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Biochemical responses in 'Kent' mango grown in Brazilian semi-arid region under different doses of triacontanol¹

Respostas bioquímicas em manga 'Kent' cultivada no semiárido brasileiro
sob diferentes doses de triacontanol

Luciana G. Sanches², Alana J. da S. Santos², Daniel de A. Carreiro², Jenilton G. da Cunha^{3*},
Jackson T. Lobo⁴, Ítalo H. L. Cavalcante² & Vespasiano B. de Paiva Neto²

¹ Research developed at Petrolina, Pernambuco, Brazil

² Universidade Federal do Vale do São Francisco, Petrolina, PE, Brazil

³ Universidade Federal do Piauí, Bom Jesus, PI, Brazil

⁴ Universidade Federal da Paraíba, Areia, PB, Brazil

HIGHLIGHTS:

Application of triacontanol favors the accumulation of organic solutes in mango plants.

Triacontanol modulates the antioxidant enzymatic activity with the greatest significant influence in the 2019 production cycle.

There are increases in the production of 'Kent' mango fruit under triacontanol application in the 2019 production cycle.

ABSTRACT: The management adopted for the mango crop requires that, at a certain stage, the irrigation depth should be reduced to promote a more uniform flowering. In regard to that, it is necessary to introduce new alternatives that mitigate the harmful effects of abiotic stress and that promote greater fruit yield. Therefore, the present study aimed to evaluate the influence of triacontanol on the levels of organic solutes, antioxidant enzymatic activity, and production of 'Kent' mango under Brazilian semi-arid conditions. The experiment was carried out for two consecutive years, 2018 and 2019, in a commercial orchard located at the DAN Farm, Petrolina-PE. The experimental design used was randomized blocks, with five treatments and four replications, evaluating four plants per plot. The treatments consisted of triacontanol doses: 0 (control treatment), 3.75; 7.50; 11.25, and 15.00 ppb per plant. Two consecutive harvests (2018 and 2019) were evaluated during the stages of branch maturation (2019 only), floral induction, full flowering, and initial fruiting. Foliar application of triacontanol positively favored the contents of total amino acids, free proline, total proteins, and activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). There was an increase in production of 50.85% (dose - 11.25 ppb) and 64.95% (dose - 10.50 ppb) in the 2018 and 2019 crop years, respectively.

Key words: *Mangifera indica* L., antioxidant enzymes, proline

RESUMO: O manejo adotado para a cultura da manga exige que, em determinada fase, a lâmina de irrigação seja reduzida para promover uma floração mais uniforme. Nesse sentido, é necessário inserir novas alternativas que atenuem os efeitos deletérios do estresse abiótico e que promovam maior produtividade de frutos. Portanto, o presente estudo teve como objetivo avaliar a influência do triacontanol sobre os teores de solutos orgânicos, atividade enzimática antioxidante e produção de mangueira 'Kent' nas condições do semiárido brasileiro. O experimento foi realizado em duas safras consecutivas, 2018 e 2019, em um pomar comercial localizado na Fazenda Dan, Petrolina-PE. O delineamento experimental utilizado foi em blocos casualizados, com cinco tratamentos e quatro repetições, sendo avaliadas quatro plantas por parcela. Os tratamentos consistiram em doses de triacontanol: 0 (tratamento controle); 3,75; 7,50; 11,25 e 15,00 ppb por planta. Duas safras consecutivas (2018 e 2019) foram avaliadas durante os estágios de maturação do ramo (somente 2019), indução floral, floração plena e frutificação inicial. Apesar das variações entre safras, observou-se que a aplicação foliar de triacontanol favoreceu positivamente os teores de aminoácidos totais, prolina livre, proteínas totais e atividade das enzimas superóxido dismutase (SOD), ascorbato peroxidase (APX) e catalase (CAT). Houve aumento na produção de 50,85% (na dose 11,25 ppb) e 64,95% (na dose 10,50 ppb) nas safras de 2018 e 2019, respectivamente.

Palavras-chave: *Mangifera indica* L., enzimas antioxidantes, prolina

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* Corresponding author - E-mail: jeniltongomes@hotmail.com

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INTRODUCTION

The São Francisco Valley stands out for the use of technology in the mango production chain, mainly the use of plant regulators, and the imposition of induced water stress, regulated by the gradual reduction of the irrigation depth, performed during the branch maturation phase (Carreiro et al., 2022; Cunha et al., 2022). This practice aims to promote the reduction of vegetative growth and leave the apical meristems more sensitive to florigenic promoters, responsible for inducing flowering (Ramírez & Davenport, 2010).

Even if managed in a controlled manner, water deficit can bring harmful consequences that have already been recognized in the literature. However, under these conditions, plants activate enzymatic and non-enzymatic defense systems in order to reduce the effects of oxidative stress caused especially by reactive oxygen species (ROS), such as superoxide ion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\bullet$) from oxidative stress, and this causes the inactivation of enzymes, degradation of pigments, lipid peroxidation, and proteolysis (Hasanuzzaman et al., 2020).

Generally, these types of damage can be mitigated by osmoregulatory substances, such as proline, soluble protein, amino acids, and soluble sugars, as well as by enzymatic system composed mainly of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) in order to protect the cellular components from the impacts of stress (Hou et al., 2021; Manjavachi et al., 2022).

An alternative to boost the antioxidant defense system is the use of triacontanol, which has the ability to positively regulate the genes involved in the photosynthetic process and those related to the perception and signaling of stress, through the modulation of the activities of different metabolic enzymes and antioxidants (Perveen et al., 2011). In addition, it improves the uptake of mineral nutrients and water, stimulating the synthesis of various organic compounds through the increase of nitrogen metabolism (Ertani et al., 2013; Naem et al., 2019), besides the retention, production, and quality of mango fruits (Dash et al., 2021).

