Physiological Response of Calendula (*Calendula officinalis* L.) plants to Gibberellic Acid (GA_3) and Forchlorfenuron (CPPU)

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الاستجابة الفسيولوجية لنباتات الأقحوان للمعاملة بحامض الجيبريللين والفوركلورفينورون

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المستخلص:

صممت الدراسة لبحث تأثير حامض الجبريللين Gibberellic Acid (GA_3) بتركيزات 50-100-150-200 جزء في المليون و الفوركلورفينورون Forchlorfenuron (CPPU) بتركيزات 25-50-75-100 جزء في المليون بالإضافة إلى معاملة المقارنة على النمو الخضري وإنتاج الأزهار والمحتوى الكيماوي لنباتات الأقحوان *Calendula officinalis*, L وتمت الإضافة رشاً على المجموع الخضري بينما تم رش نباتات المقارنة بماء مقطر. ومن أهم النتائج المتحصل عليها وجد أن استخدام تركيز 150، 200 جزء في المليون من الجبريللين أعطي نباتات أكثر ارتفاعا وكذلك أكبر عدد من الأفرع/نبات وأكبر عدد من الأوراق وأثقل الأوزان الجافة للأوراق لكل نبات. تحققت زيادة معنوية في صفات النمو الزهري والمتمثل في عدد النورات لكل نبات وقطر النورة والوزن الطازج والجاف للنورات عند جميع تركيزات حامض الجبريللين. كذلك وجد أن الرش بالفوركلورفينورون بتركيز 75، 100 جزء في المليون أعطي اكبر زيادة معنوية في صفات النمو الخضري وأوضحت نتائج التحليل الكيماوي تحسن محتوى الأوراق من الكلوروفيل والكاروتينات والمحتوى الكلى للكربوهيدارت وكذلك محتوى الأوراق من العناصر المعدنية (ن، فو، بو) نتيجة للمعاملة بكل من حامض الجبريللين والفوركلورفينورون بينما أظهرت نباتات معاملة الكنترول أقل القياسات.

الكلمات المفتاحية: *زهرة القطيفة، ارتفاع النبات، منظمات النمو، المنظمات الحيوية، الخصائص الكيميائية الحيوية.*

Abstract:

The study was conducted to evaluate the impact of different concentrations of Gibberellic Acid (GA_3) at 50, 100, 150, and 200 ppm, and Forchlorfenuron (CPPU) at 25, 50, 75, and 100 ppm, along with a control treatment, when applied as foliar sprays on the growth and flower production of Calendula (*Calendula officinalis* L.) plants. The results of the study indicate that GA_3 at concentrations of 150 and/or 200 ppm had the most significant impact on plant height (cm), number of branches per plant, leaf number per plant, and shoot dry weight per plant(g). The flowering data demonstrated that all concentrations of GA_3 significantly increased number of inflorescences per plant, inflorescence diameter (cm), and fresh and dry weight of inflorescences (g). Additionally, the highest values for vegetative growth and flowering parameters were observed with the application of Forchlorfenuron (CPPU) at concentrations of 75 and 100 ppm. Furthermore, the total chlorophyll, carotenoid content, total carbohydrate content, and leaf mineral contents (N, P, and K) were significantly enhanced by foliar spraying with various concentrations of GA_3 and CPPU compared to the control.

Keywords: *Pot marigold, plant height, growth regulators, bio-regulators, biochemical characteristics.*

Introduction:

