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# Sugar pea leaf contents and their influence on population fluctuations of *Tetranychus urticae* under field conditions at Ismailia governorate

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## ABSTRACT

A field trial on three sugar pea (*Pisum sativum* (L.), Fabaceae) cultivars was conducted during the winter season of 2022–23. These cultivars were from two distinct varieties: *macrocarpon* (cv., Snow Wind and Sugar Lace) and *saccharatum* (cv., Super Sugar Snap). The aim of the study was to investigate the influence of sugar pea leaf contents and weather conditions on the population fluctuations of *Tetranychus urticae* Koch (Prostigmata: Tetranychidae). The results showed that *T. urticae* counts were found to be related to total carbohydrate, soluble sugar, flavonoid, phenol, chlorophyll, and carotenoid contents in the three cultivars throughout the season. The study's findings revealed that mite densities, including adult, nymph, and egg counts, varied between cultivars, indicating different degrees of infestation. Surprisingly, no predators were observed on any of the three cultivars. The 'Sugar Lace' had the highest phenol content and the lowest mite densities. Furthermore, it consistently showed higher levels of flavonoids than the other cultivars. Conversely, 'Snow Wind' and 'Super Sugar Snap' have higher carbohydrate, soluble sugar, chlorophyll, and carotenoid levels, as well as higher mite population. The variation in infestation among cultivars was, therefore, attributed to changes in leaf phytochemistry. According to the study's results, including pest-resistant cultivars into Integrated Pest Management (IPM) programs could be beneficial in mite pest control. Selecting cultivars with good phytochemical profiles is a long-term solution for reducing the need for chemical control.

**Keywords:** Two-spotted spider mite; *Pisum sativum*; population density; soluble sugars; flavonoids

## INTRODUCTION

Sugar pea, *Pisum sativum*, is an important vegetable crop in Egypt that has grown in popularity due to its cultivation for both domestic and international markets (Elwan et al. 2015). There are three distinct varieties of peas, namely the English pea (var. *sativum*), the Snow pea (var. *macrocarpon*) (also known as "mange-tout"), and the Snap pea (var. *saccharatum*), a hybrid of the English and Snow peas. This leguminous vegetable is highly nutritious, containing a significant amount of minerals, carbohydrates, dietary fibre, vitamins, phytochemical substances, and various micronutrients (Ram et al. 2021). Unfortunately, this crop's vulnerability to spider mite infestation has increased, contributing to its decreased yield.

The two-spotted spider mite, *Tetranychus urticae* is a common phytophagous pest that now damages leguminous plants such as sugar peas (Abdallah et al. 2015). Its danger comes from sucking the contents of the leaf cells. As global warming continues, the negative effects of *T. urticae* will become more severe. This is primarily due to the species' increased growth rate at higher temperatures. The fundamental concern with *T. urticae* is its rapid developmental rate and large reproductive capability, which causes a rapid decline in the quality of its host plants (Kanika 2014). Pests have expanded their distribution due to global fluctuations in weather and climatic conditions, resulting in changes in their geographic ranges. As a result, population dynamics and trophic interactions with the plant species on which they feed have changed (Koshkin et al. 2021).

*Tetranychus urticae* has recently shown the ability to successfully adapt to previously resistant plant type, thus expanding its threat (Wybouw et al. 2015). The use of host plant resistance has been found as a potential alternative method in the *T. urticae* control program. This approach has been shown to have a considerable impact on different factors, including plant development, density of pest population, extent of herbivore damage, efficiency of natural enemies, and overall pesticide reliance (Zehnder et al. 2007).

Host plant resistance varies among cultivars, with each cultivar demonstrating various degrees of resistance to *T. urticae* (Scott et al. 2021). This resistance is mostly influenced by the unique qualities of each cultivar, specifically the physical and chemical properties of their leaves, as evidenced by numerous research studies. Abdallah et al. (2009) and El-Saiedy et al. (2011) demonstrated that plant cultivars have a considerable impact on *T. urticae* population density due to variations in the chemical content of their leaves. Furthermore, Potter and Anderson (1982) revealed that the existence of various secondary metabolites, including flavonoids, phenols, and carbohydrates, may explain the varying degrees of resistance observed in response to infestation by *T. urticae*. The diverse roles of secondary metabolites encompass their function as toxins, deterrents, and agents that decrease digestibility. These functions have been observed to have an impact on the performance of herbivorous arthropods, as evidenced by studies conducted by Žnidarčič et al. (2007) and Cáceres et al. (2016).

