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Impact of fulvic acid and free amino acids on paclobutrazol absorption by 'Keitt' mango

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ABSTRACT

This study evaluated the impact of fulvic acid and free amino acids on paclobutrazol soil residue, their absorption and effects on 'Keitt' mango grown in tropical semi-arid environmental conditions. The experiment was carried out from 2017 to 2018 simultaneously in two orchards with the same plants and management characteristics, located in Cabrobó, Pernambuco, Brazil. The experimental design was randomized blocks with four treatments, five replications and four plants per replication. The treatments consisted of paclobutrazol combinations with acid fulvic and free amino acids, as follows: Treatment 1: paclobutrazol + water (control); Treatment 2: paclobutrazol + fulvic acids; Treatment 3: paclobutrazol + free amino acids; and Treatment 4: paclobutrazol + fulvic acids + free amino acids. According to the results, the use of fulvic acids, free amino acids or both affects the paclobutrazol absorption by 'Keitt' mango. The addition of fulvic acid to the paclobutrazol improves the absorption of this molecule by the plant, with greater inhibition of vegetative growth of 'Keitt' mango and lower soil residues.

Keywords: *Mangifera indica* L., PBZ, soil contamination.

Impacto de ácido fúlvico e aminoácidos livres na absorção do paclobutrazol pela mangueira 'Keitt'

RESUMO

O objetivo deste estudo foi avaliar o impacto do ácido fúlvico e dos aminoácidos livres no resíduo de paclobutrazol no solo, absorção dessa molécula e seus efeitos sobre a mangueira 'Keitt' cultivada em condições do semiárido tropical. O experimento foi realizado de 2017 a 2018 simultaneamente em dois pomares com as mesmas plantas e características de manejo localizados em Cabrobó, Pernambuco, Brasil. O delineamento experimental foi em blocos ao acaso, com quatro tratamentos, cinco repetições e quatro plantas por repetição. Os tratamentos consistiram de combinações de paclobutrazol com ácido fúlvico e aminoácidos livres, sendo: Tratamento 1: paclobutrazol + água (controle); Tratamento 2: paclobutrazol + ácidos fúlvicos;

Tratamento 3: paclobutrazol + aminoácidos livres; e Tratamento 4: paclobutrazol + ácidos fúlvicos + aminoácidos livres. De acordo com os resultados, o uso de ácidos fúlvicos, aminoácidos livres ou ambos afetam a absorção de paclobutrazol pela manga 'Keitt'. A adição de ácido fúlvico ao paclobutrazol melhora a absorção desta molécula pela planta, com maior inibição do crescimento vegetativo da manga 'Keitt' e menores resíduos do solo.

Palavras-chave: contaminação do solo, *Mangifera indica* L., PBZ, substâncias húmicas.

1. INTRODUCTION

The São Francisco Valley, situated in the Brazilian semiarid, presents ideal climatic conditions for growing mangoes, but it must be associated with adequate management practices for soil fertility, irrigation, pruning and the use of plant-growth regulators (Mouco *et al.*, 2010; Santos *et al*., 2019). One important mango cultivar for São Francisco Valley is ‘Keitt’, which is grown for exportation and, according to Genú and Pinto (2002), is a very productive plant, with typical growth represented by open and arched branches and leaves facing the base of the branches, promoting an irregular canopy shape. Thus, for this mango cultivar, the use of paclobutrazol (PBZ) has been usual.

PBZ is a plant growth regulator commonly used in mango production systems in semi-arid conditions (Carneiro *et al.*, 2017; Oldoni *et al.*, 2018; Cavalcante *et al.*, 2018) to inhibit gibberellin biosynthesis and promote a better flowering uniformity (Srivastav *et al.*, 2010).

The mechanism for PBZ absorption presents difficulties, due to its low solubility in water, low soil mobility and for reversibly binding to the plant vascular system and to the soil (Lever, 1986). However, PBZ can interact with soil organic matter, due to the presence of ionic (hydrophilic) groups that have high charge densities (OH and triazole groups), and apolar (hydrophobic) groups related to the long carbon chains in their fractions (Milfont, 2008).

