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RESPONSE OF STRESSED CHINA ASTER (*CALLISTEPHUS CHINENSIS*) CV. KAMINI PLANTS TO FOLIAR APPLICATION OF BENZYLADENINE (BA) AND CYCOCEL (CCC)

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Abstract

One of the main factors limiting crops output in worldwide agriculture is soil saline-alkalization, which is a significant abiotic stressor. China aster is one of the most significant commercial flower crops. Thus, the aim of this study was to investigate the abilities of benzyladenine (BA) and cycocel (CCC) to improve China aster growth and flower quality grown under alkaline soil. In two consecutive field experiments (2018/2019 - 2019/2020), seven foliar treatments were assessed, including control (Distilled Water), two concentrations of BA [100 and 200 ppm], CCC [200 and 400 ppm], BA 150 ppm × CCC 300 ppm, and CCC 300 ppm × BA 150 ppm. BA and CCC at varying concentrations significantly improved growth, flower quality, SPAD chlorophyll units, nutrition uptake and biochemical constitutes, as compared to the control plants. Application of BA spray at 150 ppm concentration, followed by the application of CCC at concentration 300 ppm, significantly improved all attributes under the conditions of an alkaline soil.

Keywords: China aster; *Callistephus chinensis*; Ornamental plants; Alkali stress; Growth regulators; Benzyladenine (BA); Cycocel (CCC).

1. INTRODUCTION

China aster (*Callistephus chinensis* (L.) Nees) cv. Kamini is one of the most well-liked flowering annuals and is a member of the Asteraceae family. It is a resilient and freely flowering annual that is cultivated all over the world due to its simplicity in cultivation, more variety in forms and colors, and their extended vase life, which has made them popular as cut flowers among growers (Vinutha et al., 2017). Due to its widespread use in bouquets, buttonholes, and garlands, its cultivation is growing in popularity throughout the cities. It is used as a bedding plant, an edging plant for pots, and a herbaceous border in ornamental gardening (Marak et al., 2020).

In dry and semi-arid regions, soil alkalinity is one of the most prevalent issues. According to the pH value, the soil is classed as mildly alkaline (pH range: 7.1 - 8.5), moderately alkaline (pH range: 8.5 - 9.5), or severely alkaline (pH range: greater than 9.5) (Oster et al., 1999). The alkali stress-causing substances $NaHCO_3$ and Na_2CO_3 which, results from further raise the pH value. Therefore, high pH will significantly disrupt cell pH stability, ruin cell membrane integrity, and reduce root vitality and photosynthetic performance in addition to

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ion toxicity and osmotic stress (Zhang et al., 2017; Kaiwen et al., 2020). Ornamental plant may be more tolerant of alkalinity as a result of the application and uptake of plant growth regulators like BA and CCC by the plant.

The essential goals to be considered in commercial flower growing are an increase in bloom output in both quality and quantity as well as a decrease in the plant length, as tall plants lodge easily, reducing flower size and yield per plant. Exogenous application of growth regulators has a significant impact on growth, despite the fact that environment and genetic factors have a significant impact on yield and quality. In reality, horticulture and agriculture in industrialized nations have been completely transformed by the exogenous application of plant growth regulators (Pavan Kumar et al., 2015). The use of growth regulators has long been a crucial component of floriculture and the use of growth agents has been one of the most significant advancements in agrotechnology for raising flower productivity and quality standards (Sardoei 2014). Cytokinins are widely known for either promoting or restraining plant growth during a variety of physiological stages, including germination and flowering. Benzyladenine (BA) is one of the cytokinins that increases the yield of many plants either quantitatively or qualitatively (Mangena 2020; Abdel-Rahman and Abdel-Kader 2020).

Plant growth inhibitors are frequently used in ornamental plants to reduce stem elongation and create more dense, durable potted and bedding plants without altering developmental patterns or producing phytotoxic consequences (Anayat et al., 2020). On the market right now, Cycocel (CCC) is one of the most dependable and frequently utilized plant growth regulators with retardant activity. CCC is a synthetic compound which reduces the growth of stem and extended the shelf-life of flowers (Singh et al., 2018). For large-scale commercial production, it is necessary to comprehend the role of chemical regulation. These compounds still need to be properly evaluated for accelerating flowering at appropriate times and enhancing flower quality and production. Given the foregoing information, the current experiment was conducted to evaluate the effectiveness of these growth regulators mitigating the adverse effects of alkaline stress conditions on the growth, flower quality, SPAD chlorophyll value, nutritional states, and biochemical components of China aster plant.

