



Physicochemical and nutritional alterations induced by two-spotted spider mite infestation on strawberry plants



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ABSTRACT

Background: Strawberry is a pseudofruit mainly cultivated in temperate climate regions. Considering its high levels of ascorbic acid and phenolic compounds, the consumption of strawberry fruit can be beneficial to health. The Brazilian strawberry production revolves around 3000 tons per year, significantly influencing the food market and generating income to farmers. However, this production can be partially impaired by two-spotted spider mite (TSSM) *Tetranychus urticae* Koch infestations, due to decreases in the quality and quantity of fruit. Since there are no data in the literature about alterations caused by TSSM infestation in strawberry plants, our work aimed towards evaluating nutritional and physicochemical parameters of TSSM-infested strawberry plants, along with the related chemical treatment (CT) (acaricide) or biological treatment (predatory mite *Phytoseiulus macropilis* Banks).

Results: Strawberry fruit from TSSM-infested plants present the highest levels of acidity and exhibit low levels of anthocyanin and phenolic compounds, while fruit from TSSM-infested plants + biological control using predatory mite shows high levels of soluble solids, phenolic compounds and ascorbic acid, along with a high soluble solid content/titratable (SSC/TA) acidity ratio, which indicates high quality fruit.

Conclusions: Our results suggest that TSSM infestation decreases fruit quality and that the biological control of TSSM using a predatory mite is a suitable alternative to organic production, since the presence of predatory mite does not affect fruit quality and development.

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1. Introduction

Fruits play an essential role in our diet, since they contain vitamins, carbohydrates and minerals, and also non-nutrient compounds as polyphenols, all of which are necessary for a healthy life. Among them, citrus fruits stand out due to the high levels of organic acids [1]. Strawberry, a widely cultivated pseudofruit (approximately 4.6 million tons in 2011) [2] consumed in natura or processed as juice and jelly, has high ascorbic acid (vitamin C) levels [3], as

well as high levels of polyphenolic compounds (mainly ellagitannins and anthocyanins), all compounds associated with health benefits [4]. Anthocyanins, the main responsible for the characteristic red color of the strawberry fruit, influence fruit appearance [5] and display antioxidant, anti-inflammatory, anticarcinogen, and antineurodegenerative properties [6,7,8,9,10,11].

During the fruit ripening process, organic acids are degraded, decreasing the astringency and acid taste. A good indicator of fruit quality concerns the soluble solid content/titratable acidity ratio (SSC/TA). This value is enhanced by organic acid degradation, reducing the strawberry fruit acidity and producing the characteristic sweetness favored by consumers [12]. According to Resende et al. [13], the greater the SSC, the better is the flavor and taste of the strawberry fruit.

Several factors may have an effect on the nutritional and physicochemical composition of strawberry fruit, mainly the planted cultivar, the maturity stage, harvest season, planting location, climatic factors, and plant management, all of which influence fruit quality [14]. According to the Environmental Working Group [15], strawberry is one of the most chemically treated fruits, ranking second

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within the “Dirty Dozen”. Such pesticide application is necessary because strawberry fruit are widely attacked by several pests, including phytophagous mites [16].

The yield and weight of strawberry fruit can be severely affected by *Tetranychus urticae* Koch (two-spotted spider mite – TSSM) infestations. Small smudges and/or isolated discolored areas can be seen on the infested areas. Severe infestations can cause complete leaf bronzing, displaying a reddish and/or dry aspect, characteristic of older/senescent leaves. A good indicator of TSSM infestation is the production of abundant webs on both leaf faces [17]. TSSM infestations can reach alarming levels and may in fact cause the death of the plant, reducing fruit production in up to 80% [18].

Aiming to reduce the losses caused by TSSM infestations, acaricide chemical treatments are generally the main and first choice by farmers. However, organic cultivation based on clean production technologies has stood out in recent years [19]. In Brazil, farmers have recently started to adopt the inundative liberation of predatory mite *Phytoseiulus macropilis* Banks (Phytoseiidae) as an alternative to the biological control of phytophagous mites. With such a clean approach, farmers are able to efficiently control TSSM infestations without using chemical treatments [19,20].

In order to understand how strawberry fruit are nutritionally affected by TSSM infestations, the plants were subjected to different treatments: control condition (no infestation); TSSM infestation; TSSM infestation + chemical treatment (CT – acaricide); and TSSM infestation + biological treatment (BT – *P. macropilis*). This study reports the physicochemical and nutritional alterations of strawberry fruit and leaves subjected to the above mentioned conditions. The results provide evidence that TSSM infestation has deleterious effects on the nutritional parameters of strawberry fruit, while the biological control of TSSM infestations using predatory mite can be an effective method to control *T. urticae* development without negative effects on fruit quality.

