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Research article

Effects of *Carpomyia incompleta* Infestation on the Chemical Characteristics of *Ziziphus spina-christi* L. Fruits

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ABSTRACT

The results of this study showed that natural infestation by the jujube fruit fly (*Carpomyia incompleta*) induces clear physiological and chemical changes in jujube fruits (*Ziziphus spina-christi* L.). Infestation led to a decrease in the relative content of chlorophyll, carotenoids, total protein, total soluble solids (TSS), and soluble carbohydrates compared to healthy fruits, indicating impaired photosynthesis and metabolism, and a decline in the fruit's nutritional value.
Conversely, infested fruits exhibited an increase in total phenolic content compared to healthy fruits at all study sites. This suggests that the infestation stimulated the plant's chemical defense mechanisms, as phenolic compounds are among the most important secondary metabolites that accumulate in response to the biotic stress caused by the insect larvae feeding within the fruit.
The results also showed variations across all studied chemical properties among the study sites, indicating that local environmental conditions, along with insect infestation, contribute to determining the physiological and chemical response of jujube fruit. These findings suggest that the jujube fruit fly not only affects crop quantity but also reduces fruit quality and nutritional value, underscoring the importance of implementing integrated pest management programs to minimize infestation and maintain the quality and productivity of jujube fruit.

1. Introduction

The jujube tree (*Ziziphus spina-christi* L.) is one of the most important fruit trees that grows naturally in arid and semi-arid regions, belonging to the Rhamnaceae family. It is distinguished by its high tolerance to harsh environmental conditions, such as high temperatures, drought, and salinity, making it an important tree species in many countries of the Middle East and North Africa. Its fruit is also characterized by its high nutritional value, containing good levels of sugars, proteins, dietary fiber, vitamins, and minerals, in addition to phenolic compounds, carotenoids, and antioxidants, which give it significant nutritional and medicinal importance (Chen, 2017; Liu et al., 2020; Muhammad, 2022).

Jujube fruit is a rich source of bioactive compounds that contribute to promoting general health. Phenolic compounds and carotenoids play a key role in resisting oxidation and reducing the effects of free radicals, as well as contributing to improving fruit quality and increasing its shelf life and

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marketability. The accumulation of these compounds is influenced by multiple factors, including environmental conditions, genetic traits, and biotic stresses experienced by plants during different growth stages (Pareek, 2013; Rahman et al., 2018; Zhao et al., 2023).

The jujube fruit fly, *Carpomyia incompleta* (Becker), is one of the most important specialized pests attacking jujube fruits. Females lay their eggs inside the fruit, and the larvae feed on the inner pulp, leading to fruit deformation, discoloration, and a decrease in nutritional and market value. Furthermore, it increases the fruit's susceptibility to rot pathogens and causes a significant percentage of the fruit to drop prematurely. Several studies have indicated that this insect has become one of the most significant limiting factors for the productivity of jujube trees in many Arab countries, with a notable expansion in its geographical distribution in recent years (Abdel-Galil et al., 2014; Garrido-Jurado et al., 2022; Abu El-Ghiet, 2025; Al-Subhi et al., 2024).

The impact of insect infestation is not limited to quantitative crop loss, but extends to physiological and chemical changes within the fruit. Feeding by the larvae disrupts metabolic processes, reduces photosynthetic efficiency, and decreases the accumulation of chlorophyll, carotenoids, sugars, proteins, and phenolic compounds. This negatively affects the nutritional quality and sensory characteristics of the fruit (Ali et al., 2021; Al-Shammari et al., 2022; Wang et al., 2021; Zhang et al., 2021). Studying the chemical changes in infested fruit is one of the most important methods used to assess the impact of insect infestation on fruit quality. Measuring chlorophyll, carotenoids, protein, total soluble solids, phenols, and carbohydrate content provides accurate indicators of the physiological state and nutritional value of the fruit. These indicators also help evaluate the effectiveness of integrated pest management programs, which have proven successful in reducing fruit fly damage, maintaining fruit quality, and minimizing economic losses (Vargas et al., 2015; Vargas et al., 2020; Haldhar et al., 2024).

Despite the economic and environmental importance of the jujube tree in Iraq, information regarding the impact of natural infestation by the jujube fruit fly on the chemical composition of jujube fruit remains limited, particularly in Wasit Governorate. Therefore, this study aimed to estimate the effect of natural infestation by the jujube fruit fly *C. incompleta* on some chemical properties of jujube fruits, including the relative content of chlorophyll, total carotenoids, total protein, total soluble solids, total phenols, and soluble carbohydrates, and to compare these properties between healthy and infested fruits in a number of areas of Wasit Governorate.