Based on the above, the present study aimed to evaluate the influence of triacontanol on the levels of organic solutes, antioxidant enzymatic activity, and production of 'Kent' mango under Brazilian semi-arid conditions. Responses were evaluated over different phenophases of the crop and two production cycles.

MATERIAL AND METHODS

The experiment was conducted in two consecutive crop years, 2018 and 2019, with 'Kent' mango (*Mangifera indica* L.) with four years of age in the orchard of the first (2018) and second (2019) production cycles and located at DAN (*Desenvolvimento Agrícola do Nordeste - Agricultural Development of the Northeast*) Farm, municipality of Petrolina, Pernambuco, Brazil (09° 23' 08.7" S 40° 42' 00.2" W, and altitude of 365.5 m above sea level). The climate of the sub-middle region of the São Francisco Valley is classified as Bsh, with an average annual temperature of 26.0 °C and mean annual rainfall of 481.7 mm (Alvares et al., 2013). During

both crop years (2018/2019), meteorological data related to temperature (maximum, minimum, and average), relative humidity of the air, and rainfall were recorded by the Federal University of the São Francisco Valley (UNIVASF) automatic weather station, installed in the Agricultural Sciences Campus (Figure 1), 28.7 km distant from the experimental area.

The plants were arranged at the spacing of 4 × 2.5 m, with daily irrigation by drip system with double tape, with four emitters per plant with a flow rate of 2.4 L h⁻¹. During the water stress phase, the water depth was gradually reduced by up to 25% of its availability, receiving drip irrigation for only one hour in the evening, in both production cycles. We emphasize that the water depth was reduced between the months of May and June in both production cycles. The experiment was conducted under the field conditions, according to the management practiced in the region, in which the water deficit extends from the maturation of branches until the end of floral induction, with the irrigation depth being readjusted gradually during this period. Cultural practices, such as pruning, nutritional management, and phytosanitary control were performed according to the recommendations and technical standards of Integrated Mango Production defined by Lopes et al. (2003).

The experimental design used was in randomized blocks, with five treatments and four replicates, evaluating four

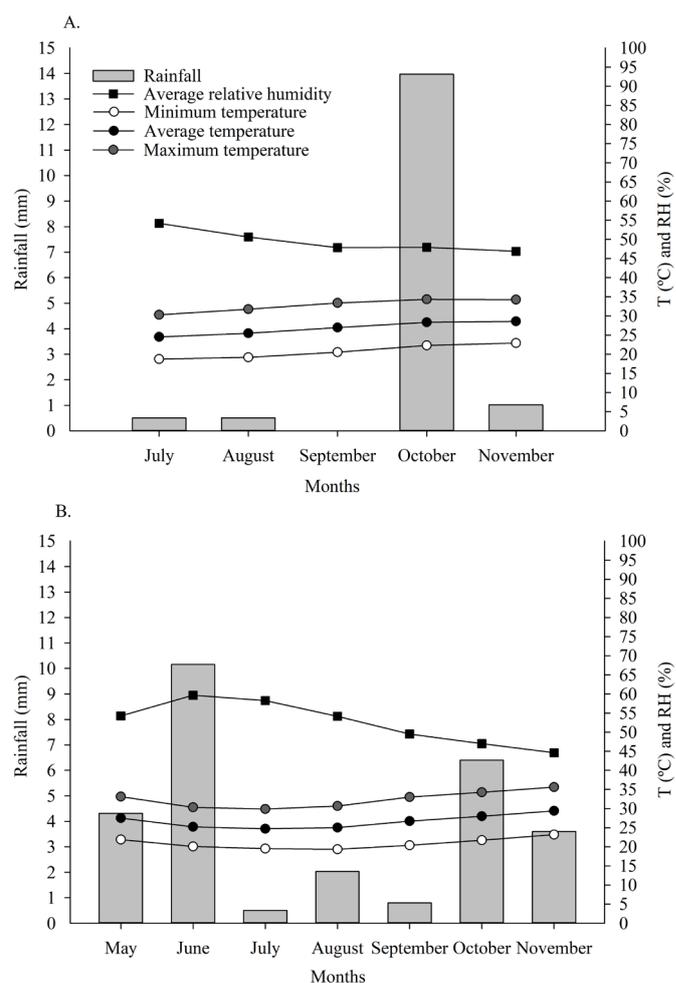


Figure 1. Maximum, minimum and average temperatures (T), relative humidity of the air (RH) and rainfall recorded during the experimental period in Petrolina-PE, 2018 (A) and 2019 (B)

plants per plot. The treatments consisted of triacontanol doses: 0 (control treatment), 3.75, 7.50, 11.25, and 15.00 ppb per plant, and 1.0 L of the solution was applied per plant. The product used containing the active ingredient of the triacontanol molecule was Revigor[®] (AQUA do BRASIL), with a concentration of 0.05 g L⁻¹ (50 µL), 5% K₂O, 0.02% B, density of 1.13 kg dm⁻³ and pH = 10. Spraying was carried out using a backpack pump sprayer (capacity 20 L) until the leaves were completely covered by the solution. The present study included the phases of branch maturation, floral induction, full flowering, and initial fruiting, but with differences in the time of evaluation in the two production cycles.

During the first experiment (2018 crop year), the applications began in the floral induction phase, and the irrigation depth practiced in the previous phase (branch maturation) had not yet been modified, so the plants remained under water deficit. The first four applications in this crop were distributed weekly to balance with the non-evaluated period and were subsequently arranged biweekly. During the second experiment (2019 crop year), the applications began in the branch maturation phase. Since in the first crop year there were no differences between the time of action of the product for the weekly and biweekly intervals of applications, in the second crop year the applications were adjusted for 15-day intervals to optimize the conduction of the experiment.

All plant material collected was taken to the Plant Physiology Laboratory of UNIVASF, to quantify the leaf contents of total amino acids, following the methodology described by Yemm et al. (1955); free proline, following the methodology described by Bates et al. (1973); and total soluble proteins according to Bradford (1976). The activities of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) were performed according to the

methodology described by Beers Jr. & Sizer (1952), Nakano & Asada (1981), and Giannopolitis & Ries (1977), respectively.