2. Materials and methods

2.1. Plant material and mite infestation

Strawberry seedlings (*Fragaria* × *Ananassa* Duch, cv. *Camarosa*) were planted in vases with peat-based substrates and carbonized rice hulls. The plants were grown in greenhouse conditions at 25°C and relative humidity was maintained at 60%. Four treatments (with four plants per treatment) were administered: Treatment 1, plants without contact with TSSM (Control); Treatment 2, TSSM-infested plants, without control (TSSM); Treatment 3, TSSM-infested plants + CT 1% abamectin (TSSM + CT); Treatment 4, TSSM-infested plants + BT using *P. macropilis* releasing (TSSM + BT). TSSM specimens used for infestations were maintained on strawberry leaves under BOD conditions at the Acarology Laboratory (Centro Universitário UNIVATES).

Urea was applied as nitrogen fertilizer at 40 d, right before the first harvest. In Treatments 2, 3 and 4, 20 d before the first harvest, ten TSSM specimens were applied per plant, while chemical and biological controls were applied 5 d before the first harvest. The fruit were harvested when their ripe area reached 2/3 of their total area. Young and mature leaves were also collected in the last fruit harvest. All the experiments were repeated twice in the same year with similar results.

2.2. Fresh strawberry fruit extract

Juice fruits were extracted according to Pelayo-Zaldívar et al. [21], with minor modifications. Each sample was homogenized using a mini-processor and the fruit mass was diluted 1:1 with distilled water. Four samples from each treatment were analyzed in triplicates.

2.3. Fruit analyses

The pH determination was performed with pH meter DM-20 (Digimed). Titratable acidity (TA) was performed by titration with NaOH 0.1 N and the results were expressed as % of citric acid equivalents.

SSC, (expressed as °BRIX) was analyzed using a refractometer (Abbé Digital, 0–95 Brix NOVA WYA-2S). The SSC/TA relates to °Brix/% of citric acid equivalents.

Ascorbic acid amounts were determined according to Terada et al. [22], with minor modifications suggested by Moretti et al. [23]. This analysis is based on the reduction of 2,6-dichlorophenol–indophenol sodium salt by an ascorbic acid solution.

Anthocyanins were quantified using the differential pH method proposed by Giusti and Wrolstad [24], in which anthocyanin pigments are structurally transformed, followed by a pH alteration that can be detected by absorbance difference.

The Folin–Ciocalteu method was used in order to assess the total phenolic content. Extracts were heated for 2 h at 85°C to eliminate vitamin C interference and assayed according to Georgé et al. [25] using gallic acid as the standard. The total phenolic content was expressed in mg of gallic acid equivalents · 100 g fruit⁻¹.

2.4. Dry strawberry leaf extract

Strawberry leaves collected at the end of the experiment were dried at 37°C for 18 h. Dried samples were ground to a fine powder in order to be used in methylxanthine and phenolic analyses.

2.5. Leaf analyses

In order to analyze methylxanthine, 250 mg of dried samples was extracted with 20 mL of 2.5% (v/v) sulphuric acid under constant agitation for 15 min. This procedure was repeated four times. The samples were spectrophotometrically analyzed at 271 nm, according to Farmacopedia Brasileira [26]. Results were expressed in mg of caffeine equivalents/g of dry weight.

Phenolic compounds in strawberry leaves were quantified using the same methodology used for quantification in fruit (Folin–Ciocalteu method) [25].

2.6. Statistical analysis

Data were subjected to variance analyses (One-way ANOVA) and the means were compared by the Tukey HSD (Honestly Significant Differences) test ($P \leq 0.05$) using SPSS Base 17.0 for Windows (SPSS Inc., USA). Levene's test (for homogeneity of variance) was used prior to ANOVA.