Calendula (Calendula officinalis L.) is commonly known as pot marigold, about 40 species of flowering plants in the aster family (Asteraceae or Compositae) belong to the Calendula genus, which is indigenous to North Africa and Southern Europe (Aliniaeifard et al., 2018). The common name of *Calendula* is pot marigold, marigold flowers or garden marigold. Calendula is an annual plant that thrives in almost any soil but can typically be found in Europe, Western Asia, and the United States. Its branching stems grow to a height of 30 to 60 cm, and it blooms from early spring., having a long tap root with numerous secondary roots; hispid, acute, oblanceolate, alternate, and sessile leaves; flower head inflorescence and cultivated for its beautiful flowers either single or double as reported by (Khalid & Teixeira da Silva, 2012). Cultivars in lovely yellow and orange hues are widely cultivated as an easy-to-grow bedding plant for mass display, pot plants, flower beds, borders, window and porch boxes and cut flowers crop especially the double flowers. The inflorescence of C. officinalis has an abundant number of carotenoids that give flowers their yellow orange color and the color shade depends on pigment content and pigment profile. The yellow flower petals of Calendula contain 19 carotenoids and orange flower contains10 unique carotenoids. These 10 carotenoids have UV-visible absorption maxima at a wavelength longer than that of flavoxanthin and provide orange color to the flower petals (Khalid & Teixeira da Silva, 2012). The pot marigold extracts possess a wide range of pharmacological effects (Pintea et al., 2003) and are used as antiseptic, stimulant, diaphoretic, antispasmodic and anti-pyretic agents (Weiner, 1990; Kirtikar & Basu, 1993). The flower extracts of the plant have anti-viral effects on HIV (Kalvatchev et al., 1997). *In-vitro*, *Calendula officinalis* (CO) plant extracts show anti-cancerous activity on various tumor cell lines derived from leukemias, fibrosacomas, melanomas, breast, cervix, prostate, pancreas and lung (Medina et al., 2006). Also, Calendulas contain volatile oil containing the carotenoids carotene, lycopin and calendulin. The plant contains saponins, resins, tannin, mucilage, sterols acid, violaxanthine and flavoxanthine. Fersh blooms contain azulenogenic sesquiterpene or sesquiterpene alcohol (Dedio et al., 1986). The leaves are diaphoretic, diuretic, oxytocic emmenagogue, astringent, sedative, antiemetic, aromatic and antianemic. They are used as herbicide, assist healing of ulcers and astringent like Hamamelis leaves. Flowers are used in case of dysmenorrheal and to produce calendulin which is used in coloring food products as jellies and jams and used to adultrate saffron flowers which are very expensive (Kotb, 1986). Seeds of calendula are used as oil seed. Its seed contains 40-60 % oil. This oil is used in cosmetics, paint and coating. Calendula oil reduces inflammation. It is used in wound healing and as an antiseptic. It is also better for skin diseases (Schiller et al., 2008). Today, calendula is often used topically, meaning it is applied to the skin. Calendula has been shown to help wounds heal faster, possibly by increasing blood flow and oxygen to the affected area, which helps the body grow new tissue. It is also used to improve skin hydration and firmness. Plant growth regulators provide effective means for the improvement and productivity of the qualitative as well as quantitative facets of growth (Zahoor et al., 2011). Since, gibberellins are essential endogenous regulators of plant growth and development, including seed germination, trichome development, stem and leaf elongation, flower induction, pollen development after anthesis, fruit and seed development (Singh et al., 2002). Gibberellins primarily

affect growth by controlling cell elongation and division, which is reflected on yield, its components and fruit quality of various grape cultivars (Pires et al., 2000). Gibberellic acid is responsible for cell elongation, rather than cell division (Kappel & MacDonald, 2002). Several researches carried out to study the effect of Gibberellic Acid (GA_3) on the growth and chemical contents of some ornamental plants as Carnation plants (Neelofar lolapori and Arora 1995), chrysanthemum (Schmidt, *et al.*, 2003) African marigold (Singh and Srivastav 2008) China aster (Sharma and Joshi 2015) and *Tagetes erecta* (Acharya *et al.*, 2021). Forchlorfenuron, a synthetic cytokinin (CPPU) with strong growth regulation activities has been found very effective in enhancing growth by stimulating cell division and cell elongation s reported by (Cruz-Castillo et al., 2002). Many researcher pointed out to the importance of CPPU in increasing growth, flowering and chemical composition of some economic plants such as (Pires et al., 2000) on grapes, (Ahmed et al., 2020) with *Gladiolus hybrida* , (Harhash et al., 2023) on *Dahlia variabilis* L.This study aimed to investigate the impact of using gibberellic acid (GA_3) and forchlorofenuronis (CPPU) as a foliar spray on vegetative growth, flowers production and chemical character of *Calendula officinalis,* L. plant.

Materials and methods:

The present work was carried out during the two successive seasons of 2020/2021 and 2021/2022 to investigate the impact the different concentration of two plant growth regulators gibberellic acid (GA_3) with Chemical Formula $C_{19}H_{22}O_6$ and forchlorofenuronis (CPPU) Chemical Formula $C_{12}H_{10}$ ClN_3O on vegetative growth, flowers production and the chemical constitutes such as chlorophyll, N, P and K contents (%) of *Calendula officinalis, L*. under the open field conditions in natural sunlight. Nine treatments consisted of four concentrations of GA_3 as a solution (50,100,150 and 200 ppm), four concentrations of forchlorofenuronis (CPPU) as a solution (25, 50, 75 and 100 ppm) and the untreated plants (control) were used.

Experimental location and culturing process:

The used seeds in this experiment were genera, i.e., *Calendula officinalis, L*. one local cultivar, orange color and double flowers were used because of its high landscape potentials in gardens. Seeds were sown on 20th September and 25th September of the growth seasons 2020/2021 and 2021/2022 respectively at private nursery in Al-Bayda city, Libya. The propagation bed is composed using a mixture of peatmoss and sand (1:3, v/v). After one month from sowing, when the seedling reached 10cm. in height with 4 leaves. These seedlings were similar in their shape and size with good root. They were transplanted to final clay pots 30 cm. in diameter with (one plant/ pot), filled using the same growing medium under the full sunny conditions (Nooh & El-Naggar, 2021). All calendula plants received the recommended 19N:19P:19K fertilization doses except for the unfertilized plants which did not receive any fertilizers. The NPK fertilization dose 3.0g/plant were applied monthly as dressing application throughout the growing seasons as mentioned by (Paparozzi & Hatterman, 1988). The first NPK fertilization dose was applied two weeks after final transplanting while the rest of doses were applied later at one-month intervals. Regular agricultural practices such as weeding and watering as basic dressing were carried out.