In the mango production system, PBZ is applied via soil dilution in water (soil drench) or directly applied in the canopy projection via the fertigation system, which is justified by the greater efficiency of the molecule applied in the soil instead of the foliar application (Mouco *et al.*, 2011). However, the continuous use of PBZ year after year can contaminate soil and water resources (Silva *et al.*, 2017), as well as negatively affect mango tree growth and development, excessively reducing branch growth and diameter (Cavalcante *et al*., 2020), and consequently the fruit production capacity of the plant, if the residue of previous applications are not considered (Vaz *et al.*, 2015).

Thus, the use of organic molecules that can increase PBZ absorption and reduce the residue that remains in the soil with its continuous use can improve PBZ absorption efficiency, reduce its dose and, consequently, cause less risks to the environment and to the mango industry. Among these organic sources, amino acids and their analogues could be used, since they have agricultural applications as complexing substances for higher efficiency of ion and molecule absorption, due to better membrane permeability (Castro and Carvalho, 2014).

Another organic source with potential use are humic substances (SHs) that induce the H + -ATPase activity that provides energy to the secondary ion carriers and promote nutrient absorption (Canellas *et al.*, 2015), activate ion metabolism and transport of different substances (Jannin *et al.*, 2012). They also stimulate protein synthesis, photosynthesis, enzymatic activity, macro- and micronutrient solubilization, microbial activity and promote hormone-like effects (Seyedbagheri, 2010). Fulvic acids, particularly, have a lower molecular mass, due to their higher content of hydrophilic components and they are considered the most bioactive fraction of the humic substances (Nardi *et al.*, 2007).

This study evaluated the impact of fulvic acid and free amino acids on paclobutrazol soil residue and their absorption and effects on ‘Keitt’ mango grown in tropical semi-arid environmental conditions.

2. MATERIAL AND METHODS

2.1. Characterization of the study area

Two and one half years-old mango (*Mangifera indica* L.) plants, of the Keitt cultivar, with uniform size and vigor in the first production cycle were used in this study. The experiment was accomplished from 2017 to 2018 in an experimental orchard located in Cabrobó (08°31' S and 39º26' W; at an altitude of 331 m above sea level), in Pernambuco, Brazil. The climate of this region is classified as Bswh (Köeppen), which corresponds to a semi-arid region. During the experiment, average air temperature and relative humidity ranged from 24.2ºC to 29.4ºC and from 57.8% and 79.2%, respectively, with accumulated precipitation of 463 mm year^{-1}.

The experiment was carried out simultaneously in two orchards with the same plant and management characteristics, in an experimental unit of 7.056 m^2. The physical and chemical characteristics of the both soil orchards are described in Table 1.

Table 1. Chemical and physical characteristics of the soil in the 'Keitt' mango orchards studied.

Orchard	Soil Layer	pH (in water)	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	SB	V
	M		mg.dm^{-3}			cmol$_c$.dm^{-3}					%
1	0.0 - 0.2	4.94	66.62	0.41	1.22	2.52	1.43	0.35	4.29	5.58	56.55
	0.2 - 0.4	5.33	31.22	0.31	1.13	2.91	1.91	0.25	3.05	6.26	67.21
2	0.0 - 0.2	5.37	70.62	0.36	0.78	3.20	1.65	0.08	3.05	5.99	66.25
	0.2 - 0.4	6.22	44.28	0.64	1.70	3.06	1.92	0.08	1.98	7.31	78.70

Orchard	Soil layer	CEC (pH7)	OM	FA	HA	HU	E.C.	Sand	Clay	Silt	Texture
	M	cmol$_c$.dm^{-3}		g.kg^{-1}			dS.m^{-1}			dag.kg^{-1}	
1	0.0 - 0.2	9.87	11.13	0.03	1.44	4.45	0.06	51.13	15.90	32.97	Loam
	0.2 - 0.4	9.31	10.68	1.01	1.30	4.02	0.07	48.32	18.10	33.58	Loam
2	0.0 - 0.2	9.04	13.13	0.07	1.61	4.95	0.15	48.94	14.10	36.96	Loam
	0.2 - 0.4	9.29	9.68	0.84	1.62	5.53	0.17	54.81	16.40	28.79	Sand Loam

SB = sum of bases (Ca^{2+} + Mg^{2+} + Na^+ + K^+); OM = Organic matter; CEC = cationic exchangeable capacity [Ca^{2+} + Mg^{2+} + Na^+ + K^+ + (H^+ + Al^{3+})]; E.C. = electrical conductivity; P, K: Melich-1; H + Al: calcium acetate (extractor) 0.5M, pH 7; Al, Ca, Mg: KCl 1 M extractor.