For biochemical evaluations, recently mature leaves were collected two days after each application, and these should be located in the four quadrants of the last vegetative flush at the median height of the crown. Then, each sample was packed in identified plastic bags (by treatment and repetition) and stored in a thermal box containing ice to preserve the structure and composition of the plant material for biochemical quantifications. The material collected to evaluate the enzymatic activity was packed in aluminum foil and stored in liquid nitrogen, in the field, to later be stored in a horizontal freezer, at a temperature of -20 °C until the moment of extraction.

The harvest of the fruits in the 2018 crop year was carried on November 20, and for 2019 on November 19, when the fruits were in Stage 2, characterized by a cream-yellowish color of the pulp (Filgueiras, 2000), weighing them for production (kg per plant).

The analysis of variance of the data was performed separately for each phase and crop year, while the doses of triacontanol were subjected to regression analysis and the fits of linear and quadratic models ($R^2 \geq 0.60$) were evaluated. Statistical analysis was performed using R software.

RESULTS AND DISCUSSION

The metabolic responses of plants were altered according to the management adopted in each phase, especially in commercial orchards. In this context, based on the analysis of variance, the contents of total amino acids, free proline, total proteins, and activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) were influenced by the doses of triacontanol (Table 1).

Table 1. Variance analysis for total amino acids, total proteins, free proline, and activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) in 'Kent' mango leaves as a function of triacontanol doses in different phenophases

Source of variation	DF	F value					
		Total amino acids (µmol g ⁻¹ FM)	Total proteins	Free proline	SOD (U mg ⁻¹ prot min ⁻¹)	APX (µM H ₂ O ₂ min ⁻¹ g ⁻¹ FM)	CAT
2018 crop year							
Floral induction	4	9.866**	0.711 ^{ns}	6.039**	0.7013 ^{ns}	0.403 ^{ns}	11.434**
Residual		0.771	0.0128	0.007	109.202	23.844	0.914
CV (%)		12.69	12.68	10.11	13.57	9.22	9.25
Full flowering	4	2.674 ^{ns}	0.518 ^{ns}	5.934 ^{ns}	1.999 ^{ns}	41.656 ^{ns}	4.949*
Residual		0.569	0.003	0.002	91.109	3.491	1.138
CV (%)		14.02	6.52	6.90	13.81	3.87	8.61
Initial fruiting	4	6.233**	0.712 ^{ns}	132.838**	0.166 ^{ns}	10.219 ^{ns}	6.099**
Residual		0.988	0.013	0.003	100.253	4.440	1.710
CV (%)		17.98	12.88	2.99	14.22	5.52	8.34
2019 crop year							
Branch maturation	4	7.755**	3.461*	15.189**	2.609 ^{ns}	14.247**	1.434 ^{ns}
Residual		0.486	0.030	0.005	67.839	19.636	0.517
CV (%)		8.17	15.04	10.44	15.91	12.61	13.69
Floral induction	4	2.754 ^{ns}	0.617 ^{ns}	1.568 ^{ns}	4.127 ^{ns}	13.755**	21.939 ^{ns}
Residual		1.212	0.010	0.005	24.450	42.033	0.753
CV (%)		16.08	8.90	9.03	9.57	9.86	12.33
Full flowering	4	5.393*	1.688 ^{ns}	4.043 ^{ns}	6.344**	234.955**	45.246 ^{ns}
Residual		0.252	0.030	0.007	43.483	6.303	0.144
CV (%)		7.65	14.39	9.58	8.93	5.86	5.56
Initial fruiting	4	38.535**	9.889**	9.894**	0.714 ^{ns}	21.259**	6.099**
Residual		0.203	0.012	0.001	55.909	71.418	1.710
CV (%)		6.03	12.38	12.37	11.41	11.45	8.34

DF - Degrees of freedom; CV - Coefficient of variation; ns - Non significant; ** - Significant at 0.01 probability level; * - Significant at 0.05 probability level

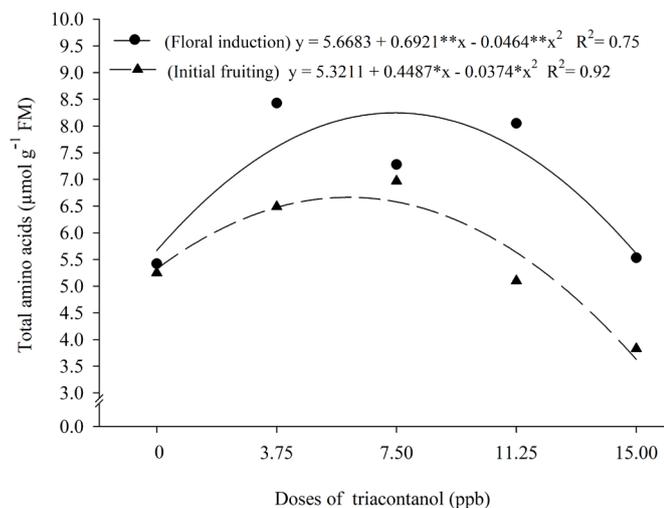
In relation to the total amino acid levels quantified in the 2018 crop year, the data were described by the quadratic model for the phases of floral induction and initial fruiting, and the maximum responses were 8.25 and 6.66 $\mu\text{mol g}^{-1}$ FM for the doses of 7.45 and 5.99 ppb of triacontanol, respectively (Figure 2). Therefore, increments of 52.21 and 26.88% higher than the value found in the control treatment could be observed.