2. MATERIALS AND METHODS

2.1. Experimental site and soil

In the two cropping seasons of 2018/2019 and 2019/2020, an experiment was carried out in an open field located in Fayoum province, Egypt (29°19'23.2"N 30°51'27.5"E). Soil samples from the experimental site were taken and examined before to the start of each experiment. Several established, published techniques were used to determine the physical and chemical characteristics of the soil under test (Klute1986; Page et al., 1982), and the results are listed in Table 1. According to the assessed data of the tested soil, it has a high pH value (7.86), indicating that it is categorized as having an alkalinity tendency. As a result, several critical nutrients, including P, Fe, Zn, Cu, Mn, and B have a tendency to fix in this soil (Abd-Alla et al., 2014).

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Table 1: Some physical and chemical properties of the used soil

Properties	Value	
	2018/2019	2019/2020
Particle size distribution %		
Coarse Sand	7.43	7.45
Fine sand	41.52	41.49
Silt	18.20	18.23
Clay	32.85	32.83
Texture class	Sandy clay Loam	
pH	7.87	7.85
ECe (dS m^{-1} soil)	3.71	3.73
Exchangeable cations (meq /l)		
Ca^{++}	5.11	5.12
Mg^{++}	4.10	4.11
Na^{+}	27.68	27.87
K^{+}	0.21	0.20
Exchangeable anions (meq /l)		
Cl^{-}	16.86	16.90
Hco_3^{-}	2.84	2.86
So_4^{--}	16.26	16.38
Co_3^{--}	1.14	1.16
SAR	13.18	13.27
ESP %	15.38	15.48
Nutrients % Available		
N	0.19	0.20
P	0.23	0.25
K	0.31	0.32

(SAR) meaning sodium adsorption ratio and (ESP) meaning exchangeable sodium percentage

2.2. Plant material

Standardized China aster seedlings were bought from the Agricultural Research Centre in Giza, Egypt, measuring 10 cm in length and having 2-3 pairs of true expanded leaves. Planting seedlings in plots took place on November 18th. Each section of an experimental plot was 3 m^2; 6 m length × 0.5 m row width and had a spacing of roughly 0.3 m among plants in each row.

2.3. Treatments and Experimental Design

This study employed a randomized complete block design (RCBD) with three replicates. In the trial there were 7 treatments distributed as follows:

(T_1) Control (Distilled Water)

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(T_2) BA 100 ppm

(T_3) BA 200 ppm

(T_4) CCC 200 ppm

(T_5) CCC 400 ppm

(T_6) BA 150 ppm × CCC 300 ppm

(T_7) CCC 300 ppm × BA150 ppm

The commercial chemical substances 6-Benzyladenine (6-Benzylaminopurine; BA) and Cycocel (2-Chloroethyl, trimethyl ammonium chloride; CCC) were purchased from the El-Gomhorya Company in Egypt. A few drops of diluted in NaOH solution were added to solubilize BA. Using a foliar spray BA and CCC were both applied. A wetting agent at 0.1% (Triton B) was added to all treatments. Whole plant leaves were sprayed over to the point of runoff. Each used medication was provided twice, at 40 and 60 days after transplantation. With the exception of treatment 6, which received a BA 150 ppm as first application and a CCC 300 ppm as second application, and treatment 7 in which, plants were sprayed a CCC 300 ppm as the first time and BA 150 ppm as the second time. Other agricultural procedures were followed exactly as recommended.

2.4. Measurements of growth and flower characteristics

In each treatment and replication, five rival plants were randomly selected and tagged to record in-depth observations. Plant height (cm), leaf area (cm^2), no. of primary branches, no. of secondary branches, days for 50 % flowering (day), number of flowers/ plant, flower diameter (cm), vase life of cut flowers (days), flower stalk length (cm) and flower weight (g), were recorded.