Chemical and mechanical analysis of growing medium:

The chemical analysis cleared that, it was containing 0.19, 0.06 and 0.15 % of N, P_2O_5 and K_2O, respectively. The mechanical analysis of the used soil revealed that it contained 0.32, 3.30 and 96.38% of clay, silt and sand, respectively. The electric conductivity (EC) was 3.02 (dsm^{-1}) with a pH of 7.88.

Treatments and experimental layout:

One month after transplanting plants were sprayed by (50,100,150 and 200 ppm) GA_3 and by (25, 50, 75 and 100 ppm) forchlorofenuronis (CPPU). The foliar spraying was conducted to run off plants. The plants were sprayed three times, the first one after 15 days from the final transplanting, the second after 15days after the first, while the third was 15 days after the second spraying. This made 8 treatments in addition to the control treatment (check treatment) without any additions in a complete randomized design without interaction in 3 replicates and each replicate contained 10 plants. The control plants were sprayed with distilled water at the same time.

Growth and flowering characteristics:

The growth data recorded at the end of experiments included:

Plant height (cm), Number of branches per plant, leaf number per plant, and shoot dry weights per plant (g).

The flowering data included:

Number of inflorescences per plant, inflorescence diameter (cm), fresh and dry weight of *inflorescence.*

Chemical Constituent:

Total chlorophyll and total carotenoids content of fresh leaf samples (mg/100g F.W.L) was determined by using the method described by (Greig et al., 1968) and Moran and Porath (1980). The total carbohydrate content in dried leaves samples was determined according to (Herbert et al., 1971).

Nitrogen content (%): At the end of the experiment, the leaves of each treatment were collected and dried at 70°C to a constant weight, then they ground and digested with H_2SO_4 and H_2O_2 (Guzman & Romero, 1988). It was determined by the distillation in micro Kjeldahl method. Phosphorus and Potassium content (%): Determination of phosphorus and potassium was done at the end of experiment. The dried leaves in each treatment were ached in muffle furnace at 550°C. The ash was then dissolved in 2N HNO_3 (Chapman & Pratt, 1961). The vanadate molybdate method was used to determine P content in the solution at 470 mn on a spectrophotometer (spectronic 20). Potassium content was measured in the solution using Flame photometer (Chapman & Pratt, 1961).

Statistical analysis:

Data were statistically analyzed according to the methods described by (Snedecor & Cochran, 1990). The data on the vegetative growth characteristics and chemical analyses were subjected to statistical analysis of variance, and the means were compared using the least significant differences ($LSD_{0.05}$) test at 95% level of confidence method (*P = 0.05).*

Results and discussion:

Impact of GA_3 and CPPU on height (cm) and number of branches per plant:

The GA_3 treatments had a significant effect compared with the control treatment the GA_3 treatment at 200 ppm for both seasons had a significant effect on *Calendula officinalis* height and branches number with the mean of 65.38, 67.68 cm in height and 16.29, 16.67

branch per plant in the 1st season and 2nd season, respectively (Table .1). The minimum plant heights (36.45 and 37.43 cm) and branches number (6.96 and 6.84) were observed under control treatment in first season and second season. In general, there was a significant impact in plant height and number of branches per plant with increasing GA_3 concentration up to 200 ppm (Table 1). The stimulatory effect of GA_3 on height and number of branches of *Calendula officinalis* plants might be due to an increase in internodes elongation, which in turn was often a consequence of increased cell wall extension. This might be explained based on striking increased in cell membrane permeability according to (Wood & Paleg, 1972; Brain & Lowe, 1959; Alvin, 1960). Also, these increases in plant height may be due to the effect of Gibberellic acid on stimulating the cell division and elongation of new growth cells formed on pot marigold (*Calendula officinalis*) plants (Wasfy, 1995). (Schmidt, et al., 2003) observed a 16.78% increase in plant height on chrysanthemum when GA_3 was treated at 300 mgl1 and (Kumar et al., 2012) noted the similar pattern in tuberose and carnations, respectively.

This data coincided with (Balbaa & Laila, 2002) on *Lavendula officinalis*, and (El-Deeb, 2023) with *Dahlia variabilis* L. Increment in branches number per plant may be due to enhancing the growth of more lateral buds leading to more branches.