2. Materials and Methods

2.1. Collection of Jujube Fruit Samples

The study was conducted during the fruiting season from October 2025 to April 2026. Jujube (*Ziziphus spina-christi* L.) fruit samples were collected from six locations in Wasit Governorate, Iraq, namely Suwaira, Kut, Numaniyah, Hay, Sheikh Saad, and Badra.

Trees exhibiting natural infestation with the jujube fruit fly, *Carpomyia incompleta*, were selected for sampling. Infestation was identified based on the presence of characteristic symptoms, including oviposition punctures, oviposition scars on the fruit surface, and discoloration of the fruit. The infestation was further confirmed by dissecting a number of fruits to verify the presence of insect larvae.

From each location, 20 healthy fruits free from visible infestation symptoms and 20 naturally infested fruits were collected. Fruit samples were selected to ensure uniformity in size, maturity stage, and physiological age. The samples were immediately transported in insulated cooling containers to the Plant Laboratory, Department of Biology, College of Science, Wasit University, where all chemical analyses were carried out as soon as possible to preserve their physiological and biochemical characteristics.

2.2. Determination of Chemical Characteristics of Healthy and *Carpomyia incompleta*-Infested Jujube Fruits

The chemical characteristics of healthy and *C. incompleta*-infested jujube fruits were evaluated at the Plant Laboratory, Department of Biology, College of Science, Wasit University. The measured parameters included relative chlorophyll content, total carotenoids, total protein, total soluble solids (TSS), total phenolic content, and total soluble carbohydrates.

2.2.1. Determination of Relative Chlorophyll Content

The relative chlorophyll content of green jujube (*Ziziphus spina-christi* L.) fruits collected from the different study locations was determined using a portable chlorophyll meter (SPAD meter) to evaluate the physiological effects of infestation by the jujube fruit fly, *C. incompleta*. Green fruits of uniform size and maturity were selected and classified into two groups: naturally infested fruits showing visible infestation symptoms and healthy fruits free of any visible signs of infestation.

Field measurements were performed directly on intact fruits using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan), which estimates relative chlorophyll content by measuring light absorbance in the red and near-infrared regions of the spectrum. The sensor was positioned at the middle portion of each fruit while avoiding severely damaged tissues and oviposition punctures to obtain representative measurements of the overall physiological condition of the fruit.

To improve measurement accuracy, three SPAD readings were recorded from different positions on each fruit, and the mean value was calculated to represent the relative chlorophyll content of each sample. All measurements were conducted during the early morning to minimize the influence of environmental factors, particularly light intensity and temperature, on SPAD readings.

2.2.2. Determination of Total Carotenoid Content

Total carotenoids were extracted from the healthy and naturally infested fruit samples described in Section 2.1 using 80% methanol as the extraction solvent. Five grams of fresh fruit tissue were homogenized in a porcelain mortar with 25 mL of 80% methanol until a homogeneous extract was obtained. The extract was then centrifuged at 4,000 rpm for 15 min, and the absorbance of the supernatant was measured at 480 nm using a UV–Visible spectrophotometer.

The total carotenoid content was calculated using the following equation:

$$\text{Total Carotenoids} = \frac{\text{O.D. at 480 nm} \times \text{S.V.} \times 100}{2500} \times 100$$

where:

O.D. at 480 nm = absorbance measured at 480 nm;

S.V. = extract volume (mL). The results were expressed as mg carotenoids per 100 g fresh weight (FW).

2.2.3. Determination of Total Protein Content

The total protein content of jujube fruits was determined using the Micro-Kjeldahl method according to the procedures described by AOAC (1980). Briefly, 0.2 g of dried fruit powder was digested with 20 mL of concentrated sulfuric acid (H_2SO_4). Total nitrogen was then determined using an Automatic Nitrogen Analyzer (Model 667) equipped with an LX-800 printer. Crude protein content was calculated by multiplying the total nitrogen content by the conversion factor 6.25, and the results were expressed as a percentage of dry weight.

2.2.4. Determination of Total Soluble Solids (TSS)

The total soluble solids (TSS) content of jujube fruits was determined using a digital refractometer according to the standard method described by AOAC (2019).

Fresh fruit samples were collected, and juice was extracted separately from 10 healthy fruits and 10 naturally infested fruits from each study location. A drop of fresh juice was placed on the prism of the digital refractometer, and the TSS value was recorded directly as °Brix. Each treatment was analyzed in three replicates, and the results were expressed as the mean ± standard deviation (SD).

2.2.5. Determination of Total Phenolic Content

The total phenolic content (TPC) of the fruit samples described in Section 2.1 was determined using the Folin–Ciocalteu colorimetric assay with slight modifications according to Waterhouse (2001). An appropriate volume of fresh fruit extract was mixed with the Folin–Ciocalteu reagent and allowed to react for 15 min. Subsequently, 1.85 M sodium carbonate (Na_2CO_3) solution was added, and the reaction mixture was thoroughly mixed and incubated at room temperature for 2 h.