2.5. Pigment content

SPAD chlorophyll index was determined using a chlorophyll meter on the top third and fourth leaves (SPAD-502, Minolta Company, Tokyo, Japan) (Jiang et al., 2017).

2.6. Determinations of N, P and K contents

Leaf nitrogen contents (in mg g^{-1} DW) were colorimetrically assessed using the Orange G dye (Hafez and Mikkelsen 1981). The concentration of phosphorus was measured using the molybdenum-reduced molybdophosphoric blue color method (Jackson 1973). Using a Perkin-Elmer model 52 flame-photometer (Gallenkamp Company, London, U.K.) with an acetylene burner, potassium was calculated (Wilde et al., 1985).

2.7. Biochemical constituents

The method used by (Shivashankar et al., 2010) was implemented to estimate the total anthocyanins. Using a methanolic extract of dry material the total phenolic content was assessed using the Folin-Ciocalteu colorimetric method (Elija Khatiwora et al., 2010). Total

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flavonoids were measured using a colorimetric test procedure (Shi et al., 2012), with some modest modifications from earlier research (Zhao et al., 2018).

2.8. Statistical analysis

The InfoStat computer software package statistically examined the collected data using analysis of variance for randomized complete block design (RCBD). Data analysis employed a two-way analysis of variance (ANOVA). The differences between the treatment means were examined using Duncan's multiple ranges test at $P \leq 0.05$ (Snedecor and Cochran 1994).

3. RESULTS

3.1. Measurements of growth and flower characteristics

BA and CCC foliar application under alkalinity soil significantly affected China aster growth measured in terms of plant height, leaf area, number of primary branches, and number of secondary branches, are shown in Table 2. The treatment 6 (BA 150 ppm × CCC 300 ppm) and treatment 7 (CCC 300 ppm × BA150 ppm) considerably outperformed the control in terms of plant height by 74.94 and 62.87, leaf area by 83.53 and 71.86, and number of primary branches by 299.73 and 217.98 %, respectively. However, there were no discernible differences between BA and CCC in the number of secondary branches. In this regard, raising BA concentration from 100 to 200 ppm considerably and gradually increased plant growth factors. We also observed that the shorter plant height and the smallest leaf area were caused by the increase in CCC concentration from 200 to 400 ppm. Contrarily, the number of primary and secondary branches increased as the CCC concentration increased.

Also, BA and CCC had a positive impact on overall flower characteristics (Table 3 and figure 1[a and b]). The application of BA 150 ppm in the first time, followed by the application of CCC 300 ppm in the second time was the most significant treatment in increasing the number of flowers/ plant, flower diameter and vase life of cut flowers. However, compared to the results from all treatments, it reduced the time required from planting to 50% flowering. Moreover, the flower stalk length and flower weight were unaffected considerably by the application of BA 150 ppm and CCC 300 ppm spraying whichever was earlier (T_6 and T_7).

Table 2: The effect of BA and CCC on the growth characteristics of China aster plants grown in alkaline soil

Treatment	Plant height (cm)	Leaf area (cm^2)	No. of primary branches	No. of secondary branches
Control	41.34 ± 0.71f	33.51 ± 1.30f	3.67 ± 0.58f	6.67 ± 0.58f
BA 100 ppm	53.42 ± 1.81d	51.86 ± 1.59c	5.67 ± 0.58e	9.67 ± 0.58e
BA 200 ppm	63.46 ± 1.52c	56.13 ± 0.66b	6.33 ± 1.15e	12.33 ± 1.15d
CCC 200 ppm	51.50 ± 0.84d	48.25 ± 1.11d	8.33 ± 0.58d	14.33 ± 0.58c
CCC 400 ppm	47.50 ± 1.16e	43.15 ± 2.89e	10.33 ± 0.58c	17.33 ± 1.53b
BA 150 ppm × CCC 300 ppm	72.32 ± 2.13a	61.50 ± 1.50a	14.67 ± 0.58a	22.33 ± 1.15a
CCC 300 ppm × BA150 ppm	67.33 ± 1.01b	57.59 ± 1.59b	11.67 ± 0.58b	21.00 ± 1.00a
	**	**	**	**

