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Growth regulators for reduction of height in potted red-yellow sunflower *Helianthus annuus* cv. 'Florenza'

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Abstract

The market for flowers and ornamental plants has been growing in Brazil. In this context, sunflower has been increasing its importance mainly as a cut flower. There are cultivars of distinct colors and shapes, but sunflower is a very high flower to be selled in pots. The objective of this research was to evaluate the effect of the growth regulators such as mepiquat chloride and paclobutrazol in the height reduction of the Sunflower known as 'Florenza' to be used it in potted flower. We adopted a completely randomized design in a 2x5 factorial scheme with 11 treatments and ten repetitions. In five samples, the mepiquat chloride (0, 2, 4, 6, 8, 10 L ha^{-1}) were used and in other five samples paclobutrazol (0, 2, 4, 6, 8, 10 L^{-1}) were applied. The applications were repeated at 15, 30 and 45 days after sowing. The plant height, stem diameter, intern diameter of the main capitulum, number of leaves and capitula, dry mass of leaves, stem, capitula and root, leaf area and chlorophyll content were evaluated. The concentration of 10 L ha^{-1} of mepiquat chloride was the most effective dose in reducing the height of a red-yellow 'Florenza' sunflower showing a height 43.2 cm smaller than the one from the control treatment.

Keywords: *Helianthus annuus* l.; mepiquat chloride; paclobutrazol; gibberellin.
Abbreviations: PBZ_paclobutrazol; DAS_days after sowing; CMT_Mepiquat chloride.

Introduction

The ornamental flowers and plant markets are the most dynamic and promising segments in Brazilian agribusiness. This distinction occurs mainly because of the market structure, diversity of species, cultivars and the dissemination of new technologies. In addition to that, the climatic diversity of Brazil benefits the cultivation of flowers from temperate and tropical climate. Therefore, it is possible to produce flowers, foliage and other derivatives during the entire year at a reduced cost (França and Maia 2008). In 2013, the Brazilian market of ornamental flowers and plants produced R$ 5.22 billion, with a growth rate of 8.3% compared to the total revenues received a year before (SEBRAE 2015).

In this context, the sunflower is gradually occupying new areas and its production is significantly increasing, mainly due to its peculiar characteristics of rusticity, resistance to drought and beauty because of its large and showy inflorescences, which are called capitula (Dantas et al., 2015). Nowadays, the sunflower gains a strong position in flower markets in virtue of having a fast cycle, which allows a return of investment in a short term by the producers. The producers may have a short term return of investment and by the possibility of using it as a cut or potted flower. As a consequence, selection of new species, cultivars and hybrids with different colorations it is often happening by breeders (Nair et al., 2009). The Sunflower (*Helianthus annuus* l.) belongs to Asteraceae family. Its origin believed to be in North America and is considered a large adaptation culture to various conditions of latitude, longitude, and photoperiod.

There are different varieties of colors and shapes of Sunflower. In this research, we highlight the 'Florenza', which is a bicolor flower with bright petals that has yellow tips around a central disk of a dark mahogany. It has many branches and can have up to five flowers with an approximate diameter of 10 cm. It grows up to 4 meters of height.

The cultivar with distinct color has a size developed by companies that are an obstacle to the use of sunflower as a potted ornamental flower, once there is no proportionality to the vase size (Wanderley et al., 2011). In the case of ornamental sunflower, its productivity should be evaluated based on plant height and quality of its inflorescences, which should be accompanied by plants with an adequate height to vases (Neves et al., 2005). According to Wanderley et al. (2014), the sunflower offers the advantage of a short cycle of about 60 days and the easiness of seeds dispersal. Plant growth regulators are generally used in floriculture industry for height control, branching and flowering. According to Taiz and Zeinger (2009) the gibberellin regulates several important activities in plants, including seed germination, and it can stimulate elongation and cell division. Gibberellic

acid treatments have been reported to promote the early flowering in different floricultural products and to increase the efficiency and the quality of them (El-Naggar et al., 2009). Wanderley et al. (2014) claimed that growth regulators can be used to control the height of the plants. Some synthetic products can inhibit the synthesis of gibberellins, such as chlormequat, daminozide, paclobutrazol and mepiquat chloride. They are used in diverse cultures to reduce the size of the plants. Mepiquat chloride (CMT) is recognised as a member of gibberellic acid biosynthesis group, i.e. an inhibitor of cell elongation and the effect of its action hinders the branches grow.