3. Results and discussion

3.1. Fruit analyses

TSSM + CT or BT presented strawberry fruit with the highest pH values (Figure 1a). The data were confirmed by the lowest titratable acidity found in these samples (Figure 1b). According to Pelayo-Zaldívar et al. [21], consumer preferences were mainly related to higher sugar (sweetness) and volatile contents. Thus, fruit derived from TSSM-infested plants displayed inferior quality. Acidity/sweetness in strawberry fruit is related to the ripening process. During this process, organic acids are degraded, astringency and acidity are reduced, sugar levels continually increase and the widely accepted sweet taste is enhanced [27,28]. Such pH alteration seems to be directly linked to plant treatment, since control and TSSM-infested plants (without any treatment) showed high acidity levels. Such data indicate that treated plants can produce higher levels of natural organic acids, or the presence of TSSM for a longer

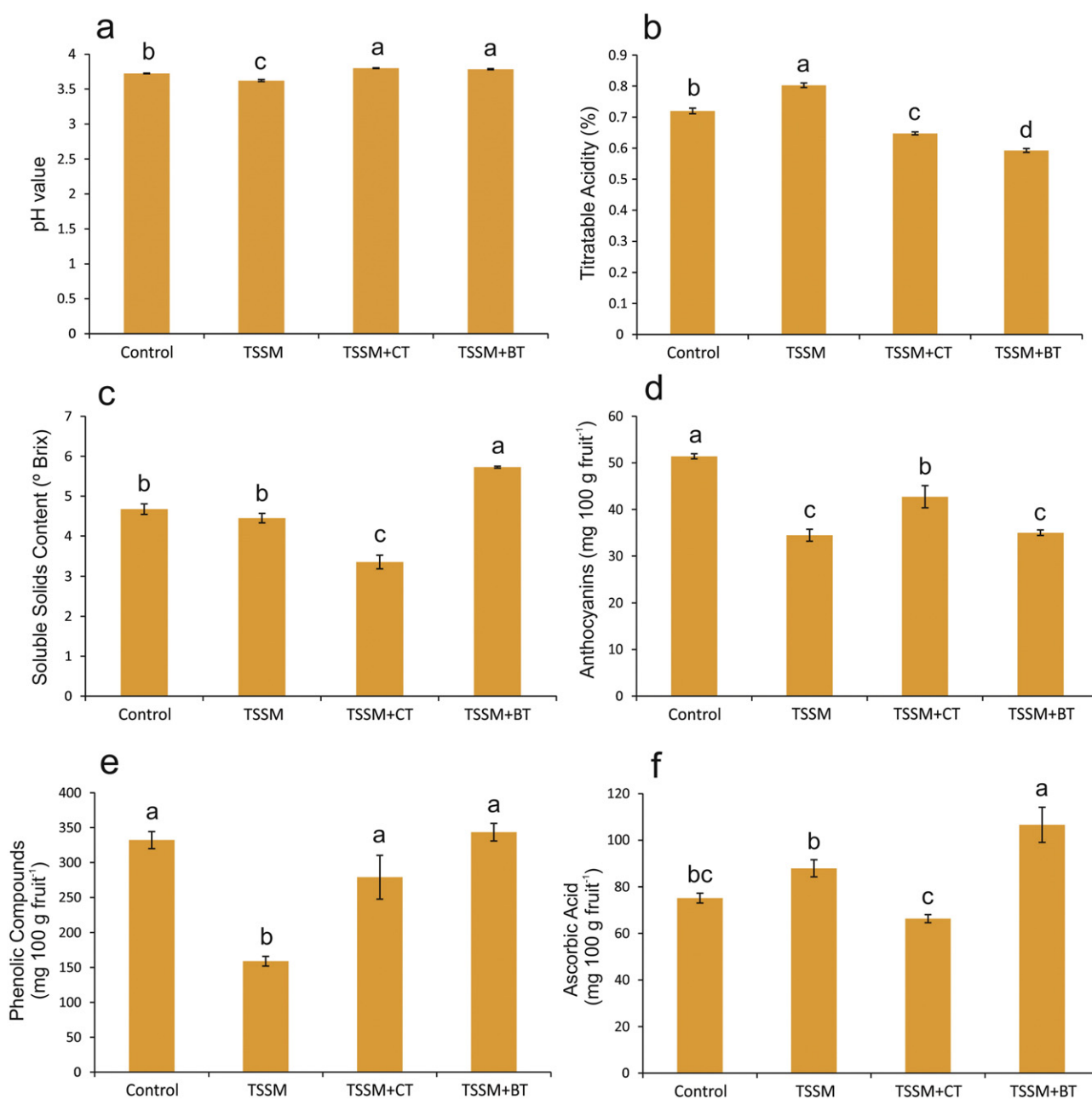


Fig. 1. Chemical and nutritional parameters in strawberry fruit. (a) pH value; (b) Titratable Acidity; (c) SSC; (d) Anthocyanins (e) Phenolic Compounds; (f) Ascorbic Acid. TSSM: Two-spotted Spider Mite; TSSM + CT: Two-spotted Spider Mite + Chemical Treatment; TSSM + BT: Two-spotted Spider Mite + Biological Treatment. The values are averages of four samples \pm SE. Different letters indicate that the means are different according to the Tukey HSD test ($P \leq 0.05$). Error bars may be too small to be visible in the figure.

period of time can inhibit the degradation of organic acids, since TSSM was extinct on the treated plants (either chemically or biologically). The sequential analysis (through time course harvesting) of these parameters could shed some light at this assumption. The pH values found in our samples (from 3.6 to 3.8) concur with previous reports [29,30].

