

Effect of Gibberellic Acid (GA) on Carrot (*Daucus carota* L.) under Cadmium Chloride Stress

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Key message (highlight):

- Investigated to find out the possible gibberellic acid effects on seed germination and foliar application on growth and some physiological aspect of carrot vegetable under cadmium chloride stress.
- Examined the effects of plants under stress and non-stress condition.
- Studied the behavior of gibberellic acid in enhances the tolerance under cadmium chloride stress.

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Abstract

Carrot (*Daucus carota* L.) is a very essential and highly nutritive vegetable of the world. It is extensively used all over the world due to its nutritional composition. Cadmium is the metal that affects various vegetables and gibberellic acid is a useful hormone against a variety of stress. In old botanical garden pot experiment was performed to check the effect of gibberellic acid under cadmium chloride stress at University of Agriculture Faisalabad. In plastic pots that contain sand the seeds of carrot T29 and red gold were sown. The experiment comprising of 3 levels of cadmium chloride (0, 200 μ M and 400 μ M) that were applied to the sand and as foliar spray two levels of gibberellic acid (0 and 0.1M) applied to plant exogenously. Under completely randomized design (CRD) the data for growth, activities of antioxidants, gas exchange, photosynthetic pigments and mineral nutrients was analysed. It was recorded that morphological parameters and photosynthetic pigments like chlorophyll a, chlorophyll b, chlorophyll ab and carotenoids decrease by the stress of cadmium chloride. The Cd stress (400 μ M) shows negative effect alongside the ionic attributes like N, P, and K ions reduced by level of 400 μ M application of cadmium chloride and spray of GAs (0.1M) shows positive effect on carrot using standard procedures. physiological parameters like Phenolics, Proline, Hydrogen peroxide and soluble sugars reduce by the stress of cadmium chloride (200 μ M). antioxidant attributes like SOD, POD, and CAT reduced by 400 μ M level of cadmium chloride. Overall Result

showed that Cd effect all the way in plant life cycle hence it is concluded that by using 0.1M gibberellic acid provide best result to overcome stress condition.

Keywords: Gibberellic acid; Cadmium Chloride; Stress; Antioxidants, photosynthetic pigments

1. Introduction

Cadmium (Cd) is a toxic element alongside stood nonessential. Human actions such as industrial, and farming practices increased weighty metal contamination that attracted great attention worldwide. To remove heavy metals, phytoremediation has become a vigorous method (Grant *et al.*, 2008) In the modern era, in soil accretion of metals aggregate is due to the rising industrial activity. A prevalent environmental matter has become exhaustive due to use of fertilizers and unfitting removal wastes (Smith, 2009). Cadmium (Cd) and copper (Cu) are amongst elements of most apprehension as in common sources of soil impurity, they can reach high level in soil due to their normal occurrence and high substances. By difference, in-plant digestion Cd has no known biological functions, except for probably require Cd for normal growth and some Cd-hyperaccumulating populations (Verbruggen *et al.*, 2009). However, Cd is mainly engaged by plants due to its chemical resemblance with important bivalent cations (Lin and Aarts 2012).

The use of growth regulators in the production of economically viable food crops has the potential to increase and improve yields. The discovery GA might not only regulate plant growth and development but also improve plant resilience to a variety of environmental stress situations has recently gained attention (Sharaf *et al.*, 2009). The low-temperature requirement in carrots indicated in several reports that gibberellin replaced during foliar applications. Carrots hastened seed stalk height during gibberellin application to vernalized. Moreover, effect of high temperature that is prevented the inhibitory on seed stalk elongation by GA3 application following vernalization. In the apical bud, an increase in endogenous gibberellin activity was found at low temperatures (Tokuji and Kuriyama, 2003). The definite objectives of current research inquiry were to find out: 1) the gibberellic acid effects on seed germination and foliar application on growth and some physiological aspect of carrot vegetable under the cadmium chloride stress. 2) to study the effect of plants under stress and non-stress condition. And 3) to study the behavior of gibberellic acid in enhances the tolerance under cadmium chloride stress.

2. Material and Methods

2.1 Field experiment site and growing season weather

This experiment was conducted at Old Botanical Garden, University of Agriculture Faisalabad.

2.2 Experimental treatments, crop husbandry, and observations

The experiment in soil was carried out to calculate the impact of cobalt chloride stress and spray of Gibberellic acid on morphological as well as biological qualities of Carrot (*Daucus carota* L.) Varieties red gold, and T29. Transplant of seed red gold and T29 sown in plastic pots that full of sand and water. Three actions of cadmium chloride (0mM, 200mM, 400mM) and two levels of gibberellic acid spray (0mM, 0.1mM) were applied in the experiment to observe the impact. It was completely randomized with three replicates and data regarding following parameters were recorded after the establishment of treatment. Seeds of carrot were provided from the botany department which was taken from Ayub agriculture research institute. Seeds were sown in 36 plastic pots, carried 10kg clean soil.8-10 seeds

were sown in 2cm deep hole in every pot. after propagation seedling sustained in such a way that can maintain 10 seedlings in each pot. Seedling were irrigated with full strength, Hoagland solution were given to the plants on weekly base and cobalt chloride stress were applied on leaves through rooting and Gibberellic acid spray through foliar. Gibberellic acid is modest gibberellin that support development and cell enlargement in carrot plants. Two levels (0, 0.1mM) in the form of gibberellic acid were applied on plants as foliar spray. Gibberellins influence the growth of plants. It was used for survivor for plants and also to reduce the effect of cobalt chloride. Two levels were used for three replicates. GA stimulate the firm growth rate of stem and root. It will make mitotic division in the leaf of some plants then rate of germination increased. Cadmium chloride is an micronutrient, its small amount required by plants because of its increased plant growth and yield. In another case if cadmium occurrences in plants become increased then its need it become harmful for plants and show bad effects on plants growth and production rates. Three levels (0, 150, 300mM) of cadmium chloride were used. 2 weeks old seedling were irrigated with cobalt chloride solution and gibberellic acid were applied as foliar. Treatments were applied after 10 days.

2.3 Morphological data

Shoot length is restrained by using an identical rod. After that, calculated the mean of all the values. A meter rod was taken to calculate the length of the root, after that, calculate the mean of all the values. First of all cut the root with the plant and use the electrical balance for the measuring roots weight. By measuring the root fresh weight, the roots of the plant were dried under the sunlight for 4-5 days. After the complete sundry, the roots of the plants were put into the oven at a temperature about 65 °C for one week. Before properly drying the roots of the plants, again using the electrical balance to measure the weights. The measuring of the shoot fresh weight picks up 2 or 3 plants then use the measuring balance to calculate their weights. After taking the fresh weight of the shoots the shoot first dry under light of the sun then place into oven. The temperature of the oven was 65 °C. When the shoots of plants were completely dried used the balance to measure their weight again. Root diameter measuring with the help of vernier calliper.

2.4 Physiological attributes

Physiological attributes such as chlorophyll "a" chlorophyll "b", carotenoid, and total chlorophyll (mg g⁻¹) were measured with the help of spectrophotometer. Determination of chlorophyll contents a, b, a/b was used in the method of Arnon (1949). Extraction of the chlorophyll take 0.1g fresh leaf and chopped these leaves into pieces and then chopped leaf pieces were put into (80%) acetone solution at room temperature of 25 over- night. After that, use the spectrophotometer for measuring the absorbance at 645, 663 and 480. Following formula was used for the measuring of the chlorophyll "a" and "b" contents.

$$\text{Chl.a (mg/g)} = V/1000 \times W \times [12.7(\text{OD } 663) - 2.69 (\text{OD } 645)]$$

$$\text{Chl.b (mg/g)} = V/1000 \times W \times [22.9(\text{OD } 645) - 4.68 (\text{OD } 663)]$$

$$\text{Total Chl. (mg/g)} = V/1000 \times W \times [20.2(\text{OD } 645) - 8.02 (\text{OD } 663)]$$

$$\text{Car. (mg/g)} = V/1000 \times W \times [(\text{OD } 480) + 0.114 (\text{OD } 663) - 0.638(\text{OD } 645)]$$

W = weight of fresh leaf in grams

V = total volume of the acetone used in extraction.

2.5 Enzymatic and non-enzymatic activity of antioxidants

For the determination of antioxidants, enzyme extract was prepared by using plant material. Take 0.5g of fresh leaf material and these materials were grind in 5 ml of 50 mM potassium phosphate buffer. The pH of the potassium phosphate buffer was maintained at 7.8. This homogenized mixture was centrifuge at 1500 rpm for 15 minutes at 4 °C. After the centrifuge the supernatant was separated from the pellet, this pellet was removed, and the supernatant was used for the determination of the antioxidants.

2.5.1 Peroxidase or peroxide reductase (POD) and catalase (CAT) activity

Chemicals used 35% H₂O₂ and 50 mM phosphate buffer (pH 7.8). Chance and Maehly (1955) given a method for the determination of the activity of the CAT and POD. This procedure was used with minor alternations for activity of the CAT and POD. In a reaction of CAT activity using 3ml of mixture that consist of 50 mM cooled potassium phosphate buffer pH 7.8, 100µl of enzyme extraction, and 5.9mM H₂O₂. 0.1ml of enzyme extraction was added to mixture for the starting of the reaction. At the wavelength of 240 nm, the absorbance was noted after the interval of 20_s, reading was decreases. The change in absorbance was 0.01 units per min.

For the activity of the POD 3ml mixture consist of 40mM H₂O₂, 50mM buffer that was potassium phosphate buffer pH (7.8) guaiacol 20mM, and the enzyme extraction was 100µl. after that the set the wavelength at 470nm and record the absorbance every 30_s. The change in absorbance is 0.01 unit per mint mg of protein. To determine the activity of superoxide dismutase (SOD) following method was used. First of all, used the 2ml cuvette and pored the 400 µl H₂O + 250 µl potassium phosphate buffer (pH 7.0) and it also added the 100 µl L. methionine, added 100 µl triton, 50 µl nitro blue tetrazolium NBT, 50 µl enzyme mixture and then added 50 µl riboflavin. When all the mixture was put into the cuvette, the cuvette was placed under the light Lampe for 15 minutes to active the enzyme activity in this procedure. After 15 min the absorbance was read at 560 nm.

