatic proteins. On the other hand, the increase in protein levels in bottle gourd and ridge gourd may be associated with the induction of stress-related proteins, such as defense proteins and pathogenesis-related (PR) proteins, in response to viral infection (Agrios, 2005).

Table-2. OD values of protein

Plants	Protein in healthy leaves	Protein in infected leaves
Tomato	0.33	0.056
Chilli	0.671	0.146
Bottle gourd	1.071	1.278
Ridge gourd	1.158	1.503

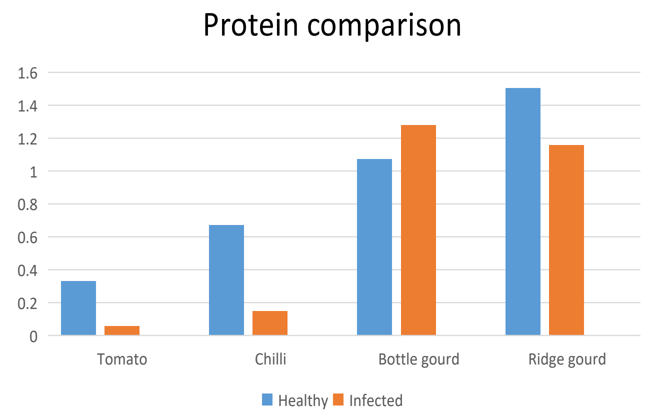

Figure-2. Variation in protein content between healthy and virus infected plants

Figure-2 shows the variation in protein content between healthy and virus infected plants. A significant decrease in protein content was observed in Solanum lycopersicum and Capsicum annuum, indicating negative impact of viral infection on protein synthesis.

In contrast, *Lagenaria siceraria* and *Luffa acutangula* exhibited an increase in protein content in infected samples, which may be attributed to the production of stress-related or defense proteins. Overall, the results indicates the viral infection alters protein metabolism in plants (Taiz et al., 2015). Chlorophyll and protein act as biochemical markers for plant stress (Frontiers Plant Science, 2023; Namthabad et al., 2014).

4. Conclusion

Viral infection significantly alters the biochemical composition of crop plants, particularly affecting chlorophyll pigments and protein content. The present study clearly demonstrates a consistent reduction in chlorophyll levels in infected plants, indicating impairment of photosynthesis due to damage to chloroplast structure and function. Protein content showed variable responses, with a decrease observed in tomato and chilli, while an increase in bottle gourd and ridge gourd suggests the possible induction of stress-related or defense proteins. These findings highlight that viral infection not only disrupts photosynthetic efficiency but also affects overall plant metabolism. Therefore, chlorophyll and protein content can serve as important biochemical indicators for assessing plant health under viral stress. The study emphasizes the need for effective disease management strategies to minimize crop losses and ensure sustainable agricultural productivity.

Declarations

Ethics approval and consent to participate: Not applicable

Competing interests:

The authors declare that they have no competing interests

References

[1] Agrios, G. N. (2005). Plant pathology (5th ed.). Elsevier Academic Press.

[2] Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Plant Physiology, 24 (1), 1-15.

[3] https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2024.1424489/full

[4] https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2025.1551123/full

[5] https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2023.1185337/full

[6] Lowry, O.H., Rosebrough, N. J., Farr, A. L., & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193(1), 265-275.

[7] Namthabad, S., & Mamidala, E. (2014). Molecular docking of HIV-1 protease using alkaloids from tinospora cordifolia. *Int J Res Appl*, *1*(1), 12-6.

[8] Plummer, D. T. (2001). An introduction to practical biochemistry (3rd ed.). Tata McGraw-Hill.

[9] Taiz, Zeiger, E., Moller, I.M., & Murphy, A. (2015). Plant physiology and development (6th ed.). Sinauer Associates.Choi SJ, Kim DU, Noh JY, et al. T cell epitopes in SARS-CoV-2 proteins are substantially conserved in the Omicron variant. *Cellular & Molecular Immunology 2022 19:3*. 2022;19(3):447-448. doi:10.1038/s41423-022-00838-5

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