For chemical characterization, the values of electrical conductivity in the saturation paste extract (E.C.), pH (H_2O), potential acidity (H + Al), exchangeable acidity (Al^{3+}) and total organic matter (OM), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and phosphorus (P) were determined, according to the methodology proposed by Silva (2009). The fractionation of humic substances was carried out according to the method suggested by the International Humic Substances Society (Swift, 1996). From this fractionation, the fractions of fulvic acids (FA), humic acids (HA) and humines (HU) were obtained, based on the solubility in acidic or alkaline solutions. Physical analyses were done by granulometry, determined by the pipette method (Donagema *et al.*, 2011).

The plants, spaced with 4.0 m between the rows and 2.0 m between the plants, were drip-irrigated daily, with four emitters per plant, for a flow of nearly 2 L h^{-1} for each emitter. All management practices such as pruning, control of weeds, pests and diseases, plant growth regulators for gibberellin inhibition (paclobutrazol, Cultar®) and dormancy break (calcium nitrate and potassium nitrate) were performed following the instructions of Genú and Pinto (2002). Nutrient management was performed through a fertirrigation system, according to plant demand (Genú and Pinto, 2002). An additional pruning was performed at 27 days after paclobutrazol treatment to keep each plant with nearly 100 shoots, and tip pruning was performed to synchronize vegetative flush events in the canopy.

2.2. Experimental design and application of treatments

The experiment followed a "randomized blocks" design with four treatments, five replications per treatment and four plants per replication. The treatments consisted of paclobutrazol (PBZ) combinations with acid fulvic and free amino acids, as follows: T1: PBZ + water (control); T2: PBZ + fulvic acids; T3: PBZ + free amino acids; and T4: PBZ + fulvic acids + free amino acids. The control treatment consisted of PBZ application, because under tropical semi-arid conditions the mango production system becomes economically viable by using this molecule (Genú and Pinto, 2002).

The PBZ source used was Cultar SC® (25% i.a. paclobutrazol). Treatments were applied through the fertigation system once at 30 days after production pruning, when plants presented a 2.0m canopy diameter. The sources of the acid fulvic and free amino acids used were, respectively: 16 ml per plant of Aminoagro Mol® (10% of N, 1% of K_2O and 8% of total organic carbon) and 16 ml per plant of Aminoplus® (11% of N, 1% of K_2O, 6% of total organic carbon, and glutamate). The treatments were applied following the recommendations of Genú and Pinto (2002) for mango tree and the product manufacturers.

2.3. Analyzed variables

PBZ analyses were performed in roots and in the last vegetative flush. Before the application of PBZ + organic acids (December 20, 2017), an orchard-soil characterization (OC) was carried out, and on later dates seven other evaluations were done, with plant material collected between 10:00 am and 11:00 am to standardize the time and minimize any potential climate effect.

The roots up to 4 mm in diameter were collected up to 0.3 m deep and 0.25 m from the mango trunk, in the projection of the irrigation system, which consisted of two drip lines (one on each side of the plant) parallel to the plant rows, with 0.5 m spacing between the drippers, totaling four plants. The last fully expanded vegetative flush was collected from the middle third of the four quadrants of the canopy, avoiding necrotic areas due to pest and disease attack.

The plant material was conducted to the laboratory, where it was washed, air dried (shade conditions) and milled in a Willey-type Knife Mill with 0.5 mm sieves. The soil PBZ residue was evaluated 272 days after PBZ application (at harvest). A soil sample was collected for each replicate of the treatments, from 0 to 0.4 m depth, transported to the laboratory, air dried (shade conditions) and then sifted with a 2.0 mm-mesh sieve.

The residual PBZ analysis was based on the extraction methodology of QuEChERS and determination by high performance liquid chromatography (HPLC) confirmed by Silva *et al.* (2017), with adaptations.

An estimation of PBZ in all plant vegetative flushes was also performed through the concentration found in the last vegetative flush of each treatment and at each evaluation date, multiplying by the total number of shoots per plant.

The shoot length (cm) and diameter (mm) were measured every 15 days, from the orchard characterization (OC) (December 13, 2017) until 75 days after the beginning of the experiment (floral induction), reaching six dates. Ten shoots were marked per plant, distributed by the four quadrants in the median portion of the canopy.