The absorbance was measured at 765 nm using a UV–Visible spectrophotometer. A calibration curve was prepared using gallic acid as the reference standard, and the results were expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE g^{-1} FW).

2.2.6. Determination of Total Soluble Carbohydrate Content

Total soluble carbohydrate content in jujube fruits was determined using the phenol–sulfuric acid method described by Dubois et al. (1956). Briefly, 3.2 g of fresh fruit tissue from each sample was thoroughly homogenized to obtain a uniform pulp and transferred into a 250-mL volumetric flask. Subsequently, 23 mL of distilled water was added, and the mixture was heated in a water bath at 80°C for 1 h. After heating, the samples were allowed to cool to room temperature and then filtered through filter paper.

An aliquot of 3 mL of the filtrate was transferred into a test tube and diluted with 73 mL of distilled water. Thereafter, 2 mL of phenol solution and 3 mL of concentrated sulfuric acid (H_2SO_4) were added. The absorbance of the reaction mixture was measured at 490 nm using a UV–Visible spectrophotometer.

The total soluble carbohydrate content was determined from a glucose standard calibration curve using the following equation:

$$\text{Total soluble carbohydrates (mg/222 g)} = \frac{C \times V_f \times D}{W}$$

Where:

C = carbohydrate concentration obtained from the glucose standard calibration curve (mg)

Vf = final volume of the extract (mL)

D = dilution factor

W = sample weight (g)

2.3. Statistical Analysis

Data were analyzed as a factorial experiment in a Completely Randomized Design (CRD), with sampling location and fruit condition (healthy and *Carpomyia incompleta*-infested) as the two experimental factors. Each treatment consisted of three replicates. Statistical analyses were performed using GenStat software, and treatment means were compared using the Least Significant Difference (LSD) test at $P \leq 0.05$.

3. Results and Discussion

3.1. Relative Chlorophyll Content

The chlorophyll content of jujube fruits was determined using a SPAD chlorophyll meter (Fig 1) for all six study locations, and the values obtained from healthy fruits were compared with those from fruits naturally infested with the jujube fruit fly, *C. incompleta*. The results revealed clear differences between healthy and infested fruits, as well as among the different sampling locations. SPAD readings ranged from 0.40 to 0.90.

Healthy fruits collected from Suwaira exhibited the highest chlorophyll content, with a mean SPAD value of 0.88, whereas infested fruits from the same location recorded a significantly lower value of 0.55, indicating the marked effect of fruit fly infestation on chlorophyll content. In Kut, the SPAD values were 0.82 for healthy fruits and 0.50 for infested fruits. Similarly, in Numaniyah, healthy and infested fruits recorded values of 0.78 and 0.48, respectively. In Hay, the corresponding values were 0.75 for healthy fruits and 0.46 for infested fruits, whereas fruits collected from Sheikh Saad recorded SPAD values of 0.70 and 0.44 for healthy and infested fruits, respectively. The lowest chlorophyll content was observed in Badra, where healthy and infested fruits recorded SPAD values of 0.68 and 0.40, respectively.

