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'Kent' mango grown in the semiarid region of Brazil under different concentrations of melissyl alcohol

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Abstract: The imposition of water deficit in mango cultivation, combined with branch maturation, favors the accumulation of essential reserves for flowering. However, simultaneous abiotic stresses such as high temperatures and low humidity, disrupt crucial physiological processes. Alternatives have been sought to mitigate these adverse effects in plants exposed to unfavorable conditions. In this scenario, this study evaluated the physiological performance of 'Kent' mango trees in the Brazilian semiarid region treated with triacontanol. The experiment was conducted over two consecutive crop years (2018 and 2019), using a randomized block design with five treatments and four replications, by evaluating four plants per plot. The treatments consisted of five concentrations of triacontanol: 0.00 (Control), 3.75, 7.50, 11.25, and 15.0 ppb. The product containing the active ingredient triacontanol was Revigor® at a concentration of 0.05 g L^1 (50 ppm). The application of Triacontanol affects photosynthetic pigments, increases the level of total soluble carbohydrates in leaves and branches, positively influences the number of panicles and leads to productivity increases in irrigated 'Kent' mango trees grown in the semiarid conditions of Pernambuco, with oscillations between factors and between harvests. There was an increase in productivity of 64.91% (estimated concentration - 10.51 ppb) in the 2019 harvest.

Index terms: *Mangifera indica* L., photosynthetic pigments, soluble sugars.

Mangueira 'kent' cultivada no semiárido brasileiro sob diferentes concentrações de melissil álcool

Resumo: A imposição do déficit hídrico na cultura da mangueira, combinada com a maturação dos ramos, permite acumular reservas essenciais à floração. Entretanto,

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estresses abióticos simultâneos, como temperaturas elevadas e baixa umidade, prejudicam processos fisiológicos cruciais. Alternativas têm sido buscadas para mitigar tais efeitos adversos em plantas expostas a condições desfavoráveis. Nesse contexto, avaliou-se o desempenho fisiológico de mangueiras 'Kent' no semiárido brasileiro, tratadas com triacontanol. O experimento foi realizado em duas safras consecutivas (2018 e 2019), em delineamento de blocos casualisados, com cinco tratamentos e quatro repetições, sendo avaliadas quatro plantas por parcela. Os tratamentos consistiram em concentrações de triacontanol: 00,00 (Controle), 3,75; 7,50; 11,25 e 15,0 ppb. O produto que contém o ingrediente ativo triacontanol foi o Revigor® a uma concentração de 0,05 g L-1 (50 ppm). A aplicação de triacontanol afeta os pigmentos fotossintéticos, aumenta os teores de carboidratos solúveis totais nas folhas e nos ramos, influencia positivamente o número de panículas e leva a ganhos de produtividade, em mangueiras 'Kent' irrigadas, cultivadas nas condições do semiárido pernambucano, havendo oscilações entre os fatores e entre as safras. Houve aumento na produtividade de 64,91% (concentração estimada - 10,51 ppb), na safra de 2019.

Termos para indexação: *Mangifera indica* L., pigmentos fotossintéticos, açúcares solúveis.

Introduction

Mango cultivation has gained prominence in the Brazilian export market of fresh fruits due to the productivity and quality of this crop, making it one of the most exported fruits in the country (KIST et al., 2022). Another factor that contributes to mango production is the adoption of technologies that have improved flowering management, e.g., pruning (LOPES et al., 2021), plant regulators (SILVA et al., 2021), and irrigation management (SANTOS et al., 2013).

Among the phases preceding floral induction, the reduction in irrigation serves as a signal for uniform branch maturation, leading to optimal carbohydrate levels directed toward the development of reproductive organs (PRASAD, 2014; CAVALCANTE et al., 2018). However, this water restriction should be implemented gradually in order to prevent severe oxidative damage to the plant when water availability falls below the basal physiological demand.

Combined with the usual high temperatures of the Brazilian semiarid region, water scarcity can compromise carbohydrate accumulation, used as an osmoregulator under abiotic stress conditions, leading to the depletion of carbohydrate reserves as a defense mechanism (WEISZMANN et al., 2018). Additionally, defense mechanisms such as stomatal closure, which reduces water loss through transpiration, have side effects that include reductions in photosynthetic activity and in the levels of photosynthetic pigments (SANTOS et al., 2013; SILVA et al., 2022).