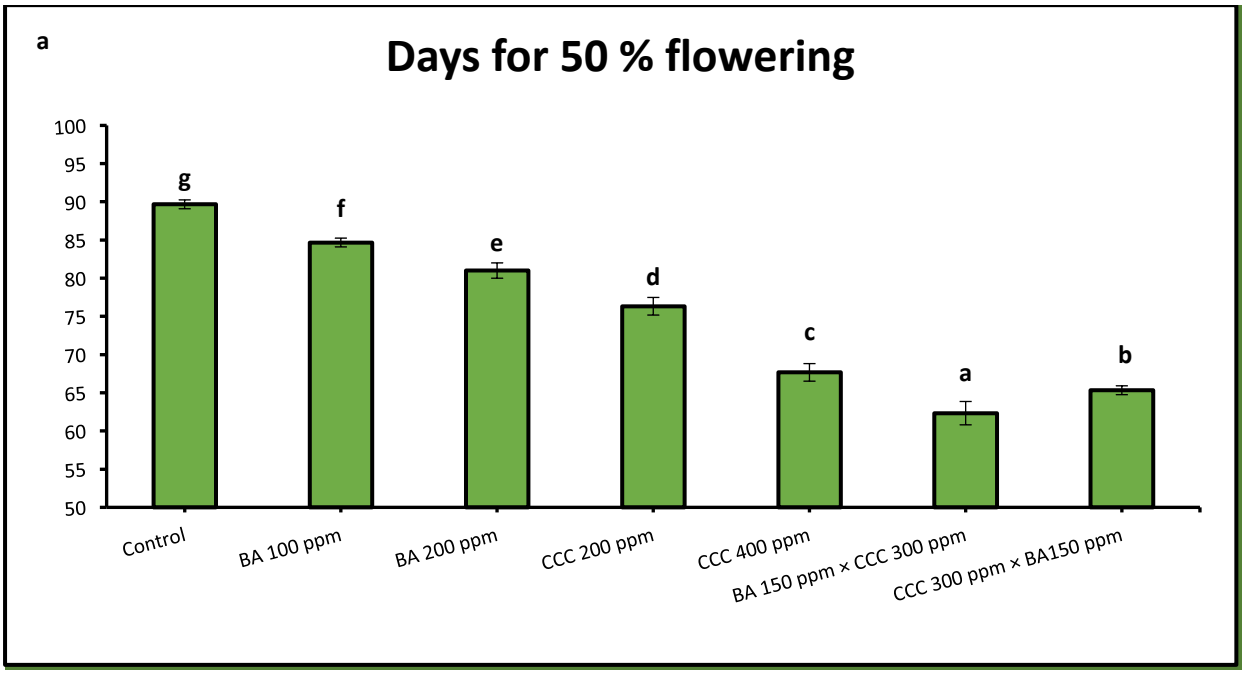
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Means (±SE) that are followed by various letters vertically column are significantly different, by using Duncan's multiple range test (** = p ≤ 0.01).

Table 3: The effect of BA and CCC on the flower characteristics of China aster plants grown in alkaline soil

Treatment	Vase life of cut flowers (day)	Flower diameter (cm)	Stalk length (cm)	Flower weight (g)
Control	3.67 ± 0.71f	4.30 ± 0.20g	21.12 ± 0.58e	4.86 ± 0.58d
BA 100 ppm	8.67 ± 0.33cd	6.51 ± 0.25d	28.21 ± 0.58c	7.64 ± 0.33bc
BA 200 ppm	10.33 ± 0.33b	7.03 ± 0.07c	31.62 ± 0.27b	9.22 ± 1.18b
CCC 200 ppm	5.67 ± 0.33e	5.18 ± 0.06f	24.30 ± 0.45d	6.88 ± 0.32c
CCC 400 ppm	7.67 ± 1.16d	6.00 ± 0.12e	26.98 ± 0.58c	8.24 ± 1.53bc
BA 150 ppm × CCC 300 ppm	11.67 ± 0.33a	8.04 ± 0.17a	35.21 ± 0.58a	12.85 ± 0.85a
CCC 300 ppm × BA150 ppm	9.67 ± 0.33bc	7.48 ± 0.06b	34.85 ± 0.35a	11.24 ± 0.57a
	**	**	**	**

Means (±SE) that are followed by various letters vertically column are significantly different, by using Duncan's multiple range test (** = p ≤ 0.01).



