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Calcium Improves Heat Cucumber Tolerance, Growth and Production by Modulating the Antioxidant Enzymes and Phytohormones

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ABSTRACT

Calcium-rich products have been shown to improve the adverse effects of heat stress on cucumber plants. It is essential to explore how to enhance the heat tolerance of cucumber using calcium rich products complexes to Sugar Alcohol. The study explained the effects of Manni-Plex Ca-Zn (C1, Ca, 2.5% Zn), Manni-Plex Ca-Zn (C2, Ca, 2.5% Zn) and Manni-Plex Cal-Mag (C3, 7.9%Ca, 4.35%Mg) at ratio 100 ml/100L water, 200 ml/100L water and 300 ml/100L water, respectively, on cucumber plants (Cicely and Viva varieties) under heat conditions by using the following parameters: plant height, leaf area, number of flowers, failed flower percent, GA3, ABA and antioxidant enzymes content. Under heat conditions, foliar application of Manni-Plex Ca-Zn, and Manni-Plex Cal-Mag were observed to increase cucumber vegetative growth and yield components. The obtained results also showed that the plant height, leaf area, number of flowers, GA3 and the activity of antioxidant enzymes (CAT and POD) content significantly improved in plants treated foliarly with Manni-Plex Ca-Zn, and Manni-Plex Cal-Mag compared to control plants. On the contrary, the highest content of ABA and failed flower percent were found in control plants compared to all the other treatments. Furthermore, the correlation study revealed that cucumber yield correlated positively with leaf area and number of leaves as well as negatively with failed flower percent.

Keywords: *Cucumis sativus* L, Sugar Alcohol, plant hormones, Catalase, peroxidase, plant growth, high temperature, total yield

INTRODUCTION

High temperature is one of the most important abiotic factors that affecting negatively on plant growth and its production. High temperature causes physiological damages in plants, including protein denaturation and oxidative injuries to membrane lipids as well as reducing total yield and quality of crops (Schlenker and Roberts, 2009 and Sung et al.2003).

Cucumber (*Cucumis sativus* L.) is an important vegetable crop that is cultivated globally, especially in tropical and subtropical regions. The optimum temperature required for the growth and development of cucumber is 20° C - 32° C. (Thompson *et al.* 1957). High temperatures (above 40°C) can often lead to heat injuries to cucumber plants, and wilt and dead tissue are the common symptoms that appeared on cucumber leaves and stems when the temperature reaches to 50°C, at short time (Talanova *et al.*, 2006).In Egypt, cucumber is planted in open fields during the warm growing seasons, where cucumber plants commonly face the composite stress of strong light and high temperature, or severe temperature increases in greenhouses. Even if ventilation systems are utilized, it is difficult to reduce heat accumulation in greenhouses. Accordingly, cucumber growth is frequently delayed by extreme daytime temperatures. This is effect on cucumber

growth, physiological characteristics as well as fruit quantity and quality (Yu *et al.*, 2018).

Calcium channel blockers stimulate survival of plants after heat treatment, additionally,calcium could act as a second messenger in various signaling pathways limiting heat-induced oxidative damage. (Larkindale and Knight, 2002). Lack of calcium causes necrosis of young leaves and reduction in the growth, leaf size, yield, and in an extreme situations (Hao *et al.*, 2004). The supplementation of calcium can play a vital role in inducing heat stress tolerance in plants by the better activity of antioxidant defense (Kolupaev *et al.*, 2005). This research work examined the effectiveness of the calcium application to enhance the heat tolerance of cucumber and provide the basis for additional improvements in the tolerance of cucumber plants to high-temperature stress.