In mango plants evaluated in the present study, the behavior observed in the increase of amino acid levels in the floral induction phase may have been influenced by the combination of the applications of triacontanol and potassium nitrate (KNO_3) during this phase; it should be pointed out that KNO_3 application is a natural management of mango cultivation in the region. Assimilation of KNO_3 induces the formation of the enzyme nitrate reductase (Anusuya et al., 2018). In summary, this enzyme acts in the production of total amino acids and other compounds such as methionine, a precursor of ethylene, and is considered the promoter of mango flowering (Sudha et al., 2012).

As for the total protein contents, linear polynomial fit was obtained for the branch maturation phase and quadratic fit was obtained for initial fruiting (Figure 3). In the maturation of branches, an increase of $0.0265 \text{ mg mL}^{-1}$ FM was observed as function of the doses of triacontanol applied, whereas for initial fruiting, the dose of 8.11 ppb of triacontanol per plant promoted maximum responses of 1.02 mg mL^{-1} FM (increase of 38.96%).

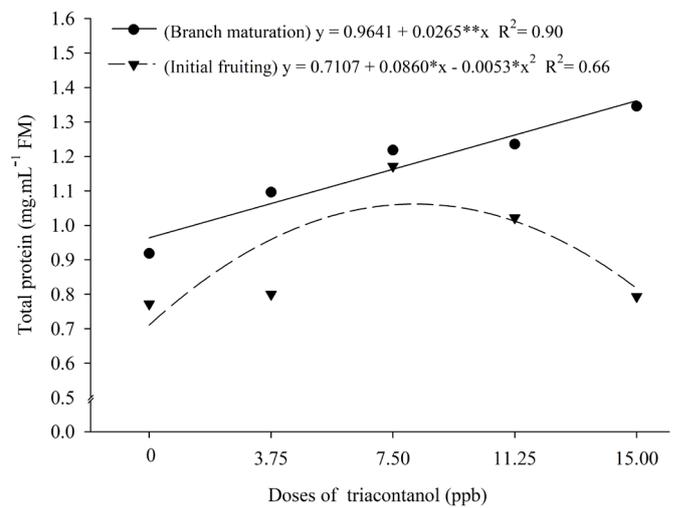
It is believed that the increase or stabilization of protein levels in plants under stress and treated with triacontanol occurs due to induction in the formation of L (+) - adenosine (acting as secondary messenger), capable of moving and transmitting signals throughout the plant, inducing the production of solutes such as proteins, even under the action of stressors (Khan et al., 2014).

As for the free proline levels in the 2018 crop year, the fit of the quadratic polynomial model revealed an increase in the levels of this amino acid in the phases of floral induction and initial fruiting (Figure 4A), with the doses of 6.30 and 0.53 ppb triacontanol, with responses of 0.29 and 0.20 $\mu\text{mol g}^{-1}$ FM,



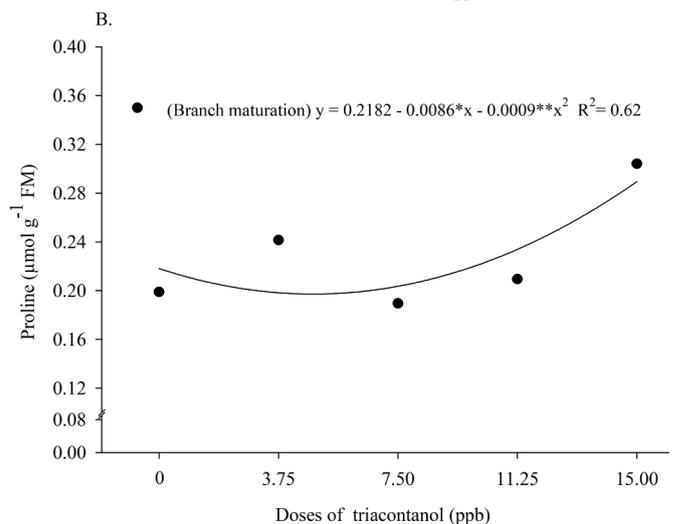
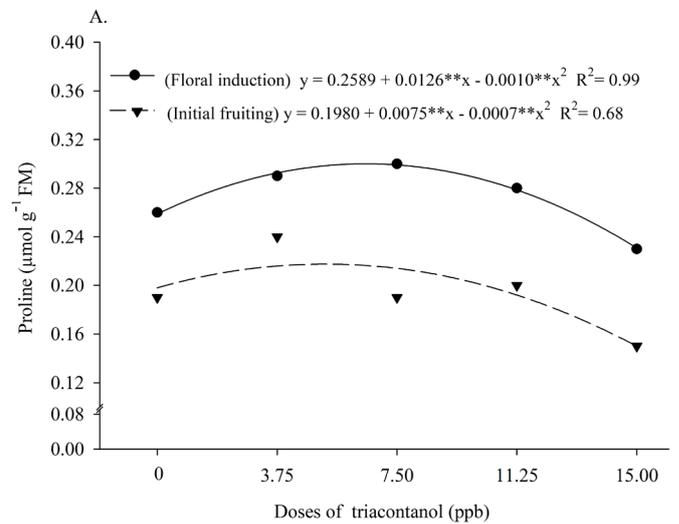
** - Significant at $p \leq 0.01$ by F test; * - Significant at $p \leq 0.05$ by t test

Figure 2. Total amino acid content in 'Kent' mango leaves as a function of the doses of triacontanol, in the crop year 2018



** - Significant at $p \leq 0.01$ by F test; * - Significant at $p \leq 0.05$ by t test

Figure 3. Total protein content in 'Kent' mango leaves as a function of triacontanol doses, in the crop year 2019



** - Significant at $p \leq 0.01$ by F test; * - Significant at $p \leq 0.05$ by t test

Figure 4. Free proline contents in 'Kent' mango leaves as a function of the doses of triacontanol, in the crop years 2018 (A) and 2019 (B)

obtaining percentage increments of 11.53 and 5.26% compared to the control treatment. For the 2019 crop year, the fit of the quadratic model was only for the branch maturation phase, and,

unlike what occurred in 2018, it was possible to observe that the highest dose was responsible for maintaining the high average values ($0.30 \mu\text{mol g}^{-1} \text{FM}$) observed for proline (Figure 4B).