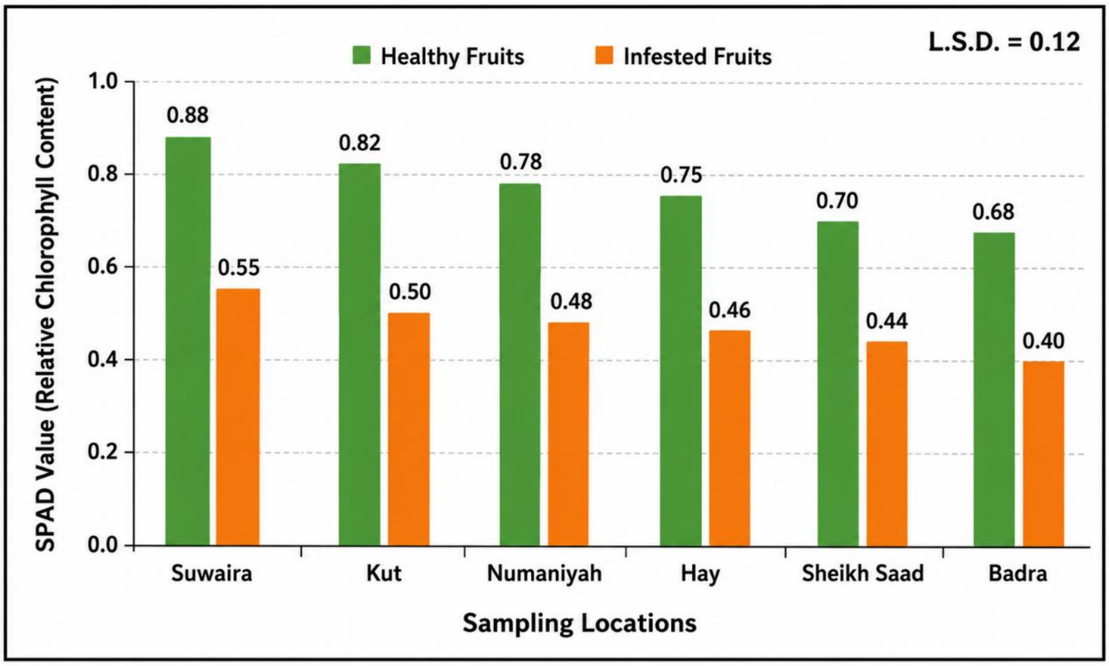

Fig. 1. Relative chlorophyll content (SPAD) of healthy and *C. incompleta*-infested jujube fruits

Among the study locations, healthy fruits from Suwaira and Kut exhibited the highest chlorophyll content, whereas those from Badra and Sheikh Saad showed the lowest values, suggesting that environmental conditions associated with different locations may influence chlorophyll accumulation in jujube fruits. Furthermore, fruit fly infestation consistently reduced chlorophyll content across all locations. These findings are consistent with previous studies demonstrating that insect infestation impairs photosynthetic activity and decreases chlorophyll accumulation in plant tissues (Wang et al., 2021; Chen et al., 2020).

The observed variation among locations may be attributed to differences in climatic conditions and site characteristics that influence chlorophyll biosynthesis and accumulation, which is in agreement with the findings of Li et al. (2019). Moreover, the consistently lower chlorophyll content observed in infested fruits indicates that infestation by *C. incompleta* imposes physiological stress on the fruit, resulting in reduced chlorophyll synthesis and diminished utilization of light energy. These findings support those reported by Zhang et al. (2022), who concluded that biotic stress caused by insect infestation adversely affects fruit quality.

3.2. Total Carotenoid Content

The results showed a clear difference in total carotenoid content between healthy and *C. incompleta*-infested jujube fruits across all study locations (Figure 2). Total carotenoid content in infested fruits ranged from 0.40 mg g^{-1} in Suwaira to 0.65 mg g^{-1} in Kut, whereas healthy fruits contained higher levels, ranging from 0.70 mg g^{-1} in Suwaira to 0.90 mg g^{-1} in Badra. These findings indicate that locations with higher insect infestation levels, such as Suwaira, exhibited a greater reduction in carotenoid content than locations with lower infestation levels, such as Badra and Sheikh Saad.

The reduction in carotenoid content observed in infested fruits can be attributed to the tissue damage caused by C. incompleta, which accelerates the degradation of sensitive biochemical compounds, including carotenoids. This effect was more pronounced in locations with higher infestation intensity, suggesting a negative relationship between infestation severity and carotenoid accumulation (Zhang et al., 2021).