Under these conditions, plant regulators that mitigate abiotic stresses and improve the functioning of metabolic processes have been adopted to facilitate the adaptation and expression of the productive potential of plants. Such plant regulators include triacontanol (Melissyl alcohol), which has been reported to increase nutrient acquisition, chlorophyll indices, photosynthetic activity, growth parameters, enzyme activity, and various organic compounds in leaf tissues. It has also been shown to enhance production and quality in different plant species under normal and stressful conditions (PERVEEN et al., 2017; ISLAM et al., 2020; VERMA et al., 2022), triacontanol representing an innovative alternative for fruit-bearing species such as mango.

From this perspective, this study aimed to evaluate the levels of photosynthetic pigments, total soluble carbohydrates, and productivity of 'Kent' mango trees cultivated in the Brazilian semiarid region under the influence of melissyl alcohol.

Material and Methods Study Area Description

The experiment was conducted over two consecutive crop years, 2018 and 2019, using four-year-old trees of the mango cv. 'Kent' (*Mangifera indica* L.). The orchard was in the first (2018) and second (2019) production cycles and was located at the DAN Farm (Desenvolvimento Agrícola do Nordeste) in the municipality of Petrolina (Latitude 9°18'19.2"S, Longitude 40°33'55.9"W, and an elevation of 365.5 meters above sea level), Pernambuco, Brazil. The climate of the region where the experiment was carried out (Sub-middle region of the São Francisco River Valley) is classified as *Bsh*, with an average annual temperature of 26.0 °C and an average annual rainfall of 481.7 mm (ALVARES et al., 2013).

Throughout both crop years (2018/2019), meteorological data including temperature (maximum, minimum, and average), relative humidity, and rainfall were recorded using the automatic weather station of the Federal University of Vale do São Francisco (UNIVASF), located at the Agricultural Sciences Campus (Figure 1).

Figure 1. Maximum, minimum, and average temperatures; relative humidity, and rainfall recorded during the experimental periods in the 2018 (A) and 2019 (B) crop years. Petrolina, Pernambuco, Brazil.

The experimental orchard had a spacing of 4 x 2.5 meters, with daily irrigation using a double-line drip system through four emitters per plant operating at a flow rate of 2.4 L h^{-1} . During the water stress phase, irrigation was gradually reduced to 25% (100 L per day), with drip irrigation provided for only one hour per day during nighttime in both crop years. Crop management practices such as pruning, nutritional management, and pest control were carried out according to the Recommendations and Technical Standards for Integrated Mango Production provided by Lopes et al. (2003).

Experimental Design and Treatment Application

The experiment was set up in a randomized complete block design with five treatments and four replications, in which four plants per plot were evaluated. The treatments consisted of different concentrations of triacontanol: 0.00 (Control), 3.75, 7.50, 11.25, and 15.0 ppb. The product containing the active ingredient triacontanol was Revigor® (AQUA do BRASIL), at a concentration of 0.05 $g L⁻¹$ (50 ppm). The product was applied using a 20-L backpack sprayer until full leaf coverage was achieved. The applications were done bi-weekly and followed through the branch

maturation phase until the beginning of fruiting. Applications were continued during the stages of branch maturation, floral induction, full flowering, and initial fruiting. The plant material for biochemical evaluations was collected 48 hours after each application.

For the first evaluation cycle (2018 crop year), applications began during the floral induction phase, while the irrigation level from the previous phase (branch maturation) had not yet been modified, so that the plants remained under water deficit. The first four applications in this season were distributed weekly to account for the unassessed period, and subsequent of tiacontanol applications were made in two-week intervals. During the second experiment, applications started during the branch maturation phase. In the first crop year, no difference was observed between the duration of the product's action for weekly and bi-weekly application schedules. Therefore, in the second crop year, applications were adjusted to a 15-day interval to optimize the experiment.

For the biochemical evaluations, freshly mature leaves were collected two days after each application from the four quadrants of the most recent vegetative growth at middle canopy height. Each sample was then placed in labeled plastic bags (according to treatment and replication) and stored in a cooler containing ice to preserve the structure and composition of the plant material for quantification of the following variables: chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, following the methodology described by Lichtenthaler and Buschmann (2001), as well as total soluble carbohydrates, according to Dubois et al. (1956).

Before the application of treatments, the plants were characterized for their content of chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids (Carot), and total soluble carbohydrates in the leaves (TSCL) and branches (TSCB) (Table 1).

Table 1. Initial contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, and total soluble carbohydrates in the leaves and branches of the mango cultivar Kent.