The increase in free proline concentrations as a function of triacontanol doses in the floral induction phase (2018 crop year), when water stress management remained with only 25% availability of the irrigation depth, indicates that the exogenous application of triacontanol provided greater comfort for plants subjected to water deficit. The results corroborate those of Perveen et al. (2016), who reported that the exogenous application of triacontanol in maize cultivars under water stress increased the concentration of free proline.

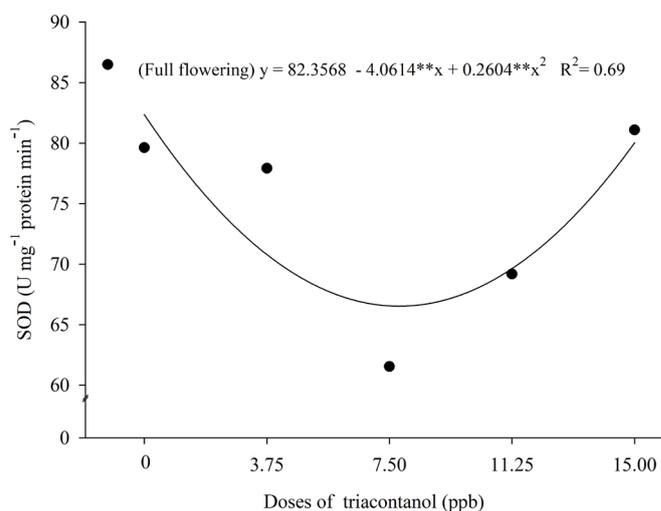
Positive effects of proteins in the branch maturation phase may be linked to the fact that the contents of free proline in the branch maturation phase were also increased (2019 crop year), and this made it possible to verify the mitigating effect in plants treated with triacontanol since proline is one of the main total amino acid constituents of proteins (Saikia et al., 2020).

Proline levels are maintained at sufficient levels to promote osmotic adjustment due to the rapid action of triacontanol in the tissues after its application. The decrease in proline levels related to the increase in doses (11.25 and 15.00 ppb of triacontanol per plant) in the initial fruiting phase may be a natural response since irrigation in this phase had already been normalized. This trend was also observed in maize by Perveen et al. (2016), who found that, upon rehydration, all plants reduced the concentration of proline because under ideal conditions of water availability the plants rapidly express genes responsible for the production of enzymes that degrade proline, such as Δ^1 -pyrroline-5-carboxylate dehydrogenase (P5CDH) and proline dehydrogenase (ProDH) (Yoshiba et al., 1997). Due to that, the reduction of proline in rehydrated plants may indicate that they have established normal levels of this amino acid, recovering from stress, regardless of the doses of triacontanol.

The responses of plants when grown under stress conditions have been monitored not only by the variation of molecules such as total amino acids and proteins, but also by the responses of enzymes related to the elimination of toxic compounds resulting from such conditions, especially SOD, APX, and CAT. The activity of the SOD enzyme was described by the quadratic polynomial model only for full flowering, showing a reduction caused by the introduction of triacontanol and consequently increase under the highest dose (Figure 5).

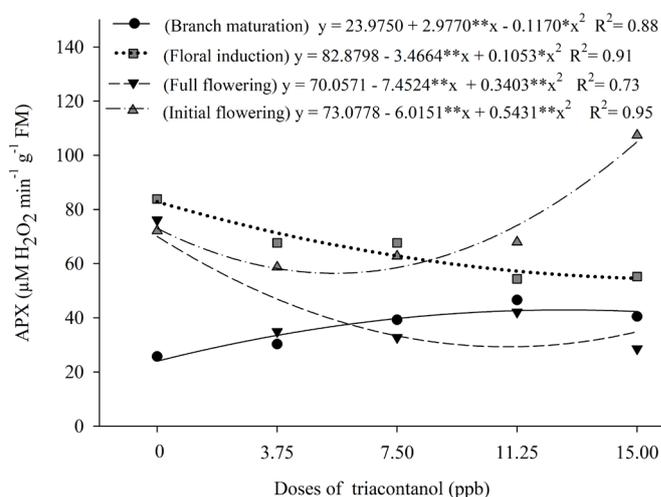
As for the activity of the APX enzyme, there was a satisfactory fit of the quadratic model for the phases of branch maturation, floral induction, full flowering, and initial fruiting (Figure 6), with an increasing effect on the enzymatic action as a function of the doses in the phase of branch maturation and subsequent reduction in the other phases, with only a significant increase at the dose of 15.00 ppb, when evaluated in the initial fruiting phase. However, the estimated dose that promoted an enhanced effect in the branch maturation phase corresponds to 12.72 ppb of triacontanol, resulting in $42.91 \mu\text{M H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{FM}$ in APX activity, being 67.11% higher than the value found in the control treatment.

It is evident that the exogenous application of triacontanol favored the increase in the activity of the APX enzyme,



** - Significant at $p \leq 0.01$ by t test

Figure 5. Activity of the enzyme superoxide dismutase (SOD) in 'Kent' mango leaves as a function of the doses of triacontanol, in the crop year 2019