Results achieved by using foliar application with forchlorofenuronis (CPPU) at 100 ppm showed highest significant effect on plant height number of branches (59.81cm in height and 12.71 branch plant^{-1}) in the 1st season and (61.02 cm and 12.45 branch plant^{-1}) in the 2nd season (Table 1). The effect of (CPPU) can probably be related to the action of cytokinin on the differentiation of areal parts since the stimulation of shoot growth because of (CPPU) treatments. Cytokinins appeared to play an important role in the regulation of cell division, differentiation and organogenesis in developing plants (Skoog & Armstrong, 1970; Hall, 1973). Additionally, the cytokinin-like activity that causes rapid cell division and cell elongation may have contributed to the increases in several growth aspects with CPPU therapy (Thomas & Katterman, 1986). Strong cytokinin CPPU slows apical dominance, which promotes the formation of lateral shoots. Similarly, results recorded by Hafez, 1990) on marjoram plant, (Balbaa, 2002) on *Tagets minata* plant, (Taheri & Haghighi, 2018) in bell pepper(*Capsicum annum* cv. PS301) and (Nambiar et al., 2012) in *Dendrobium hybrid.*

Impact of GA_3 and CPPU on leaf number plant^{-1} and shoot dry weights plant^{-1} (g):

The resulting showed that GA_3 200 ppm had a statistically high effect on number leaves and shoot dry weights of marigold (*Calendula officinalis*) plants in the 1st season and 2nd season with the means of (51.83, 51.47) for leaves number, and (75.14and 76.87 g) for dry weights of shoot, respectively as compared to control treatment Data in (Table .1).which noted the lower number of leaves (26.11and 24.18), shoot dry weights (37.30and 36.78g), respectively, during both seasons. The stimulating effects of gibberellic acid may be due to activating apical meristems, enhancing the biosynthesis of the protein and carbohydrates. These together lead to enhancing the initiation of leaves primordial and consequently more leaves would be formed (EL-Naggar & Sharaf 2002). (Shedeed et al., 1990), found similar results on *Mentha piperita*, (Brose & Dhumal, 2001) on *Solanum khasianum*, (Pablo, 2001) on *Eryngium foetidum* and (Vijayakumari, 2002) on *Andrographis paniculata*.

From the results shown in (Table. 1) it was cleared that the of (CPPU) treatments present the significant effect on leaf number and dry weights of shoot per plant of *C. officinalis*

with increase CPPU concentration up to 100 ppm with the means of (43.55 and 44.08) for leaves number and (64.95and 64.98g) for dry weights of shoot.

This increase was a result of enhancing the growth of more lateral buds leading to more branches and more leaves number. The effect of (CPPU) can probably related to the action of cytokinin on the differentiation of areal parts since, the stimulation of shoot growth as a result of (CPPU) treatments has been occurred in *Asparagus officinalis* (Yang & Clore, 1973; Milbocker, 1972) on Poinsettia. Cytokinins appeared to play an important role in the regulation of cell division, differentiation and organogenesis in developing plants (Skoog & Armstrong, 1970; Hall; 1973). This results in an agreement with (Shedeed et al., 1990) on basil plant, (Farooqi et al., 1996) on *Artimisia arzura*. Also, concentrations 50 and 100 mgL^{-1} of GA_3 gave the best results in the two cuts of both seasons compared with the other concentrations and control. These results may be due to the favorable effects of GA_3 on the vegetative growth, which lead to an increase in the photosynthesis efficiency and accumulation of dry matter in the leaves (EL-Nagaar & Sharaf, 2002). The increase leaves dry weight is a result of enhancing the growth of more lateral buds leading to more branches; more leaves number and exogenous cytokinins resulted in promotion of protein, RNA, lipid and starch synthesis in the plant (Mothes, 1964). Similar trend of results was obtained by (El-Sharkawy, 1981) on *Origanum majorana* and (Balbaa, 2002) on *Tagets minata* plant.

Table (1): Impact of gibberellic acid (GA_3) and forchlorofenuronis (CPPU) on vegetative growth characteristics of *Calendula officinalis*, L. during 2020/2021 and2021/2022 seasons.