MATERIALS AND METHODS

The experiments were carried out at a private Greenhouse located at the Maghaghah, Minya Governorate, Upper Egypt during two summer seasons from April to September of years 2019 and 2020. Two different commercial cucumber cultivars seeds (Viva and Cicely) were transplanted in the greenhouse. Seedlings of cultivar were planted in both sides of 2 rows, of 12.4 m length and 0.6 m width, with 0.4 m distance intervals between seedlings. (Figure1). Foliar application of Manni-Plex Cal-

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Zn at the rate (C1: 100 ml/L water and C2: 200 ml/L water) and Manni-Plex Cal-Mag (C3: 300 ml/L water) have been tested on the Viva and Cicely cucumber varieties. Control was untreated cucumber plants. These products were purchased from BRANDT International, Florida, USA. The three foliar dosages were applied every 14 days within the season on the Viva and Cicely varieties.

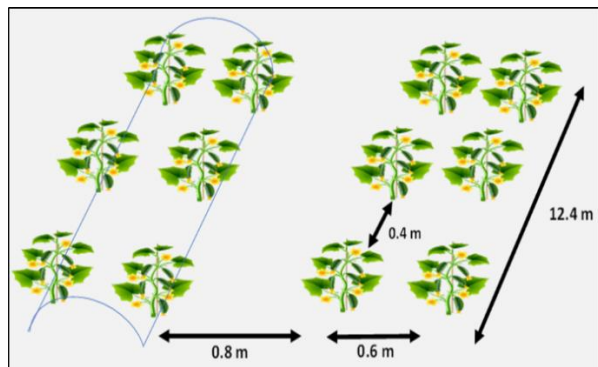


Figure 1. The schematic diagram of the experimental unit.

All treatments were applied at the vegetative growth stage until the start of the flowering stage. The treatment of distributed randomly and each treatment was repeated four times. The meteorological data has been recorded during the experimentation (Figure 2). Agronomical and physiological data including plant height, number of flowers, leaf area, number of falling of flowers, failed flowers, yield plot⁻¹, gibberellic acid (GA3), abscisic acid (ABA), catalase (CAT) and peroxidase (POD) were collected from 12 plants of each treatment.

Phytohormones were determined in plant leaves. Briefly, freeze-dried cucumber leaves were mild to fine

powder and 10 mg of fine powder was washed three times with 80% methanol (v/v) and 2,6-bis (1,1-dimethylethyl)-4-methylphenol at 4°C in the dark. The final extract was filtrated and dehydrated according to the method described by Fales et al. (1973). The quantification of abscisic acid (ABA) and gibberellic acid (GA3) were determined using pure standards of the hormones and a Microsoft program to calculate the concentrations of the identified peaks.

Antioxidant enzymes were assessed using the method stated by Polle *et al.* (1994). Briefly, approx. 1 g of the fourth fresh leaf were ground in liquid nitrogen and homogenized in 5ml potassium phosphate buffer. The homogenates were then centrifuged at 14,000 ×g for 18 min at 5°C and the supernatants were used to assess the activity of superoxide dismutase (POD) according to Tian and Yu (2009). and catalase (CAT) by Aebi (1984).

Statistical analysis

The experimental data of dual successive seasons, 2019 and 2020, were subjected to combined analysis after carrying out the normality distribution test (Shapiro and Wilk, 1956) and homogeneity test (Hartley, 1950). Furthermore, means were compared at the 0.05 level according to Duncan's test using the Statistica 7 program.