NBT solution preparation: Took 7.45 ml formamide and mixed with 2.55 ml buffer. Added 0.1 mg NBT. L-methionine solution preparation: Took 0.444g L-methionine and dissolved in 30 ml buffer. Triton X solution preparation: Took 75µl and dissolved in 30 ml buffer. Riboflavin solution preparation: Took 0.0264 riboflavin and added in 30 ml buffer.

2.5.2 Determination of H₂O₂ Hydrogen peroxide

Velikova, V. and Edreva (2000) method were used for the determination of the hydrogen peroxidase. Take 0.5 g of fresh plant material put into chilled pestle and mortar for the grinding by adding 5.0ml of 0.1% (w/v) trichloroacetic acid TCA. At 12000 × g for 20 minutes the homogenate was centrifuge. Then take the 0.5 ml of enzyme extraction and added into 0.5 ml of potassium phosphate buffer (pH

7.0) and also added 1 ml of potassium iodide to extraction. Using a spectrophotometer, thoroughly vortex the entire mixture and determine its absorbance at 390 nm (IRMECO U2020).

2.6 Total soluble proteins (TSP) estimation

Total soluble proteins were measured by using Bradford method (1976). In an ice bath, a fresh leaf sample (0.1 g) was extracted in 5 mL potassium phosphate buffer (pH 7.8). Then the homogenate was centrifuged at 10000 rpm for 15 min at 4°C. Took 0.1 ml sample in test tubes and added 5 mL of Bradford reagent. After vortex was kept for half an hour and then measured the absorbance at 595 nm using the spectrophotometer (IRMECO U2020).

Bradford reagent: Bradford reagent contained 0.1g Coomassie brilliant blue which was mixed into 100ml of phosphoric acid (85%). Then poured into 50ml of ethanol (95%). Now it became 150ml. By adding distilled water increased its volume up to 1L and filtered it 3 to 4 times.

2.7 Mineral ions determination

Allen, Grimshaw, and Rowland (1986) were followed to determine mineral ions. Took 0.1g dry ground material of roots and shoots in digestion flasks and added 2mL sulfuric acid in each flask then covered them with aluminium foil for 24 hr at room temperature. Next day the samples were heated at 200°C on a hot plate until fumes were produced. Then with the help of a pipette 1-2 ml of hydrogen peroxide was added drop by drop until the material became colourless. Then with the help of deionized water, the volume of extract was maintained to 50 ml. The extract was then filtered and utilized to determine the concentrations of Na⁺, Ca²⁺, and K⁺ ions. The sodium (Na⁺), potassium (K⁺), and calcium (Ca²⁺) concentrations were then measured using a flame photometer (Jenway, PFP7). The values of K⁺, Ca²⁺ and Na⁺ from flame photometer were compared with standard curves and then calculated the final amounts.

2.8 Phenolics (mg/g fresh wt.)

The phenolic content was evaluated using the Julkunen-Titton technique (1985). Each sample contained 0.25g of fresh leaf material. The leaves were ground in 3mL of 80 percent acetone using a pestle and mortar. Extracted leaves were centrifuged for 15 minutes at 12000 rpm. Supernatant was isolated using a micropipette. About 0.1mL (100 µl) supernatant was placed in a microfuge tube, and 1mL (1000 µl) of distilled water was added for dilution and placed in a microfuge tube. In this tube, add 2.5mL (500 µl) of folin-ciocalteu phenol reagent and aggressively shake. Alongside added 2.5ml of 20% Na₂CO₃ and 100ml of distilled water, then fill to 5ml and vortex quickly for 5 to 10 seconds. After leaving the homogenate for 20 minutes, the values at 750nm were analysed using a spectrophotometer.

2.9. Proline

Leaf samples were fostered and 20ml. of filtrate was taken in a test tube that was mixed in 2.0 ml. acid ninhydrin solution. Acid ninhydrin was prepared to mix 1.25 g ninhydrin with 30ml. GA. The reaction took under at 100 degrees and then terminated. After this mixture cooled and then vortexed.

2.10 Statistical analysis

Statistical analyses of collected data of all growth, oil yield, and bio-diesel yield parameters were done by employing Fisher's analysis of variance and LSD test at 5% level of probability for comparison of means of treatments (Steel *et al.*, 1997).

3. Result and Discussion

Cadmium chloride caused significant ($p \leq 0.05$) effect on shoot weight while GA also caused significant ($p \leq 0.05$) effect on shoot Fresh weight of both cultivars. However minimum reduction was observed in cv. Red gold under control condition and maximum reduction were observed under stress conditions. When 0mM gibberellic acid was applied. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress (Fig 3.1). Cadmium Chloride caused significant ($p \leq 0.05$) growth of root fresh weight of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress. (Fig 3.2). Cadmium Chloride caused significant ($p \leq 0.05$) increase in Shoot length of both cultivars. However, reduction maximum was observed in cv. Red gold was under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect significantly to this variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress (Fig 3.3). Cadmium Chloride caused significant ($p \leq 0.05$) increase in root length of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.4). Cobalt Chloride caused non-significant ($p \leq 0.05$) increase in Root diameter of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect is non-significant to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.5). Cadmium Chloride caused significant ($p \leq 0.05$) increase in Shoot dry weight of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.6). Cadmium Chloride caused significant ($p \leq 0.05$) increase in Root dry weight of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.7). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of shoot sodium (mg/g dry wt.) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significant to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.8). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase root

sodium (mg/g dry wt.) of both cultivars. However reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.9). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of shoot potassium (mg/g dry wt.) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.10). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of potassium root (mg/g dry wt.) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.11). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of shoot calcium (mg/g dry wt.) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.12). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of root calcium (mg/g dry wt.) Of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.13). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of Proline of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.14). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of soluble proteins of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.15). Cadmium Chloride caused significant ($p \leq 0.05$) increase of SOD (U mg/g protein) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.16). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of POD (U mg/g protein) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.17). Cadmium Chloride caused significant ($p \leq 0.05$) increase of CAT (U mg/g protein) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this

variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.18). Cobalt Chloride caused non-significant ($p \leq 0.05$) increase of Phenolics of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.19).

Photosynthetic pigments

Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of Chl. a (mg/g fresh wt.) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significant to this variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.20). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of Chl.b (mg/g fresh wt.) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.21). Cadmium Chloride caused significant ($p \leq 0.05$) increase of Chl. ab (mg/g fresh wt.) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect significantly to this variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.22). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of total Chl. (mg/g fresh wt.) of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significant to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.23). Cadmium Chloride caused significant ($p \leq 0.05$) increase of carotenoid (mg/g fresh wt.) of both cultivars. However reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.24). Cadmium Chloride caused non-significant ($p \leq 0.05$) increase of hydrogen peroxide of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Non-significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.25). Cadmium Chloride caused significant ($p \leq 0.05$) increase of hydrogen peroxide of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect non-significantly to this variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.26). Cadmium Chloride caused significant ($p \leq 0.05$) increase of hydrogen peroxide of both cultivars. However, reduction maximum was observed in cv. Red gold under stress when 0mM gibberellic acid was applied. Application of gibberellic acid effect

significantly to this variable. Significant variety difference was observed in this regard. Overall cv.T29 perform better than cv. Red gold under stress and non-stress condition (Fig 3.27).

4. Conclusion

Morphological parameters and photosynthetic pigments like chlorophyll a, chlorophyll b, chlorophyll ab and carotenoids decrease by the stress of cadmium chloride. The Cd stress shows negative effect and spray of GAs shows positive effect on carrot and these results are resembled with previous results of (Vassilev *et al.*, 2005) in which the rate of Cd reduces the effect of photosynthesis rate. In my present study, the ionic attributes like N, P, and K ions reduced by different application of cadmium chloride. The stress of cadmium chloride shows negative effects and GA shows positive effect on carrot and these results are matched with previous results of (Hasan *et al.*, 2007) in which the rate of cadmium decreases the ionic effects on carrot. In the latest study the physiological parameters like Phenolics, Proline, Hydrogen peroxide and soluble sugars reduce by the stress of cadmium chloride. The stress of cadmium shows negative effect on these parameters and results are matched with previous results (Singh and Tiwari, 2003) in which the rate of cadmium decreases the physiological parameters in carrot. In the present study the antioxidant attributes like SOD, POD, and CAT reduced by different level of cadmium chloride. The stress of Cd shows negative effect on these parameters and results are matched the previous results (Bishnoi *et al.*, 1993) in which the rate of cadmium reduces the antioxidant parameters in carrot.

Authors Contribution

Hira Hafeez and Faran Muhammad Conceived the whole research idea and followed standard procedures for the methodology. Muhammad Mansoor analysed, interpretation the data of whole study. All authors revised the manuscript.