An exhaustive understanding of pest ecology and host-plant physiology is necessary to understand host-plant relationships and detecting the elements responsible for pest buildup. The development of an effective and sustainable IPM strategy is critical in order to minimize yield loss. Therefore, the objectives of the present study is to 1) determine if *T. urticae* populations varied between sugar pea cultivars; 2) investigate the influence of leaf contents, including total carbohydrate, soluble sugar, flavonoids, phenol, chlorophyll, and carotenoid contents of three sugar pea cultivars on *T. urticae* abundance; 3) investigate the influence of weather parameters on population fluctuation of *T. urticae* on three cultivars of sugar pea at Ismailia governorate.

## MATERIALS AND METHODS

### Experimental site

The field trial was conducted in sandy loamy soil at Abu Sultan (30°22′47″N, 32°18′02″E), Fayed, Ismailia governorate, during the winter season of 2022–23.

### Plant materials

Three sugar pea cultivars from two varieties were used: *macrocarpon* (cv., Snow Wind and Sugar Lace) and *saccharatum* (cv., Super Sugar Snap). The seeds were purchased from Syngenta Company, the US.

### Experimental design

Sugar pea seeds were sowed on Sept. 2022 at a spacing of 10 cm within rows and 1 m between rows with a drip irrigation system. A randomized complete block design was employed to represent each cultivar, with three replicates assigned to each. The cultural practices followed were in compliance with the Egyptian Ministry of Agriculture's requirements. There were no chemicals used.

### *Tetranychus urticae* sampling

Bi-monthly samples of 60 leaves were collected randomly one month after plantation, encompassing the top, middle, and bottom of 20 plants (20 leaves per each cultivar in three replicates). The collected leaves were transferred to the laboratory in polythene bags for examination. The various developmental stages of mites, i.e., adults, eggs, and nymphs, were counted separately in a 2.5 $cm^2$ area on the underside of the leaf using a stereo microscope (Olympus SZ-PT, Japan). Temperature and relative humidity were monitored at regular intervals during the study to assess the correlation between environmental factors and mite abundance (Figure 1). The meteorological data was obtained from the Central Laboratory for Agricultural Climate, Agricultural Research Center, Dokki, Giza, Egypt.

### Chemical analysis

During the first month, the respective parameters were estimated using non-infested leaves as the corresponding control. Following the appearance of infestation, the leaves were systematically picked monthly until the culmination of the season. The samples were dried in an oven at 60°C before being ground into a fine powder.

The powdered samples were then extracted and filtrated separately. The filtrates were collected for further analysis. For the estimation processes, the extraction assays were carried out according to Crompton and Birt (1967) and Chaplin and Kennedy (1994) for total carbohydrates and soluble sugars, respectively; Karawya and Aboutable (1982) for total flavonoids; and Kähkönen et al. (1999) for total phenols. All activity measurements were monitored using a UV-Vis Spectrophotometer Thermo, Nicolet Evolution 300 coupled with vision pro software, the US.

**Estimation of total carbohydrate and soluble sugar contents**

The content of total carbohydrates and soluble sugars was estimated colorimetrically using phenol-sulfuric by the method of Dubois et al. (1956). The absorbance was measured spectrophotometrically at 480 nm.

**Estimation of total flavonoids content**

The total flavonoid content was determined using the method of Karawya and Aboutable (1982). The absorbance was measured spectrophotometrically at 445 nm. For the calibration curve, quercetin was used as a standard. Total flavonoid was expressed as quercetin equivalents (QE) in mg per gram dry weight (DW) (mg QE $g^{-1}$ DW).

**Estimation of total phenols content**

The total phenol content was evaluated using the Folin-Ciocalteu technique with gallic acid as the standard (Singleton and Rossi 1965). The absorbance was spectrophotometrically measured at 650 nm and expressed as gallic acid equivalents (GAE) in mg per g DW (mg GAE $g^{-1}$ DW).

**Estimation of chlorophyll and carotenoid contents**

Chlorophyll (Chl.) a, b, and carotenoid (Car.) contents in leaf samples were determined according to Arnon (1949) and Wettstein (1957), respectively. The absorbance was measured spectrophotometrically (Unico spectrophotometer, UV/VIS-2100, the US) at 622 and 644 nm absorbance for Chl. a, b, and 440 nm for Car. The values were expressed as mg $g^{-1}$ fresh weight (FW).

**Statistical analysis**

A two-way analysis of variance (ANOVA) was performed using the 23rd version of the SPSS software to independently determine the significant influence of months (sampling date) and cultivars on each parameter. Tukey's honestly significant difference (HSD) test was used to compare means, with a significance level of $P < 0.05$. The correlation coefficient (R) between temperature, relative humidity, and total number of *T. urticae* was determined using Pearson's correlation analysis.