Strawberry fruit from TSSM-infested + BT plants presented the highest sugar levels, while TSSM + CT fruit presented the lowest levels (Figure 1c – SSC, °BRIX). This index is directly related to fruit quality, since the main indicators of pleasant flavors are SSC [31]. According to Resende et al. [13], SSC are responsible for the pleasant taste and flavor of strawberry fruit and, in addition to organic acids and soluble pectins, sugars are the main contributors of SSC in strawberries [21]. Again, fruit from TSSM-infested plants displayed lower levels than fruit from

TSSM + BT, suggesting that the presence of TSSM leads to low quality fruit.

One of the widely accepted parameters used to verify strawberry fruit quality and consumer acceptability [32] concerns the relation between SSC/TA. As shown in Figure 2, TSSM + BT plants presented the highest fruit quality (9.60), while TSSM and TSSM + CT plants yielded fruit with the lowest quality levels (5.5 and 5.2, respectively). According to Green [33], a sugar/acid ratio of 5.3 is considered the lowest acceptable level for strawberry fruit. Cordenunsi et al. [34] showed that cv. Oso Grande, which is considered an excellent option for fresh consumption in terms of nutritional value and quality parameters, has a sugar/acid ratio of 9.2. Recently, Reganold [35] found a difference of approximately 16% in SSC/TA ratio between chemically treated and organically cultivated strawberries. Yet, Camargo

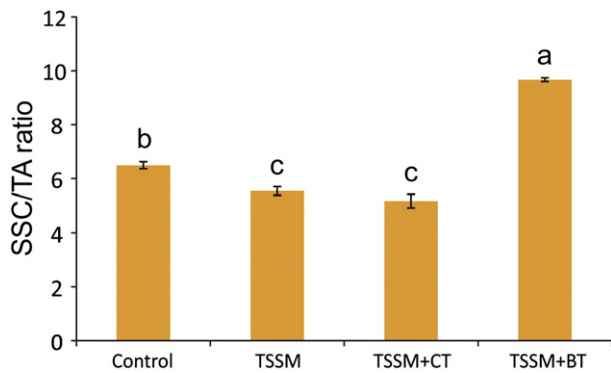


Fig. 2. Quality assessment of strawberry fruit through SSC/TA ratio. TSSM: Two-spotted Spider Mite; TSSM + CT: Two-spotted Spider Mite + Chemical Treatment; TSSM + BT: Two-spotted Spider Mite + Biological Treatment. The values are averages of four samples \pm SE. Different letters indicate that the means are different according to the Tukey HSD test ($P \leq 0.05$). Error bars may be too small to be visible in the figure.

et al. [36] found that cv. Aromas has a higher (approximately 9%) SSC/AT ratio when organically cultivated in comparison with traditional cultivation using chemical treatment. Our data point to an extremely high difference (approximately 78%) between TSSM + BT and TSSM + CT.

Besides size and shape, color is another important component of the appearance of strawberry fruit, and it is mainly defined by their anthocyanin content [34]. Anthocyanins are quantitatively the most important type of phenolic compound present in strawberries [37]. All strawberry fruit analyzed in our work presented anthocyanin values in agreement with previous studies (Figure 1d) (from 13 to 60 mg \cdot 100 g of fruit⁻¹), according to Cordenunsi et al. [34], Cordenunsi et al. [38] and Blando et al. [39]. However, TSSM-infested plants still produced the lowest quality fruit (34.4 mg \cdot 100 g of fruit⁻¹), containing a low quantity of red pigments, giving an unhealthy impression, and, consequently, less attractiveness to consumers. According to Naumann and Seipp [40], color and texture are the most important characteristics for the retail market and can strongly influence consumer choices. Fruit from control plants (no contact with TSSM) showed the highest anthocyanin level (51.4 mg \cdot 100 g⁻¹), suggesting that TSSM infestation can reduce anthocyanin concentration in strawberry fruit, even when followed by chemical or biological treatment. According to Musilová et al. [41], one of the main factors affecting anthocyanin accumulation in strawberry fruit is the cultivar, since they showed different anthocyanin levels for seven cultivars under the same soil, management and harvesting conditions. Our results (from 34.4 to 51.4 mg \cdot 100 g of fruit⁻¹) are in agreement with Castro et al. [42], which found 48 mg \cdot 100 g of fruit⁻¹ in the same cultivar used in our work.