PGR (ppm)	Plant ht (cm)	Branch No. /Plant	leaf No./Plant	Shoot dry wt. /Plant
	First Season			
Control	36.45	6.96	26.11	37.30
GA350	44.86	9.85	36.75	42.`6
GA3100	53.54	12.32	39.17	54.11
GA3150	57.30	14.19	41.58	61.93
GA3200	65.38	16.29	51.83	75.14
CPPU 25	37.97	8.20	35.88	39.20
CPPU 50	44.65	8.73	38.38	47.29
CPPU 75	49.45	11.18	40.22	53.19
CPPU 100	59.81	12.71	43.55	64.95
L.S.D $_{(0.05)}$	4.12	2.14	2.17	7.03
	Second Season			
Control	37.43	6.84	24.18	36.78
GA350	44.93	9.89	36.38	43.70
GA3100	55.51	12.57	38.80	54.14
GA3150	56.98	14.28	41.22	62.23
GA3200	67.68	16.67	51.47	76.87
CPPU 25	38.38	8.00	35.42	39.19
CPPU 50	43.88	8.99	37.92	48.36
CPPU 75	53.00	11.19	39.75	53.61
CPPU 100	61.02	12.45	44.08	64.98
L.S.D $_{(0.05)}$	5.72	1.98	2.08	8.20

L.S.D $_{(0.05)}$ = Least significant differences at 0.05 level of probability; PGR, Plants Growth Regulators; GA_3, Gibberellic Acid; CPPU, Forchlorofenuron (N-(2 – chloro-4-pyridyl)-N`-phenylurea.

Impact of GA_3 and CPPU on number of inflorescences and diameter (cm):

The concerned data in (Table.2) showed that spraying pot marigold with most concentrations of used GA_3 and CPPU increased the number of inflorescences and diameter compared with the control during both seasons. Moreover, spraying GA_3 at

200 ppm gave the highest value of number of inflorescences (14.01and14.53) and inflorescence diameter (7.97 and 8.29 cm) of *Calendula officinalis* in both growing seasons as compared with other treatments. On the other hand, the lowest number of inflorescences (6.13 and 6.22) and diameter of inflorescence (3.76 and 3.64 cm) were found for the untreated plant (control) in the two growing seasons of this study. This may be due to adding gibberellic acid (GA_3) at suitable level leading to the increase and activation of the formed roots. This stimulates absorption of the essential elements for flowers initiation and development. According to (Singh & Srivastava, 2008), the enhanced flower production as a result of GA_3 treatment can be linked to the fact that GA_3 improves induction of flower bud break, which is the differentiation of floral primordial in the apical growth zone. Early flower induction as a result of GA_3 replacing some vernalization could be the cause of the increased blooming length under GA_3 treatment. The outcomes are consistent with those from (Kumar et al., 2003; Sharma and Joshi, 2015) in aster. The same trend was reported by (El-Naggar, 2002) on tuberose and (El-Naggar, et al., 2020) on *Matricaria chamomilla*, L.

The Data clarified that number of inflorescences and inflorescence diameter increased by (CPPU) treatments at (75 and 100 ppm) in two seasons as compared with the other treatments of (CPPU) (Table. 2). This increase is a result of enhancing the growth of more lateral buds leading to more branches consequently, more inflorescences number of Calendula plants and there is a connection between higher levels of endogenous cytokinin and the generation of more inflorescence meristematic cells. According to research by (Blanchard & Runkle, 2008), the application of exogenous cytokinin results in elevated levels of endogenous cytokinin.These results were in agreement with the study of (Mohamed, 2008) with *Origanum majorana*, L. and (Harhash, et al., 2023) on Dahlia (*Dahlia variabilis* L.)

Impact of GA_3 and CPPU on fresh and dry weight *inflorescence* (g):

As for marigold inflorescence fresh and dry weight (g) data presented in (Table.2) indicated that fresh and dry weight *inflorescence* were significantly affected by applications of some plant growth regulators and concentrations as a foliar spraying. In both seasons, the highest values of *inflorescence* fresh weight (10.59 and 11.46 g) and inflorescence dry weight (0.86 and 0.88) were obtained from *Calendula officinalis* plants treated with gibberellic acid (GA_3) at 200 ppm, respectively. On the second rank, applying GA_3 at 150 ppm also record a higher value than most other treatments. Such treatment increased inflorescence fresh weight to (9.93and 10.92 g) and increased total inflorescence dry weight to (0.79 and 0.82 g) in the both seasons, respectively, without any significant differences between it and the previous superior treatments for the second season. However, the highest concentration of 200 ppm gibberellic acid (GA_3) was more effective than 50 and/or100 ppm GA_3. Whereas the lowest inflorescence fresh and dry weights in both seasons were obtained from the control. The suitable concentrations of gibberellic acid (GA_3) led to increase the activity of growing regions of the plant by increasing the leaves development and the size of photosynthesizing surface, thus total inflorescence fresh and dry weights could be increased. These results are in accordance with those obtained by (Abdelgawad, 2020) on *Matricaria chamomilla*, L. Generally, all (CPPU) treatments had a significant effect on total fresh and dry weight of inflorescence per plant of Calendula compared with the control treatment. These increases were a result of enhancing the growth of more lateral buds leading to more branches and inflorescence

number consequently, increased fresh and dry weight inflorescence. The effect of kinetin can probably relate to the action of cytokinin on the differentiation of areal parts (Yang & Clore, 1973). These results were in agreement with (Balbaa, 2002) on *Tagetes minata* and (Harhash, et al., 2023) on Dahlia (*Dahlia variabilis* L.) plant.