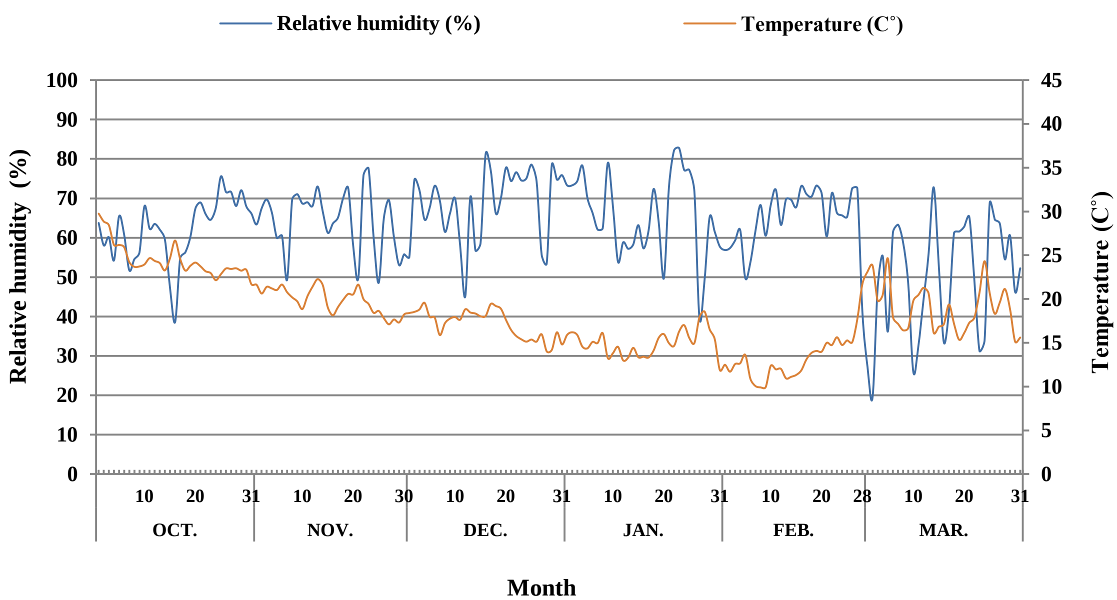



**Figure 1.** Average temperature degree and relative humidity percentage at Abu Sultan, Fayed, Ismailia governorate during the winter season of 2022–23.

## RESULTS AND DISCUSSION

### Population fluctuation of *T. urticae* on three sugar pea cultivars

Throughout the trial, no predators were sighted on any of the three sugar pea cultivars. The density of *T. urticae* adults, nymphs, and eggs was significantly influenced by month. The manifestation appeared in Dec. 2022, about two months after the seeds were sown, with an initial population density of 5.08, 13.16, and 59.79 for adults, nymphs, and eggs/leaf, respectively (Table 1). The density of mites gradually increased, peaking during Jan. for both nymphs and eggs. The density of adults, on the other hand, peaked in Feb. *Tetranychus urticae* density was highest on 'Snow Wind', followed by 'Super Sugar Snap' and lastly 'Sugar Lace'. Our findings indicated that the type of sugar pea cultivars cultivated in Egypt lately influences the performance of *T. urticae*. This result was consistent with previous studies on different crops in the same Fabaceae family. According to the findings of Sedaratian et al. (2008), different cultivars of soybean (*Glycine max* (L.) Merr., Fabaceae) can influence the bionomics of *T. urticae*; the authors noted a significant difference in *T. urticae* population density between seven soybean cultivars studied. Furthermore, Najafabadi et al. (2014), found that the development and survive of *T. urticae* were influenced by quality of the host plant. This finding was observed through an evaluation conducted on six different cultivars of bean (*Phaseolus vulgaris* L., Fabaceae).

**Table 1.** Monthly fluctuation in density of *T. urticae* different stages on three sugar pea cultivars during the winter season of 2022–23.

| Parameters | *Tetranychus urticae* stages | | |
|---|---|---|---|
| | Adult | Nymph | Egg |
| | No./leaf | | |
| **Cultivar (C)** | | | |
| Snow Wind | 14.32±2.40a | 25.56±4.00b | 89.74±13.31a |
| Sugar Lace | 7.97±1.67c | 23.41±5.00b | 55.90±10.77c |
| Super Sugar Snap | 12.72±2.25b | 33.35±6.15a | 81.05±13.25b |
| **Month (M)** | | | |
| Nov. | 0±0c | 0±0d | 0±0e |
| Dec. | 5.08±1.19b | 13.16±2.41c | 59.79±11.04d |
| Jan. | 17.23±1.25a | 51.69±3.45a | 133.57±7.20a |
| Feb. | 19.07±1.72a | 37.49±1.17b | 97.81±4.40b |
| Mar. | 16.96±1.25a | 34.86±3.09b | 86.65±7.74c |
| **F-value** | | | |
| Cultivar (C) | 237.48*** | 609.12*** | 1105.50*** |
| Month (M) | 58.90*** | 65.25*** | 228.90*** |
| Interaction C*M | 5.90*** | 20.90*** | 51.60*** |

Means followed by the same letters in the same column are not significantly different by Tukey's HSD ($P < 0.05$).