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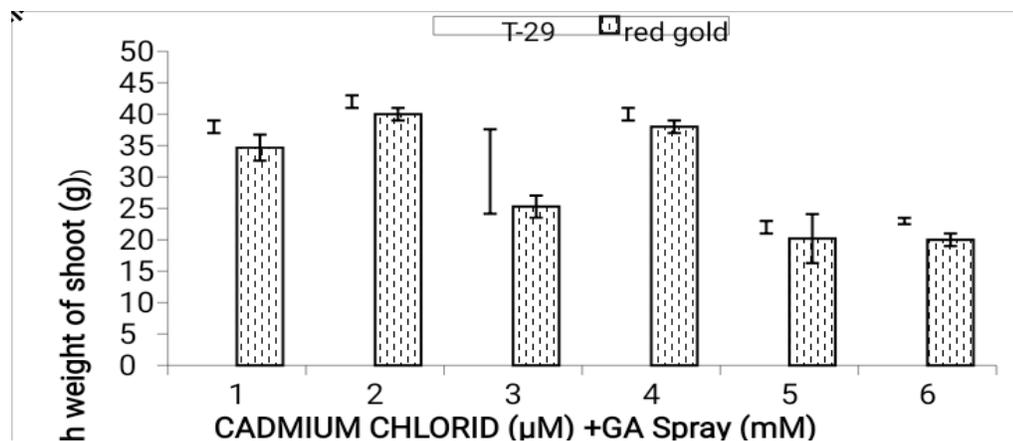
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REFERENCES

1. Adem, G. D., Roy, S. J., Zhou, M., Bowman, J. P., Shabala, S.
2. (2014): Evaluating contribution of ionic, osmotic and oxidative
3. stress components towards salinity tolerance in barley. BMC Plant
4. Biol. 14. DOI: <https://doi.org/10.1186/1471-2229-14-113>
5. Bishnoi, N. R., I. S. Sheoran and R. Singh. 1993. Influence of cadmium and nickel on photosynthesis and water relations in wheat leaves of differential insertion level. *Photosynthetica*, 28: 473-479.
6. Chance, B., & Maehly, A. C. 1955. Assay of catalases and peroxidases. 136.
7. FAO, 2014. 2018. Food and Agriculture Organization. <http://www.fao.org/platformfood-loss-waste/background/es/>, Accessed date: 2 September 2018.
8. Grant, C.A., J.M. Clarke, S. Duguid and R.L. Chaney. 2008. Selection and breeding of plant cultivars to minimize cadmium accumulation. *Sci. Total Environ.*, 390: 301-310.
9. Hasan, S.A., B. Ali, S. Hayat and A. Ahmad. 2007. Cadmium induced changes in the growth and carbonic anhydrase activity of chickpea. *Turkish J. Biol.*, 31, 137-140.
10. Lin, Y.F. and G.M. Aarts. 2012. The molecular mechanism of zinc and cadmium stress response in plants. *Cell. Mol. Life Sci.*, 69: 3187-3206.

11. Singh, P., K. and R. K. Tewari. 2003. Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. *J. Environ. Biol.*, 24:107-112.
12. Smith, S.R. 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge: Review article. *Environ. Int.* 35:142–156.
13. Steel, R.G.D., Torrie, J.H., Dicky, A.D. (1997): *Principles and Procedures of Statistics, A biometrical approach*. 3rd Ed. McGraw Hill, Inc. Book Co. N.Y. (U.S.A.). pp. 352-358.
14. Tokuji, Y. and K. Kuriyama. 2003. Involvement of gibberellin and cytokinin in the formation of embryogenic cell clumps in carrot (*Daucus carota*). *J. Plant Physiol.*, 160: 133-141.
15. Varzakas, T., G. Zakynthinos and F. Verpoort. 2016. Plant food residues as a source of nutraceuticals and functional foods. *Foods*, 5: 88-96.
16. Vassilev, A., Perez-Sanz, A., Semane, B., Carleer, R., & Vangronsveld, J. 2005. Cadmium accumulation and tolerance of two *Salix* genotypes hydroponically grown in presence of cadmium. *Journal of plant nutrition*, 28(12), 2159-2177.
17. Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant science*, 151(1), 59-66.
18. Verbruggen, N., C. Hermans and H. Schat. 2009. Mechanisms to cope with arsenic or cadmium excess in plants. *Curr. Opin. Plant Bio.*, 12: 1–9.
19. Sharaf, A. E. M. M., Farghal, I. I., & Sofy, M. R. 2009. Role of gibberellic acid in abolishing the detrimental effects of Cd and Pb on broad bean and lupin plants. *Res. J. Agric. Biol. Sci*, 5, 668-673.

Fig 3.1 Analysis of variance for Shoot fresh weight (g) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid



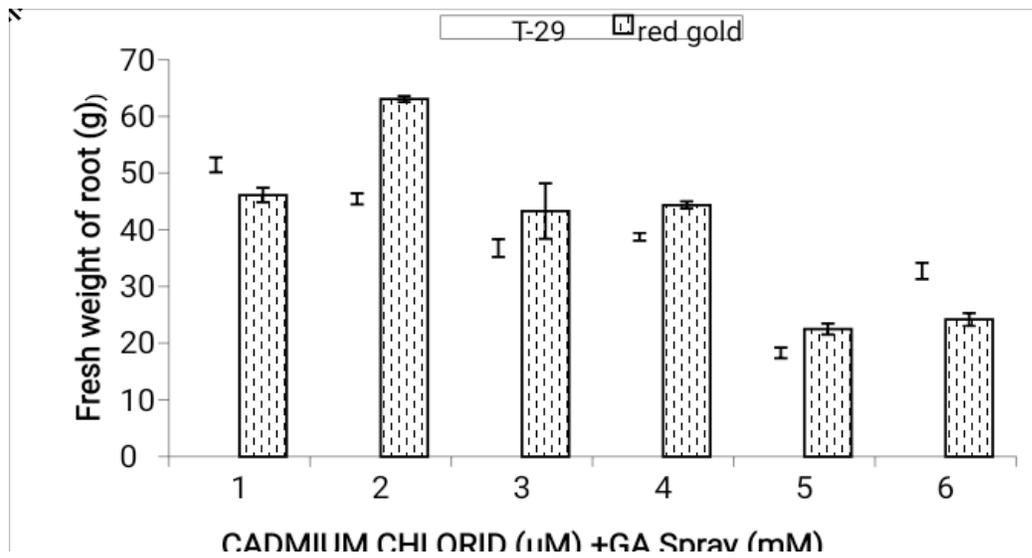
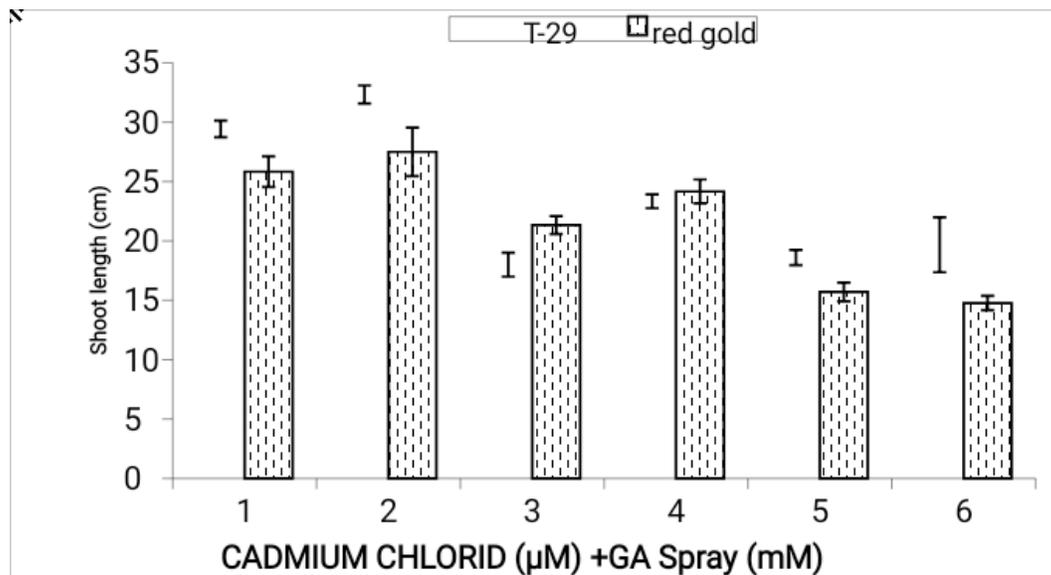


Fig 3.2 Analysis of variance for root fresh weight (g) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

Fig 3.3 Analysis of variance for shoot length (cm) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid



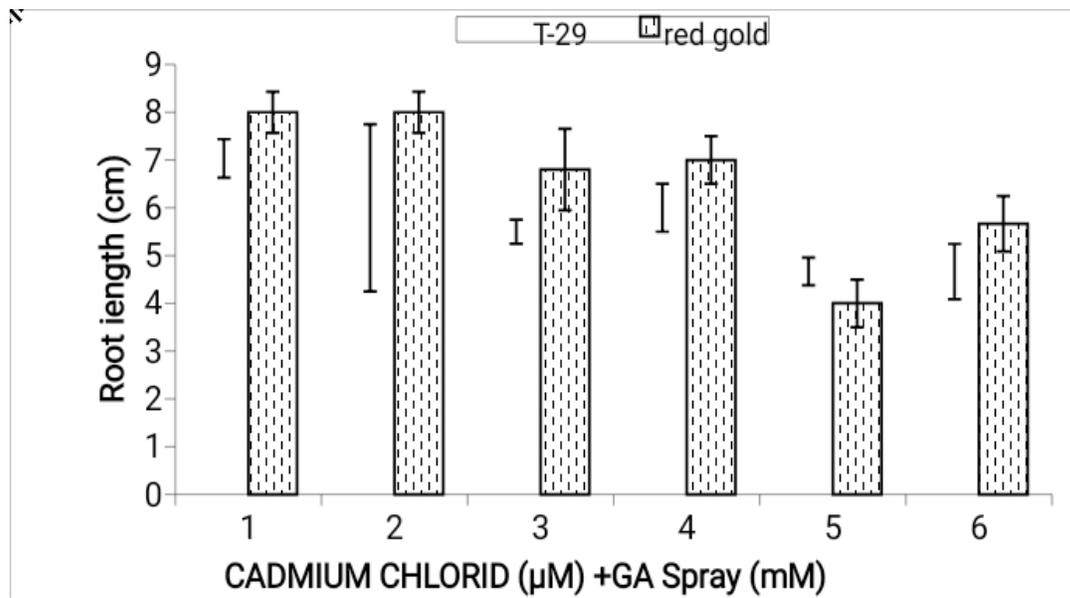


Fig 3.4 Analysis of variance for Root length (cm) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