Several studies have identified a wide range of phenolic compounds in strawberry fruit [37,43], but anthocyanins remain quantitatively the most important type. Fruit from TSSM-infested plants showed extremely low phenolic compound levels (158.9 mg \cdot 100 g of fruit⁻¹ – Figure 1e), while TSSM + BT fruit had the highest phenolic level (343.5 mg \cdot 100 g of fruit⁻¹, not statistically different from control and TSSM + CT treatments). It is clear that the presence of TSSM for longer periods of time results in low phenolic levels in strawberry fruit, since TSSM-infested plants showed lower levels than the other samples. We can suggest that TSSM extinction (or the absence of contact with TSSM in control condition) stimulates the synthesis of phenolics, allowing higher levels of these compounds in strawberry fruit. Luczynski et al. [44] demonstrated that the development of TSSM *T. urticae* Koch is negatively correlated to concentrations of phenolic compounds in strawberry leaves, and that mite development is delayed on leaves with higher concentrations of phenolic compounds. Based on these data obtained in leaves [44], we could also hypothesize that the low level of phenolic compounds seen on TSSM-infested plants could be related to a higher catabolism of phenolic compounds, instead of stimulation on the

control or treated plants. To the best of our knowledge, the relation between phenolic compounds in strawberry fruit and the presence of TSSM has not yet been reported. However, more in-depth studies would be needed to check which of the above mentioned hypotheses is correct.

In the ascorbic acid analysis (AA, Figure 1f), fruit from TSSM-infested + BT plants showed the highest AA levels (106.6 mg \cdot 100 g of fruit⁻¹). On the other hand, fruit from TSSM-infested + CT plants presented the lowest AA levels (66.3 mg \cdot 100 g of fruit⁻¹, not statistically different from control treatment). Thus, our results suggest that TSSM extinction using CT (acaricide) is not the most suitable alternative. The presence of phytophagous and/or predatory mites on the strawberry plants could be linked to high levels of ascorbic acid as a defense mechanism, since we can clearly note a relation between mite presence and high ascorbic acid levels. Krajnc [45] showed that concentrations of total AA were slightly increased by moderate attack of *Ips typographus* in *Picea abies*, while total AA values remained at control levels after massive attack. According to Schijlen et al. [46], ascorbic acid is an efficient oxygen radical scavenger, acting in plants as antioxidants and protective agents against several sources of damage (including mite infestation). Our data also suggest that biological control was highly satisfactory, generating fruit with high ascorbic acid levels. It seems that biological control using predatory mite can influence ascorbic acid accumulation, allowing the development of high quality fruit. Amodio et al. [47] verified that organically cultivated kiwis accumulate higher ascorbic acid levels than chemically treated ones. However, Cayuela et al. [48] were not able to find any differences in ascorbic acid accumulation in organically or conventionally cultivated strawberry fruit. According to Lee and Kader [49] the amount of vitamin C in fruits and vegetables depends on various factors such as genotypic differences, preharvest climatic conditions, and postharvest handling procedures.

3.2. Leaf analyses

Most of the studies on nutritional parameters and chemical composition in strawberries are restricted to fruit analyses, with few exceptions [44,50,51]. However, strawberry leaves are important for photosynthesis and their role in sugar accumulation is well known, since sugars are primarily accumulated in the developing fruit by translocation from leaves [52]. In addition, strawberry leaves are also known for their efficient antioxidant capacity [50].

As seen in Figure 3a, young strawberry leaves showed higher phenolic compound levels than mature (older) leaves in all treatments. The same pattern is also seen in young green tea leaves, which are the choice leaves used in the production of black tea due to their high polyphenol content [53]. We hypothesize that younger leaves produce higher levels of phenolics as a defense mechanism, since mature strawberry leaves are in fact more attacked and damaged by TSSM. Young and mature leaves under the control condition presented the highest phenolic levels, and all other plants which had contact with TSSM (TSSM, TSSM + CT and TSSM + BT) presented low levels of phenolic compounds. These data partially agree with the abovementioned report [44], which verified a negative correlation between phenolic compounds and TSSM development.