### Physiological changes in sugar pea leaf contents affecting the *T. urticae* infestation

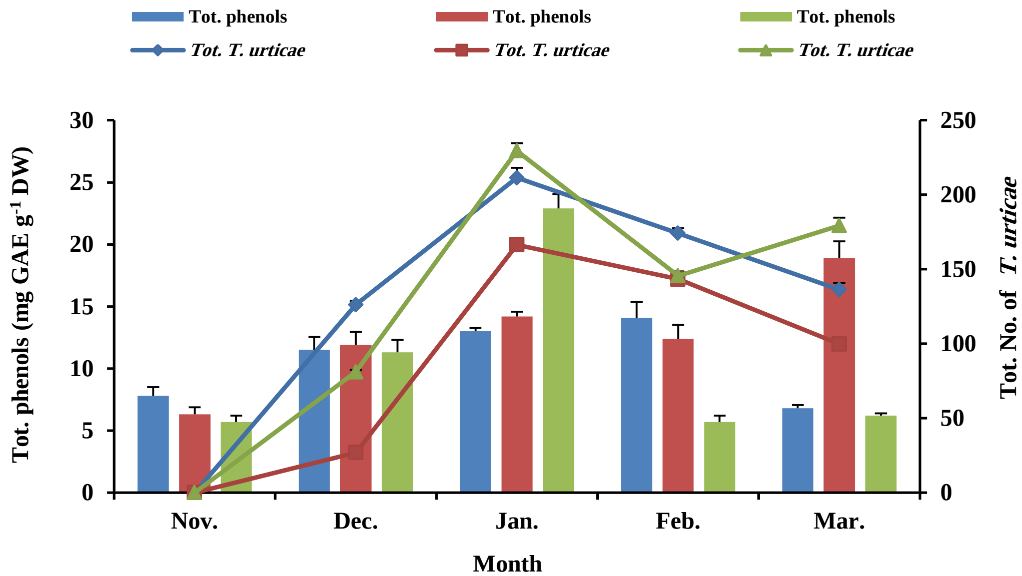
Except for carotenoids, the data revealed substantial differences in the effects of cultivar, month, and their interactions on total carbohydrate, soluble sugar, flavonoid, phenol, and chlorophyll contents. The total phenol and soluble sugar contents peaked in Jan., while the total flavonoid, carbohydrate, and chlorophyll contents peaked in Nov. and Dec. (Table 2). The 'Snow Wind' had the highest content of total carbohydrate and leaf-soluble sugar between the cultivars studied, while the ‘Sugar Lace’ had the highest content of total phenol and flavonoid (Table 2).

Fewer studies have been focused on how the leaf content of each cultivar affects the population dynamics of *T. urticae* on sugar peas. The lowest level of total phenol was observed in Nov. Subsequently, the phenol content gradually increased, peaking in Jan., corresponding with the highest population density of *T. urticae*. The overall phenol content then declined as the density of *T. urticae* reduced during the following months (Figure 2A). In the absence of *T. urticae*, the total flavonoid content in ‘Snow Wind’, ‘Sugar Lace’, and ‘Sugar Snap’ was 22.6, 35.6, and 29.8 mg QE $g^{-1}$ DW, respectively. Upon the emergence of infestation in Nov., the total flavonoid content began to increase, with percentage increase of 120.5, 56.4, and 49.9% in ‘Snow Wind’, ‘Sugar Lace’, and ‘Sugar Snap’, respectively (Figure 2B). However, after the peak of *T. urticae* population density in Jan., the total flavonoid content in ‘Snow Wind’ and ‘Super Sugar Snap’ declined by 77 and by 81% in ‘Sugar Lace’. Furthermore, ‘Sugar Lace’ had the lowest density of *T. urticae*, as well as the highest phenol and flavonoid content in its leaves (Figures 2A and B). This observation is agree with the findings of Luczynski et al. (1990), who demonstrated that increased foliar phenolic compounds in resistant strawberry (*Fragaria* × *ananassa* Duchesne, Rosaceae) cultivars efficiently inhibited *T. urticae* growth. The study conducted by Kielkiewicz and van de Vrie (1990) revealed that mite infestation was inversely related to the concentration of phenolic compounds in chrysanthemum (*Dendranthrema grandiflora* Tzvelev, Asteraceae) leaves. According to Wermelinger et al. (1991), an increase in plant phenolic content resulted in a decrease in the reproductive potential of *T. urticae*. Similarly, flavonoids have been shown to restrict the feeding, development, and oviposition behaviour of insects due to their variable distribution among plant species (Simmonds 2003). Furthermore, Ali et al. (2015) observed a negative correlation between total flavonoid content and *T. urticae* density on tomato (*Solanum lycopersicum* L., Solanaceae) leaves.