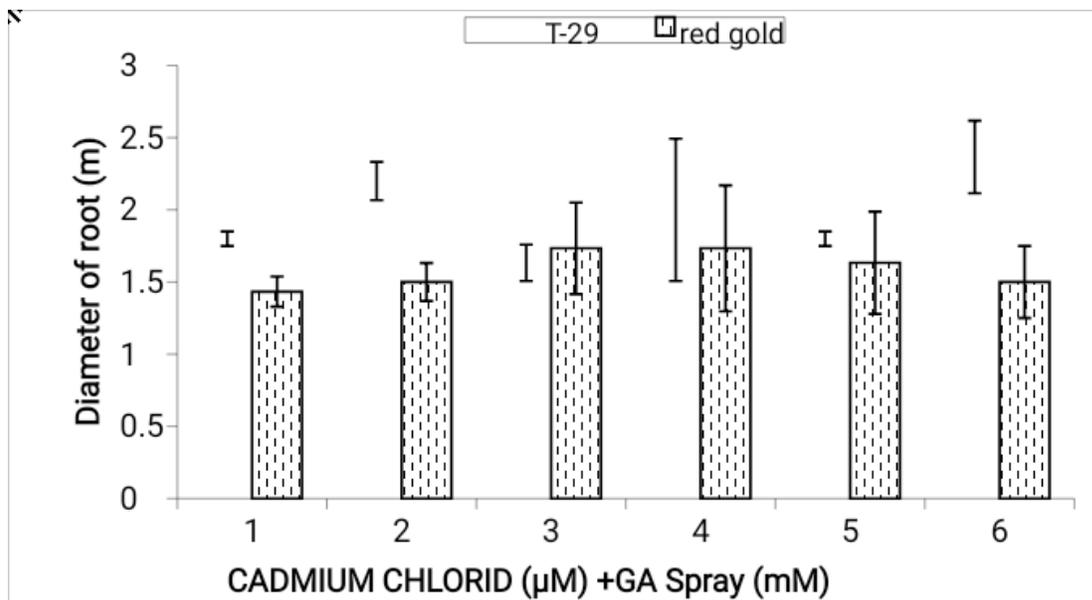


Fig 3.5 Analysis of variance for Root diameter (cm) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

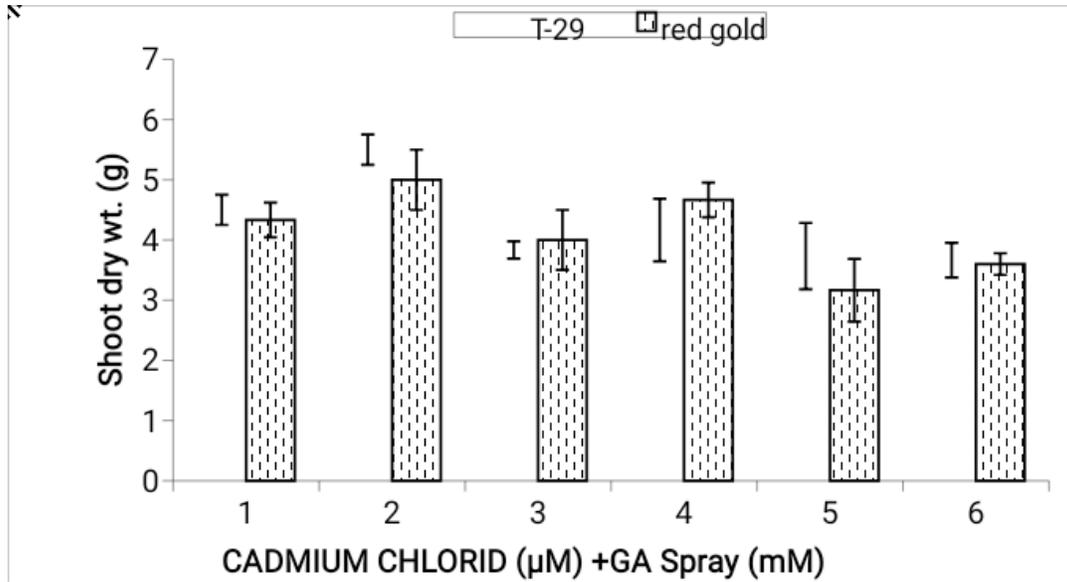


Fig 3.6 Analysis of variance for Shoot dry weight (g) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

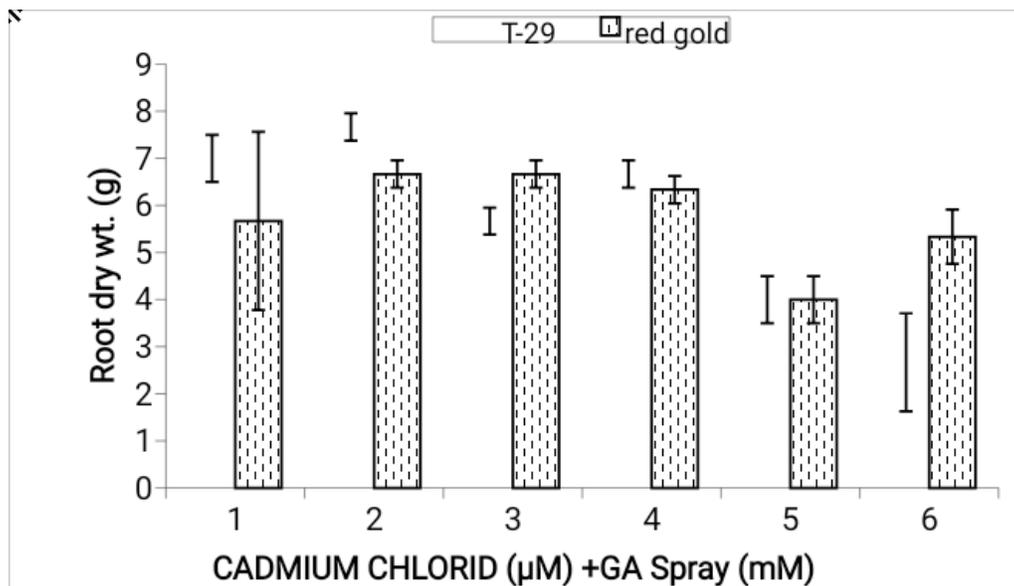


Fig 3.7 Analysis of variance for Root dry weight (g) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

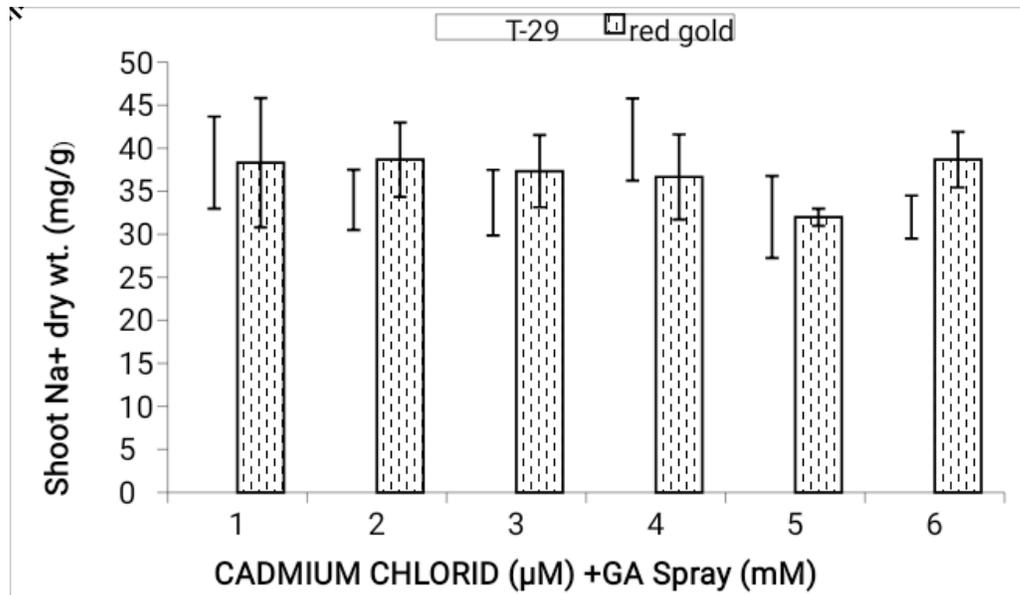


Fig 3.8 Analysis of variance for Shoot Na⁺ (mg/g dry wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

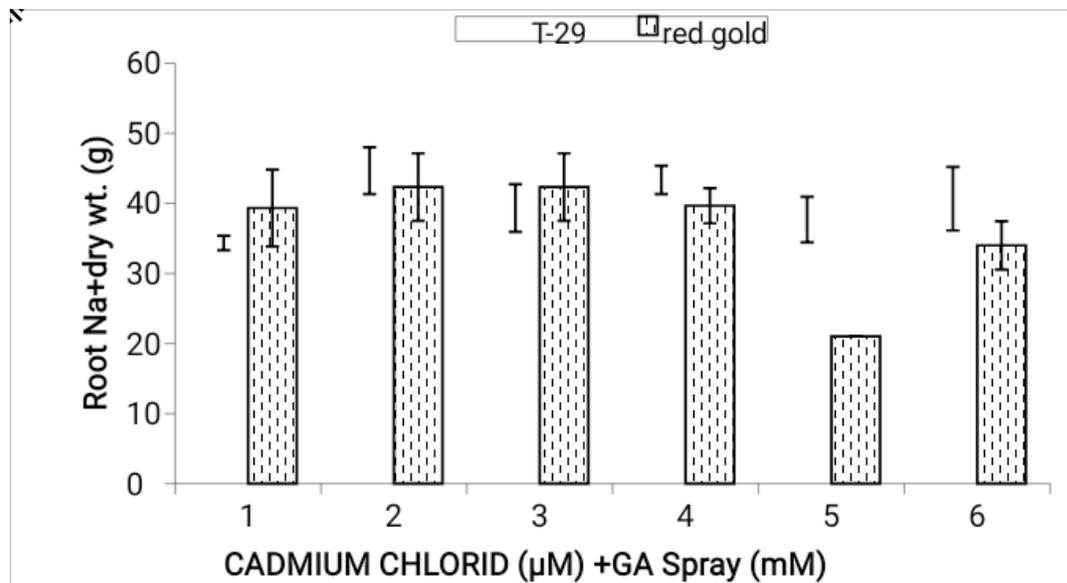


Fig 3.9 Analysis of variance for Root Na⁺ (mg/g dry wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