Table (2): Impact of gibberellic acid (GA_3) and forchlorofenuronis (CPPU) on flowering characteristics of *Calendula officinalis*, L. plant during 2020/2021 and 2021/2022 seasons.

PGR treatment (ppm)	No. inflorescences per plant	Inflorescence diameter (cm)	Fresh wt. inflorescences (g)	Dry wt. inflorescences (g)
		First Season		
Control	6.13	3.76	7.2	0.57
GA3 50	8.32	5.15	7.86	0.64
GA3100	11.43	6.25	9.37	0.74
GA3150	13.16	7.02	9.93	0.79
GA3200	14.01	7.97	10.59	0.86
CPPU 25	8.04	4.28	7.74	0.53
CPPU 50	8.82	4.87	8.2	0.62
CPPU 75	10.13	5.14	8.75	0.68
CPPU 100	12.35	6.57	7.74	0.69
L.S.D $_{(0.05)}$	1.39	0.05	1.34	0.09
		Second Season		
Control	6.22	3.64	7.03	0.56
GA3 50	8.45	5.27	8.65	0.66
GA3100	10.97	6.37	10.31	0.72
GA3150	13.39	6.98	10.92	0.82
GA3200	14.53	8.29	11.64	0.88
CPPU 25	7.94	4.17	7.15	0.54
CPPU 50	8.78	4.9	8.52	0.63
CPPU 75	10.49	5.21	9.02	0.67
CPPU 100	12.16	6.12	9.63	0.71
L.S.D $_{(0.05)}$	1.94	0.07	1.52	0.09

L.S.D $_{(0.05)}$ = Least significant differences at 0.05 level of probability; PGR, Plants Growth Regulators; GA_3, Gibberellic Acid; CPPU, Forchlorofenuron (N-(2 – chloro-4-pyridyl)-N'- phenylurea.

Influence of GA_3 and CPPU on chemical constituents of *Calendula officinalis:*

1- Impact on total chlorophyll and carotenoides (mg/100 g F.W.):

Total chlorophyll (a+b) and carotenoides of *Calendula officinalis* L. in the leaves were shown in (Table. 3) as a mg/100 g F.W. GA_3 significantly increased the chlorophyll (a+b) and carotenoides. Generally, GA_3 at the rate of 200 ppm gave the highest content in both seasons. which equal (193.65 and 197.90 mg/100 g L. F.W). of chlorophyll content and (68.49 and 69.15 %) of carotenoides content in the first and second seasons as compared to the other treatments during the two growing seasons. The use of GA_3 significantly increased the amount of chlorophyll in *Calendula* leaves, which may have slowed down chlorophyll oxidation, increased chlorophyll production, or stabilized the thylakoid membrane. By enhancing membrane stability and preventing chloroplasts from senescing, growth regulators may also delay floral senescence and hence reduce chlorophyll loss (Amin et al., 2011). These results may be attributed to the enhancing impact of GA_3 at suitable rate on the assimilation and accumulation of the majorelements specially nitrogen (NH_4), phosphorus (P), iron (Fe^{++}), magnesium (Mg^{++}) cations, which are found in many metabolically active compounds, including chlorophylls and carotenoides and these elements are necessary for enzymes activation and formation of chloroplasts and chlorophyll (Hassouna & Madkour, 1991) Besides, using GA_3 led to increase the green pigments in the plants by stimulating the production of chlorophyll in leaves (Wasfy, 1995) Similar trend of results was obtained by

(Jhone et al, 1997; EL-Naggar, 1999) on gladiolus, (Abdelgawad, 2020) on *Matricaria chamomilla*, L. and (El-Deeb, 2023) on Dahlia (*Dahlia variabilis* L.) plant.

The results in (Table. 3) showed that, the total chlorophyll (a+b) and carotenoides of *Calendula* plants was increased by increasing (CPPU) concentrations in both seasons, whereas the highest value was obtained by using CPPU at the rate of 100 ppm. These results are in harmony with those of (George and Shemington, 2008). who reported that the increases in chlorophylls formation and protein synthesis in tissues were a result of treating with synthetic cytokinins. In addition, cytokinins protects chlorophylls against the photo-oxidation process by enhancing the concentration of carotenoids (Petrenko & Biryukova, 1977). Also, Cytokinins appeared to play an important role in the regulation of cell division and increase plastids number, differentiation and organogenesis in developing plant (Skoog & Armstrong, 1970; Hall, 1973). The application of cytokinin may increase the content of chlorophylls in leaf tissues because it reduced chlorophyll degradation and delays the aging process (Xu et al., 2011). These findings corroborated those of (Jeffecoat, 1977) about *dianthus caryophyllus* and (Azza et al., 2014) on *Schefflera arboricola* plants.

