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RESEARCH ARTICLE

MEPIQUAT CHLORIDE IN RED SUNFLOWER CULTIVATION AS FLOWER VASE

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ABSTRACT

The objective was to evaluate the effect of mepiquat chloride in red sunflower cultivation as flower vase. A completely randomized design was adopted with ten repetitions and five concentrations of mepiquat chloride (250; 500; 750; 1000 and 1250 mg L^{-1}), was used as a control sprayed plants with only distilled water. The applications were performed at 15; 30 and 45 days after sowing. We evaluated the plant height parameters (PG), stem diameter (SD), internal diameter of the main inflorescence(IDMI), number of leaves (NL) and inflorescences (NI), dry mass of leaves (DML), mass dry stem (MDS), dry mass of inflorescence(DMI) and root dry weight (RDW), leaf area (LA), contents of chlorophyll a, b and carotenoid. Increasing the concentration of mepiquat chloride reduced the PG, IDMC, MDS, DMC, RDW and LA, but increased the SD values, DML, chlorophyll a, b and carotenoids. The NL and MSF did not significantly change the application of growth reducer. The application of mepiquat chloride increased the number of inflorescences per plant and reduced height sunflower cv. Sol Vermelho, but these reductions were not enough to obtain plants suitable for marketing in pots.

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INTRODUCTION

The sunflower (*Helianthus annuus* L.) is a native plant from North America, belonging to Asteraceae botanic family, which shows a vegetal behavior known heliotropism (Embrapa, 2002). Beyond its recognized importance in the production of grains, in recente years this specie has been highlighted as an ornamental plant, cultivated in vase and cutting flower (Schoellhorn*et al*., 2003), mainly in the south-central region of Brazil (Maringoni *et al*., 2001). In the early 90s, Japan produced the first F1 sunflower hybrid which did not emit polen. This fact has become the sunflowers more attractive for cultivation in vase or as cutting flower, because the fall of polen is an undesirable feature commom in other cultivars (Schoellhorn *et al*., 2003; Sloan & Harkness, 2006). Furthermore, currently there are cultivars with a variety of tones and shapes (Schoellhorn *et al*., 2003). The high size of the cultivars developed by Brazilian seed companies has been an obstacle for further exploration of sunflower as an ornamental plant, since there is disproportion to the vase size (Wanderley *et al*., 2011).

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In the case of ornamental sunflower, the productivity should be evaluated based on plant height and quality of inflorescences, i.e, the beautiful inflorescences must be associated by plants with adequate heights for the vases (Neves *et al*., 2005). Commercially, the sunflower shows the advantage of its short cycle (about 50 days) and easy propagation by seeds. On the other hand, the plant has large size, which makes it difficult for ornamental purposes (Wanderley *et al*., 2014). An instant strategy to control the size of plants is the use of growth regulators, which are typically synthetic products that inhibit gibberellin synthesis, such as chlormequat, paclobutrazol, daminozide, mepiquat chloride, among others, which have been used in ornamental plants for the control of vigor and height. The mepiquat chloride is mostly absorbed by the green parts of the plant and can be included in the group of gibberellic acid biosynthesis inhibitor, which makes it an inhibitor of cell elongation (Lamas, 2001). It belongs to the chemical group Quaternary Ammonia, which have the mode of action characterized by the inhibition of branches growth (Wanderley *et al*., 2011). In cotton plants, mepiquat chloride helps the culture establishment, since its potential benefits are: reduce the vegetative growth, improve plant architecture, earlier fruit opening, improving efficiency of mechanical harvest and higher qualities of the final products (Barbosa & Castro, 1983; Reddy *et al*., 1995).