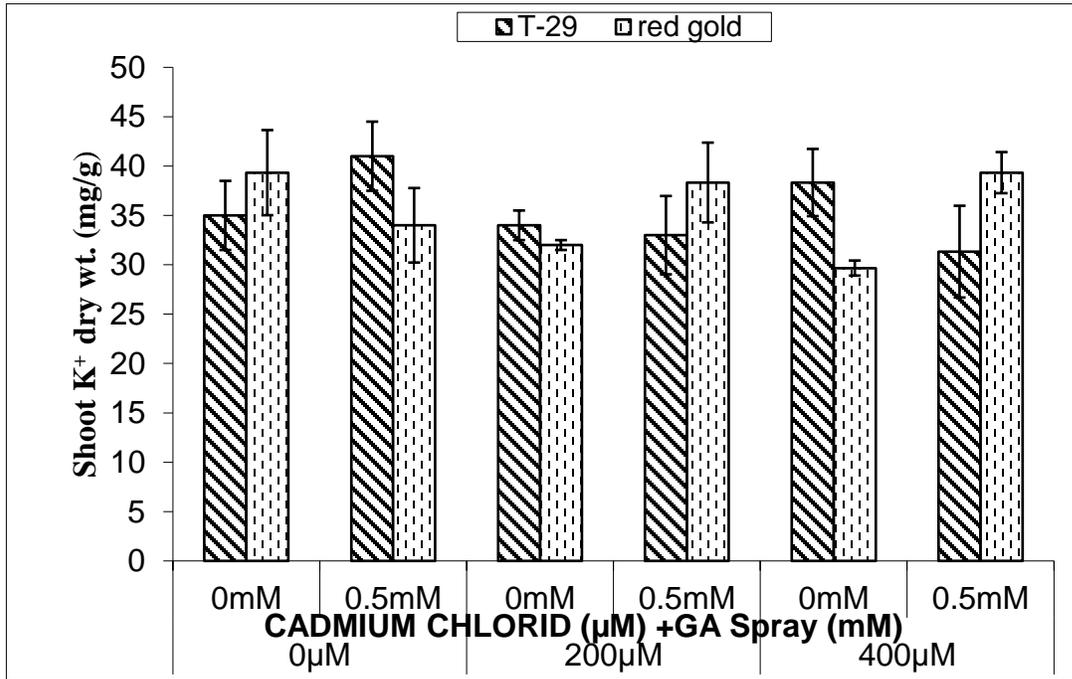


Fig 3.10 Analysis of variance for Shoot K⁺ (mg/g dry wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

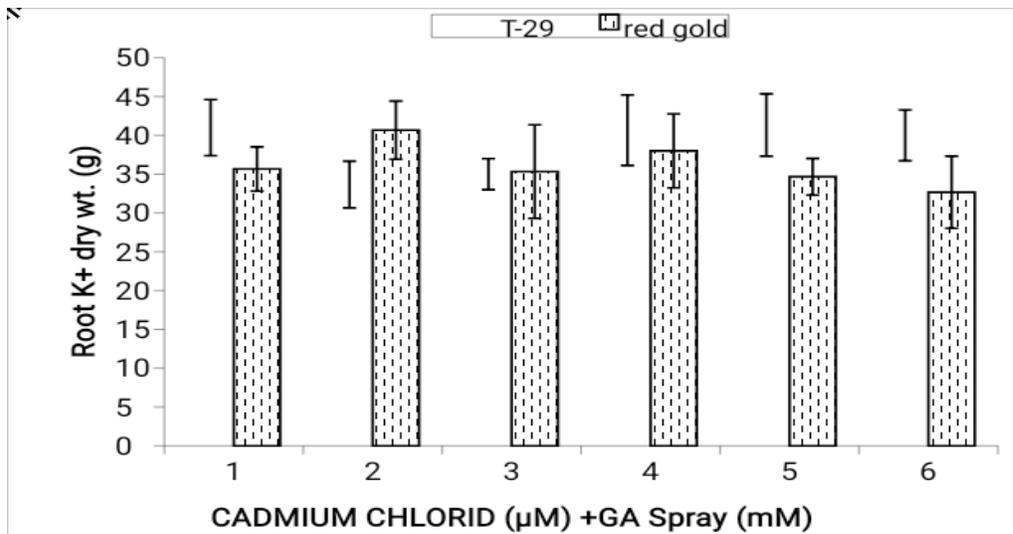


Fig 3.11 Analysis of variance for Root K⁺ (mg/g dry wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

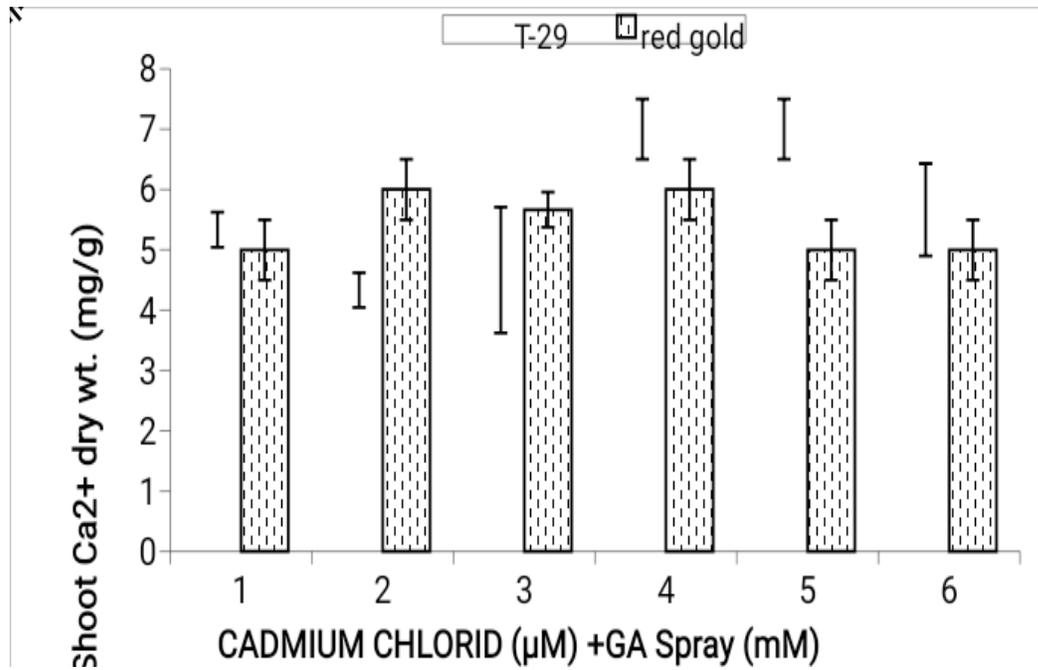


Fig 3.12 Analysis of variance for Shoot Ca⁺ (mg/g dry wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

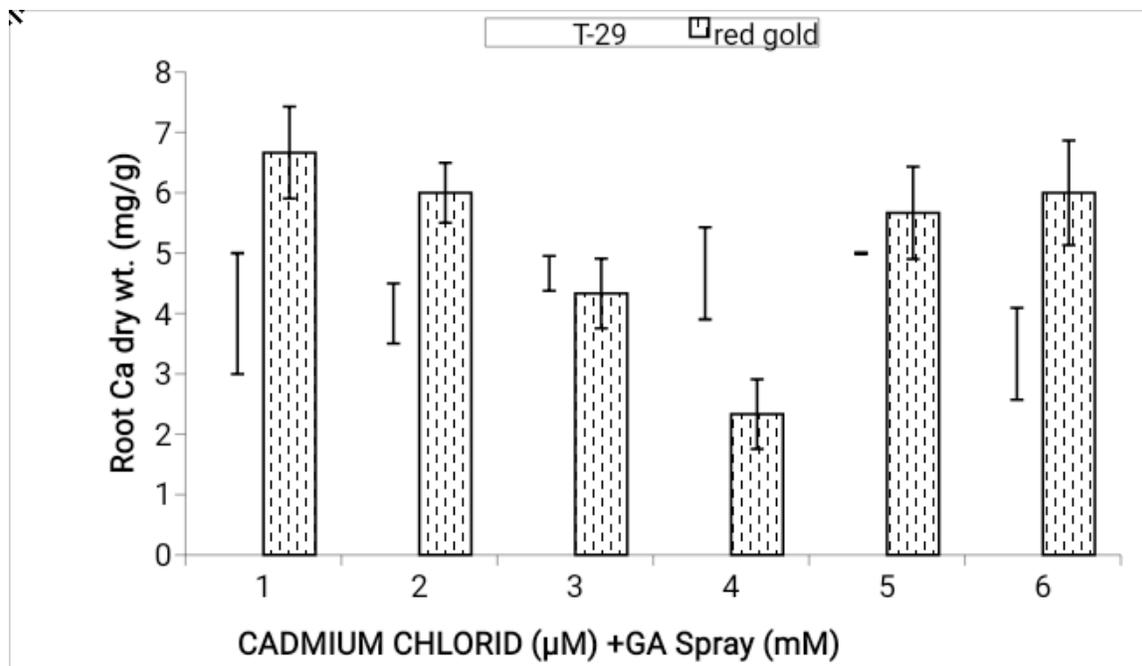


Fig 3.13 Analysis of variance for Root Ca⁺ (mg/g dry wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