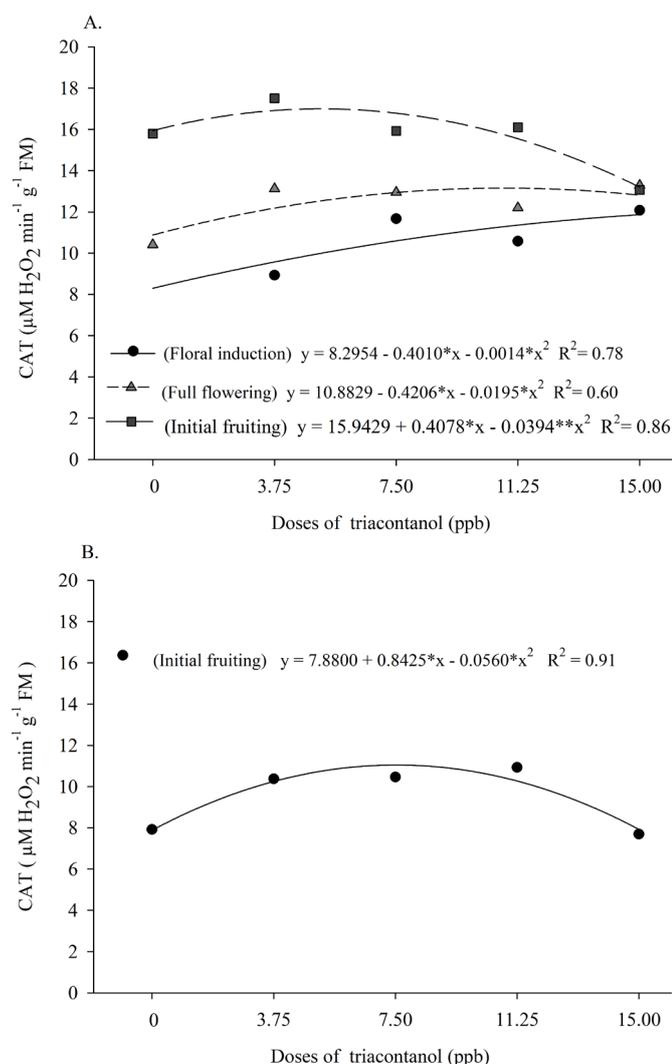


** - Significant at $p \leq 0.01$ by F test; * - Significant at $p \leq 0.05$ by t test

Figure 6. Activity of the enzyme ascorbate peroxidase (APX) in 'Kent' mango leaves as a function of the doses of triacontanol, in the crop year 2019

mitigating the oxidative stress resulting from water stress in the branch maturation phase. Furthermore, the reduction of APX in the other phases, when compared with the control treatment plants, demonstrates that the plants treated with triacontanol showed greater comfort since the irrigation depth had already been resumed. Positive modulations in APX activity were also observed in a study by Karam & Keramat (2017), who reported that foliar sprays of 10 and 20 mM of triacontanol proved to be effective in reducing the toxic effects of salt stress on *Coriandrum sativum* L. by modulating the activities of CAT, APX, SOD, and POD enzymes.

With regard to CAT activity for the 2018 crop year, a quadratic model was fitted for the phases of floral induction, full flowering, and initial fruiting (Figure 7A), with the doses of 14.32, 10.78, and 5.17 ppb of triacontanol, promoting maximum responses of 11.16, 13.15, and $16.99 \mu\text{M H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{FM}$, generating increases in CAT activity of 32.44, 26.22, and 6.25%, respectively. In the 2019 crop year, a quadratic model was



** - Significant at $p \leq 0.01$ by F test; * - Significant at $p \leq 0.05$ by t test

Figure 7. Catalase enzyme (CAT) activity in 'Kent' mango leaves as a function of the doses of triacontanol, in the crop years 2018 (A) and 2019 (B)

fitted for initial fruiting, with an enhanced effect depending on the treatments and a reduction at the 15.00 ppb dose of triacontanol (Figure 7B). The maximum estimated dose was 7.52 ppb of triacontanol, which led to a value of 11.05 µM H₂O₂ min⁻¹ g⁻¹ FM, increasing CAT activity by 39.70% compared to control treatment.

In this context, there are reports that triacontanol positively modulates the mechanisms involved in the increase of antioxidant enzymes, functioning as a good antioxidant mediator, besides inhibiting the breakdown of lipid peroxidation of non-enzymatic and enzymatic reactions (Ertani et al., 2013). The activation of the CAT enzyme verified in the initial fruiting phase (2018 crop year) comes from the thermal stress caused by the increase in temperature and decrease in the relative humidity of the air recorded in the month of August (Figure 1A), when the maximum temperature was 33.36 °C and RH dropped to 47.86%.

Similarly, it occurred in the 2019 crop year (Figure 1B), with a maximum temperature of 33 °C and RH of 49.52%. In addition, there was a relationship between CAT activity and APX activity (Figure 7) for the phase in question; for APX, dose

0 (control) and dose 15.00 (maximum) ppb of triacontanol per plant showed higher activity of this enzyme, while both treatments showed reductions in catalase activity. This behavior is also consistent with the other treatments, and they showed reductions in APX and consequently an increase in CAT.

Thus, it is known that thermal stress limits the efficiency of photosynthetic assimilation of carbon (carboxylation), because the progressive increase in temperature may have induced photorespiration (oxygenation) (Timm & Hagemann, 2020). In this case, the production of hydrogen peroxide (H₂O₂) in the peroxisome can easily oxidize and destroy other compounds, but catalase is the most abundant enzyme in the peroxisome, converting H₂O₂ into water and releasing oxygen (Al-Hajaya et al., 2022). Therefore, the exogenous application of triacontanol promoted a mitigating effect with increased catalase activity in 'Kent' mango at the doses mentioned above.

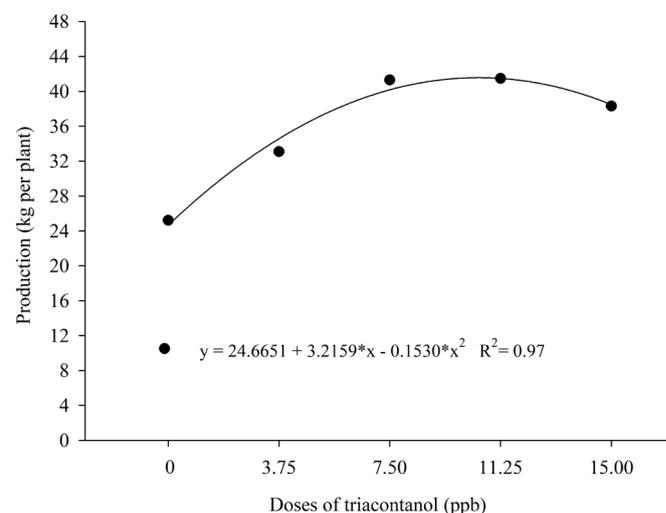
According to the summary of the analysis of variance (Table 2), triacontanol doses positively affected the production of 'Kent' mango only in the 2019 crop year.