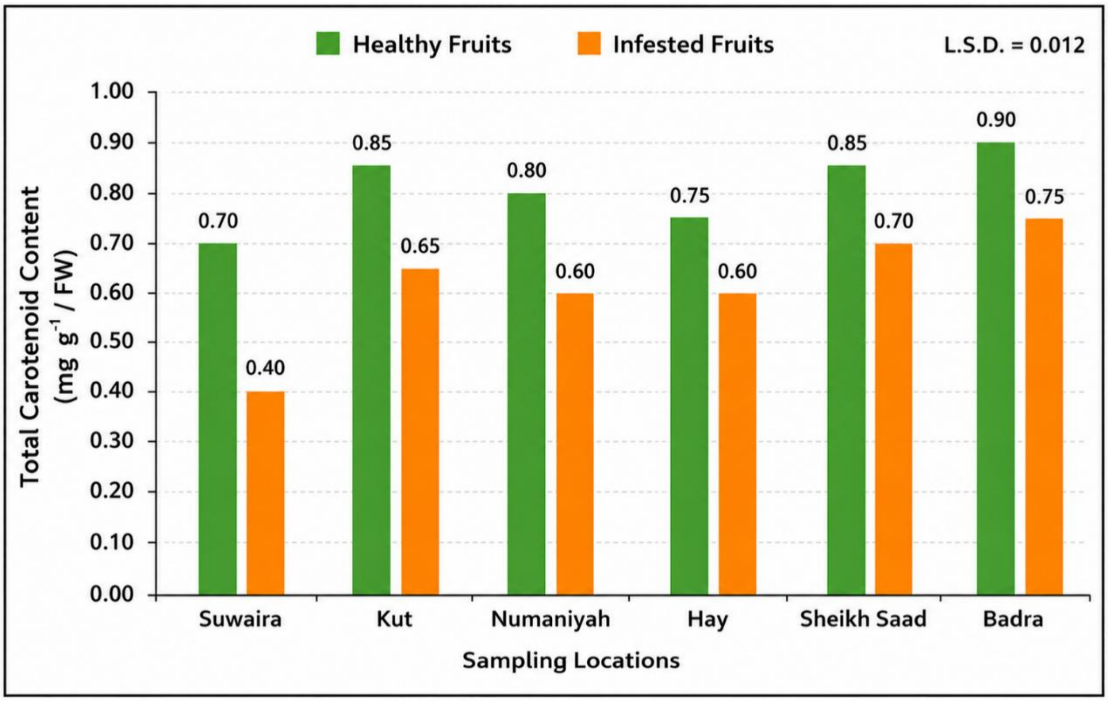

Fig. 2. Total carotenoid content in healthy and *C. incompleta*-infested jujube fruits.

These findings are consistent with previous studies demonstrating that insect infestation reduces the concentration of important phytochemicals, including carotenoids, phenolic compounds, and total sugars, thereby decreasing the nutritional quality of fruits (Hossain et al., 2020). Carotenoids play an essential role in antioxidant defense and in maintaining fruit quality during postharvest storage. Therefore, the marked decline observed in infested fruits suggests that infestation may accelerate fruit deterioration.

Furthermore, fruits collected from locations with lower infestation pressure, particularly Badra and Sheikh Saad, contained higher carotenoid levels than those collected from heavily infested locations such as Suwaira. This pattern indicates that both environmental conditions and insect infestation influence carotenoid accumulation in jujube fruits. These results are in agreement with recent studies reporting a clear inverse relationship between insect infestation and carotenoid content (Li et al., 2022; Zhang et al., 2021).

3.3. Determination of Total Protein Content

The results showed that the total protein content of jujube fruits (Figure 3) was markedly affected by infestation with the jujube fruit fly, *C. incompleta*, and that the extent of this effect was closely associated with the level of insect infestation at each study location. In Suwaira, which exhibited the highest infestation level, infested fruits recorded the lowest protein content compared with healthy fruits, with protein values ranging from 13.2% to 15.1%. In contrast, fruits collected from locations with lower infestation levels, such as Badra and Sheikh Saad, exhibited considerably higher protein contents, ranging from 21.5% to 23.2%, indicating a pronounced reduction in protein content in heavily infested areas.

In locations with moderate infestation levels, including Kut, Numaniyah, and Hay, the protein content of infested fruits ranged from 13.8% to 15.0%, whereas healthy or less infested fruits consistently maintained higher protein levels. These findings indicate a negative relationship between infestation intensity and protein content in jujube fruits.

The present results suggest that infestation by *C. incompleta* adversely affects the nutritional quality of jujube fruits. Feeding by the larvae on the fruit tissues depletes nutrients and damages internal tissues, resulting in a reduction in total protein content. These findings are consistent with those reported by Liu et al. (2019) and Singh et al. (2020).

Previous studies have demonstrated that fruit-feeding and piercing–sucking insects negatively affect fruit quality and crop productivity by depleting plant nutrients and inducing changes in the chemical composition and major nutritional constituents, including proteins, amino acids, carbohydrates, and mineral elements, thereby reducing both the nutritional and economic value of the crop (Bateman, 1972; Aluja & Mangan, 2008; Ouaarous et al., 2025). Furthermore, studies have shown that reducing infestation levels through integrated pest management (IPM) programs contributes to maintaining fruit quality and minimizing nutritional losses caused by insect infestation (Vargas et al., 2015; Haldhar et al., 2024).