Chl: chlorophyll; Carot: carotenoids; TSCL: Total soluble carbohydrates in leaves; TSCB: Total soluble carbohydrates in leaves in branches.

Fruit harvest was carried out on November 20th and November 19th, in the 2018 and 2019 crop years, respectively, when the fruits were at maturity stage 2, characterized by a creamy-yellowish pulp color (Filgueiras et al. 2000). Subsequently, the fruits were weighed to estimate the yield of each treatment.

Statistical analysis was performed using the R software. Analysis of variance of the data obtained was conducted using the F-test to check for significant effects. If significance was observed, the means of the triacontanol concentration factor were subjected to regression analysis, and adjustments to linear and quadratic polynomial models were evaluated ($R^2 \geq 0.6$). Graphs were created using Sigmaplot® version 14.

Results and Discussion Photosynthetic Pigments

According to the analysis of variance, the triacontanol concentrations influenced the variables analyzed, with fluctuations observed between crop years and evaluation phases (Table 2).

Table 2. Summary of analysis of variance for foliar contents of photosynthetic pigments and total soluble carbohydrates in leaves (TSCL) and branches (TSCB) of the mango cultivar Kent, based on different phenophases and triacontanol concentrations.

SV: Source of Variation; CV: Coefficient of variation; ns: not significant; **: significant at 1% probability ($p < 0.01$); *: significant at 5% probability (p < 0.05).

 The relationship between chlorophyll *a* levels and triacontanol concentrations determined in the 2018 crop year was defined by a quadratic adjustment for the floral induction and initial fruiting phases (Figure 2A). There was a decrease in chlorophyll *a* levels with increasing triacontanol concentrations during the induction phase, followed by an increase during the initial fruiting phase at

higher triacontanol concentrations. In the 2019 crop year, this relationship showed a quadratic adjustment in three phases (Figure 2B). During the initial fruiting phase, the maximum chlorophyll content was estimated at 4.10 ppm of triacontanol per plant, resulting in a response of 0.96 mg g^{-1} , representing a 3.22% increase compared to the control treatment.

Figure 2. Chlorophyll *a* contents in leaves of the mango cv. Kent as a function of triacontanol concentrations in the2018 (A) and 2019 crop years (B).

For the chlorophyll *b* levels, a quadratic adjustment was observed for the 2018 crop year (Figure 3A), indicating a decreasing effect with triacontanol concentrations during the floral induction phase. Conversely, during the initial fruiting phase, there was an increase in chlorophyll *b* levels for the triacontanol concentrations of 11.25 and 15 ppb triacontanol per plant. In the 2019 crop year, quadratic

adjustments were found for the branch maturation and full flowering phases (Figure 3B). These adjustments showed an increasing effect up to the estimated maximum concentrations of 7.65 and 8.78 ppb of triacontanol per plant, resulting in chlorophyll *b* responses of 0.35 and 0.25 mg per gram of fresh mass, with increments of 52.17% and 13.64% compared to the control treatment.

Figure 3. Chlorophyll *b* contents in leaves of the mango cv. Kent as affected by triacontanol concentrations, in the 2018 (A) and 2019 crop years (B).

With regard to the total chlorophyll levels for the 2018 crop year (Figure 4A), the data showed quadratic adjustments for the floral induction and initial fruiting phases. During the floral induction phase, there was a reduction in total chlorophyll levels with increasing triacontanol concentrations. Conversely, during the initial fruiting phase, there was an increase in total chlorophyll levels at higher concentrations of triacontanol. For the 2019 crop year, the branch maturation phase did not show a quadratic adjustment (Figure 4B), while the other phases did not show significant differences.

While the action of triacontanol resulted in fluctuations in the contents of chlorophyll *a*, *b*, and total among different concentrations and phenophases, there are positive results from other research studies that reinforce its effect in increasing chlorophyll levels in crops such as rice (*Oryza sativa*) (LI et al., 2016), maize (*Zea mays*) (PERVEEN

et al., 2017), and peppermint (ZAID et al., 2019).

Figure 4. Total chlorophyll contents in leaves of the mango cv. Kent as a function of triacontanol concentrations in the 2018 crop year.