The paclobutrazol (PBZ) is a product which prevents the synthesis of gibberellic acid by regulating the growth and by reducing the elongation of the internodes. Fonseca et al. (2005) described the order of effects on the plant. The first one is an interruption of the plant growth, which affects the new branches and reduces the branches length; the second is the bloom anticipation that in some cases increases its precocity as high doses are used (Oliveira et al., 2014).

One of the main difficulties in recommendations of regulatory factors is the definition of an appropriate dose of application. In many cases, the expected results have not been achieved following the recommendations (Bogiani and Rosolem 2009). Many sunflower cultivars show low predictability after application of these compunds mainly due to genotypic differences and their phyto-regulator sensitivity.

The objective of this research was to evaluate the effect of mepiquat chloride and paclobutrazol growth regulators in the height reduction of red-yellow sunflower called 'Florenza' to be used as a potted flower.

Results and discussion

Plant growth

The results showed a significant reduction in the height of the plants with the increase in concentration of growth regulators, either Mepiquat chloride or Paclobutrazol. The Mepiquat chloride showed a greater efficiency in time control than the other one. The reduction of the height of the plants was caused by the application of mepiquat chloride at concentrations of 2, 4, 6, 8, and 10 L ha^{-1}, which indicated reductions of 21.2, 28.8, 30.4, 40.8 and 43.2 cm, respectively. The treatment with paclobutrazol has caused the reduction of 0.6, 2.6, 11.4, 16.6 and 22.0 cm, with the same concentration respectively, when compared to the control plants.

Comparison of two growth regulators showed that treatments at different concentrations of mepiquat chloride have greater efficiency on the reduction of plant height, by which the height reduced up to 43% (Fig.2A). The reduction of plant height is related to the balance of the hormones caused by the application of mepiquat chloride, which interferes with the biosynthesis of gibberellic acid and inhibits the formation of this hormone in plant. This reduction in growth of the plant is due to physiological changes (Marur, 1998).

Kappes et al. (2011) worked with three growth regulators in their experiment (mepiquat chloride, ethyl-trinexapac, and placobutrazol) and they verified that the applications of these regulators had a direct influence on the vegetative development of crotalaria, what caused physiological responses of the plants as the decrease in cell stretching. The height of the plant was influenced separately by the growth regulators, application rates and by the interaction between the factors. The authors suggested that growth regulators act as chemical signals in the regulation of plant growth and development. The regulator that was the most efficient in height reduction was paclobutrazol, which reduced 70.8 cm of the plant in the highest tested dose of 300 g ha^{-1}.

A similar result was obtained by Coutinho et al. (2014), whose researched application of daminozide growth regulator. They showed the height reduction of ornamental Sunflower (*Helianthus annuus* cv. Dwarf Sunbright kids) with the elevation in concentration of regulators. The biosynthesis reduction of gibberellin can also promote the decrease of plant metabolism, which causes the decrease of respiratory rate and of ATP availability and reduction in plant growth (Bai and Chaney 2001).

Stem development

In relation to the stem diameter, a negative correlation with elevation of the growth regulator concentration was happened. The decrease in stem diameter at rates of 7.0, 14.0, 13.3, 14.0 and 24.2% happened using 0, 2, 4, 6, 8, 10 L^{-1} mepiquat chloride, respectively. Similarly, reduction in stem diameter happened at rates of 0.9, 2.5, 4.8, 7.5 and 16.9% using 0, 2, 4, 6, 8, 10 L^{-1} paclobutrazol, respectively. Bertoncelli et al. (2016) tested the dosages of paclobutrazol and obtained an increase in stem diameter on a rate of 1.69, 5.74, 7.73, 9.24 and 10.59% in dosages of 0.25; 0.5; 0.75; 1.0 and 1.25 g $^{L-}$, respectively. The same authors emphasized that the lower synthesis of gibberellin reduces the height and consequently there is an increase in the diameter of the internodes without causing deformation of the plant. However, this result was just possible due to the low dosage of the applied regulator.

Capitulums

The sunflower capitulum diameter showed a negative correlation with the elevation of the growth regulator concentration. both growth regulator mepiquat chloride and for paclobutrazol caused downsizer, with reductions of up to 1.56 cm and 0.65 cm, respectively (Fig. 2B). Equivalent results were found by Pallez et al. (2002), who observed reductions in the size of the plant capitulum, which was treated with paclobutrazol growth regulators, but these decrease in diameter had no impact or commercial loss (about 2 cm shorter than the control plant). Lima et al., (2013) emphasized that sunflower varieties must have small capitula when destined as cut flowers. Very large capitula may deform the floral stems due to its weight. This should be considered for potted flowers.