Our results indicated that the peak of total carbohydrate content was observed during Nov. and Dec., when the density of *T. urticae* ranged from 0 to 126 individuals/leaf (Figure 3A). In contrast, a rise in the density of *T. urticae* was associated with a decline in the total carbohydrate content in Jan. The extent of this decrease was 57.4, 57.3, and 26.6% in ‘Snow Wind’, ‘Sugar Lace’, and ‘Super Sugar Snap’, respectively. Moreover, the results showed that a higher level of soluble sugars in the leaves was correlated with a higher population density of *T. urticae*, and vice versa (Figure 3B). As compared to ‘Sugar Lace’, the ‘Snow Wind’ consistently had the highest content of carbohydrates and soluble sugar, as well as the highest density of *T. urticae*. This was in conformance with previous studies on other crops, which found that the soybean cultivars most susceptible to *T. urticae* infestation had the highest levels of total carbohydrate when compared to the tolerant cultivars (El-Sanady et al. 2008). Similarly, Ali et al. (2015) demonstrated that the increased presence of carbohydrates in tomato leaves positively correlated with mite infestation severity. Furthermore, Wermelinger et al. (1991) found a positive correlation between the augmentation in leaf carbohydrate content and *T. urticae* fecundity. Also, El-Saiedy et al. (2011) and Abdallah et al. (2009) indicated that soluble sugar is a key factor in *T. urticae* abundance. In contrast, Nain and Rathee (2017) recorded the highest sugar content in the *T. urticae*-resistant okra (*Abelmoschus esculentus* (L.) Moench, Malvaceae) cultivar.

In the absence of *T. urticae* infestation, the Chl. a content in the leaf of ‘Snow Wind’, ‘Sugar Lace’, and ‘Super Sugar Snap’ was 114.7, 111.3, and 112.7 mg 100 $g^{-1}$ FW, respectively. However, when *T. urticae* attacked the leaves and peaked in Jan., the Chl. a level began to decline, with the degree of reduction varying according to the cultivar type. The ‘Snow Wind’ with the highest *T. urticae* density had the highest content of Chl. a, while the ‘Sugar Lace’ with the lowest *T. urticae* density had the lowest Chl. a content (Figure 4A). A similar pattern was noted for Chl. b and Car. contents, which followed the same cultivar-dependent trend as Chl. a in terms of *T. urticae* infestation (Figures 4B and C).

(A)




(B)

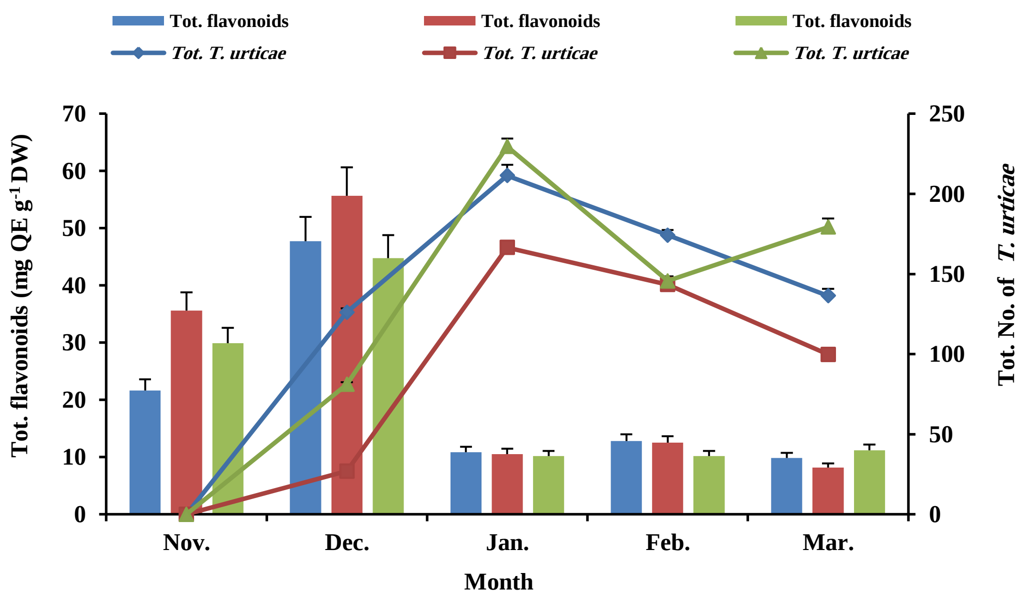



**Figure 2.** Relationship between the total phenol (A) and flavonoid (B) contents of the leaf of three sugar pea cultivars (**SW**- 'Snow Wind', **SL**- 'Sugar Lace', and **SSS**- 'Super Sugar Snap') and the total number of *T. urticae* during the winter season of 2022–23.