2.4. Statistical analysis

The data obtained of shoot length and diameter was subjected to the analysis of variance (ANOVA). All statistical analyses were performed using the SISVAR and SIGMAPLOT, and averages were compared by the Tukey test at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Paclobutrazol residue in the mango tree

In Figure 1 are shown the average results of the PBZ residue in roots and in the last vegetative flush of the 'Keitt' mango in different phenological phases in the two orchards studied (Orchard 1 and Orchard 2).

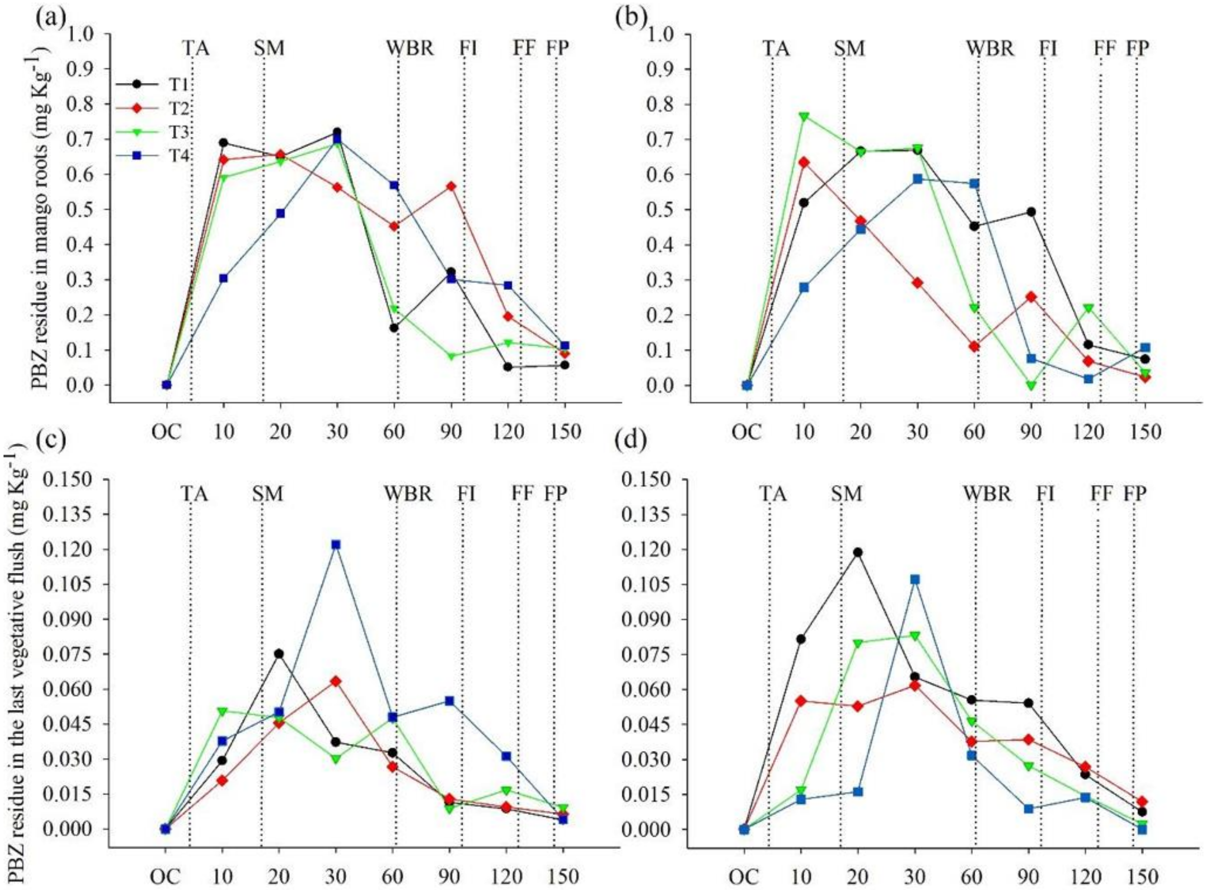

Figure 1. PBZ residue in mango roots [orchard 1 (a); orchard 2 (b)] and in the last vegetative flush [orchard 1 (c); orchard 2 (d)] of mango 'Keitt' as a function of fulvic acids and free amino acids.T1: PBZ + water (control); T2: PBZ + fulvic acids; T3: PBZ + free amino acids; and T4: PBZ + fulvic acids + free amino acids. OC: orchard characterization (12/09/2017); TA: treatment application; SM: shoot maturation; WBR: water blade reduction; FI: flowering induction; FF: full flowering; FP: fruit size – 'pellet'.