In relation to the number of capitula, there was a reduction in all treatments, showing a decrease of up to 50% and 70% of mepiquat chloride concentrations and paclobutrazol, respectively (Fig 2C). Karlovic et al. (2004) applied different concentrations of daminozide and clormequat regulators during chrysanthemum cycle once and observed that in

Fig 1. Sunflower 'Florenza' 60 DAS.

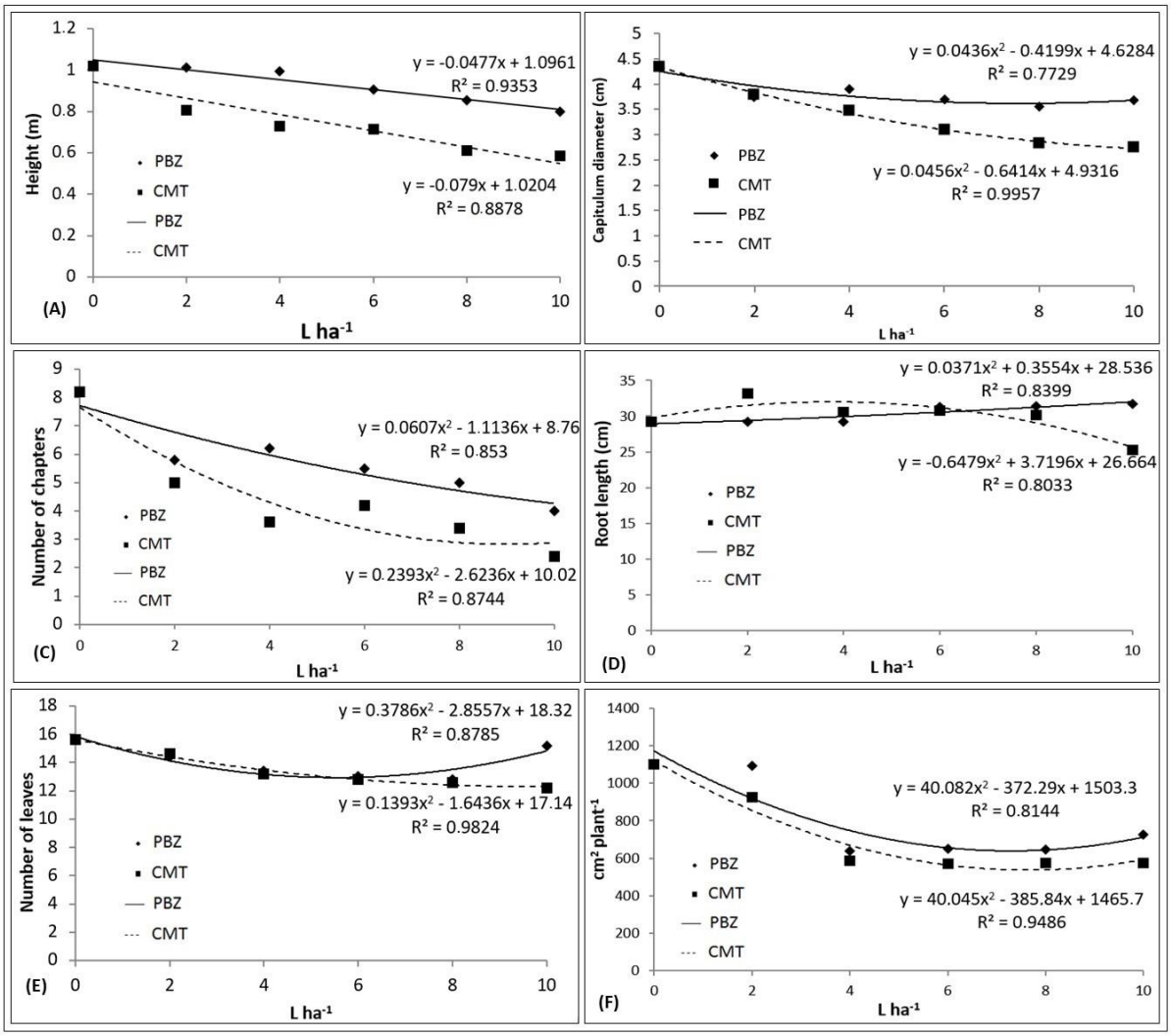

Fig 2. Sunflower 'Florenza' submitted the application of different concentrations of growth reducers, mepiquat chloride (CMT) and paclobutrazol (PBZ), 60 DAS – (A) height (m); (B) internal diameter of the capitulum (cm); (C) number of capitulum (D) root length (cm); (E) number of leaves; (F) leaf area.