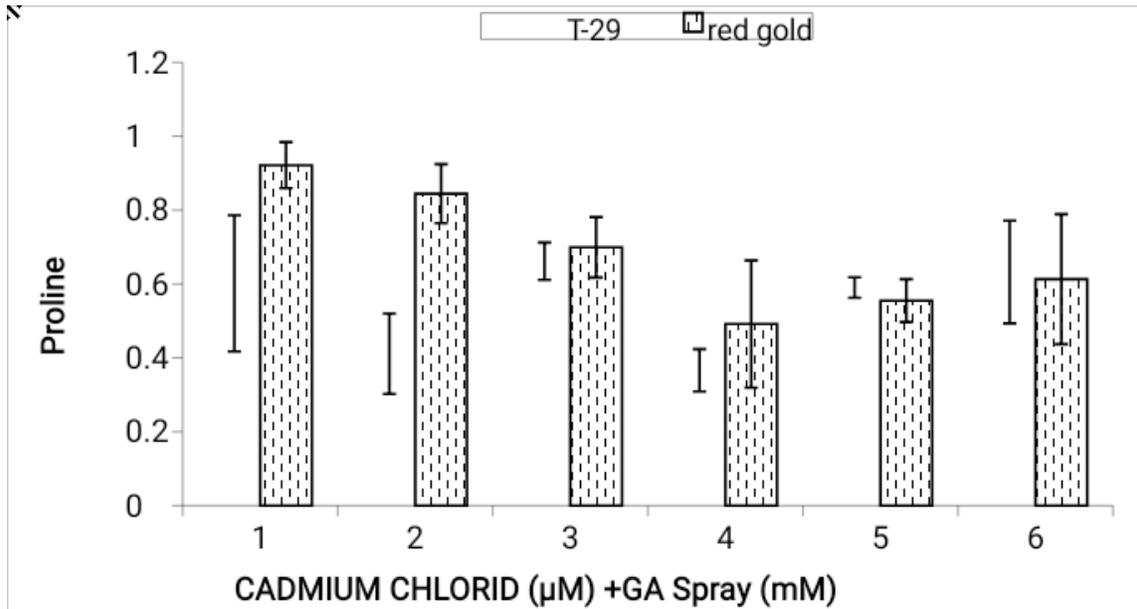


Fig 3.14 Analysis of variance for Proline of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

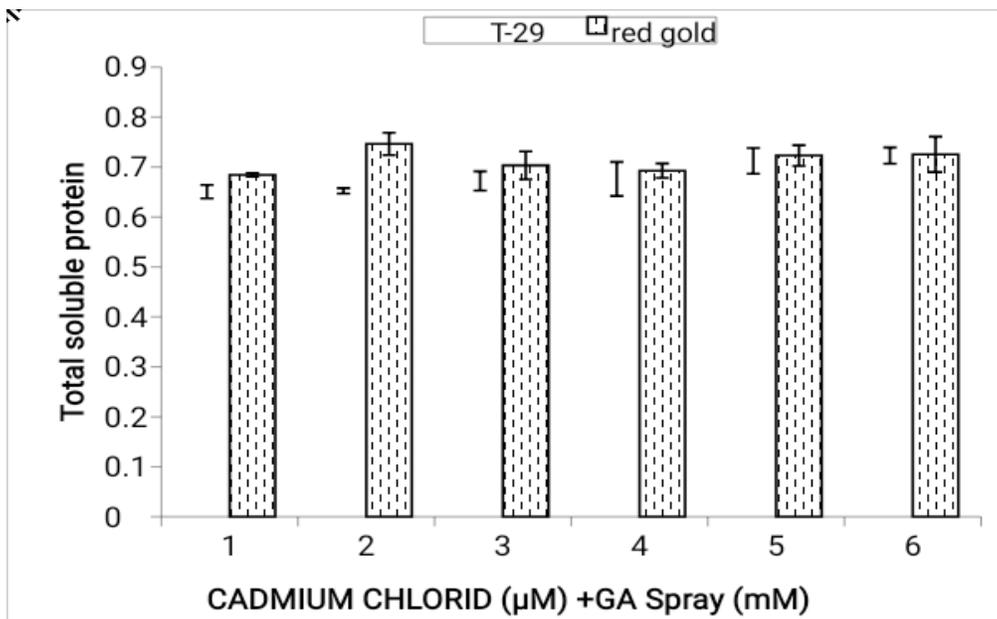


Fig 3.15 Analysis of variance for Soluble Proteins of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

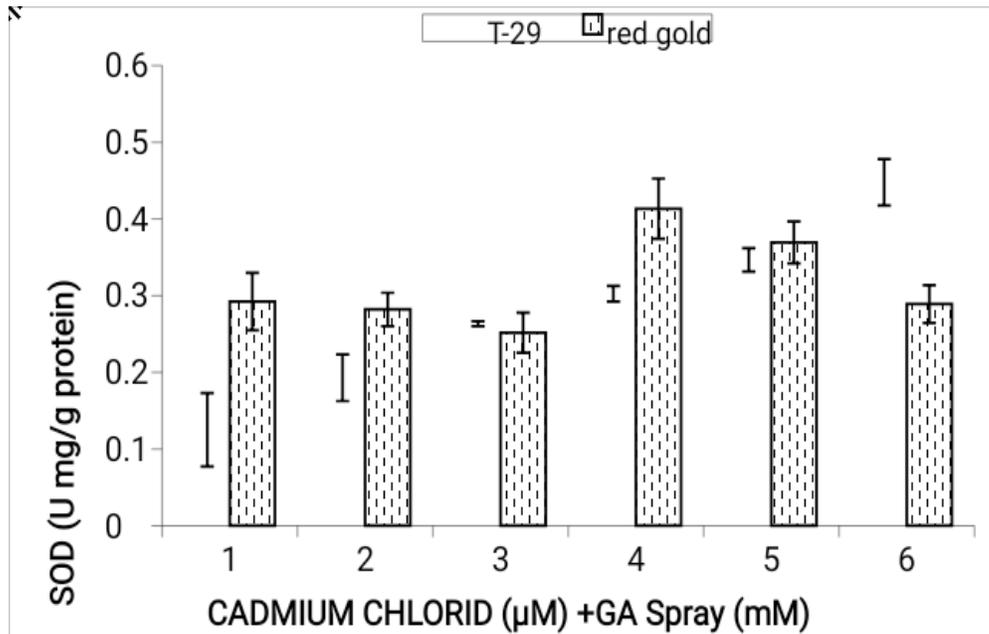
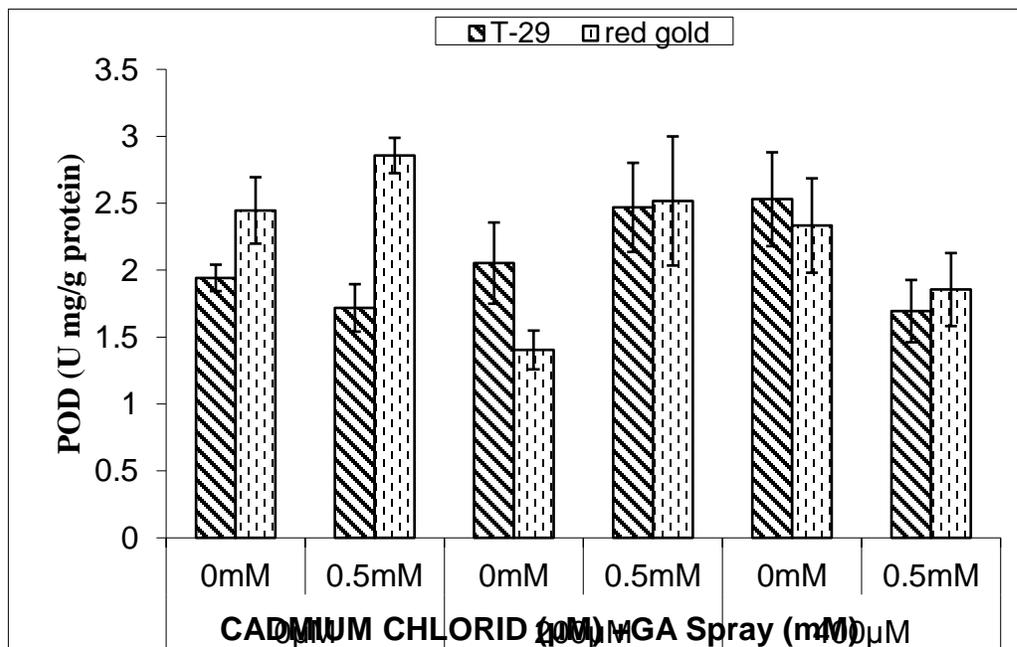


Fig 3.16 Analysis of variance for SOD (U mg/g protein) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid



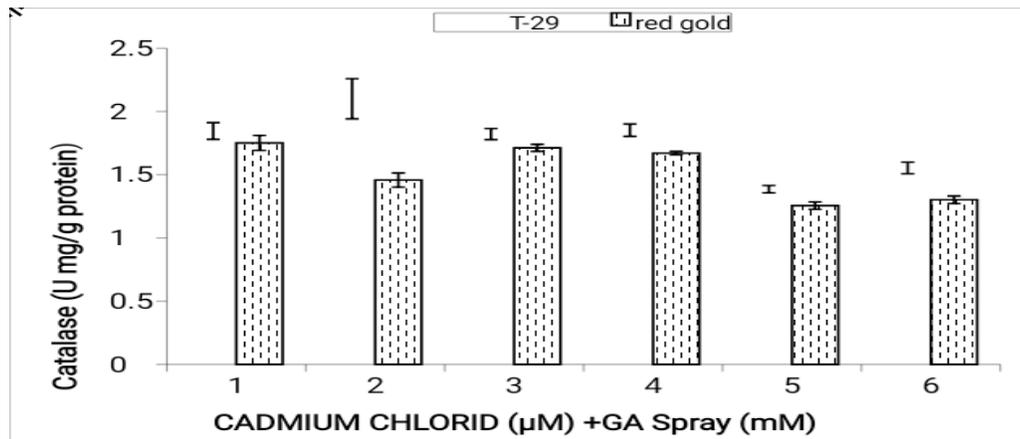


Fig:3.17 Analysis of variance for POD (U mg/g protein) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