The effects of mepiquat chloride on the overgrowth of plants is associated to the reduction stem extension, fewer nodes, shorter branches and decreased leaf area (Fernandez *et al*., 1991). Regarding to the dose to be used, according Wallace *et al.* (1993), its subdivision has more marked effect compared to the single dose application on plant height, number of nodes and length of internodes. The definition of an appropriate dosage to be applied is one of the main difficulties in growth regulators recommendation (Athayde & Lamas, 1999), since the results expected using the recommended doses are not always achieved. Assessing the size reduction of two sunflower cultivars, Wanderley *et al*. (2014) observed that the dose 2mgL^{-1} of paclobutrazol reduces the sunflower height, but doesn't affect the quality of the inflorescences. This aspect can provide the opportunity of its use in the production of flowers of sunflower as ornamentals, being that the sensitivity paclobutrazol is higher to He lio 358 genotype compared to BRS Oasis. The objective of this work was to evaluate the effect of mepiquat chloride in red sunflower cultivation as flower de vase.

MATERIALS AND METHODOS

The work was conducted in a greenhouse located at 23º23' Latitude South and 51º11' Longitude West, at an altitude of 566m above the sea level. There were used in the experiment seeds of the specie *Helianthus annuus* cv. red sun. This cultivar is characterized by the presence of red flowers with dark center, the flowers shows an internal diameter between 15 and 18 cm (main flower) and between 10 and 12 cm (secondary flowers), it reaches a height between 2 and 3 meters and it's average life cycle is 70 days. The seeds were sown in black polystyrene trays with 144 divisions with internal capacity of 17 cm³ per cell, which contained vegetal soil substrate, where we verified the seedling emission four days after sowing (DAS). For planting the seedlings there were used hard pots of polyethylene in black, with drains at the bottom end, which measured 15 cm height, 17 cm diameter, 14 cm in bottom diameter and 3 L of capacity. After fill the vases with vegetal soil substrate, the sunflower seedlings were transplanted at 12 DAS. At this time, we carried out a fertilization with 10 g of slow-release fertilizer (N-P-K formula: 15-09-12). During the experiment the plants were watered manually using a hose, once a day until the total saturation of the substrates.

Treatments consisted of five concentrations of the regulator mepiquat chloride (0.25; 0.5; 0.75; 1.0 e 1.25 g L^{-1}), and as control plants were used sprayed with distilled water only. A completely randomized design with ten repetitions was adopted, and the experimental unit consisted of one pot, which contained only one plant. For treatments applications, we used a hand sprayer with 500 ml of capacity to spray uniformly the regulator, aiming to give a complete coverage of the leaves and stems. The concentrations tested were applied at 15; 30 and 45 DAS, always at 18 hours. At the first application, the plants showed two pairs of final leaves and 1.70 cm of average height. At the end of the experiment, the plants were evaluated to characterize them as to their growth. At 60 DAS we carried the evaluation of growth parameters: plant height (PH), defined as the distance from the neck to the inflorescence insertion point; stem diameter (SD), measured in the region above the substrate surface and below the intersection of the 1st inter node by using a digital caliper with precision of 0.01 mm; internal diameter of the main inflorescence (IDMI), measured with the same digital caliper. It was also counted the number of leaves (NL) and inflorescences (NI) per plant, being considered inflorescence the showing ligules open (regardless of its size).
After these evaluations, the plants were cut next to the substrate and separated into leaves, stems, chapters and roots (washed for complete removal of the substrate). To obtain the dry mass of leaves (DML), stems (DMS), inflorescence (DMI) and roots (DMR), the materials were placed in paper bags, properly identified, and dry in an oven with forced air circulation, at 70°C until constant mass. After this period, the samples were weighed using an analytical balance.

The leaf area (LA) was evaluated with a bench leaf area meter (Licor® LI-3100) where the leaves were placed on the equipment conveyor for carrying out readings. It was also evaluated the levels of chlorophyll a, b and carotenoids, by using 0.2 g of fresh leaf tissue meshed in liquid N and then placed in tubes with covers containing 10 ml of 100% acetone (v /v), following the methodology described by Meschede *et al*. (2011).The extracts were filtered and the readings were performed in a spectrophotometer at wavelengths of 663, 645 and 434 nm for chlorophyll a, b, and carotenoids, respectively. The measurements of chlorophyll levels (mg gmf^{-1}) were based on the following equations performed byWhitham *et al*. (1971): Chlorophyll a = (11.24 x A663 - 2.04 x A645), Chlorophyll b = (20.13 x A645 - 4.19 x A663) and Carotenoids = (1000 x A434 – 1.90Clorofila a - 63.14 Chlorophyll b)/214, where A is the absorbance at the wavelength indicated. The data were submitted to analysis of variance, using the ASISTAT® statistical software. The significant data were subjected to regression analysis at the level of 5% probability.