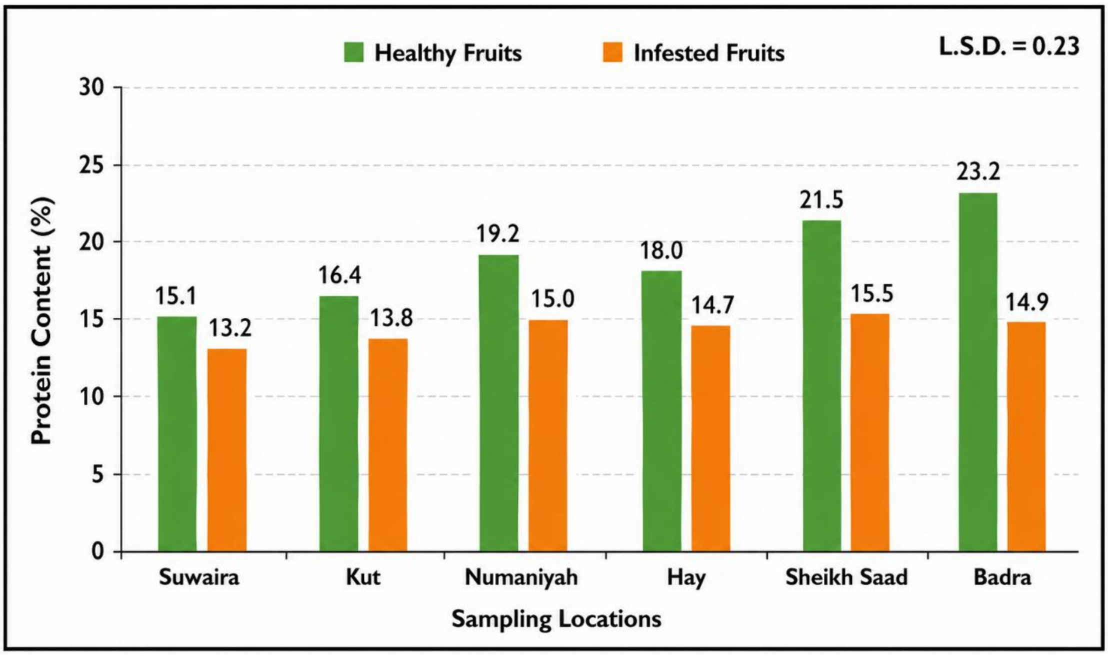

Fig. 3. Total protein content in healthy and *C. incompleta*-infested jujube fruits.

3.4. Determination of Total Soluble Solids (TSS)

Sugars constitute the principal component of total soluble solids (TSS), together with organic acids, amino acids, and soluble pectins. Sweetness, which is closely associated with the sugar content of the fruit, is considered one of the most important quality attributes of jujube fruits. Although consumer preferences may vary, fruits with higher sugar content and lower acidity are generally preferred.

The results revealed clear variation in total soluble solids (TSS) among the different study locations (Figure 4). The highest TSS values were recorded in fruits collected from locations with lower levels of insect infestation, whereas the lowest values were observed in locations with the highest infestation levels, reflecting the adverse effects of feeding damage and tissue degradation caused by the jujube fruit fly. In infested fruits, the TSS values were 12.2 ± 0.6% in Suwaira (the location with the highest infestation level), 13.0 ± 0.5% in Kut, 13.5 ± 0.4% in Numaniyah, 12.8 ± 0.3% in Hay, 15.2 ± 0.5% in Sheikh Saad, and 15.5 ± 0.4% in Badra, which had the lowest infestation level. In contrast, no significant differences in TSS were observed among healthy fruits collected from the different study locations.

The variation in TSS among locations also reflected differences in infestation intensity (Figure 4). Locations with higher infestation levels, such as Suwaira, exhibited the lowest sugar content, whereas locations with lower infestation levels, particularly Badra and Sheikh Saad, maintained relatively higher sugar concentrations. These findings emphasize the importance of integrated pest management in preserving fruit quality.

The present results indicate that insect infestation adversely affects sugar accumulation in jujube fruits, resulting in lower TSS values in infested fruits than in healthy fruits. These findings are consistent with previous studies reporting that fruit-feeding insects, including fruit flies, disrupt sugar metabolism and reduce soluble sugar accumulation in fruits (Singh et al., 2021; Al-Shammari et al., 2022).

