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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.

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 SCC, 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-diclorophenol–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