RESULTS AND DISCUSSION

The variables number of leaves and dry mass of leaves were similar (P>0.05) between the contrasting concentrations of mepiquat chloride applied in the plants. The increase in the concentration of mepiquat chloride reduced the height of the sunflower plants at 7.6; 12.7; 17.5; 25.8 and 29.2% (Figure 2) at concentrations of 250; 500; 750; 1000 and 1250mg L^{-1}, respectively, when compared to control plants (Figure 1).

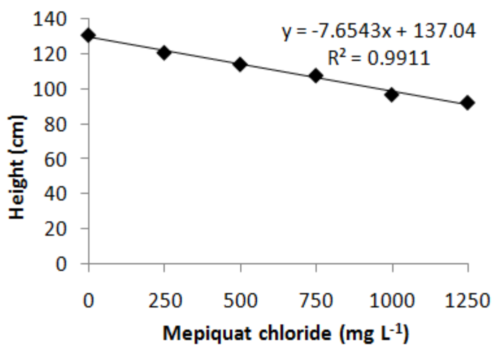

Figure 1. Height (cm) of sunflower plantscv. Sol Vermelho submitted to application of contrasting concentrations of mepiquat chloride

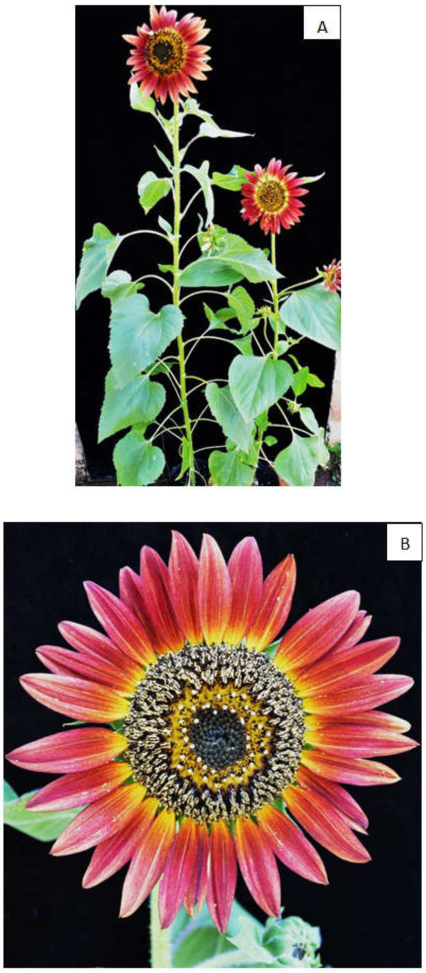

Figure 2. A) Comparation between the control plant (in the left) and the plant with 1250mg $L^{-1}$of mepiquatchloeide (in the right) and B) Detailofthe inflorescenceof sunflower cv. Sol Vermelho

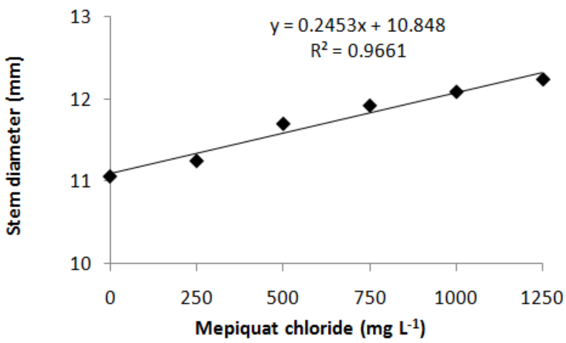

Figure 3. Stem diameter (mm) of sunflower plants cv. Sol Vermelhoin response to contrasting concentrations of mepiquat chloride

Similar results was obtaied by Coutinho *et al.* (2014), who observed reduction of the size of ornamental sunflower (*Helianthus annuus* cv. Dwarf Sunbright kids) with increasing concentration of the daminozide growth regulator. This reduction in plant height is related to the hormone balance caused by application of mepiquat chloride, which interferes in the gibberellic acid biosynthesis by inhibiting the formation of the plant hormone, which results in reduced growth and physiological changes (Reddy *et al.*, 1995; Marur, 1998).