Methylxanthines – namely caffeine, theobromine and theophylline – inhibit insect feeding and are pesticides at concentrations known to occur in plants, functioning as natural insecticides [54]. In our experiments, young and mature leaves showed similar methylxanthine concentrations (Figure 3b). However, TSSM-infested leaves presented the highest values, suggesting that the synthesis of methylxanthines can be stimulated by the presence of TSSM, since the only treatment with live mites at the harvesting period concerned the TSSM-infested plants. In fact, high concentrations of caffeine in young leaves of tea and coffee species act as a defense mechanism in order to protect young soft tissues from pathogens and herbivores [55]. It has been demonstrated that spraying tomato leaves with caffeine deters feeding by tobacco

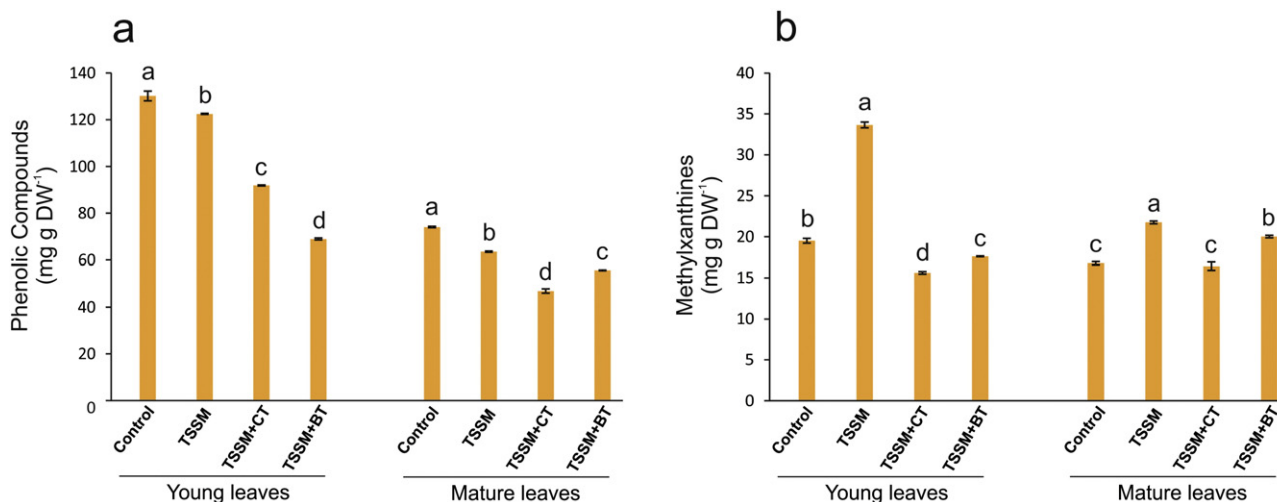


Fig. 3. Phenolic Compounds (a) and Methylxanthines (b) in strawberry leaves. TSSM: TSSM + CT: Two-spotted Spider Mite + Chemical Treatment; TSSM + BT: Two-spotted Spider Mite + Biological Treatment. The values are averages of four samples \pm SE. Different letters indicate that the means are different according to the Tukey HSD test ($P \leq 0.05$). Error bars may be too small to be visible in the figure. DW = dry weight.

hornworms, and caffeine acts as a neurotoxin while treating cabbage leaves and orchids, killing or repelling slugs and snails [56].

In summary, this study suggests that the infestation of strawberry plants with TSSM can alter several physicochemical and nutritional properties, decreasing the quality of the fruit. On the other hand, plants infested with TSSM and then treated with the predatory mite *P. macropilis* actually showed even better performance than the control plants, displaying excellent fruit quality. Therefore, the biological control of TSSM is a safe and efficient manner to reduce the infestation of phytophagous mites and also to increase the quality of the strawberry fruit. It is important to consider that only one cultivar was used. A study with a larger number of cultivars could reveal other significant changes in physicochemical and nutritional parameters. However, the changes presented in this study do provide a starting point for a more in-depth analysis of nutritional and physicochemical changes caused by mite infestation on strawberry plants.

Conflict of interest

None.

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Author contributions

Proposed the theoretical frame: EL, RAS, NJF, CFVS; Conceived and designed the experiments: RAS, NJF, CFVS; Contributed reagents/materials/analysis tools: EL, RAS, NJF, CFVS; Wrote the paper: EL, RAS, NJF, CFVS; Performed the experiments: EL, RAS; Analyzed the data: EL, RAS.

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