The PBZ uptake was quite rapid for treatments T1, T2 and T3, because at 10 days after PBZ application (DAP) all were above 0.50 mg kg^{-1}, whereas T4 contained less than 0,31 mg kg^{-1} in Orchards 1 and 2, respectively (Fig. 1 a-b). At 30 DAP the PBZ levels of T1, T3 and T4 remained high and T2 declined.

PBZ in T2 was more rapidly translocated to the plant aerial part, while the other treatments (T1 and T3) continued practically with the same root PBZ amount, and the T4 continued to be absorbed slowly because fulvic acids (FA) promoted a greater mobility to PBZ molecules in the soil, mainly due to their large specific surface area, high density of loads and smaller size (Canellas *et al.*, 2015).

The negative charges of the humic substances react with the organic compounds containing N, in this case with the triazole (PBZ), forming a complex (Sposito, 2008), resulting in less load resistance in comparison to the non-complexed molecule.

It is also necessary to emphasize that the PBZ residue amount in roots is much higher than the concentrations in the last vegetative flush, and even with time elapsing this difference persists.

Despite the low mobility of this molecule, the highest PBZ absorption level occurred shortly after its application, since it was directed to the most active root zones through fertigation. However, from BWR to FI, root PBZ increased for T1 and T2 in both orchards (Figure 1 a-b), which may have been caused by the water potential reduction caused by the accumulation of solutes near the root zones (Taiz *et al.*, 2017). However, T3 and T4, both with amino acids, did not maintain PBZ available for the plant until 90 DAP, proving to be less effective with time elapsing.

PBZ receives oxygen from the cytochrome P450, avoiding the *ent*-caurene oxygenation (Srivastav *et al.*, 2010). There must be a ratio between the amount of *ent*-caurenoic acid that is not formed and the number of PBZ molecules that are effectively used in inhibiting gibberellin formation.

After the water blade reduction (WBR), the levels of PBZ in the last vegetative flush began to decline for all treatments until the 'pellet' fruit size (FP) phase, with values below 0.02 mg kg^{-1}. Higher values were reported by Bhattacherjee and Singh (2015) in mango leaves cv. Dashehari (0.8 and 1.6 g i.a. $plant^{-1}$), which promoted 0.32 and 0.56 mg kg^{-1} at 120 DAP. These authors performed fourteen readings from 0 to 360 DAP and on seven reading dates (almost totally intercalated), PBZ was not detected in leaves, and the authors attributed this result to the molecule biodegradation in leaves or to the reduction of PBZ hydraulic conductivity by leaves.

Figure 2 contains the PBZ estimation in all last vegetative flushes of the plants. A very similar tendency to that recorded for the last vegetative flush (Figure 1 c-d) can be observed, because the plants were quite uniform, having approximately 100 productive shoots per plant.