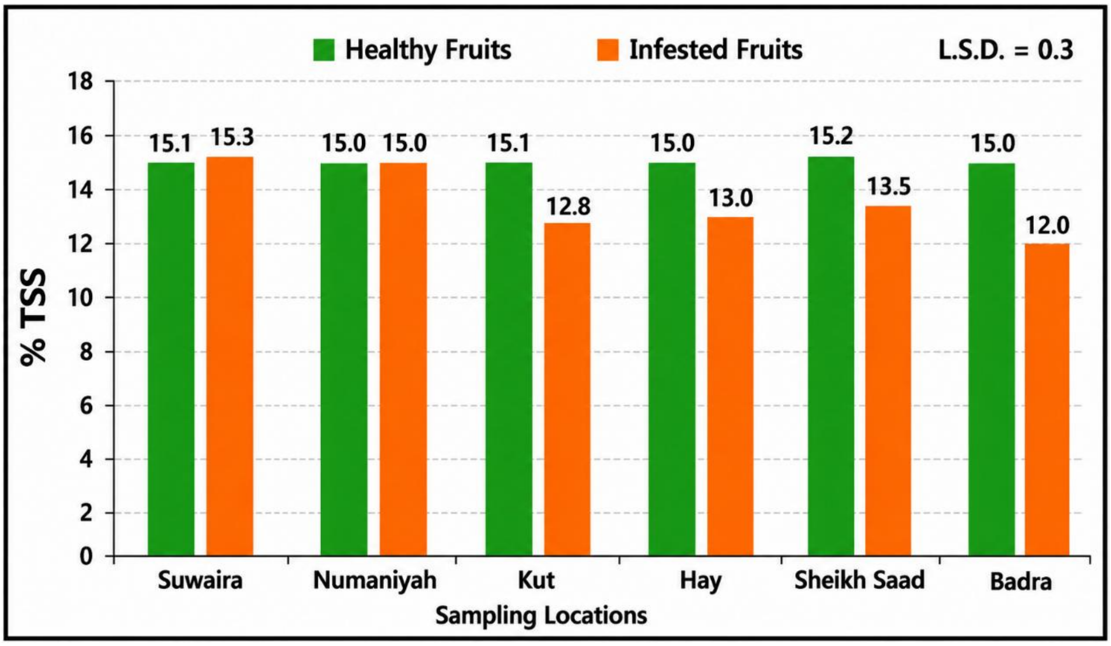

Fig. 4. Total soluble solids (TSS) in healthy and *C. incompleta*-infested jujube fruits

3.5. Total Phenolic Content

The results revealed clear differences in total phenolic content between healthy and *Carpomyia incompleta*-infested jujube fruits (Figure 5). Infested fruits consistently exhibited higher phenolic contents than healthy fruits at all sampling locations, indicating that insect infestation induced the accumulation of phenolic compounds as part of the plant's biochemical defense response.

The highest total phenolic content in infested fruits was recorded in Badra (13.0 mg GAE/100 g FW), followed by Sheikh Saad (12.6 mg GAE/100 g FW), Hay (12.2 mg GAE/100 g FW), Numaniyah (11.8 mg GAE/100 g FW), Suwaira (11.6 mg GAE/100 g FW), whereas the lowest value was observed in Kut (11.3 mg GAE/100 g FW). In contrast, healthy fruits showed relatively stable phenolic contents across all study locations, ranging from 9.7 to 10.1 mg GAE/100 g FW, with no significant differences among locations.

The increase in phenolic content observed in infested fruits is attributed to the activation of induced defense mechanisms following insect attack. Phenolic compounds are important secondary metabolites that contribute to antioxidant activity, strengthen cell walls, and protect plant tissues against herbivores and pathogens. The accumulation of these compounds therefore represents a biochemical response of jujube fruits to larval feeding by *C. incompleta*.

These findings agree with previous studies indicating that insect infestation can stimulate the biosynthesis of phenolic compounds as part of the induced defense response in fruits and other plant organs (Shahzad et al., 2020; Ali et al., 2021). Accordingly, total phenolic content may serve as a useful biochemical indicator of the physiological response of *Ziziphus spina-christi* fruits to infestation by *C. incompleta*.

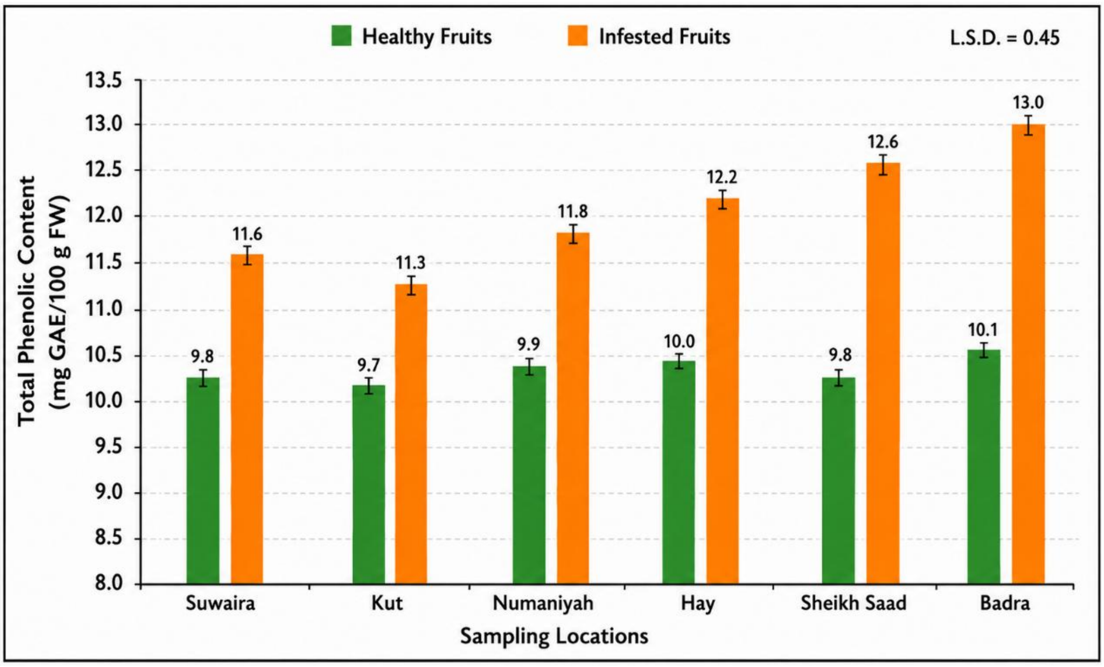

Fig. 5. Total phenolic content in healthy and *C. incompleta*-infested jujube fruits

3.6. Determination of Total Soluble Carbohydrate Content

The results showed that the total soluble carbohydrate content of jujube fruits varied among the six study locations (Figure 6). The highest carbohydrate content was recorded in fruits collected from locations with lower levels of insect infestation, particularly Badra and Hay, whereas the lowest content was observed in Suwaira, the location with the highest infestation level. These findings indicate that infestation by the jujube fruit fly, *C. incompleta*, adversely affects carbohydrate accumulation in the fruits.