The reduction in gibberellin biosynthesis may also promote decreasing in plant metabolism (Marshall *et al.* 2000; Fletcher *et al.* 2000), i.e., the respiration rate becomes lower, which reduces the available ATP and consequently the plant growth(Bai& Chaney 2001). Although the application of 1250mg $L^{-1}$of mepiquat chloride reduced the plant height to 92cm (reduction of approximately 30%), this is still not considered an adequate height for marketing sunflower in vase, since the target plant height for this sector is around 35 cm (Wanderley *et al.*, 2014). The stem diameter was positively correlated with the growth regulator concentration, which demonstrated increases of 1.69; 5.74; 7.73; 9.24 and 10.59% at the concentrations 250; 500; 750; 1000 and 1250mg L^{-1}, respectively (Figure 3). According Taiz & Zeiger (2013) the lowest plant gibberellin synthesis reduces the cell elongation (growth), resulting in internodes thickening, but without morphological deformation of the stem. Alternatively, Coutinho *et al.* (2014) verified significant reduction in stem diameter due the application of daminozide, with losses of 0.15 mm each 1 g $L^{-1}$of the product applied. The stems diameters reduced 3.5; 6.86; 10.29 and 13.73% at concentrations 2; 4; 6 and 8 g L^{-1}, respectively.

The internal diameter of sunflower chapter showed a negative correlation with the concentration of the plant growth regulator applied, by reducing 1.0; 2.1; 2.7; 3.1 and 3.4% at concentrations of 250; 500; 750; 1000 and 1250mgL^{-1}, respectively, compared to control plants (Figure 4).Similar results were found by Pallez *et al.* (2002), who verified reductions in chapter sizes in plants with growth regulators paclobutrazol, but these decreases in chapter diameter had no impact or commercial loss (around 2 cm lower than the control). On the other hand, the use of paclobutrazol growth regulator showed no difference by chapter diameter for the cultivars Helio 358 e BRS Paixão (Wanderley *et al.*, 2011)

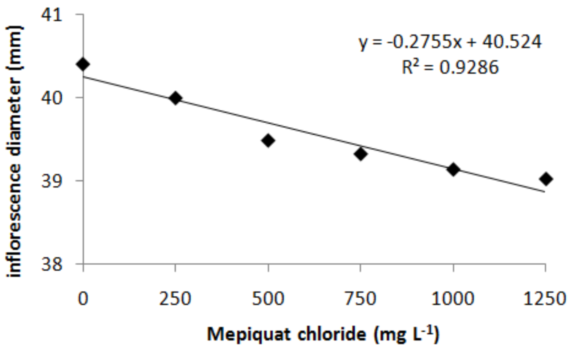

Figure 4. Internal inflorescence diameter (mm) of sunflower plants cv. Sol Vermelhoin response to contrasting concentrations of mepiquat chloride

The number of inflorescences presented a quadratic response with increasing concentration of mepiquat chloride, showing an increase in the order of 159.37; 165.62; 181.25; 187.50 and 203.12% at concentrations 250; 500; 750; 1000 and 1250mg L^{-1}, respectively, when compared to control plants (Figure 5). This response may be due the relationship between source/drain, wherein the reduction of elongation caused by growth regulators decreases the demand of photo assimilates for growth, which are used in higher proportion by the plant reproductive organs. According to Taiz & Zeiger (2013), plant hormones play an important role in regulating the drain-source relationships, because they affect the photo-assimilates partition by controlling the drain growth, leaf senescence and other developmental processes.