These findings align with those of Hildebrand et al. (1986), who evaluated two soybean cultivars for resistance to *T. urticae* infestation; whereas they found that when *T. urticae* population increased, the leaf Chl. and Car. contents decreased by 55.26 and 79.3%, respectively. Similarly, Ali et al. (2015) and Abdallah et al. (2018) observed that increasing the density of *T. urticae* reduces the Car. content of the leaves. As a result, the variation in *T. urticae* infestation among cultivars is attributed to changes in leaf content.

Environmental factors such as temperature and humidity, in addition to leaf content, can also impact *T. urticae* population fluctuations. *Tetranychus urticae* oviposition, development, and movement are supported by high temperature and low humidity (van de Vrie et al. 1972). There is no published data on the influence of these factors on the sugar pea crop, although similar studies on other members of the same family have been undertaken.

**Table 2.** Summary of leaf contents of three sugar pea cultivar during the winter season of 2022–23.

| Parameters | Tot. Phenols | Tot. Flavonoids | Tot. Carbohydrates | Soluble Sugar | Chl. (a) | Chl.(b) | Carotenoids |
|---|---|---|---|---|---|---|---|
| | mg GAE $g^{-1}$ DW | mg QE $g^{-1}$ DW | mg $g^{-1}$ | | mg 100 $g^{-1}$ FW | | |
| **Cultivar (C)** | | | | | | | |
| Snow Wind | 10.64±0.83b | 20.57±3.89b | 198.03±23.37a | 35.42±6.13a | 106.87±4.3a | 36.61±1.2a | 59.29±1.16a |
| Sugar Lace | 12.74±1.14a | 24.48±5.04a | 168.46±19.2b | 20.44±2.05c | 91.83±3.07c | 29.20±1.2c | 56.47±1.12a |
| Super Sugar Snap | 10.36±1.79b | 21.23±3.8ab | 173.13±18.9b | 26.15±3.65b | 98.62±3.34b | 32.88±1.4b | 58.09±1.18a |
| **Month (M)** | | | | | | | |
| Nov. | 6.60±0.43c | 29.04±2.42b | 273.77±14.23a | 13.13±0.65c | 112.91±2.7a | 35.49±0.5a | 60.96±1.25a |
| Dec. | 11.57±0.53b | 49.38±2.76a | 247.80±26.54a | 13.84±0.83c | 109.46±5.2a | 37.18±2.3a | 59.84±1.67a |
| Jan. | 16.70±1.60a | 10.50±0.48c | 121.83±7.05b | 41.92±8.18a | 95.62±5.23b | 32.48±1.9b | 56.84±1.19a |
| Feb. | 10.73±1.38b | 11.83±0.68c | 117.71±4.48b | 32.61±2.02b | 91.24±2.31b | 28.15±0.8c | 56.60±1.48a |
| Mar. | 10.63±2.11b | 9.72±0.62c | 138.26±6.63b | 35.18±5.2ab | 86.31±1.34b | 31.18±1.7b | 55.51±1.35a |
| **F-value** | | | | | | | |
| Cultivar (C) | 54.01*** | 149.84*** | 79.22*** | 62.24*** | 22.15*** | 55.22*** | 2.64ns |
| Month (M) | 11.69*** | 3.70* | 5.96** | 34.54*** | 15.64*** | 99.51*** | 1.63ns |
| Interaction C*M | 32.17*** | 2.67* | 9.20*** | 23.52*** | 3.88** | 29.56*** | 0.62ns |

Means followed by the same letters in the same column are not significantly different by Tukey's HSD ($P < 0.05$).

Our findings revealed a substantial, significant negative correlation between *T. urticae* density and average temperature. The ‘Snow Wind’ had the highest correlation coefficient value, followed by ‘Sugar Lace’, while the lowest was found in ‘Super Sugar Snap’. Conversely, there was a slight, non-significant negative correlation between *T. urticae* density and relative humidity in ‘Super Sugar Snap’ and ‘Sugar Lace’, while there was a non-significant positive correlation in ‘Snow Wind’ (Table 3). These findings are in agreement with those of Tabasum and Buhroo (2022), who found that *T. urticae* population on peas, was negatively correlated with relative humidity. Similarly, Sathua and Singh (2023) showed a negative correlation between *T. urticae* population and relative humidity in six French bean cultivars. In contrast, Chauhan and Shukla (2016), found a significant positive correlation between temperature and relative humidity and the population of *T. urticae* on beans.

Understanding the population density and activity patterns of *T. urticae* on sugar pea crop might help with cultivar selection, monitoring, and determining the best time to implement control measures. Utilizing pest-resistant cultivars offers an alternate option for pest management that does not rely on chemical-based methods.