The total soluble carbohydrate content in fruits collected from Suwaira was approximately 24.0%, while values in Kut and Numaniyah ranged from 24.5% to 25.2%. Fruits collected from Hay and Sheikh Saad recorded values of approximately 25.5% and 25.8%, respectively, whereas the highest carbohydrate content (26.4%) was recorded in Badra. In contrast, no significant differences in total soluble carbohydrate content were observed among healthy fruits collected from the different study locations.

These results indicate that increasing insect infestation reduces carbohydrate accumulation in jujube fruits, probably because part of the soluble sugars is consumed by the developing larvae during feeding. Conversely, fruits subjected to lower infestation levels retain higher concentrations of soluble sugars due to reduced insect feeding activity. These findings highlight the importance of effective insect management in maintaining fruit quality with respect to soluble sugars and carbohydrates, emphasizing the value of early intervention through appropriate control measures, including insecticides or entomopathogenic nematodes, to improve fruit quality (Vargas et al., 2019). The present findings are consistent with previous studies reporting that insect infestation significantly alters the total sugar content of fruits (Singh et al., 2021).

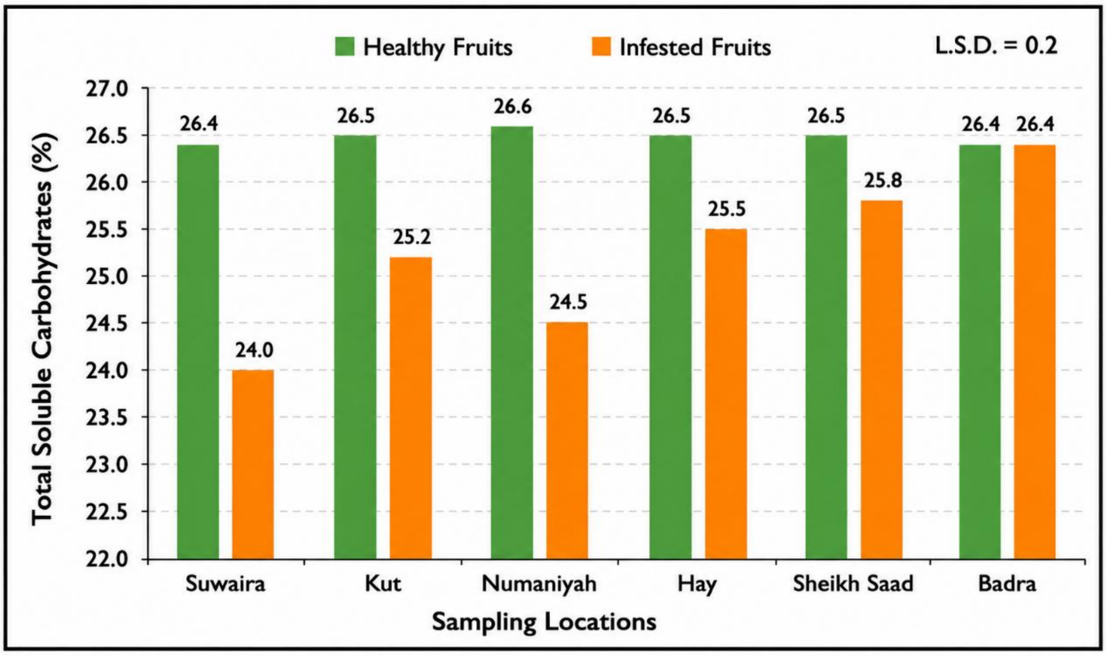

Fig. 6. Total soluble carbohydrate content in healthy and *C. incompleta*-infested jujube fruits

4- Conclusion

The study showed that natural infestation by the jujube fruit fly (*Carpomyia incompleta*) led to a significant decrease in the chlorophyll, carotenoids, protein, total soluble solids (TSS), and carbohydrate content of jujube fruits, negatively impacting their nutritional value. Conversely, the total phenol content in infested fruits was higher compared to healthy fruits, indicating the activation of the plant's chemical defense mechanisms in response to the insect infestation. These results underscore the importance of pest control to maintain the quality and nutritional value of jujube fruits.

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