RESULTS AND DISCUSSION

Metrological data

The climate data was recorded during the experimentation (Figure 2). According to the climate data are shown in table 1, the area of the experiment is classified as a hot desert by the Köppen-Geiger climate classification system. The highest average temperatures recorded during the cucumber growing season were from May to September of 2019 and 2020.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Record high °C (°F)	30.7 (87.3)	33.8 (92.8)	41.4 (106.5)	45.2 (113.4)	48.0 (118.4)	47.5 (117.5)	43.6 (110.5)	44.6 (112.3)	43.4 (110.1)	42.0 (107.6)	37.6 (99.7)	31.0 (87.8)	48.0 (118.4)
Average high °C (°F)	20.4 (68.7)	22.4 (72.3)	25.8 (78.4)	31.2 (88.2)	35.4 (95.7)	36.9 (98.4)	36.6 (97.9)	35.9 (96.6)	34.0 (93.2)	31.3 (88.3)	25.9 (78.6)	21.4 (70.5)	29.8 (85.6)
Daily mean °C (°F)	11.7 (53.1)	13.5 (56.3)	16.8 (62.2)	21.9 (71.4)	26.2 (79.2)	28.4 (83.1)	28.7 (83.7)	28.0 (82.4)	26.1 (79.0)	23.0 (73.4)	17.7 (63.9)	13.0 (55.4)	21.3 (70.3)
Average low °C (°F)	3.9 (39.0)	5.2 (41.4)	8.2 (46.8)	12.6 (54.7)	16.4 (61.5)	19.3 (66.7)	20.4 (68.7)	20.3 (68.5)	18.4 (65.1)	15.5 (59.9)	10.2 (50.4)	5.8 (42.4)	13.0 (55.4)
Record low °C (°F)	-3.4 (25.9)	-0.3 (31.5)	1.0 (33.8)	4.5 (40.1)	9.0 (48.2)	14.3 (57.7)	16.0 (60.8)	16.2 (61.2)	13.5 (56.3)	10.0 (50.0)	3.0 (37.4)	-0.8 (30.6)	-3.4 (25.9)
Average precipitation mm (inches)	0 (0)	0 (0)	1 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.1)	1 (0.0)	1 (0.0)	5 (0.2)
Average precipitation days (≥ 1.0 mm)	0.1	0.1	0.1	0	0	0	0	0	0	0	0	0	0.3
Average relative humidity (%)	63	56	52	43	37	40	46	51	53	55	61	67	52
Mean monthly sunshine hours	251.5	253.4	283.8	304.2	339.1	367.2	382.0	363.3	315.9	309.4	260.9	235.7	3,666.4
Source 1: NOAA ^[20]													
Source 2: Climate Charts ^[21]													

Figure 2. Shows the estimated averages of the temperature records for Minya Governorate since 2019 and 2020

Effect of treatments on agronomical traits of cucumber

Data in figure 3 shows the effect of commercial products, containing on Ca, on agronomical attributes (leaf area, number of flowers, failed flower and yield per m²) of cucumber plants in Viva variety. Significant differences were observed between agronomical attributes, except plant height (table 2). Moreover, the maximum leaf area, number of flowers, and yield per plot was recorded in cucumber plants treated with Manni-Plex Cal-Zn at a rate of 200

ml/100L (C2) followed by Manni-Plex Cal-Mag at a rate of 300 ml/100L water (C3) and Manni-Plex Cal-Zn at a rate of 100 ml/100L (C1) compared to control. On the other hand, the maximum value of flowers failed percent was recorded in control treatment while the minimum values were in cucumber plants treated with Manni-Plex Cal-Zn and Manni-Plex Cal-Mag at ratio 200 ml/100L and C3, 300 ml/100L water, respectively.