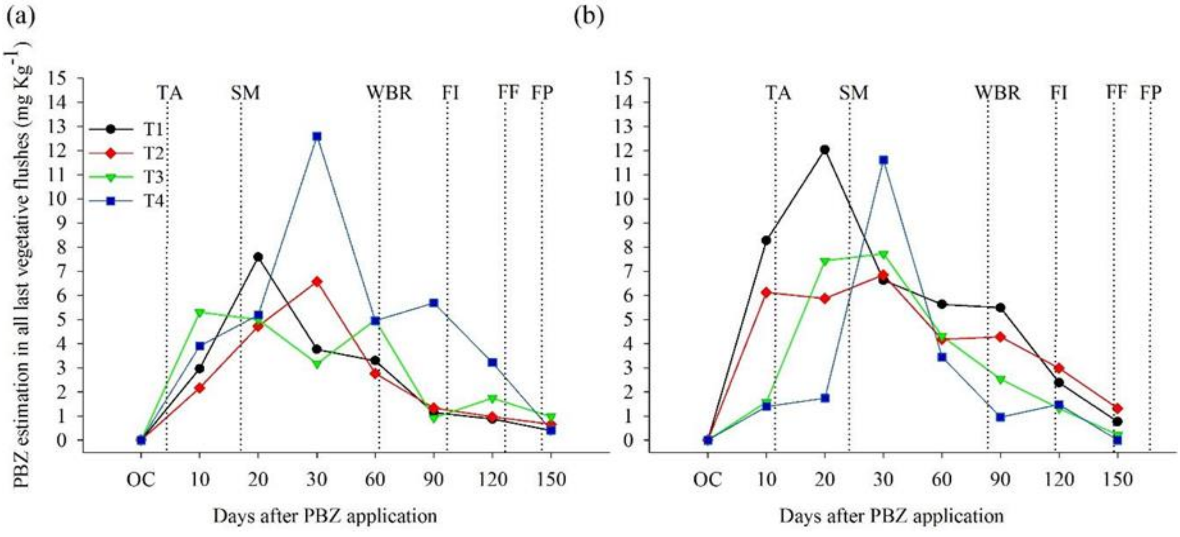

Figure 2. PBZ estimation in all last vegetative flushes [orchard 1 (a); orchard 2 (b)] of 'Keitt' mango as a function of fulvic acids and free amino acids.
T1: PBZ + water (control); T2: PBZ + fulvic acids; T3: PBZ + free amino acids; and T4: PBZ + fulvic acids + free amino acids. OC: orchard characterization (12/09/2017); TA: treatment application; SM: shoot maturation; WBR: water-blade reduction; FI: flowering induction; FF: full flowering; FP: fruit size – 'pellet'.

In spite of the notorious effects of FA on T2, treatment T4, which also has FA, presented less efficient results, such as slow root absorption (Figure 1 a-b) and the late peak of PBZ at 30 DAP (Figure 2 a-b), probably because the FA can react with the amino acids (containing two protonatable NH_2 groups) by cation exchange and form some complex between them (Baldotto and Baldotto, 2014), resulting in a smaller amount of complexes with PBZ, delaying its soil transport, and consequently its absorption, due to the competition for absorption of other molecules.

The best PBZ absorption was promoted by the use of fulvic acid (FA) individually (T2) that can be explained by the hydrophobic and hydrophilic interactions between PBZ and organic matter (OM), which increases PBZ adsorption (Milfont *et al.*, 2008). Thus, a greater amount of PBZ remains available to the plant for longer, because it is translocated along with the OM, mainly by FA, since these have lower molecular mass and thus greater ease of movement in the soil, near the root zones and inside the plant (Nardi *et al.*, 2007).

3.2. Paclobutrazol residue in soil

At the end of the experiment, there was a remarkable difference for soil PBZ residue, emphasizing that T2 promoted the lowest soil PBZ residue amount (Figure 3). This result indicates that T2 promoted an efficient PBZ use and it reduces environmental impacts.

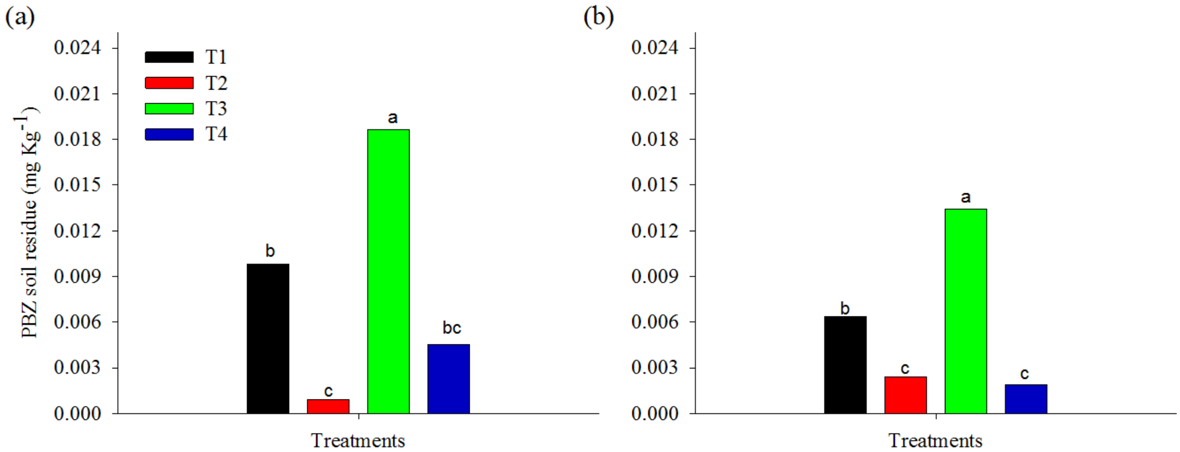

Figure 3. PBZ soil residue [orchard 1 (a); orchard 2 (b)] in orchards of 'Keitt' mango as a function of fulvic acids and free amino acids, at 272 after treatments.
T1: PBZ + water (control); T2: PBZ + fulvic acids; T3: PBZ + free amino acids; and T4: PBZ + fulvic acids + free amino acids. Bars with the same letters do not differ among themselves by Tukey's test at 5% probability error.