Fig 3.18 Analysis of variance for Catalases (U mg/g protein) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

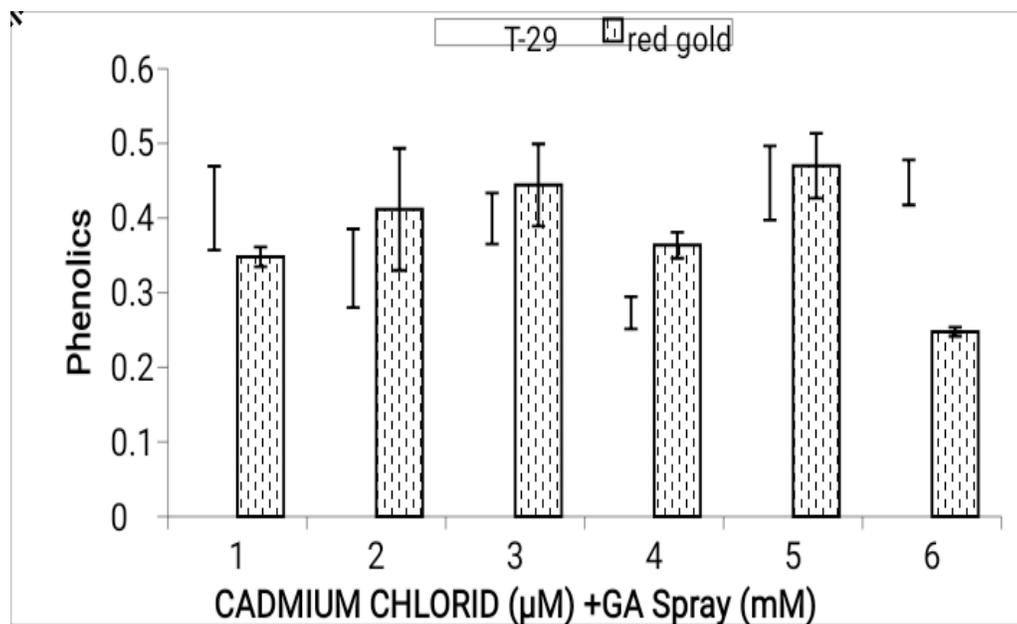


Fig 3.19 Analysis of variance for Phenolics of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

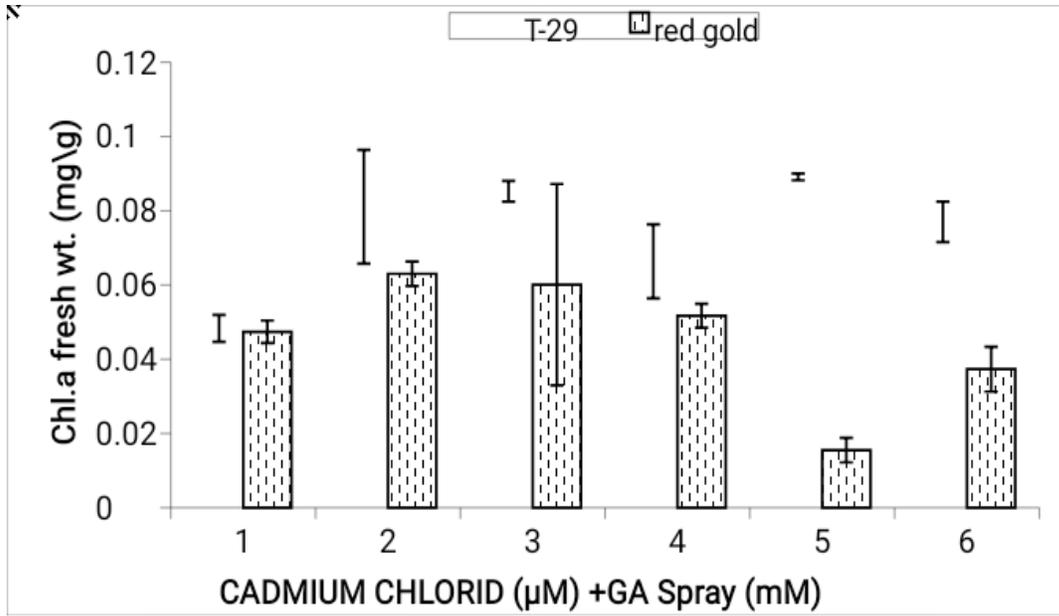
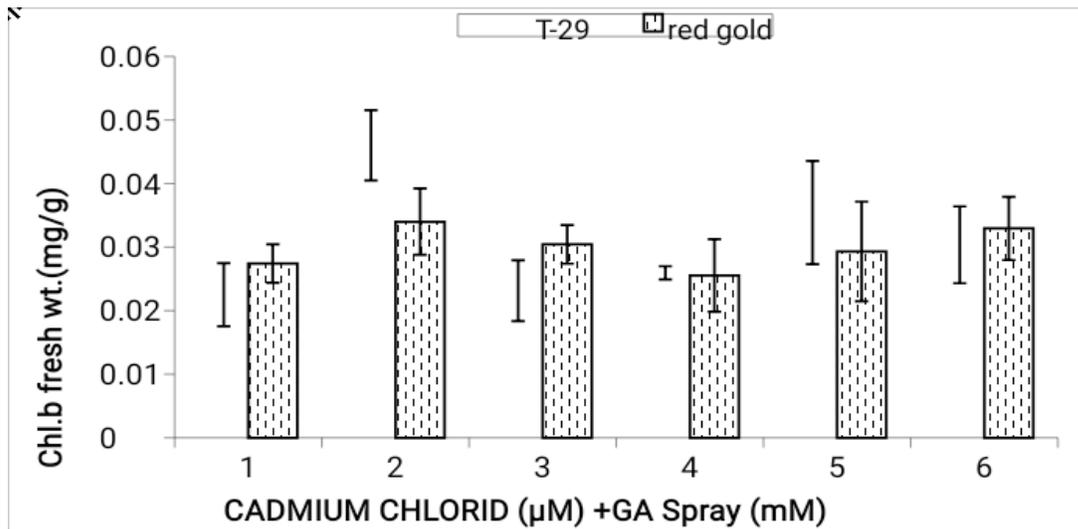


Fig 3.20 Analysis of variance for Chl.a (mg/g fresh wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

Fig 3.21 Analysis of variance for Chl.b (mg/g fresh wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid



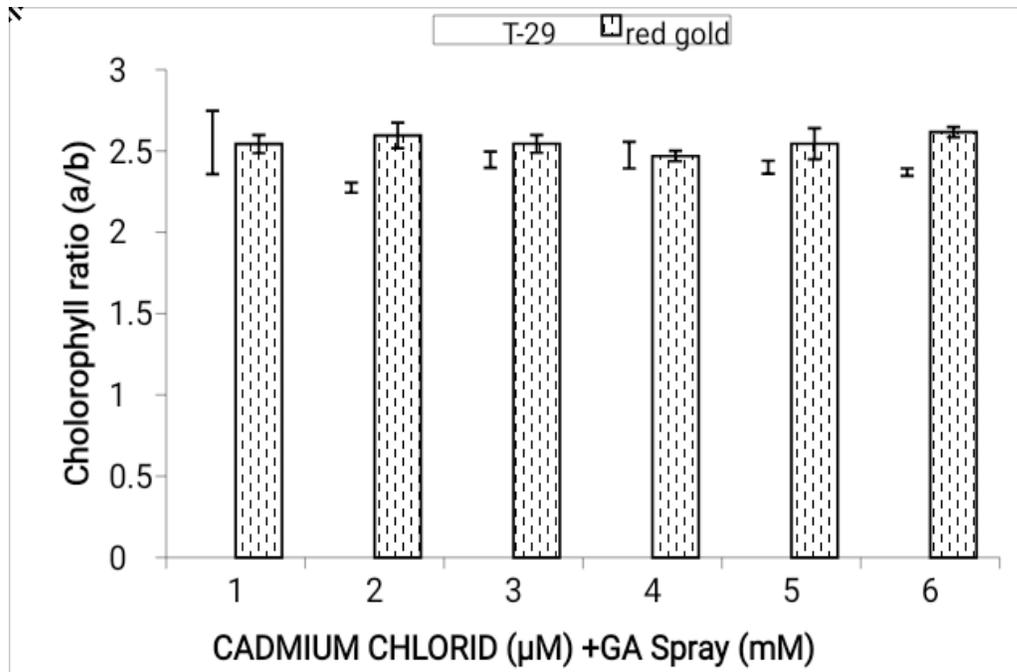


Fig 3.22 Analysis of variance for Chl.ab (mg/g fresh wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

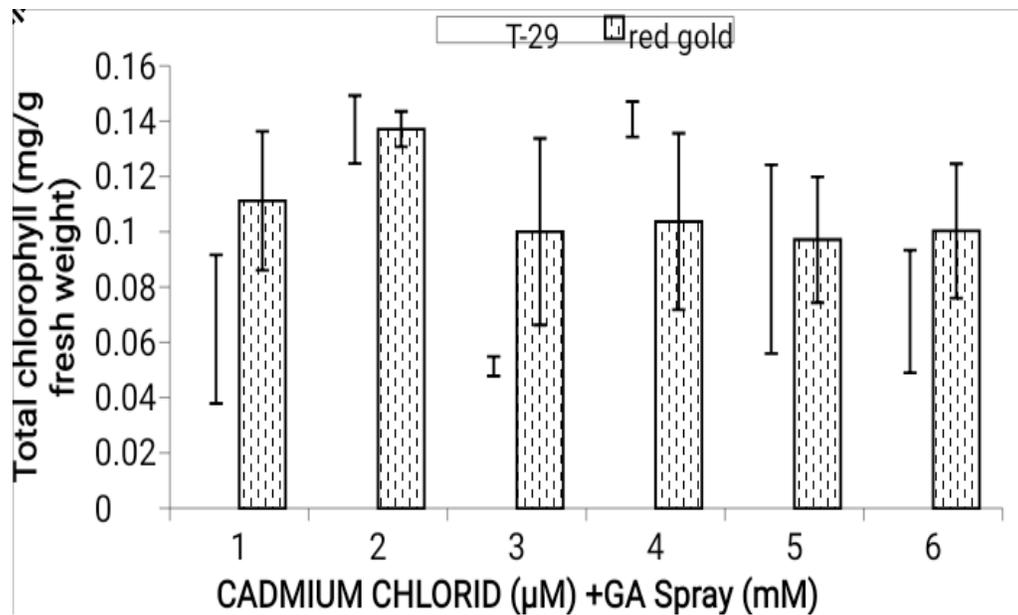


Fig 3.23 Analysis of variance for total Chl. (mg/g fresh wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