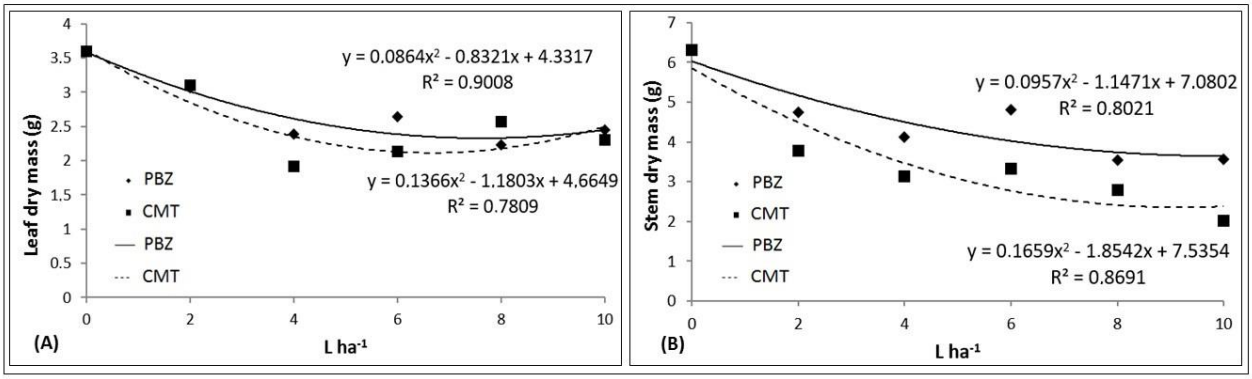

Fig 3. Dry mass of sunflower 'Florenza' plants treated with different concentrations of two growth regulators, mepiquat chloride (CMT) and paclobutrazol (PBZ), 60 DAS. (A) Leaf dry mass; (B) Stem dry mass.

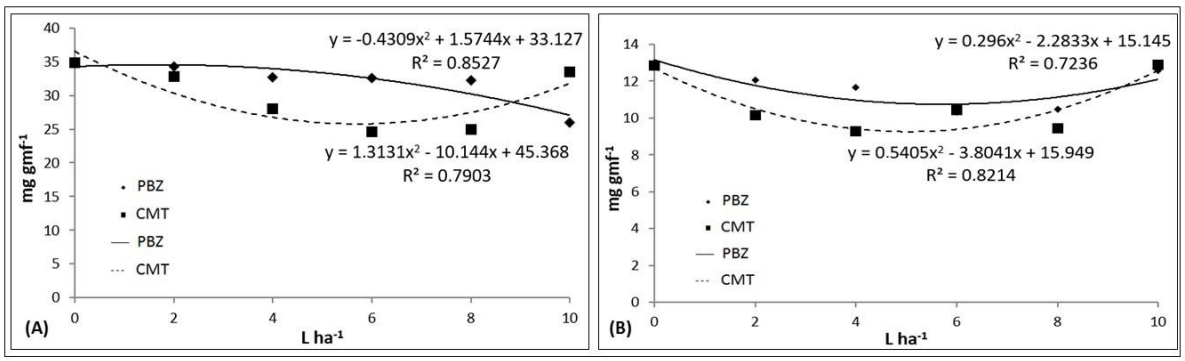

Fig 4. Levels of chlorophyll in plants of sunflower 'Florenza' submitted to the application of growth reducers in different concentrations, mepiquat chloride (CMT) and paclobutrazol (PBZ), 60 DAS. (A) – levels of chlorophyll a; (B) levels of chlorophyll b.

concentration of 3 g L^{-1} a significant reduction of the number of inflorescences per plant happened.