Regarding the production of 'Kent' mango for the 2018 crop year, there was no satisfactory fit of the quadratic model, as shown by the equation: $20.5590 + 1.7149^{ns}x - 0.1108^{ns}x^2$, with $R^2 = 0.27$. However, for the 2019 crop year, according to the fit of the quadratic model, there was a higher fruit production in 'Kent' mango as a function of doses of triacontanol (Figure 8), and the estimated dose was 10.50 ppb of triacontanol per plant, corresponding to a production of 41.56 kg per plant, an increase of 64.95% compared to the control treatment. Thus,

Table 2. Summary of variance analysis of production (kg per plant) of 'Kent' mango a function of the doses of triacontanol

Source of variation	F value	
	Production (kg per plant)	
	2018 crop year	2019 crop year
Doses	14.75 ^{ns}	71.23 ^{**}
Average	24.07	35.98
DF	4	4
Residual	8.59	7.59
CV%	12.18	4.55

DF - Degrees of freedom; CV - Coefficient of variation; ns - Non significant; ** - Significant at 1% probability level ($p \leq 0.01$)



* - Significant at $p \leq 0.05$ by t test

Figure 8. Production of 'Kent' mango as a function of triacontanol doses, in the crop year 2019

triacontanol promoted 16.38 kg more mango per plant. This better performance in the 2019 crop year may be a consequence of the long-time application of the product, which in the 2019 crop year began already in the branch maturation phase.

The low production verified in the 2018 crop year (average of 22.8 kg per plant) may be linked to the age of the plants that were in their first production cycle. On the other hand, it is noteworthy that the number of fruits in the two harvests was similar (65 fruits per plant), but the production verified in the 2019 crop year was higher (Figure 8), indicating that the fruits were certainly heavier. In this regard, it is suggested that triacontanol influenced the weight of the fruits, a fact also already recorded in fruits of mango cv. Arka Neelachal Kesri (Dash et al., 2021).

In addition, the increments observed in this second crop, such as an increase in total amino acids, total proteins, free proline, and APX and CAT activity during phenophases, led to better conditioning for plants to keep their metabolic functions active, resulting in higher production in plants treated with triacontanol. These characteristics come from the mechanisms triggered in the physiological system of plants through the introduction of triacontanol, including improvements in their growth and physicochemical attributes, in addition to improving the quality, content of organic molecules, and yield of many crops (Islam et al., 2021).

Based on the results presented, the hypothesis of this study was proven, and triacontanol has the potential to be introduced in the production management of mango cultivated in the Brazilian semi-arid region. However, due to the variations presented, further research is needed on the use of triacontanol in mango crops to demonstrate its biosynthesis in plants and how it regulates plant metabolism and what transcription factors are involved in the metabolism.

CONCLUSIONS

The use of triacontanol increases the contents of total amino acids, free proline, total proteins, and activity of the enzymes superoxide dismutase, ascorbate peroxidase, and catalase, attenuating the effect of oxidative stress and increasing the production of 'Kent' mango fruits by 50.85% (dose of 11.25 ppb) and 64.95% (dose of 10.50 ppb) in the 2018 and 2019 crop year, respectively.

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LITERATURE CITED

Al-Hajaya, Y.; Karpinska, B.; Foyer, C. H.; Baker, A. Direcionamento nuclear e peroxissomal da catalase. *Plant, Cell & Environment*, v.45, p.1096-1108, 2022. <https://doi.org/10.1111/pce.14262>

- Alvares, C. A.; Stape, J. L.; Sentelhas, P. C.; Gonçalves, J. L. de M.; Sparovek, G. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, v.22, p.711-728, 2013. <https://doi.org/10.1127/0941-2948/2013/0507>
- Anusuya, R.; Vijayakumar, R. M.; Srividhya, S.; Sivakumar, R. Comparison of physiological and flowering parameters of main and off season by using different plant nutrients and growth hormone in mango (*Mangifera indica* L.) cv. Bangalora. *Journal of Agriculture and Ecology Research*, v.5, p.76-82, 2018. <http://doi.org/10.53911/JAE>
- Bates, L. S.; Waldren, R. P.; Teare, I. D. Rapid determination of free proline for water-stress studies. *Plant and Soil*, v.39, p.205-207, 1973. <https://doi.org/10.1007/BF00018060>
- Beers Jr., R. F.; Sizer, I. W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, v.195, p.133-140, 1952. [https://doi.org/10.1016/S0021-9258\(19\)50881-X](https://doi.org/10.1016/S0021-9258(19)50881-X)
- Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, v.72, p.248-254, 1976. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Carreiro, D. de A.; Amariz, R. A.; Sanches, L. G.; Lobo, J. T.; Paiva Neto, V. B. de; Cavalcante, Í. H. L. Gas exchanges and photosynthetic pigments of 'Tommy Atkins' mango as a function of fenpropimorph. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.26, p.239-247, 2022. <https://doi.org/10.1590/1807-1929/agriambi.v26n4p239-247>
- Cunha, J. G. da; Cavalcante, Í. H. L.; Silva, L. dos S.; Silva, M. A. da; Sousa, K. Â. O. de; Paiva Neto, V. B. de. Algal extract and proline promote physiological changes in mango trees during shoot maturation. *Revista Brasileira de Fruticultura*, v.44, p.1-13, 2022. <https://doi.org/10.1590/0100-29452022854>
- Dash, A.; Samant, D.; Dash, D. K.; Dash, S. N.; Mishra, K. N. Influence of *Ascophyllum nodosum* extract, homobrassinolide and triacontanol on fruit retention, yield and quality of mango. *Journal of Environmental Biology*, v.42, p.1085-1091, 2021. <https://doi.org/10.22438/jeb/42/4/MRN-1541>
- Ertani, A.; Schiavon, M.; Muscolo, A.; Nardi, S. Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. *Plant and Soil*, v.364, p.145-158, 2013. <https://doi.org/10.1007/s11104-012-1335-z>
- Filgueiras, H. A. C. Colheita e manuseio pós-colheita, In: Filgueiras, H. A. C.; Cunha, A. Frutas do Brasil: Manga pós-colheita. Fortaleza: Embrapa Agroindústria Tropical, 2000. p.22-25.
- Giannopolitis, C. N.; Ries, S. K. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, v.59, p.309-314, 1977. <https://doi.org/10.1104/pp.59.2.309>
- Hasanuzzaman, M.; Bhuyan, M. H. M. B.; Zulfiqar, F.; Raza, A.; Mohsin, S. M.; Mahmud, J. A.; Fujita, M.; Fotopoulos, V. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, v.9, p.1-52, 2020. <https://doi.org/10.3390/antiox9080681>
- Hou, P.; Wang, F.; Luo, B.; Li, A.; Wang, C.; Shabala, L.; Ahmed, H. A. I.; Deng S.; Zhang, H.; Song, P.; Zhang, Y.; Shabala, S.; Chen, L. Antioxidant enzymatic activity and osmotic adjustment as components of the drought tolerance mechanism in *Carex duriuscula*. *Plants*, v.10, p.1-20, 2021. <https://doi.org/10.3390/plants10030436>