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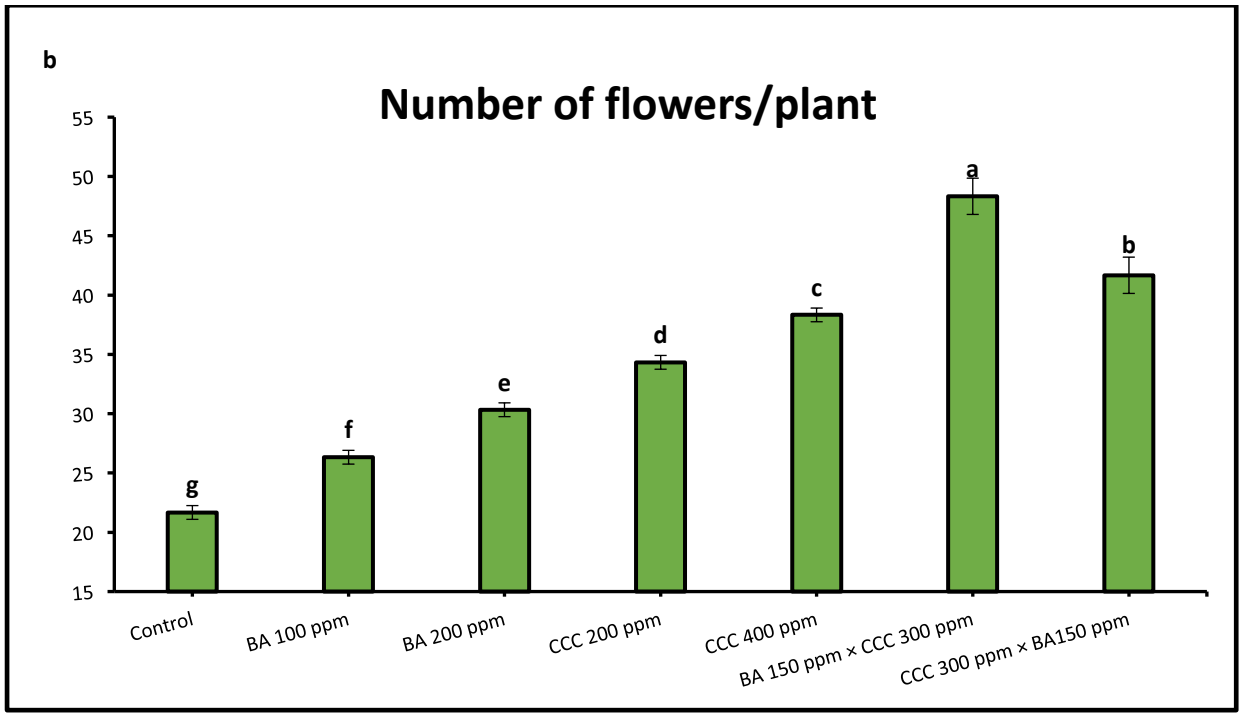


Figure 1 (a and b): The effect of BA and CCC on days for 50 % flowering and number of flower plant^{-1} of China aster plants grown in alkaline soil. The difference between bars with various letters is significant by using Duncan's multiple range test at the 0.05 level

3.2. Pigment content

Foliar applying of BA and CCC at varying concentrations SPAD chlorophyll content significantly increased (Table 4). These improvements were more pronounced under (T_6) in which BA150 ppm was used as the first application and CCC 300 ppm as the second one, followed by (T_7) in which CCC 300 ppm was sprayed as the first time and BA 150 ppm as the second one.

3.3. Determinations of N, P and K contents

Table 4 lists the content of macronutrients (N, P, and K) found in plant leaf tissues. N, P, and K contents showed statistically significant variations among the application of BA and CCC concentrations. In this regard, plants treated with BA at 150 ppm followed by CCC at 300 ppm exhibited the maximum N content. Spraying plants with BA 150 ppm and CCC 300 ppm whichever was earlier did not significantly influence the P and K contents in contrast to the control plants (T_6 and T_7).