With the initial increase and final reduction of PBZ levels in the plant, it is possible to observe that the PBZ was gradually used during mango phenological phases, then the fastest decrease in T2 shows that it was used more rapidly during the shoot maturation phase in both orchards (Figure 2), which is quite interesting for the crop, due to the need to accumulate carbohydrates as a function of PBZ use (Prasad *et al.*, 2014).

Another possibility is the effect of humic substances on the stimulation of auxin synthesis, responsible for cell expansion and stretching (Trevisan *et al.*, 2010). The expansion is related to the synthesis of H^+*-ATPase* pumping protons to form ATP, causing a variation in the electrochemical potential, reducing the apoplast pH, making the cell wall more flexible, thereby facilitating root growth (Zandonadi *et al.*, 2010), mainly lateral roots, which are more important for water and nutrient absorption (Caron *et al.*, 2015). This may explain the lower soil PBZ residue promoted by the treatments with FA.

The PBZ remains active in the soil for a long period, but it is affected by the soil type and climatic conditions (Costa *et al.*, 2012).

The soil PBZ residues of the present study were below those found by Bhattacherjee and Singh (2015), where 300 days after the application of 0.8 and 1.6 g a.i. PBZ plant^{-1} detected 0.01 and 0.03 mg kg^{-1}, respectively, and residue in leaves was detected after 60 days (30 d interval measurements), showing that it was translocated more slowly to the shoots than have shown the results of the present study.

It is emphasized that Bhattacherjee and Singh (2015) applied the PBZ in a soil drench in a radial diameter of 1 m from the tree trunk (conventional application), which is significantly less effective than the fertigation used in the present study (Souza *et al.*, 2018). Silva *et al.* (2017) evaluated soil PBZ concentrations using the same method of the present study ('QuEChERS' and determination by HPLC) and found average values varying from 0.14 to 6.86 mg kg^{-1} in soils with different mango cultivars, finding that 'Keitt' orchards presented concentrations from 1.49 to 0.18 mg kg^{-1} at 150 DAP, which are higher values than the present study (Figure 3).

3.3. Shoot vegetative growth of the mango tree

In relation to the shoot vegetative growth, the PBZ action was very fast, since it presented a significant effect at 15 DAP in both orchards, reducing the shoot length of all treatments (Figure 4 a-b). However, there were significant differences between T2 and T3 in Orchard 1 from day 30 onwards, with T2 having the lowest values (Figure 4 a-b).