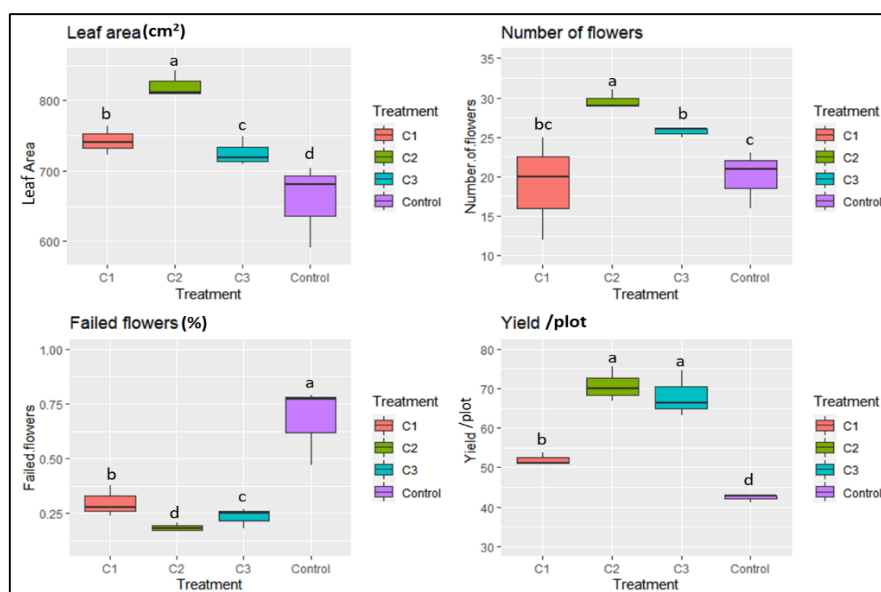


Figure 3. Effect of Manni-Plex Ca-Zn , Manni-Plex Cal-Mag and control on Leaf area (cm²), Number of flowers, Failed flowers (%) and yield of Viva variety. Bar indicating to stander Error.

Similar results were observed on the Cicely variety. Data in figure 4 and table 2 showed that significant differences were recorded regarding all the agronomical traits, containing, plant height, leaf area, number of flowers, failed flower percent and yield. Moreover, the maximum leaf area, number of flowers, and yield per plot was achieved in plants treated with Manni-Plex Cal-Mag (C3, 300

ml/100L water) followed by Manni-Plex Cal-Zn (C2, 200 ml/100L) and Manni-Plex Cal-Zn (C2, 100 ml/100L) compared to control. On the other hand, the maximum value of flowers failed percent was observed in control treatment while the minimum values were in plants treated with Manni-Plex Cal-Mag (C3) and Manni-Plex Cal-Zn (C2 & C1)

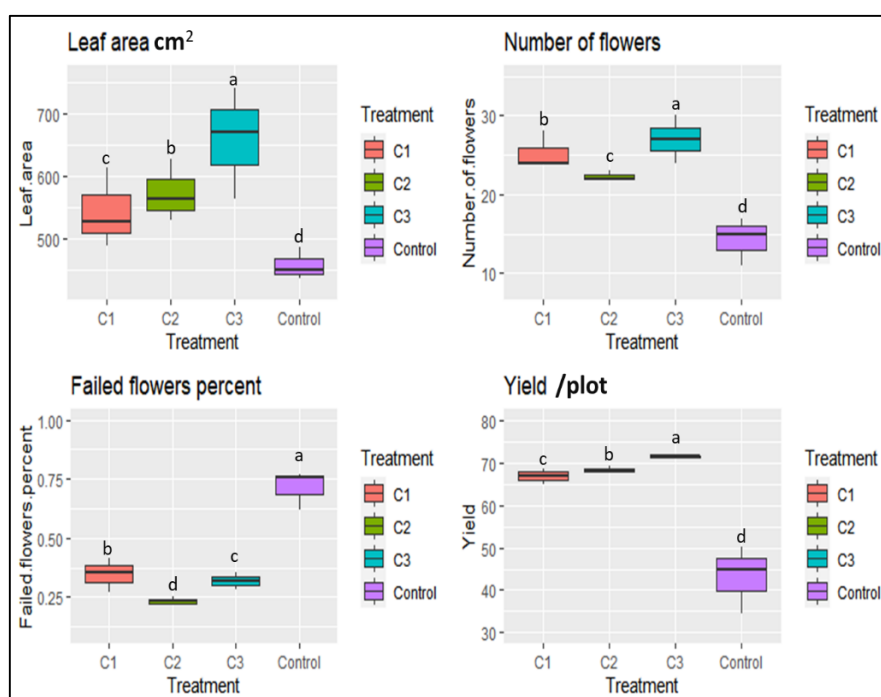


Figure 4. Effect of Manni-Plex Ca-Zn , Manni-Plex Cal-Mag and control control on Leaf area (cm²), Number of flowers, Failed flowers (%) and yield of Cicely variety. Bar indicating to stander Error.

Furthermore, the correlation matrix showed a strong positive correlation between the yield and the leaf area and the number of flowers. However, the yield was strongly negatively correlated with failed flowers %. (Fig.5). The enhancement in vegetative growth and productivity of cucumber plants treated with Manni-Plex Ca-Zn or Manni-Plex Cal-Mag could be associated with calcium and other

micronutrients (Zn). Whereas these products contain high content of calcium and an adequate amount of zinc and magnesium.