**Table 3.** The correlation between the temperature degrees, relative humidity, and the total number of *T. urticae* during the winter season of 2022–23.

| Cultivars | T°C | R.H. |
|---|---|---|
| Snow Wind | -0.782** | 0.042 |
| Sugar Lace | -0.748** | -0.123 |
| Super Sugar Snap | -0.543* | -0.279 |

(**) Correlation is significant at the 0.01 level, (*) Correlation is significant at the 0.05 level.

## CONCLUSION

To successfully achieve the goal of sustainable agriculture, it is necessary to maintain a balance between the highest possible yield and product quality while minimizing environmental impact. Our findings provide essential information regarding the resistance of three sugar pea cultivars to spider mite infestation. The 'Sugar Lace' had significantly positive traits and a relatively high level of resistance, whilst the other two cultivars indicated moderate susceptibility. As a result, using partly resistant cultivars can improve both biological and chemical control methods. When combined with other ecological parameters such as temperature, humidity, and other environmental conditions. This information becomes useful for developing and planning of IPM programs for spider mites on sugar pea intended for export.

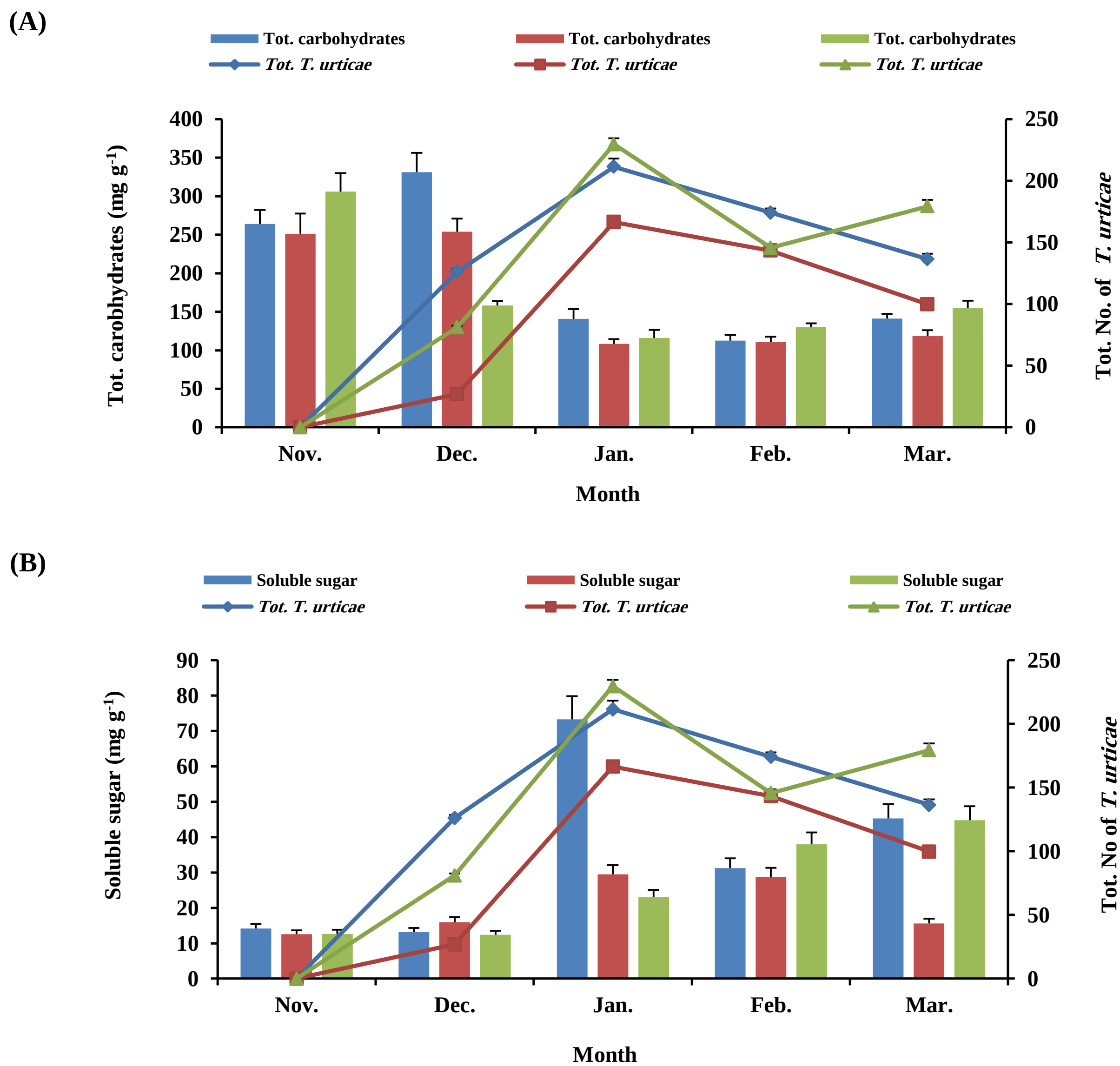



**Figure 3.** Relationship between the total carbohydrate (A) and soluble sugar (B) contents of the leaf of three sugar pea cultivars (**SW**- 'Snow Wind', **SL**- 'Sugar Lace', and **SSS**- 'Super Sugar Snap') and the total number of *T. urticae* during the winter season of 2022–23.