2- Impact of GA_3 and CPPU on Total carbohydrates (mg/ g D.W):

As for the influence of the studied GA_3 and CPPU on total carbohydrates as mg/g D.W in the dried leaves of *Calendula officinalis* L plants, it was clear from data in (Table. 3) that applying GA_3 and CPPU significantly, enhanced the total carbohydrate as compared with the control plants. In addition, the highest value of total carbohydrate in both seasons were achieved using the GA_3 at 200 ppm (259.93and 254.03 mg/ g D.W) in the both seasons, respectively., as compared with the other treatment. This improvement in the total carbohydrate contents as a result of foliar spraying with GA_3 could be attributed to physiological role of GA_3 in enhancing leaf production (Table.3), which probably had higher chlorophyll content (Table.3) , which led to increase assimilation of leaf Co_2 (Kandil et al., 2011) and consequently more carbohydrates production, beside the effect of GA_3 on metabolism and enzyme levels responsible for biosynthesis and the mode of action of GA_3 in the activation of enzymes of carbohydrates transformation or in the regulation of the consumption sugars and the promotion of water and Co_2 absorption, which can be led to increase the capacity of plants in building metabolites. These results agree with those reported by (Eraki et al., 1993) on rose plants, (El- Naggar, 1999) on gladiolus plants, Gawade, *et al.*, (2019) on chrysanthemum and (El-Deeb, 2023) on Dahlia (*Dahlia variabilis* L.) plant.

Data presented in (Table. 3) cleared that, all CPPU treatments in two seasons significantly increased the total carbohydrate contents. The highest total carbohydrate contents found by CPPU 100 ppm with the mean of (231.79 and 233.86)and kinetin at the rate of 100 mgL^{-1} in the second season with the mean of (0.467- 0.431 mg/ g D.W). The increase in total carbohydrate may be due to increase the number of leaves and leaves weight/plant and to the role of cytokinins in increasing biosynthesis. Similar results were found by Hafez (1990) on marjoram plants and (Mahmoud, 1996) on *Ocimum basilicum* and (El-Deeb, 2023) on Dahlia plant.

Table (3): Impact of gibberellic acid (GA_3) and forchlorofenuronis (CPPU) on chlorophylls, carotenoids (mg/100g F.W.L) and total carbohydrates (mg/ g D.W) of *Calendula officinalis*, L. plant during 2020/2021 and 2021/2022 seasons.

PGR treatment (ppm)	Total chl (a +b)	Carotenoids	Total carbohydrates
		First Season	
Control	133.46	28.65	181.93
GA3 50	155.43	39.96	212.23
GA3 100	168.16	44.7	230.87
GA3 150	180.16	58.88	245.82
GA3 200	193.65	68.49	259.93
CPPU 25	146.47	30.1	197.79
CPPU 50	149.82	36.89	213.52
CPPU 75	154.36	47.34	226.68
CPPU 100	159.94	48.16	231.79
L.S.D $_{(0.05)}$	3.15	1.53	3.17
		Second Season	
Control	134.15	28.40	193.00
GA3 50	156.73	38.57	231.27
GA3 100	167.75	44.72	242.59
GA3 150	183.53	56.83	251.88
GA3 200	197.90	69.15	254.03
CPPU 25	148.40	30.82	198.29
CPPU 50	151.84	37.53	214.74
CPPU 75	156.93	47.12	228.03
CPPU 100	160.70	48.80	233.86
L.S.D $_{(0.05)}$	3.07	1.6	2.48

L.S.D $_{(0.05)}$ = Least significant differences at 0.05 level of probability; PGR, Plants Growth Regulators; GA_3, Gibberellic Acid; CPPU, Forchlorofenuron (N-(2 – chloro-4-pyridyl)-N'- phenylurea.

3- Impact of GA_3 and CPPU on macronutrients N, P and K (%):

The concerned data in (Table.4) showed that spraying *Calendula* plants with most concentrations of used GA_3 increased the nitrogen (N), phosphorous (P) and potassium (K) percentages in leaves compared with the control plants during the (2021 and 2022) seasons. Moreover, spraying GA_3 at (150 and/or 200 ppm) gave the highest percentages of nitrogen (3.61 and 3.60 %) in both seasons as compared with other treatments. While the highest percentage of P (0.38 and 0.39 %) and K (3.21and 3.25 %) was recorded when GA_3 at 200 ppm in both seasons were applied. On the other hand, the lowest N, P and K percentages in leaves were recorded for the untreated plant in both growing seasons of this study. GA_3 at optimum rate (150 and/or200 ppm) increased the amount of N, P and K in leaves compared with the other GA_3 concentrations. This may be due to the role of GA_3 in promoting active roots and these results may be attributed to the enhancing impact of GA_3 at suitable rate on the assimilation and accumulation of the major elements specially nitrogen (NH4), phosphorus (P). Similar results were obtained by Farima and Flupi (1987) on carnation, Abdelgawad (2020) on *Matricaria chamomilla*, L. and Harhash, *et al* (2023) on Dahlia.