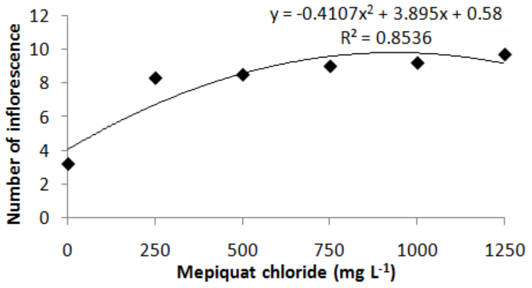

Figure 5. Number of inflorescenceof sunflower plants cv. Sol Vermelhoin response to contrasting concentrations of mepiquat chloride

Karlovic *et al*. (2004) verified significant reductions in the number of inflorescences per plant by applying different concentrations of daminozide and chlormequat regulators, once during the chrysanthemum cycle observed in concentration 3 g $L^{-1.}$ The root length was negatively correlated with the concentration of mepiquat chloride applied, which was reduced 0.83; 3.24; 8.49; 9.96 and 13.53% at concentrations of 250; 500; 750; 1000 and 1250mgL^{-1}, respectively, when compared to control plants (Figure 6). This response is also related to the concentration of gibberellin in the root cells, because gibberellins can both promote the elongation of the aerial component and the root system (Taiz&Zeiger, 2013).

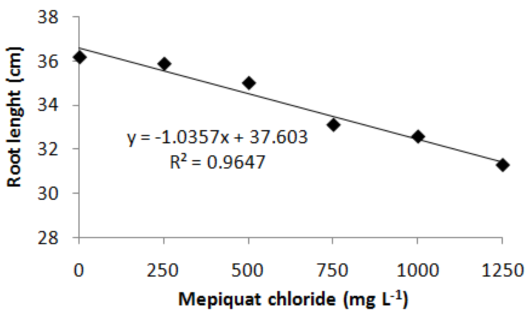

Figure 6. Root lenght(cm) of sunflower plants cv. Sol Vermelhoin response to contrasting concentrations of mepiquat chloride

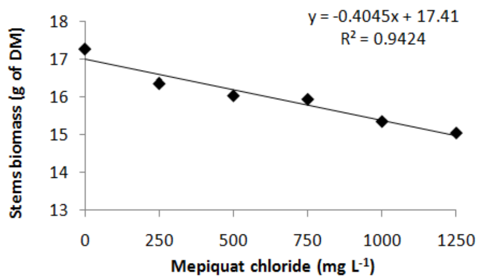

Figure 7. Stems biomass (g of DM) of sunflower plants cv. Sol Vermelhoin response to contrasting concentrations of mepiquat chloride

The stems biomass decreased with increasing concentration of growth regulator applied at 5.20; 7.06; 7.61; 11.03 and 12.81% at concentrations of 250; 500; 750; 1000 and 1250mg L^{-1}, respectively, when compared to control plants (Figure 7). A similar result was observed by Coutinho *et al*. (2014), who verified lower accumulation of stems biomass of ornamental sunflower in response to increasing daminozide concentration. Bogiani *et al*. (2011) also observed reductions in dry mass of cotton plants due the administration of the inhibitor of gibberellin and mepiquat chloride. The authors related that the reduction of dry matter is an indicative of the control of the excessive plant vegetative growth, which enables a different regulation on reproductive and vegetative growth. The dry matter accumulation by chapter also showed a negative correlation with increasing concentration of mepiquat chloride. Chapters biomass decreased 3.42; 5.43; 7.85; 9.73 and 11.47% at concentrations 250; 500; 750; 1000 and 1250mg L^{-1}, respectively, when compared to control plants (Figure 8). Similar results were observed by Wanderley *et al*. (2014), who verified for both sunflower cultivars BRS Oasis and Helio 358 lower chapters biomass with increasing doses of paclobutrazol.