Calcium (Ca) play important role in the improvement of the growth, leaf size and the yield of several plants (Jiang and Huang, 2001). It also actively contributes to membrane function cell wall structure and cellular

signaling responses (White and Broadly, 2003; Hepler, 2005) under normal and abnormal (environmental stresses) conditions. A similar finding was observed by several researchers who found that Foliar application of Calcium

significantly improved growth parameters including, vine length, number of branches, fruit length, fruit diameter, number of fruits/ plant and yield of cucumber plants (Siddique *et al.*, 2017).

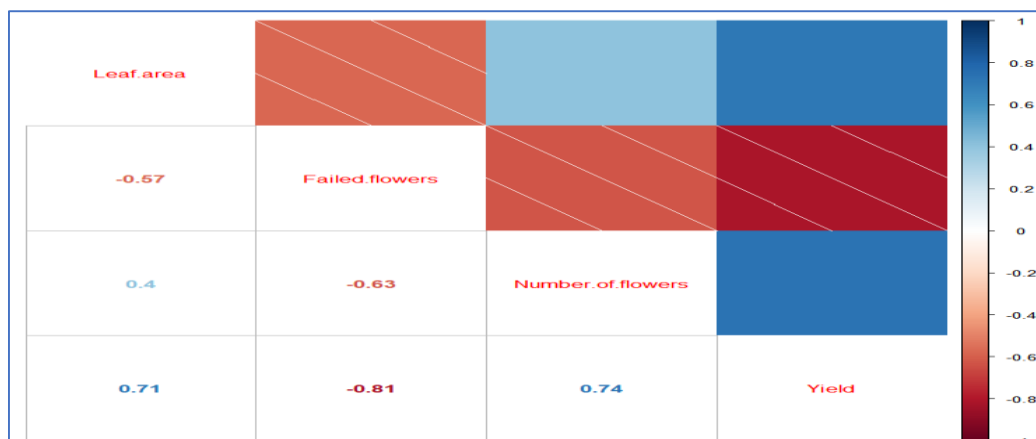


Figure 5. The correlation matrix between yield, number of flowers, failed flowers and leaf area

Effect of treatments on phytohormones and antioxidant enzymes

Plant hormones content and activity of antioxidant enzymes are significantly affected by applied treatments, in both cultivars of cucumber (Cicely and Viva varieties), as shown in table 2. Compared to control, the maximum concentration of GA3, CAT and POD were observed in cucumber plants treated with Manni-Plex Cal-Zn and

Manni-Plex Cal-Mag. On the other side, the maximum values of abscisic acid (ABA) were obtained in control while the minimum values were observed in plants treated with Manni-Plex Cal-Zn and Manni-Plex Cal-Mag. Similar results were observed by numerous authors who found that wheat plants treated with Ca²⁺ under heat stress had higher GA3, CAT and POD activities than untreated plants (Jiang and Huang, 2001; Tian, and Yu, 2009 and Xu *et al.*, 2013).

Table 2. Effect of Manni-Plex Ca-Zn , Manni-Plex Cal-Mag and control and control on gibberellic acid (GA3), abscisic acid (ABA), catalase (CAT), and peroxidase (POD).