However, chlorophyll degradation is one of the consequences of excessive radiation stress, acting through photoinhibition, which subsequently reduces photosynthetic efficiency (WEISZMANN, 2018). Nevertheless, plants have defense mechanisms that maintain control over the rate of light energy absorption by chlorophyll *b* and its subsequent transfer to the reaction center of chlorophyll *a*, ensuring the regular functioning of the photochemical phase, which is the initial step of the photosynthetic process (ALTON, 2017). Thus, plants under stressful conditions may respond with lower pigment concentrations to capture less light energy and avoid potential photooxidative damage.

With regard to the carotenoid levels, the data showed a quadratic adjustment for the initial fruiting phase in the 2018 crop year

(Figure 5A), with reductions in carotenoid levels at lower triacontanol concentrations and subsequent increases at the concentrations of 11.25 and 15.0 ppb triacontanol per plant. The carotenoid levels obtained in the 2019 crop year showed quadratic adjustments for the floral induction, full flowering, and initial fruiting phases (Figure 5B). The estimated maximum triacontanol concentrations of 7.45, 6.44, and 5.31 ppb triacontanol per plant led to the accumulation of 0.39, 0.36, and 0.37 mg g^{-1} , respectively. This resulted in carotenoid increases of 25.80%, 15.22%, and 18.22% compared to the control treatment.

Figure 5. Carotenoid contents in leaves of the mango cv. Kent as a function of triacontanol concentrations in the 2018 (A) and 2019 crop years (B).

The results of the present study align with the findings of Naeem et al. (2019), where the authors observed that a lower concentration (1 μM) of TRIA led to increases in the carotenoid content, as well as in the total chlorophyll index and gas exchange attributes in *Catharanthus roseus* L. compared to untreated plants. From this perspective, increases in the carotenoid content imply positive effects on the photosynthetic apparatus, as it serves as an important accessory pigment for light absorption at the wavelengths of 450-570 nm, where chlorophylls do not efficiently absorb light energy (PORCAR-CASTELL et al., 2014). Moreover,

plants typically accumulate carotenoids to mitigate stressful conditions, assisting in dissipating excess absorbed energy in the form of heat, especially through the zeaxanthin – violaxanthin cycle, which balances the cell's redox state (KUCZYNSKA et al., 2020).

Carbohydrates

The content of total soluble carbohydrates in leaves for the 2018 crop year showed a linear adjustment during the full flowering phase (Figure 6A). This resulted in increasing leaf TSC levels at higher triacontanol concentrations. The triacontanol concentration of 15.00 ppb per plant led to the maximum response (123.50 mg g^{-1}), representing a 9.82% increase compared to the control treatment. For the 2019 crop year, a quadratic adjustment was observed during the full flowering phase (Figure 6B), resulting in decreased leaf TSC levels in plants subjected to triacontanol.

Figure 6. Total soluble carbohydrate contents in leaves (TSCL) of the mango cv. Kent as a function of triacontanol concentrations in the 2018 (A) and 2019 crop years (B).

Houtz et al. (1985) suggested that triacontanol induces an increase in the specific activity of RuBisCO and phosphoenolpyruvate carboxylase, as well as malate dehydrogenase activity in photorespiration. In this regard, improvements in the activity of these photosynthesis-related enzymes might have contributed to the higher accumulation of leaf TSC levels in triacontanol-treated plants in the current study. Carbohydrates are accumulated in leaves in optimal amounts resulting in a vigour of reproductive phase, especially during the formation of inflorescences (SILVA et al., 2021).

In the 2019 crop year, there was a decreasing effect on leaf TSC levels due to triacontanol concentrations, could be attributed to the full flowering phase in which the plants were. During this period, there is an increase in the activity of carbohydrate-hydrolyzing enzymes, and mobilization of metabolites from leaves to developing reproductive organs, which is in line with the findings of Prasad et al. (2014).

The increased concentration of total soluble carbohydrates in plants after triacontanol

application (ISLAM et al., 2019) might be related to gains achieved through the activation of the antioxidant defense system, e.g., the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) (SANCHES et al., 2023).

The leaf TSC levels found in the current study (2019 crop year) were higher (ranging from 120.08 to 190.34 mg g^{-1}) than those reported by Cunha et al. (2022), whose results ranged from 115.36 to 138.47 mg g^{-1} when evaluating the effect of proline and seaweed extract supply to mitigate water stress in 'Tommy Atkins' mango trees under semiarid conditions. This further reinforces the potential of this molecule for such purposes.