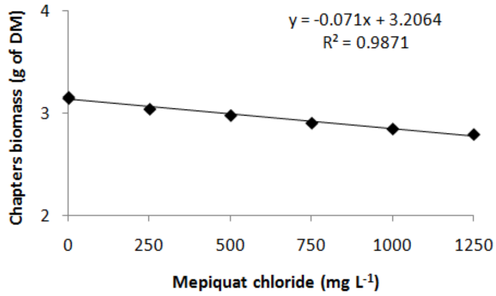

Figure 8. Chapters biomass (g of DM) of sunflower plants cv. red sun in response to contrasting concentrations of mepiquat chloride

The dry mass of the root system decreased 4.26; 8.69; 13.83; 17.61 and 20.95% at concentrations of 250; 500; 750; 1000 and 1250mgL^{-1}, respectively, when compared to control plants (Figure 9). A similar result was observed by Coutinho *et al*. (2014), who verified that increasing daminozide concentration reduces the root dry matter accumulation of ornamental sunflower. These same authors state that the reduction effect of dry matter accumulation in the stem and root section, caused by application of growth regulator, shows a preferred carrying of carbohydrates to produce chapters.

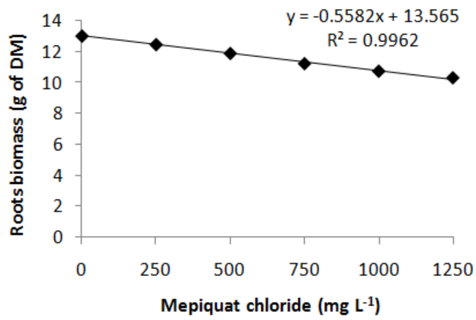

Figure 9. Roots biomass (g of DM) of sunflower plants cv. red sun in response to contrasting concentrations of mepiquat chloride

Wanderley *et al*. (2014) observed for the genotype Helio 358 that growing doses of paclobutrazol significantly reduced the root dry matter, while for BRS Oasis it was only verfied reduction of root dry mass accumulation from the dose 2 mg $L^{-1}$and above. The increasing concentration of mepiquat chloride reduced the leaf area index in 6.4; 8.6; 11.8; 13.2 and 16.6% at concentrations of 250; 500; 750; 1000 and 1250mgL^{-1}, respectively, when compared with the control (Figure 10).

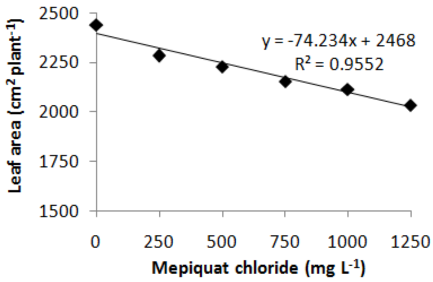

Figure 10. Leaf areaof sunflower plants cv. red sun in response to contrasting concentrations of mepiquat chloride. 2015

Coutinho *et al*. (2014) states that the reduction in leaf area (LA) causes a reduction in the plant's ability to do photosynthesis, necessary for its development. This reduction in LA provides the plants compactation, which becomes smaller and shows more dense leaves, due to the shortening of the internodes caused by the application of the growth regulator daminozide. Bonacin *et al*. (2006) found that the application of thegrowth regulator daminozide (0.5 g $vaso^{-1}$) was more effective in reducing the height, leaf area and diameter of the chapters of three sunflower hybrids when compared with chlormequat and placobutrazol growth regulators. However, the authors highlight that such reductions were not sufficient to obtain adequate plans for marketing.

The specific leaf area (SLA) was negative influenced by the increase in concentration of mepiquat chloride, which reduced 7.99; 11.37; 16.01; 17.57 and 21.73% at concentrations250; 500; 750; 1000 and 1250mg L^{-1}, respectively, when compared with the control (Figure 11). According to Benincasa (2003), the SLA is expressed by the ratio between LA and dry mass of leaves, since the LA is a morph physiological component and the leaf mass is an anatomical component of a plant specie, because it is related to the internal structure (number and size) of mesophyll cells, thereby inferring that the inverse of the SLA reflects the thickness of the leaves.