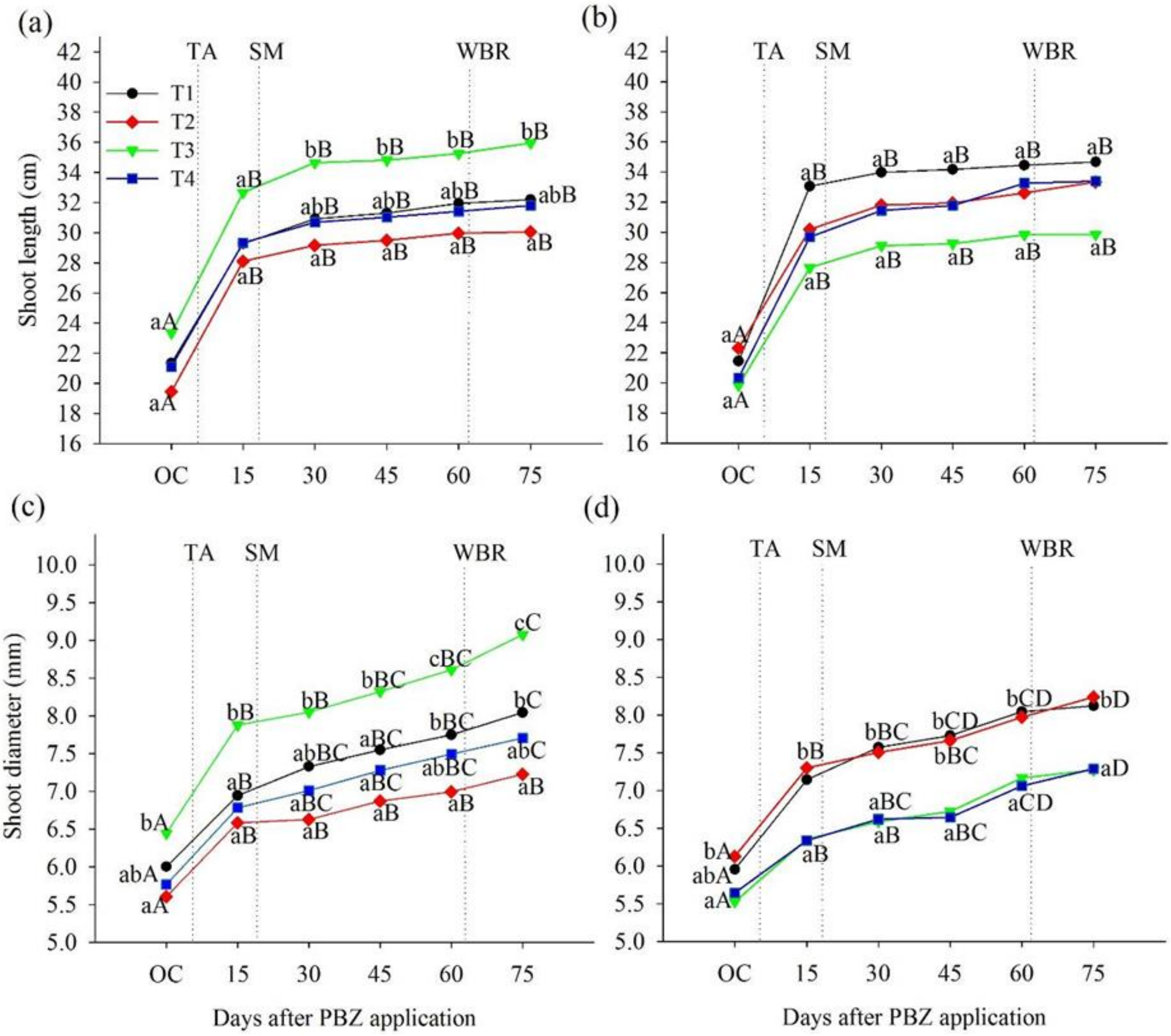

Figure 4. Shoot length [orchard 1 (a); orchard 2 (b)] and shoot diameter [orchard 1 (c); orchard 2 (d)] of 'Keitt' mango as a function of fulvic acids and free amino acids. T1: PBZ + water (control); T2: PBZ + fulvic acids; T3: PBZ + free amino acids; and T4: PBZ + fulvic acids + free amino acids. OC: orchard characterization (12/09/2017); TA: treatment application; SM: shoot maturation; WBR: water blade reduction.

Mouco *et al.* (2011) registered shoots 4.98 cm shorter as a function of PBZ use (4.0 g a. i. plant^{-1}) when compared to the control in 'Kent' mango cultivar. Meena *et al.* (2014) states that the shorter shoot length recorded in PBZ treated plants occurs due to gibberellin suppression, since they act in the cell elongation, and in these conditions, although cell division continues to occur, the new cells are not elongated, resulting in shortened internodal lengths.

For the diameter of the last vegetative flush, in Orchard 1, only T3 was different from the control, although this has already been observed even before PBZ application (AC) (Figure 4 c-d). In Orchard 2, T3 and T4 were lower than T1, and similar to each other. In relation to dates, shoot growth was slow but progressive for all treatments in both orchards.

It has been reported that the PBZ does not act or has little influence on the secondary growth responsible for the increase of plant diameter, due to the activity of the vascular exchange and the felogen (Meena *et al.*, 2014; Hegde *et al.*, 2018). However, this is a pioneer assessment, and perhaps the concentration of the sources used was not enough to express greater efficiency on this variable.

4. CONCLUSION

The use of fulvic acids, free amino acids or both affects PBZ absorption by the 'Keitt' mango.

The addition of fulvic acid to the PBZ improves the absorption of this molecule by the plant, with greater inhibition of vegetative growth of the 'Keitt' mango.

At the end of the productive cycle, there is a lower soil PBZ residue when it is applied together with fulvic acid, and therefore this treatment can be recommended for mango crop management in semi-arid conditions.

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