With regard to TSC levels in branches, the quadratic model did not fit the observed data for the 2018 crop year, in contrast with the results presented for the 2019 crop year during the full flowering and initial fruiting phases (Figure 7). The triacontanol concentrations positively affected the TSC levels in branches during full flowering, achieving a maximum response of 248.27 mg g^{-1} with 11.56 ppb of triacontanol per plant, equivalent to a 49.0% increase compared to the control. However, during the initial fruiting phase, an opposite behavior was observed with lower average TSC values in branches.

Figure 7. Carbohydrate contents in branches (branch TSC) of the mango cv. Kent as a function of triacontanol concentrations in the 2019 crop year.

The increase in branch TSC levels during the full flowering phase might be associated with the reduction in leaf TSC levels (Figure 5B). According to Santos-Villalobos et al. (2013), who evaluated carbohydrate levels in 'Ataulfo' mango, the carbohydrate concentrations decreased over time, suggesting that these components were consumed for panicle and fruit development. Therefore, it is suggested that the reduction in branch TSC concentrations during the initial fruiting phase resulted from translocation to the sink, which corresponds to the early fruit development phase. However, the positive effect of triacontanol in increasing the carbohydrate concentration in branches led to an increase in essential energy sources for flowering. These sources are greatly required for fruit growth and subsequent development (CARREIRO et al., 2022).

According the analysis of variance, triacontanol concentrations positively affected the number of panicles, number of fruits in the 2018 crop year, and the productivity of the mango cv. Kent in both seasons (Table 3).

Table 3. Number of panicles per plant and productivity of the mango cv. Kent as a function of triacontanol concentrations (MA).

SV: Source of Variation; CV: Coefficient of variation; **: significant at 1% probability (p < 0.01); *: significant at 5% probability ($p < 0.05$).

Among the agronomic variables that showed significant differences for the 2018 crop year, only the number of panicles showed a quadratic adjustment (Figure 8), with a pronounced effect on triacontanol -treated plants. The maximum response was observed at 70.63 panicles per plant with an estimated dose of 11.79 ppb of triacontanol per plant, representing a 26.44% increase compared to the control treatment.

Figure 8. Number of panicles of the mango cv. Kent as a function of triacontanol concentrations in the 2018 crop year.

The increase in the number of panicles in the 2018 crop year could have been caused by the rise in leaf TSC levels since the leaf carbohydrate content is directly involved in panicle development and the intensity of flowering induction (SANTOS-VILLALOBOS, 2013). Therefore, being plants in their first

productive cycle (2018 crop year), the effect of triacontanol responded more efficiently. This is because, in the 2019 crop year, there was no significant influence on the increase of panicle emission.

For the 2019 crop year, based on the quadratic adjustment, the yield was positively

affected by treatments containing triacontanol (Figure 9B), resulting in a maximum response of 41.59 t ha⁻¹ for the concentration of 10.51 ppb of triacontanol per plant. This increase resulted in a 64.91% gain, equivalent to 16.37 t of fruits per hectare, compared to the control treatment.

Figure 9. Yield (t ha⁻¹) of the mango cv. Kent as a function of triacontanol concentrations in the 2018 (A) and 2019 crop years (B).

As a reference to the mango yield in the region of this study, the mentioned yields were similar to those found by Lobo (2019), who obtained values ranging from 22.05 to 53.32 t ha⁻¹ for 'Kent' mango in the sixth year of production. It is worth emphasizing here the age difference between orchards, with the plants in this study being much younger and therefore having lower productive capacity.

For the 2018 crop year, the lower yield can be attributed to the fact that the plants were in their first production cycle and because fruit weight was higher in the 2019 crop year. This increase in fruit weight could be related to the triacontanol treatment, an effect already observed in other experiments involving the application of this molecule, including with the mango cultivar 'Arka Neelachal Kesri' (Dash et al., 2021).

From this perspective, despite fluctuations throughout phenophases and cultivation cycles, a general influence of triacontanol was observed on the physiological parameters of 'Kent' mango, which is a positive aspect as the molecules were capable of modulating plant performance during the experiment. Alongside this factor, there are also climatic interferences, the initial timing of applications, and biochemical modulations that enhance the productive potential of mango.

The fluctuations observed may be associated with plant performance during the experiment, tied to climatic interferences, the onset of applications, and biochemical modulations that favor the productive potential of mango.

Conclusion

Triacontanol application affects the photosynthetic pigments, increase total soluble carbohydrate levels in leaves and branches, positively influence the number of panicles, and lead to yield gains in irrigated 'Kent' mango trees cultivated under the conditions of the semiarid region of Pernambuco.

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