Treatment	Doses	Plant Height (cm)		GA3 $\mu\text{g g}^{-1}$ FW		ABA $\mu\text{g g}^{-1}$ FW		CAT(units mg^{-1} protein)		POD(units mg^{-1} protein)	
		Viva cultivar	Cicely cultivar	Viva cultivar	Cicely cultivar	Viva cultivar	Cicely cultivar	Viva cultivar	Cicely cultivar	Viva cultivar	Cicely cultivar
(C1)	100 ml/100Lwater	161.3 ^a	168.3 ^b	0.9 ^b	0.11 ^b	0.11 ^b	0.13 ^b	12.1 ^a	12.5 ^b	7.02 ^a	9.2 ^c
(C2)	200 ml/100Lwater	162.2 ^a	170.2 ^b	0.12 ^a	0.12 ^a	0.09 ^b	0.12 ^b	9.8 ^b	10.9 ^b	7.9 ^a	10.1 ^b
(C3)	300 ml/100Lwater	163.7 ^a	201.7 ^a	0.13 ^a	0.12 ^a	0.10 ^b	0.13 ^b	10.3 ^b	11.1 ^b	7.1 ^a	11 ^b
Control	100L Water	161.9 ^a	142.3 ^c	0.05 ^c	0.07 ^c	0.15 ^a	0.18 ^a	7.1 ^c	8.2 ^c	5.1 ^b	6.5 ^c

CONCLUSION

The heat stress could adversely affect the physiological and agronomical characteristics of cucumber plants, moreover, heat stress causes severe calcium and zinc deficiency that leads to deterioration in the plant's potential to form healthy and productive flowers. For this reason, the controlled application of the high-tech complexes sugar alcohol with calcium zinc and calcium magnesium could boost the marketable yield by 67% and 65% in Viva and Cicely varieties, respectively. Additionally, the application of BRANDT products complexed to sugar alcohol and rich in Calcium Manni-Plex Ca-Zn and Manni-Plex Cal-Mag increase the level of gibberellic acid in cucumber leaves as well as regulating the levels of ABA and antioxidant enzymes (CAT and POD).

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يحسن الكالسيوم تحمل نباتات الخيار للحرارة من خلال تعديل انزيمات الاكسدة و الهرمونات النباتية

حسن علي حسن^١ ، سوزى محمد كامل عبد العزيز^٢ ، هاشم محمد^٣ و محمود جعفر^٤

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لقد ثبت أن المنتجات الغنية بالكالسيوم تعمل على تحسين الآثار الضارة للإجهاد الحراري على نباتات الخيار. من الضروري استكشاف كيفية تعزيز تحمل الحرارة للخيار باستخدام المنتجات الغنية بالكالسيوم. أوضحت الدراسة تأثير الرش الورقي لبعض المغذيات الغنية بالكالسيوم مثل Manni-Plex Cal-Zn (C1) بمعدل 100 مل / ١٠٠ لتر (C2)، Manni-Plex Cal-Zn بمعدل 200 مل / ١٠٠ لتر و Manni-Plex Cal-Mag (C3) بمعدل 300 مل / ١٠٠ لتر ماء على ارتفاع النبات، مساحة الورقة، عدد الأزهار، نسبة سقوط الأزهار و محتوى الجبرلين (GA3)، الأبيسيسك (ABA) ومحتوى الإنزيمات المضادة للأكسدة [الكاتاليز (CAT) والبروكسيداز (POD)] لنباتات الخيار لصنفى Viva و Cicely تحت ظروف الحرارة المرتفعة. لوحظ أن الرش الورقي Manni-Plex Cal-Zn (C2) و Manni-Plex Cal-Mag (C3) زاد من النمو الخضري للخيار ومكونات المحصول. كما أظهرت النتائج المتحصل عليها أن ارتفاع النبات ومساحة الورقة وعدد الأزهار و GA3 ونشاط محتوى الإنزيمات المضادة للأكسدة (الكاتاليز والبروكسيداز) تحسنت بشكل ملحوظ في النباتات المعاملة بالمركبات الغنية بالكالسيوم مثل Manni-Plex Cal-Mag (C3)، Manni-Plex Cal-Zn (C2) مقارنة بالكنترول. على العكس من ذلك، قد لوحظ أن أعلى محتوى للأبيسيسك (ABA) ونسبة الأزهار المتساقطة في نباتات الكنترول مقارنة بجميع المعاملات الأخرى. علاوة على ذلك، أوضحت دراسة الارتباط أن محصول الخيار يرتبط ارتباطاً إيجابياً بمساحة الورقة وعدد الأوراق وكذلك ارتباطاً سلبياً بنسبة الزهرة المتساقطة.