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Table 4: The effect of BA and CCC on SPAD chlorophyll and macronutrients contents of China aster plants grown in alkaline soil

Treatment	SPAD (Unit) chlorophyll	N (mg g^{-1} DW)	P (mg g^{-1} DW)	K (mg g^{-1} DW)
Control	30.78 ± 0.30g	9.91 ± 0.11f	0.10 ± 0.0e	10.71 ± 0.30f
BA 100 ppm	43.53 ± 0.88d	12.56 ± 0.22c	0.15 ± 0.01bc	13.11 ± 0.15c
BA 200 ppm	49.45 ± 0.34c	13.10 ± 0.07b	0.13 ± 0.01cd	14.19 ± 0.20b
CCC 200 ppm	35.34 ± 0.29f	10.66 ± 0.23e	0.12 ± 0.01de	11.70 ± 0.10e
CCC 400 ppm	39.20 ± 0.54e	11.45 ± 0.27d	0.14 ± 0.01cd	12.56 ± 0.25d
BA 150 ppm × CCC 300 ppm	60.76 ± 0.27a	14.09 ± 0.11a	0.18 ± 0.01a	14.83 ± 0.34a
CCC 300 ppm × BA150 ppm	57.20 ± 0.58b	13.14 ± 0.04b	0.16 ± 0.0ab	14.67 ± 0.27a
	**	*	*	*

Means (±SE) that are followed by various letters vertically column are significantly different, by using Duncan's multiple range test (* = $p \leq 0.05$ and ** = $p \leq 0.01$).

3.4. Biochemical constituents

Following the previously described trends, total anthocyanin, total phenolic and total flavonoid contents of flowers increased with the application of BA and CCC (Table 5). Additionally, plants that treated with BA at 150 ppm and CCC at 300 ppm, regardless of which was sprayed first, showed a significant increase in all biochemical components. A significant and gradual increase in the total anthocyanin, total phenolic, and total flavonoid contents was also seen when BA concentration was raised from 100 to 200 ppm and CCC concentration from 200 to 400 ppm as compared to the control plants.

Table 5: The effect of BA and CCC on biochemical constituents of China aster plants grown in alkaline soil

Treatment	Total Anthocyanin (mg/100 g FW)	Total phenol (mg/ g DW)	Total flavonoid (mg/ g DW)
Control	167.90 ± 0.30g	40.68 ± 0.11g	25.24 ± 0.0g
BA 100 ppm	182.64 ± 1.23d	52.55 ± 1.19d	40.57 ± 0.83d
BA 200 ppm	189.23 ± 0.67c	58.28 ± 0.55c	45.17 ± 0.68c
CCC 200 ppm	171.62 ± 0.37f	44.66 ± 0.35f	30.06 ± 0.43f
CCC 400 ppm	175.32 ± 0.54e	47.07 ± 0.27e	34.21 ± 0.01e
BA 150 ppm × CCC 300 ppm	210.22 ± 0.55a	87.65 ± 0.35a	68.05 ± 0.95a
CCC 300 ppm × BA150 ppm	203.25 ± 01.09b	83.86 ± 0.31b	63.90 ± 34b
	**	**	**

Means (±SE) that are followed by various letters vertically column are significantly different, by using Duncan's multiple range test (** = $p \leq 0.01$).

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4. DISCUSSION

The growth regulators utilized in this experiment produced fruitful results due to soil alkalinity's detrimental effects being countered by the BA and CCC. Stressed plants growing in alkaline soil treated with growth regulators had healthy metabolic status, which led to healthy plant growth as seen by a rise in plant height, leaf area, number of primary and secondary branches. Moreover, according to the findings plant height, leaf area, the number of primary and secondary branches all increased with increasing BA concentration. Also, it appears that applying BA has demonstrated a superior benefit than using CCC in terms of overall growth. These results align with those attained by Vijayakumar et al. (2017) on China aster, El-Ghamery and Mousa (2017) on black seed, Kumar et al. (2019) on Nerium, Kumar et al. (2020) on Marigold and Mangena (2020) on soybean. The stimulatory effects of BA on plant growth may be attributed to their stimulating effects on cell division, cell elongation, shoot initiation, nutrient mobilization, as well as, delaying of senescence and apical dominance alternation in plants (Werner and Schmülling 2009). While, CCC application may result in a reduction in plant height and leaf area because it prevents the production of gibberellin and cell division in apical meristematic cells (Demura and Ye 2010). The plants sprayed with BA 150 ppm followed by CCC 300 ppm produced the tallest plants, the largest leaf area and formed, the greatest number of primary and secondary branches. This combined treatment may have the effect of stimulating cytokinin formation earlier preventing premature leaf senescence, and promoting more vegetative growth with higher levels of photosynthetic pigments. This may help to explain why the BA and CCC had positive effects on flower characteristics and chlorophyll content in alkaline soil, which ultimately had a positive impact in the growth of China aster plants.