There was a significant effect of (CPPU) treatments on N, P and K % in leaves of *Calendula*. The highest percentage of leaf macro-mineral content (N, P and K) was produced by using (CPPU) at the rate of 100 ppm in the two seasons (2.56, 0.32, 2.27 and 2.60, 0.50, 2.25 %, respectively). This result was supported by the findings of Ruffel *et*

al. (2011), as they cleared that cytokinins appear to increase the elements content in plants. Similarly, (Singh & Paliwal, 2017) revealed that utilization of kinetin expanded phosphorus, nitrogen and protein content in okra fruit, and this might be because of plant senescence. Also, (Moatshe et al. 2011) found that leaf mineral content of Morula tree sprayed with BAP had significantly higher than control trees.

Table (4): Impact of gibberellic acid (GA_3) and forchlorofenuronis (CPPU) on Macro-nutrients (N, P and K%) of *Calendula officinalis*, L. plant during 2020/2021 and 2021/2022 seasons.

PGR treatment (ppm)	N %	P %	K %
	First Season		
Control	1.6	0.16	1.42
GA3 50	1.93	0.23	1.61
GA3 100	2.29	0.29	2.12
GA3 150	3.59	0.33	3.19
GA3 200	3.61	0.38	3.21
CPPU 25	1.72	0.2	1.55
CPPU 50	1.98	0.22	1.8
CPPU 75	2.16	0.3	2.07
CPPU 100	2.56	0.32	2.27
L.S.D $_{(0.05)}$	0.13	0.05	0.06
	Second Season		
Control	1.62	0.18	1.45
GA3 50	1.94	0.25	1.65
GA3 100	2.31	0.3	2.32
GA3 150	3.58	0.34	3.12
GA3 200	3.6	0.39	3.25
CPPU 25	1.74	0.21	1.59
CPPU 50	2.97	0.22	1.88
CPPU 75	2.27	0.31	2.1
CPPU 100	2.6	0.32	2.3
L.S.D $_{(0.05)}$	0.12	0.05	0.06

L.S.D $_{(0.05)}$ = Least significant differences at 0.05 level of probability; PGR, Plants Growth Regulators; GA_3, Gibberellic Acid; CPPU, Forchlorofenuron (N-(2 – chloro-4-pyridyl)-N‘- phenylurea.

Conclusion:

The findings from this study provide significant insights into the influence of Gibberellic Acid (GA_3) and Forchlorofenuron (CPPU) on the growth, flowering characteristics, and chemical composition of *Calendula officinalis*, L.

Where, The application of GA_3 and CPPU resulted in notable improvements across various growth parameters.The treatment with GA_3 at 200 ppm significantly increased plant height, number of branches, leaf number, and shoot dry weight in both seasons. The GA_3-treated plants exhibited superior growth compared to the control, with the highest values observed at the 200-ppm concentration. This enhancement can be attributed to GA_3's role in promoting cell division, elongation, and membrane permeability, leading to greater vegetative growth. CPPU also demonstrated significant positive effects, particularly at 100 ppm, enhancing plant height, branch number, leaf number, and shoot dry weight. The cytokinin activity of CPPU promoted lateral bud growth and differentiation,contributing to these improvements.GA_3 at 200 ppm led to the highest number of inflorescences and largest inflorescence diameters. This treatment also resulted in the highest fresh and dry weights of inflorescences, likely due to increased nutrient uptake and photosynthetic efficiency facilitated by GA_3.CPPU treatments, particularly at 75 and 100 ppm, significantly increased the number and diameter of inflorescences, as

well as the fresh and dry weights of inflorescences. The cytokinin effect of CPPU likely enhanced meristematic activity and delayed senescence, promoting prolonged flowering. GA_3 at 200 ppm significantly increased the total chlorophyll (a+b) and carotenoid content, as well as total carbohydrate content in the leaves. This suggests enhanced photosynthetic capacity and metabolic activity, which are essential for plant growth and development. CPPU at 100 ppm also improved chlorophyll and carotenoid content and increased total carbohydrates, indicating a similar enhancement in photosynthetic and metabolic processes. Overall, the application of GA_3 and CPPU demonstrated considerable potential in enhancing the growth, flowering, and chemical properties of Calendula officinalis, L. The results indicate that these plant growth regulators can be effectively utilized to optimize the cultivation of Calendula for both ornamental and medicinal purposes. Future studies could further explore the underlying mechanisms and long-term effects of these treatments to refine application strategies for maximum benefit.

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