(A)

(B)

(C)

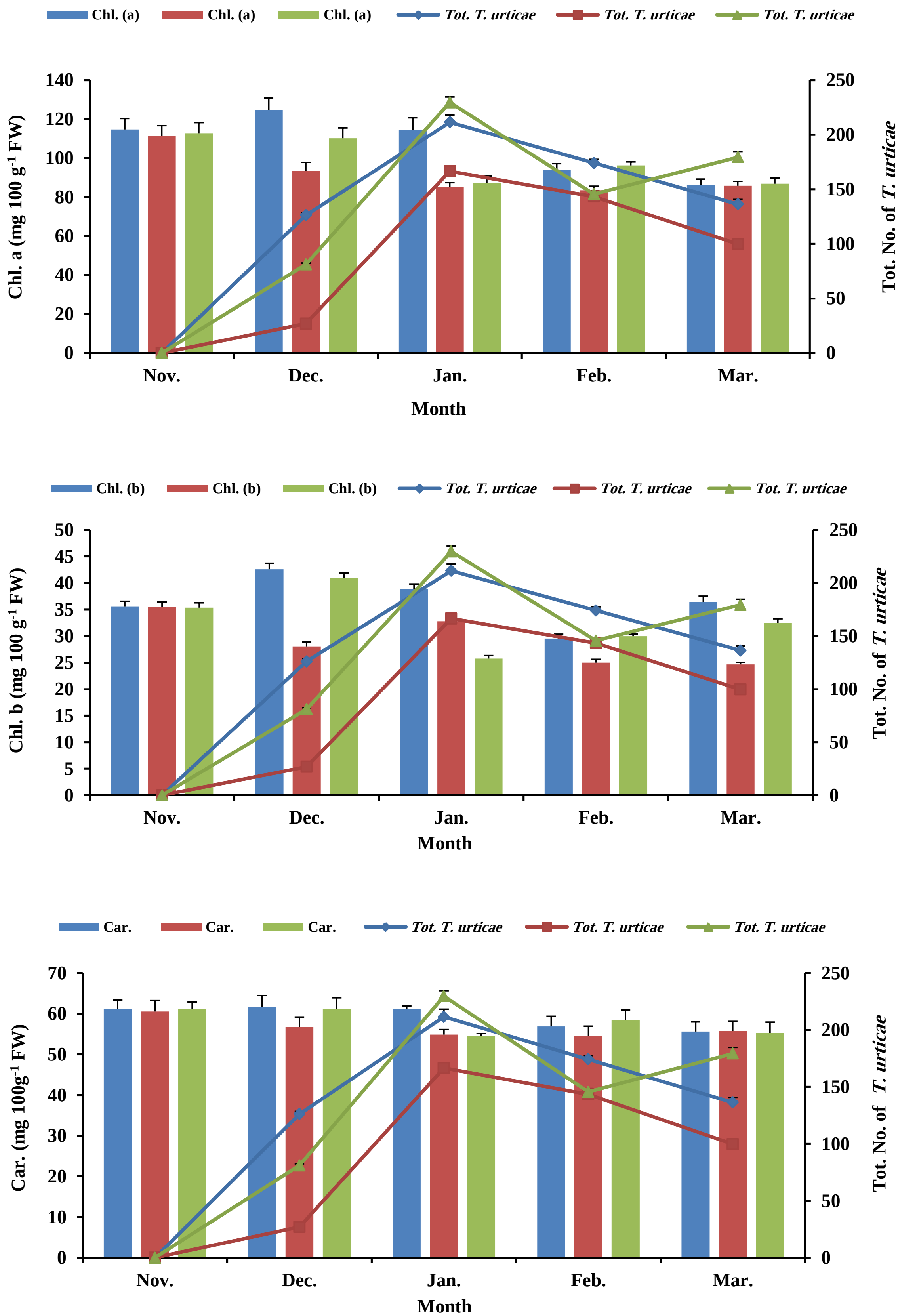



**Figure 4.** The relationship between the Chl. a (A), Chl. b (B), and Car. (C) contents of the leaf of three sugar pea cultivars (**SW**- 'Snow Wind', **SL**- 'Sugar Lace', and **SSS**- 'Super Sugar Snap') and the total number of *T. urticae* during the winter season of 2022–23.

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