- Islam, S.; Zaid, A.; Mohammad, F. Role of triacontanol in counteracting the ill effects of salinity in plants: a review. *Journal of Plant Growth Regulation*, v.40, p.1-10, 2021. <https://doi.org/10.1007/s00344-020-10064-w>
- Karam, A. E.; Keramat, B. Foliar spray of triacontanol improves growth by alleviating oxidative damage in coriander under salinity. *Indian Journal of Plant Physiology*, v.22, p.120-124, 2017. <https://doi.org/10.1007/s40502-017-0286-z>
- Khan, Z. H.; Mohammad, F.; Khan, M. M. A. Enhancing the growth, yield and production of essential oil and citral in lemongrass by the application of triacontanol. *International Journal of Agricultural Research*, v.4, p.113-122, 2014.
- Lopes, P. R. C.; Haji, F. N. P.; Moreira, A. N.; Mattos, M. A. de A. Normas técnicas e documentos de acompanhamento da produção integrada de manga. Petrolina: Embrapa Semi-Árido: 2003. 72p. Documentos 183
- Manjavachi, M. K. de P.; Silva, T. A.; Silva, E. A. A. da; Guimarães, C. C.; Sartori, M. M. P. Physiological and biochemical responses of osmo-primed parsley seeds subjected to saline stress. *Acta Scientiarum-Agronomy*, v.44, p.1-11, 2022. <https://doi.org/10.4025/actasciagron.v44i1.54364>
- Naeem, M.; Ansari, A. A.; Aftab, T.; Shabbir, A.; Alam, M. M.; Khan, M. M. A.; Uddin, M. Application of triacontanol modulates plant growth and physiological activities of *Catharanthus roseus* (L.). *International Journal of Botany Studies*, v.4, p.131-135, 2019.
- Nakano, Y.; Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*, v.22, p.867-880, 1981. <https://doi.org/10.1093/oxfordjournals.pcp.a076232>
- Perveen, S.; Iqbal, M.; Nawaz, A.; Parveen, A.; Mahmood, S. Induction of drought tolerance in *Zea mays* L. by foliar application of triacontanol. *Pakistan Journal of Botany*, v.48, p.907-915, 2016.
- Perveen, S.; Shahbaz, M.; Ashraf, M. Modulation in activities of antioxidant enzymes in salt stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol. *Pakistan Journal of Botany*, v.43, p.2463-2468, 2011.
- Ramírez, F.; Davenport, T. L. Mango (*Mangifera indica* L.) flowering physiology. *Scientia Horticulturae*, v.126, p.65-72, 2010. <https://doi.org/10.1016/j.scienta.2010.06.024>
- Saikia, B.; Singh, S.; Debbarma, J.; Velmurugan, N.; Dekaboruah, H.; Arunkumar, K. P.; Chikkaputtaiah, C. Multigene CRISPR/Cas9 genome editing of hybrid proline rich proteins (HyPRPs) for sustainable multi-stress tolerance in crops: the review of a promising approach. *Physiology and Molecular Biology of Plants*, v.26, p.857-869, 2020. <https://doi.org/10.1007/s12298-020-00782-6>
- Sudha, R.; Balamohan, T. N.; Soorianathasundaram, K. Effect of foliar spray of nitrogenous chemicals on flowering, fruit set and yield in mango (*Mangifera indica* L.) cv. Alphonso. *Journal of Horticultural Sciences*, v.7, p.190-193, 2012.
- Timm, S.; Hagemann, M. Photorespiration - how is it regulated and how does it regulate overall plant metabolism? *Journal of Experimental Botany*, v.71, p.3955-3965, 2020. <https://doi.org/10.1093/jxb/eraa183>
- Yemm, E. W.; Cocking, E. C.; Ricketts, R. E. The determination of amino-acids with ninhydrin. *Analyst*, v.80, p.209-214, 1955. <https://doi.org/10.1039/AN9558000209>
- Yoshida, Y.; Kiyosue, T.; Nakashima, K.; Yamaguchi-Shinozaki, K.; Shinozaki, K. Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiology*, v.38, p.1095-1102, 1997. <https://doi.org/10.1093/oxfordjournals.pcp.a029093>