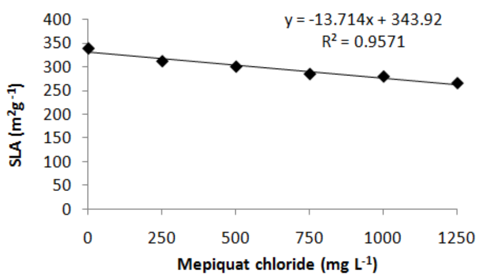

Figure 11. Specific leaf areaof sunflower plants cv. red sun (SLA, m^2g^{-1}) in response to contrasting concentrations of mepiquat chloride

Decreases in SLA indicate an increase in thickness of the leaves, resulted from the increase in cells plant size and number. This justifies the absence of statistically significant change in leaves biomass even with reduction in LA, because the lower leaves biomass were offset by an increase in the thickness of the leaves. It is known that gibberellins act in cell elongation, so this hormone deficiency caused by application of growth regulators can lead to more flat cells, which may have influenced the increase of the leaves thickness at the expense of reduction of LA. Chlorophyll A, B and carotenoids contents were positively correlated with concentration of mepiquat chloride applied in sunflower leaves, which increases 4.29; 28.86; 39.27; 68.24 and 84.8% for chlorophyll A, 40.53; 57.25; 86.46; 113.34 and 98.18% for chlorophyll B, and 9.90; 13.24; 32.13; 53.09 and 85.90% for carotenoids, in concentrations of 250; 500; 750; 1000 and 1250mg L^{-1}, respectively, when compared to control plants (Figure 12). According Chaney (2004) the reduction of the synthesis of gibberellins caused by the action of the growth regulator favors the methabolic pathway for phytolproduction, a terpenoid present in the chlorophyll molecule, thus resulting in increase in the chlorophyll content in the leaf.

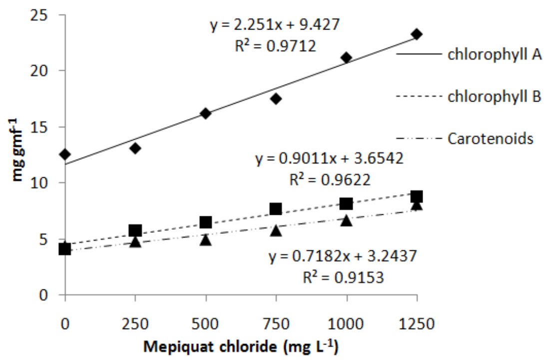

Figure 12. Levels of chlorophyll A, B and carotenoidsof sunflower plants cv. Sol Vermelhoin response to contrasting concentrations of mepiquat chloride

Another hypothesis is related to the fact that mepiquatchloride inhibit ethylene synthesis, a molecule that promotes an increase in the activity of enzyme schlorophyllase and oxidases (Yamauchi *et al*., 1997), which are responsibles for the chlorophyll degradation and consequently the disappearance of the green color. Coutinho *et al*. (2014) verified higher relative index values of chlorophyll with increasing concentrations of the growth regulator daminozide, revealing leaves with more intense green color. The use of paclobutrazol regulator in ornamental sunflower provided major enhancements in the green color of its leaves, ensuring that such effect favored a greater contrast between the green color of the leaves and the yellow color of the chapters, increasing the visual, ornamental and commercial attractiveness of these plants (Barbosa *et al*., 2009). Regarding to carotenoids, they are integral components of thylakoid membranes and are in general closely associated with proteins that form the photosynthetic system, and also help to protect the plant from damage caused by light (Taiz & Zeiger, 2013). Therefore, the increase in chlorophyll concentration caused by both the increase in thickness of the leaves as the production of phytol, due to reduced synthesis of gibberellins, can be indirectly led to higher carotenoid production to protect the photosynthetic apparatus from damage caused by light.

Conclusion

The application of mepiquat chloride increased the number of flower chapters per plant and reduced height of sunflower cv. Sol Vermelho, but in the doses tested the such reductions were not sufficient to obtain adequate plants for marketing in vase.

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