Fig 3.24 Analysis of variance for carotenoids (mg/g fresh wt.) of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

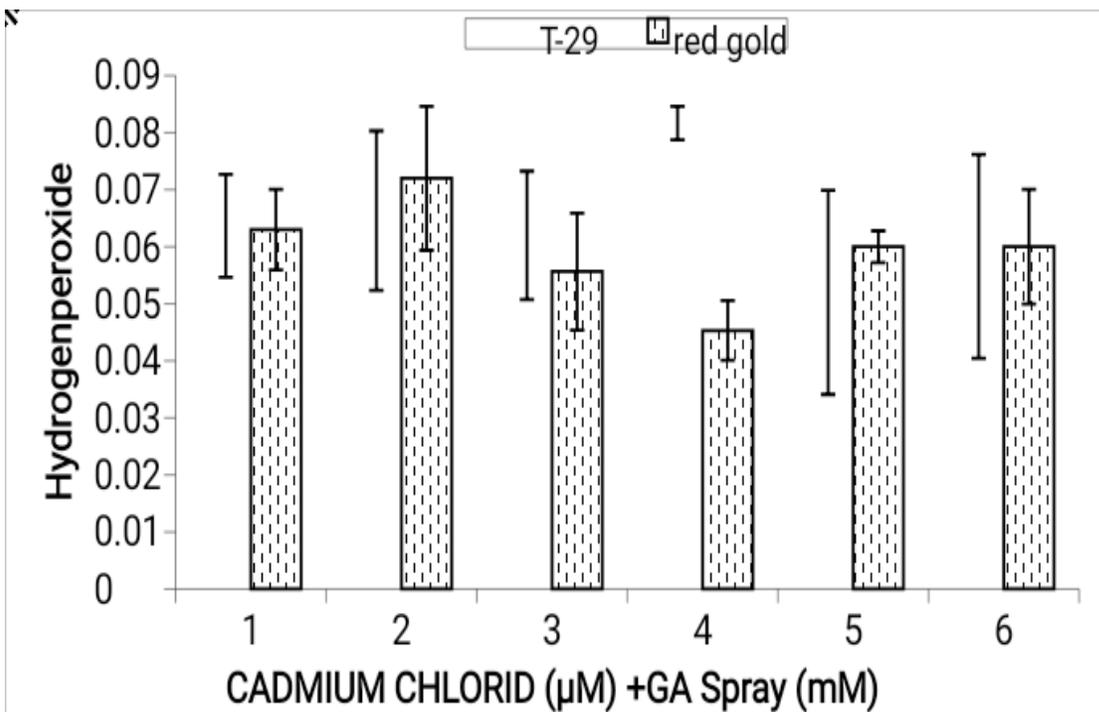
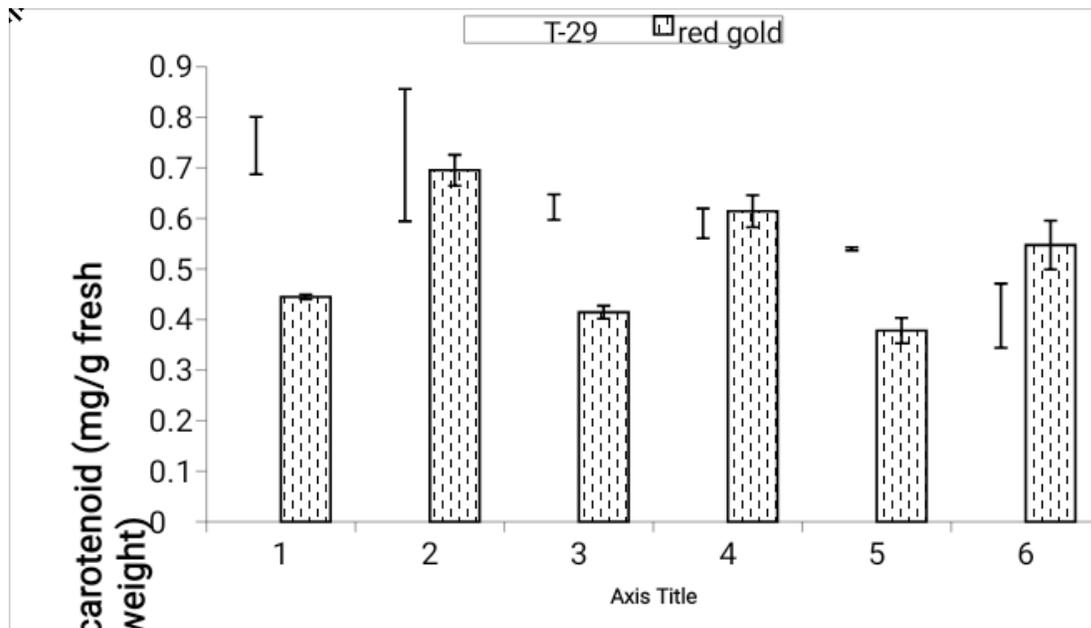


Fig 3.25 Analysis of variance for Hydrogen peroxide of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

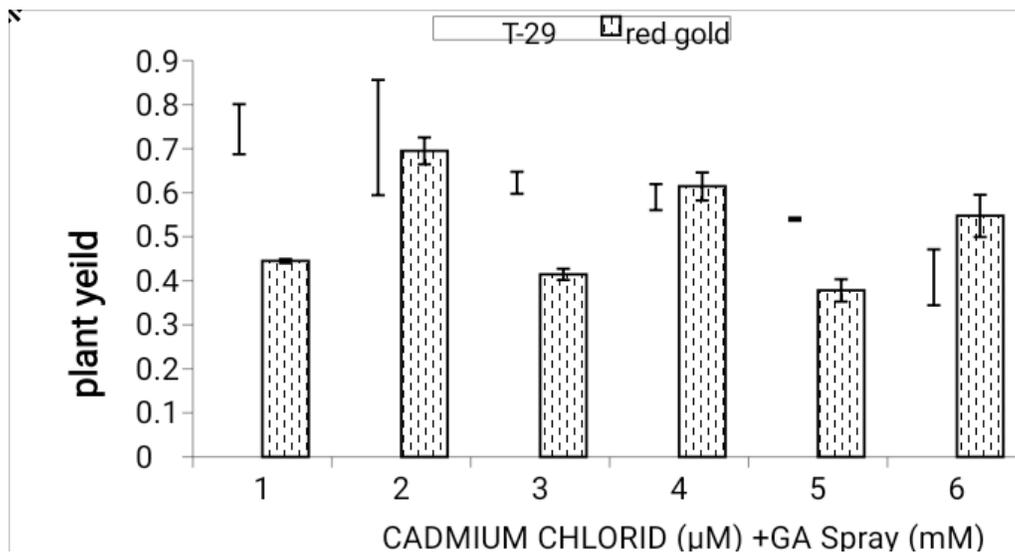


Fig 3.26 Analysis of variance for Plant Yeild of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid

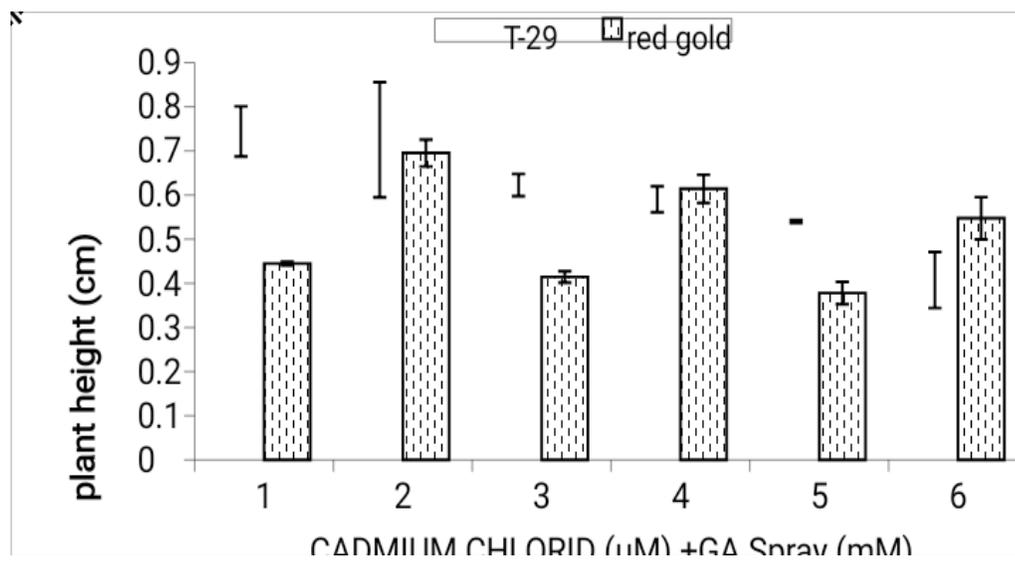


Fig 3.27 Analysis of variance for plant height of two carrot varieties grown under stress cadmium chloride and non-stress control with foliar spray of gibberellic acid