Root development

There was a positive correlation between the dosages of paclobutrazol and root length, in which the root length was increased by up to 8.7%. This behavior is also related to concentration of gibberellin in the root's cells, according to Taiz and Zeiger (2009). The gibberellin can promote the stretching of the aerial part and of the root system. The treatments composed of mepiquat chloride caused a decrease in the root length of 13.42% in the reduction of 10 L ha^{-1} dosage (Fig. 2D). If the gibberellin production occurs primarily in the roots, the use of practices that provide the lowest growth of the root system will influence the production of this hormone and consequently in the length of the root (Oliveira 2012).

Leaf area

Treatment with mepiquat chloride caused a decrease in the number of leaves in all dosages, which decreases number of leaves after increasing the regulator concentration. Treatment with different doses of paclobutrazol induced decrease in the number of leaves dosage until it reached approximately 5 L ha^{-1}. A later increase was observed after this dose (Fig. 2E). The dose of 10 L ha^{-1} did not differ from what was observed previously. It was observed that the increase of concentration in mepiquat chloride reduced the plant leaf area. The leaf area was decreased by 17.0, 47.0, 48.0, 47.7 and 47.9% at concentrations of 2, 4, 6, 8 and 10 L ha^{-1}, respectively, with a sharp decline at 4 L ha^{-1} dosage. However, it remained constant as the doses increased. Similarly, application of paclobutrazol growth regulator showed a great decline in leaf area at the dosage of 4 L ha^{-1}. However, it remained uniform as this dosage was increased. The decreases of 1.0, 42.2, 41.5, 41.3, and 34.33% were observed by the authors in dosages of 2, 4, 6, 8 and 10 L ha^{-1}, when compared with the control plant (Fig 2F).

Coutinho et al. (2014) confirmed that reduction in leaf area leads to a decrease in the plant ability to perform photosynthesis, which is necessary for development. The reduction in leaf area created a smaller plant with smaller leaves and more densely populated due to shortening of internodes by the application of daminozide growth regulator.

Bonacin et al. (2006) applied 0.5 g $vase^{-1}$ of daminozide on three hybrids of ornamental sunflower via foliar application and verified that this regulator proved to be more effective in the height reduction of the AF and of the capitula diameter of those plants, when compared to chlormequat. However, they emphasized that such reductions were not sufficient to obtain appropriate plants for marketing.

Dry mass of plants

There was no significant difference in root dry mass. There was a decrease in stem and leaf dry mass, as the regulator's concentration was increased (Fig. 3). A similar result was observed by Coutinho et al. (2014) who observed decreased in accumulation of MSC in ornamental sunflower by increase in concentration of daminozide. Bogiani and Rosolem (2011) also verified reductions in dry mass of cotton plants treated with gibberellin and mepiquat chloride inhibitor and reported the decrease in the dry mass matter, which is an indication of excessive plant vegetative growth control, enabling a differentiated regulation on reproductive and vegetative growth. These same authors affirmed that the effect of reduction in accumulation of stem dry mass, capitulum and root were caused by the application of growth regulator, which shows a preferred carbohydrate washed to produce capitula. Wanderley et al. (2014) observed that growing doses of Helio paclobutrazol significantly reduced the amount of root dry mass in sunflower genotype 358. For BRS Oasis, the reduction of accumulated dry mass was found only in the stem with a 2 L ha^{-1} dose or higher.

Variation in chlorophyll concentration

The quantities of chlorophyll *b* showed a similar behavior to other traits, when it was submitted to mepiquat chloride regulator. The two tested regulators induced a decrease in chlorophyll concentration until it reached approximately 5 L ha^{-1}. The chlorophyll content was increased with the increase of the dosages, as shown in Fig. 4B. According to Chaney (2004) the reduction of gibberellins caused by growth regulator favors the route of final production of phytol, a terpenoids present in chlorophyll molecules. It results in elevation of chlorophyll levels in the foliage. Another possibility is related to the fact that mepiquat chloride inhibits ethylene synthesis, which promotes an increase in the activity of clorofilase enzymes and oxidases (Yamauchi et al., 1997) and is responsible for chlorophyll degradation and the disappearance of the green color.

Coutinho et al. (2014) verified that elevation in chlorophyll index is occurred in higher concentrations of regulators. That is the reason for plants to have dark green leaves due to the effect of daminozide concentrations. The use of paclobutrazol regulator in Sunflower *Florenza* induced a darker green color of leaves and such effect favored a bigger contrast between the green from the leaves and the yellow from the capitula, which increases the visual, ornamental and commercial attractiveness of these plants (Babu et al., 2009).