Likewise, the increase in flowering characteristics found in our experiment may be attributable to the plants' overall vegetative growth, which allowed for more photosynthetic area and metabolic activity, which in turn increased the transport and utilization of the photosynthetic product (Conti 2017), resulting in higher flower quality. Also, early flowering in BA and CCC treated plants may be attributable to the fact that these plants initially accumulated enough food reserves as a result of an increase in the number and area of leaves or to reducing the apical dominance (Singh et al., 2018). On Matthiola and Althaea plants, Hamza and Helaly (1983) reported that CCC accelerated flowering and enhanced growth as well as mineral contents. Moreover, Atteya and El-Gendy (2018) stated that CCC can change the endogenous levels of phytohormones and induce early flowering by promoting roots, which increases the amount of nutrients reaching the plant. By changing C/N ratio this reserve food has been used for reproduction while restricting vegetative development (Singh and Bala 2020), resulting in earlier flowering. Similarly, Mandal et al. (2018) demonstrated that prior to flowering, litchi plants treated with ethrel (2 ml/L) had the highest C/N ratio in both leaves and shoots.

Our findings showed that macronutrients and biochemical constituents (Tables 4 and 5) were identical to those reported by Mahmoud et al. (2016) on lupine, Bessonova et al. (2019) on China aster, Kumar et al. (2020) on Marigold and Mangena (2020) on soybean. The steadily

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rising macronutrients suggest that foliar BA or CCC application may be caused by activating enzymes that control carbohydrate metabolism and encourage the production of RNA and proteins (Hopkins and Huner 2004). Also, it increases cell permeability which allows nutrients to enter root cells more quickly and increases plant nutrient uptake (Ibrahim et al., 2010). Therefore, the increase in total anthocyanin, total phenolic, and total flavonoid contents were correlated with the increases in IAA contents in shoots, which suggested that the majority of phenolic compounds are diphenols and polyphenols, which may inhibit IAA oxidase activity and cause auxin accumulation (Ciura and Kruk 2018), which would then reflect in stimulating the biochemical components of China aster.

Anthocyanins, phenol, and flavonoids are the main types of antioxidants. To maintain a healthy balance of reactive oxygen species (ROS) within cells, these chemicals are dispersed throughout the cell. According to the roles of antioxidant enzymes and antioxidants, the cooperation of the two can reduce the damage to cells brought on by alkali stress (Sun et al., 2019; Wang et al., 2020). Hence, biochemical modulation can be used to explain how BA and CCC are able to modulate the detrimental consequences of alkali stress on plants.



Figure 2: **Summary graphic illustrating the proposed mechanism for how BA and CCC influence growth, yield and resistance to alkalinity stress**

5. CONCLUSION

Our findings indicate that China aster plants cultivated in alkaline soil (pH = 7.86) and treated with BA and CCC at various concentrations experienced significantly greater growth and flower quality. Applying BA, followed by CCC resulted in higher levels of SPAD chlorophyll unites, N, P and K (nutrient uptake) in the leaf tissues, as well as antioxidants (total anthocyanins, total phenol, and total flavonoids) in the flower. This suggests that these

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characteristics, at least in part, increased the tolerance of China aster plants to alkalinity stress, like that protecting the photosynthetic mechanism and plant growth. Hence, this treatment may be preferred for quality flower production. Therefore, in alkaline soil conditions, we recommend spraying the China aster plants with BA and CCC.

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