Materials and methods

Experimental site and climate conditions

The work was conducted in Glasshouses with plenty of sun, which is located at 23° 23 ' South latitude and 51° 11 ' West longitude and an altitude average of 566m. The climate of the region is Cfa type according to Köppen classification. The experiment was conducted between October and November 2015.

Experimental design and plant material

The seeds used in the experiment were from *Helianthus annuus* cv. *Florenza* species from Harri Seeds®'s company with a cycle of 70 days on average (Fig. 1). They were sown directly in hard-black polyethylene plastic pots with drains at the bottom, which had 15 cm of height, 17 cm in top diameter, 14 cm of bottom diameter and a 3L of capacity. They were filled with a vegetal soil substrate, in which the seedling growth for four days after the sowing (DAS) was observed. The Sunflower seedlings were fertilized with 10g of the slow-release fertilizer called Basacote® 3M (15-9-12) at 12^{th} DAS. The plants were manually watered once a day until the substrates totally saturated during the experiment.

A completely randomized design in a 2x5 factorial scheme with ten repetitions and 11 treatments was adopted. Five Mepiquat Chloride concentrations (2, 4, 6, 8, 10 L ha^{-1}) and similarly five paclobutrazol (2, 4, 6, 8, 10 L ha-1) were assigned as treatments. We sprayed plants with distilled water as control treatment. The paclobutrazol treatments were prepared as follows: 4 mL of a commercial product diluted (Hide 250 SC) in 1 L of water, which resulted in a stock solution of paclobutrazol in a concentration of 1 g L^{-1}. We prepared dosages of paclobutrazol (2, 4, 6, 8 and 10 liters per hectare) from this solution. The experimental unit consisted of vase with one plant per pot.

For Mepiquat Chloride treatments, we used a manual spray with capacity of 500 mL. Plants were sprayed evenly with a complete coverage of leaves and stems. For the paclobutrazol, we applied 50 mL of the substance to the substrate per pot. The concentrations tested were repeated on the 15^{th}, 30^{th}, and 45^{th} DAS at 6:00 p.m. At the time of the first application, the plants had two pairs of final leaves and an average height of 25 cm.

Harvest and evaluation of the characteristics

On 60th DAS, evaluation of the following growth parameters was carried out: plant height, defined as the distance from the neck to capitulum insertion point; stem diameter, defined as the area above the substrate surface and below the first internode intercession, which was measured using a digital caliper with accuracy of 0.01 mm; internal diameter of the main capitulum, measured with the same digital caliper. We also counted the number of leaves and flower heads per plant, which were considered capitula those that had open ligule regardless of its size.

After completion of nondestructive evaluations, the plants were cut close to the substrate, with leaves, stems, and roots separations. The last ones were washed with water for a complete substrate withdrawal. Then, the length of the largest root was measured. To obtain the dry mass of the leaves, stem, root and capitula, the materials were placed in paper bags duly identified and dried in an oven with forced air circulation at 60° C until it reached a constant mass. After this period, the samples were weighed using an analytical balance.

For the evaluation of the foliar area, a leaf area measurer of Licor brand, model LI-3100 was used. The leaves were detached from the plants and then arranged in the wake of the equipment to read the results. The levels of chlorophyll *a* and chlorophyll *b* were also evaluated following the methodology described by Meschede et al. (2011), in which

samples with 0.2 g of fresh leaf tissue were pounded in N liquid and then placed in tubes with a cap containing 10 mL of acetone 100% (v/v). The extracts were filtered and the readings were done in a spectrophotometer with wavelengths of 663, 645 and 434 nm for chlorophyll *a* and chlorophyll b, respectively. The determination of chlorophyll levels (mg gmf^{-1}) were based on the following related equations, accordingly to Whitham et al. (1971): chlorophyll a = (x 2.04 x 11.24 Mellon-A645) and Chlorophyll b = (20.13 x A645-4.19 x Mellon).

Statistical analysis

The data were subjected to the variance analysis, with statistical program ASISTAT®. The results with significant data were submitted to a regression analysis with 5% of probability level.

Conclusion

The concentration of 10 L ha^{-1} of mepiquat chloride was most effective in reducing the red-yellow sunflower height showing a value of 43% lower, compared to control treatment. This treatment is recommended for the reduction of red-yellow sunflower. Paclobutrazol was less efficient in reducing the size of the red-yellow sunflower when compared to the mepiquat chloride.

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